ISSN 1007-9327
CN 14-1219/R

World Journal of Gastroenterology®

Indexed and Abstracted in:

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

Volume 15 Number 6
February 14, 2009
World J Gastroenterol
2009 February 14; 15(6): 641-768

Online Submissions
wjg.wjgnet.com
www.wjgnet.com
Printed on Acid-free Paper
世界胃肠病学杂志

A Weekly Journal of Gastroenterology and Hepatology

World Journal of Gastroenterology ®

Editorial Board

2007-2009

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

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

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

Austria

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

Belarus

Yury K Marakhouski, *Minsk*

Belgium

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

Brazil

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

Bulgaria

Zahariy Krastev, *Sofia*

Canada

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

Chile

Silvana Zanlungo, *Santiago*

China

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

Croatia

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

Cuba

Damian C Rodriguez, *Havana*

Czech

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

Denmark

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

Egypt

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

Estonia

Riina Salupere, *Tartu*

Finland

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

France

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

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

Germany

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

Greece

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

Hungary

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

Iceland

Hallgrimur Gudjonsson, *Reykjavik*

India

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

Iran

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

Ireland

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

Israel

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

Italy

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

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

Japan

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

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

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

Lithuania
Limas Kupcinskas, *Kaunas*

Macedonia
Vladimir C Serafimoski, *Skopje*

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

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

Monaco
Patrick Rampal, *Monaco*

Morocco
Abdellah Essaid, *Rabat*

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

New Zealand
Ian D Wallace, *Auckland*

Nigeria
Samuel B Olaleye, *Ibadan*

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

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

Peru
Hector H Garcia, *Lima*

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

Portugal
Miguel C De Moura, *Lisbon*

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

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

Serbia
Dusan M Jovanovic, *Sremska Kamenica*

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

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

Slovenia
Sasa Markovic, *Ljubljana*

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

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

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

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

Sweden

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

Switzerland

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

Turkey

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

United Arab Emirates

Sherif M Karam, *Al-Ain*

United Kingdom

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

United States

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

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

Uruguay

Henry Cohen, *Montevideo*

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

World Journal of
Gastroenterology®
Weekly Established in October 1995
Volume 15 Number 6
February 14, 2009

Contents

Contents

World Journal of Gastroenterology
Volume 15 **Number** 6 **February** 14, 2009

Contents

World Journal of Gastroenterology
Volume 15 **Number** 6 **February** 14, 2009

INTRODUCTION

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

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

NAME OF JOURNAL
World Journal of Gastroenterology

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

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

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

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

PRINTING
Beijing Kexin Printing House

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

PUBLICATION DATE
February 14, 2009

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

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

CSSN
ISSN 1007-9327
CN 14-1219/R

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

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.641

World J Gastroenterol 2009 February 14; 15(6): 641-646
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

A shield against a monster: Hepatitis C in hemodialysis patients

Seyed-Moayed Alavian

Seyed-Moayed Alavian, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Tehran 1998136194, Iran
Author contribution: Alavian SM contributed all to this paper.
Correspondence to: Seyed-Moayed Alavian, Professor, Internal Medicine, Gastroenterology and Hepatology, Baqiyatallah Research Center for Gastroenterology and Liver Disease, Vanaq Square, Mola Sadra St., PO Box 14155-3651, Tehran 1998136194, Iran. alavian@thc.ir
Telephone: +98-21-88945186 Fax: +98-21-88945188
Received: May 12, 2008 Revised: July 15, 2008
Accepted: July 22, 2008
Published online: February 14, 2009

Abstract

Hepatitis C virus (HCV) infection is highly prevalent among patients on hemodialysis (HD). The prevalence of HCV infection in HD patients varies markedly from country to country. Some factors are especially related to these high prevalence rates, such as blood transfusions and length of dialysis time. Nosocomial routes of transmission including the use of contaminated equipment and patient-to-patient exposure is considered more important. Several prophylactic measures have been suggested to avoid infection by HCV in the HD environment.

Key words: Dialysis; Epidemiology; Hepatitis C virus; Incidence; Isolation; Nosocomial transmission; Prevalence; Prevention; Universal precaution

Alavian SM. A shield against a monster: Hepatitis C in hemodialysis patients. *World J Gastroenterol* 2009; 15(6): 641-646 Available from: URL: http://www.wjgnet.com/1007-9327/15/641.asp DOI: http://dx.doi.org/10.3748/wjg.15.641

INTRODUCTION

Hepatitis C virus (HCV) infection is considered a major public health problem worldwide. Patients with chronic renal failure who are on hemodialysis (HD) have a high prevalence of HCV. They are among the highest risk groups for the acquisition of HCV infection[1-3]. Prevalence of HCV infection has decreased in this group in recent years[4], but still remains a significant public health concern. HCV-infected patients on HD have significant liver disease and a decreased life expectancy[5,6]. The relative risk for death in HCV-infected patients on HD compared with non-infected patients is greater than 1.4[6-8]. In addition, HCV infection leads to decreased graft and patient survival in renal transplant recipients[9,10]. On the other hand, due to the increased prevalence of non-communicable diseases such as diabetes mellitus and hypertension, and their complications, chronic renal failure has become a more serious health issue throughout the world[11]. Therefore, the clinical importance of HCV has been increasingly recognized in the dialysis community.

PREVALENCE AND INCIDENCE

HCV prevalence in HD varies geographically, both within and between countries[12]. The reported anti-HCV seropositivity since 1999 ranges from low (1.9%) in the Slovenian 2001 Annual Report[13] to high (80%) in Senegal[14]. HCV seroprevalence in the HD population was 59% in Bosnia and Herzegovina[15] , 6.8% in Belgium[16], 16.3% in France[2], 6.1% in Germany[17], 10%-29% in Greece[18-20], 22.5%-32.1% in Italy[21,22], 75% in Moldavia[23], 3.4% in the Netherlands[24], 1.9% in Slovenia[13], 11% in Sweden[25], 7%-23.3% in the USA[26-30], 4% in the UK[31] 20.5% in Libya[32], 71% in Kuwait[31] 80% in Senegal[14], 23.7% in Sudan[33], 19%-41.7% in Tunisia[34,35], 8.4%-43.2% in Brazil[36-39], 6.7% in Mexico[40], 59.3% in Peru[41], 3.5% in Puerto Rico[42] and 13.2% in Iran[43]. Some investigators have suggested a decline in HCV prevalence among HD patients in recent years, mostly attributable to strict adherence to universal precautions with[16,44-49] or without[50,51] observing isolation measures. This decrease is more significant in the USA and European countries[4,16,47,52]. Studies that have prospectively followed HD patients for their HCV serostatus have yielded annual incidence rates of de novo HCV infection of 0.4% in France[53], 0.5% in Tunisia[54], 0.5% in the Netherlands[24], 0.83% in Italy[55], 1.38%[56] and 2.1%[57] in the USA, 0.33%[58], 2.59%[59], 3.1% in Japan[60], 3.7%[61] 5.5% in Brazil[62], and 6.2% in Greece[20]. This variation in different countries and different centers underlines the importance of infection

control. New infection was evidently more frequent at centers that had higher anti-HCV prevalence and failure in infection control measures. In some countries, both prevalence and incidence remain very high, indicating major ongoing nosocomial transmission, probably due to the limited resources available to treat a rapidly growing HD population[63,64].

RISK FACTORS FOR HCV TRANSMISSION

The high prevalence of HCV seropositivity among HD patients was initially attributed to blood transfusions for the treatment of uremic anemia in this population, which were often necessary[41,65-68]. Historically, the number of blood transfusions received was consistently reported in the literature to be associated with an increased prevalence of HCV-positive dialysis patients[31]. However, several recent reports have failed to recognize blood transfusion as an independent risk factor in HCV spread among HD subjects[2,20,23,24,30,33,62,69-74]. Indeed, from the late 1980s onward, the prescription of erythropoietin reduced the need for blood transfusion in HD patients. Furthermore, the introduction of sensitive tests for screening blood donors has markedly reduced the risk of HCV transmission through blood product transfusion. These two reasons may explain recent findings on the lack of association between blood transfusion and HCV infection. Nonetheless, considering the fact that new HCV infections do still occur in patients without a history of transfusion, the duration of HD is increasingly being considered as a risk factor for HCV infection[75,76]. Almost all recent surveys on the subject have congruently suggested that the length of time on HD is a risk factor for HCV seropositivity[17,20,23,29,30,33,36,39,43,60,69-71,77-80]. Nosocomial patient-to-patient transmission of HCV infection among HD patients has been suggested by several investigators who performed phylogenetic analysis of HCV viral isolates[24,25,53,54,81-84]. Lack of strict adherence to universal precautions by staff and sharing of articles such as multidose drugs might be the main mode of nosocomial HCV spread among HD patients[54,82,84-86]. Although some studies found that nosocomial spread of HCV declined when HCV-infected patients were treated in dedicated HD units[44-49,87,88], other investigators could control nosocomial spread of HCV among HD patients by the strict application of hygienic precautions, without isolation of HCV-infected subjects or machine segregation[12,50,89]. Indeed, the observed efficacy of isolation might simply be due to the prevented sharing of articles between patients and might reflect a better implementation of other hygienic precautions.

The spread of HCV infection in HD units is mainly due to nosocomial transmission between patients[53,88,90-94]. The importance of this route of transmission is demonstrated by the high HCV prevalence in some HD units and by the lower infection rate in patients on peritoneal dialysis compared with those on HD. A high prevalence of patients with HCV infection in HD facilities has been considered a risk factor for transmission of the infection. However, there is no consensus on the necessity for infection control isolation of HCV-positive patients for at least two reasons: firstly, the infectivity of HCV is lower than that of the hepatitis B virus; and secondly, the criteria for patients to be isolated remain to be defined. On the contrary, some HD patients are infected with HCV but do not have antibodies. Detection of viral RNA by reverse-transcription polymerase chain reaction (RT-PCR) is the only method to confirm HCV infection, however, this technique is not available at all centers.

PREVENTIVE STRATEGIES

Several prophylactic measures have been suggested to avoid infection by HCV in the HD environment, and range from isolating patients with HCV infection[44,47,48,88,95], to adopting a series of biosafety measures specific for HD, such as preparing medications in a separate area, cleaning and disinfecting dialysis station surfaces, washing hands and changing gloves between patient contacts, and items dedicated for use only with a single patient[12,39,50,96]. Strict adherence to universal infection control precautions seems to be enough to control the spread of disease in HD units[12,50,89,97-99]. Some reports have recommended the adoption of infection control isolation measures at centers with a high HCV prevalence[47,87,100,101] or if the staff/patient ratio at the center is lower than 28/100[102]. At centers with a high prevalence of HCV infection and in developing countries, universal precautions may not always be possible to implement. Thus isolation measures for HCV-positive patients should be implemented[47]. The CDC recommends that special precautions be observed in dialysis units including the wearing and changing of gloves and water-proof gowns between patients; systematic decontamination of the equipment, circuits, and surfaces after each patient treatment; no sharing of instruments (e.g. tourniquets) or medications (e.g. multiuse vials of heparin) among patients; and the assignment of patients to specific HD units[97]. Clearly, it is necessary to attempt, one step at a time, to minimize intradialytic or intracenter HCV transmission.

CONCLUSION

In summary, despite the marked decrease in anti-HCV prevalence in HD patients in many countries, the disappearance of HCV from HD units should not be expected for decades. Universal infection control precautions are the keystone in the prevention of nosocomial HCV transmission in HD units; however, isolation measures, including health care monitoring of infected patients and providing care in a dedicated section of the unit, improve prevention results. Those HD units with a high HCV prevalence or in which there is no fulltime infection control personnel dedicated to the infected patients during HD sessions may have a greater risk of seroconversion. Therefore, isolation in

special units or dialyzing patients in specific sessions must be considered[44]. As HCV-infected HD patients serve as a reservoir of infection for other patients, HD staff, and the transplant team[28,103,104] at HD centers must be aware of new HCV infections in order to review their practices and increase vigilance. Public health authorities should be aware about the prevalence and incidence of HCV infection in HD patients in different cities in their respective countries, so that changes can be proposed and the risks of infection among patients can be assessed. Implementation of surveillance systems and continuing education of the unit's personnel on recommended infection control measures in HD units are necessary. The treatment of most HCV-infected patients with interferon alpha can significantly contribute to decreasing HCV infection in this group in the future[105]. Successful control of infection requires further studies to assess the effectiveness of different preventive policies.

REFERENCES

1 **Barril G**. Hepatitis C virus-induced liver disease in dialysis patients. *Nephrol Dial Transplant* 2000; **15** Suppl 8: 42-45
2 **Salama G**, Rostaing L, Sandres K, Izopet J. Hepatitis C virus infection in French hemodialysis units: a multicenter study. *J Med Virol* 2000; **61**: 44-51
3 **Alavian SM**, Hosseini-Moghaddam SM, Rahnavardi M. Hepatitis C among hemodialysis patients: a review on epidemiologic, diagnostic, and therapeutic features. *Hep Mon* 2007; **7**: 153-162
4 **Espinosa M**, Martn-Malo A, Ojeda R, Santamara R, Soriano S, Aguera M, Aljama P. Marked reduction in the prevalence of hepatitis C virus infection in hemodialysis patients: causes and consequences. *Am J Kidney Dis* 2004; **43**: 685-689
5 **Mathurin P**, Mouquet C, Poynard T, Sylla C, Benalia H, Fretz C, Thibault V, Cadranel JF, Bernard B, Opolon P, Coriat P, Bitker MO. Impact of hepatitis B and C virus on kidney transplantation outcome. *Hepatology* 1999; **29**: 257-263
6 **Stehman-Breen CO**, Emerson S, Gretch D, Johnson RJ. Risk of death among chronic dialysis patients infected with hepatitis C virus. *Am J Kidney Dis* 1998; **32**: 629-634
7 **Pereira BJ**, Natov SN, Bouthot BA, Murthy BV, Ruthazer R, Schmid CH, Levey AS. Effects of hepatitis C infection and renal transplantation on survival in end-stage renal disease. The New England Organ Bank Hepatitis C Study Group. *Kidney Int* 1998; **53**: 1374-1381
8 **Hanafusa T**, Ichikawa Y, Kishikawa H, Kyo M, Fukunishi T, Kokado Y, Okuyama A, Shinji Y, Nagano S. Retrospective study on the impact of hepatitis C virus infection on kidney transplant patients over 20 years. *Transplantation* 1998; **66**: 471-476
9 **Gentil MA**, Rocha JL, Rodríguez-Algarra G, Pereira P, López R, Bernal G, Muñoz J, Naranjo M, Mateos J. Impaired kidney transplant survival in patients with antibodies to hepatitis C virus. *Nephrol Dial Transplant* 1999; **14**: 2455-2460
10 **Knoll GA**, Tankersley MR, Lee JY, Julian BA, Curtis JJ. The impact of renal transplantation on survival in hepatitis C-positive end-stage renal disease patients. *Am J Kidney Dis* 1997; **29**: 608-614
11 **Aghighi M**, Heidary Rouchi A, Zamyadi M, Mahdavi-Mazdeh M, Norouzi S, Rajolani H, Ahrabi S, Zamani M. Dialysis in Iran. *IJKD* 2008; **2**: 11-15
12 **Jadoul M**, Cornu C, van Ypersele de Strihou C. Universal precautions prevent hepatitis C virus transmission: a 54 month follow-up of the Belgian Multicenter Study. The Universitaires Cliniques St-Luc (UCL) Collaborative Group. *Kidney Int* 1998; **53**: 1022-1025
13 **Buturović-Ponikvar J**. Renal replacement therapy in Slovenia: annual report 2001. *Nephrol Dial Transplant* 2003; **18** Suppl 5: v53-v55
14 **Diouf ML**, Diouf B, Niang A, Ka EH, Pouye A, Seck A, Raphenon G, Moreira-Diop T. [Prevalence of hepatitis B and C viruses in a chronic hemodialysis center in Dakar] *Dakar Med* 2000; **45**: 1-4
15 **Ahmetagić S**, Muminhodzić K, Cickusić E, Stojić V, Petrović J, Tihić N. Hepatitis C infection in risk groups. *Bosn J Basic Med Sci* 2006; **6**: 13-17
16 **Jadoul M**, Poignet JL, Geddes C, Locatelli F, Medin C, Krajewska M, Barril G, Scheuermann E, Sonkodi S, Goubau P. The changing epidemiology of hepatitis C virus (HCV) infection in haemodialysis: European multicentre study. *Nephrol Dial Transplant* 2004; **19**: 904-909
17 **Hinrichsen H**, Leimenstoll G, Stegen G, Schrader H, Fölsch UR, Schmidt WE. Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: a multicentre study in 2796 patients. *Gut* 2002; **51**: 429-433
18 **Rigopoulou EI**, Stefanidis I, Liaskos C, Zervou EK, Rizos C, Mina P, Zachou K, Syrganis C, Patsidis E, Kyriakopoulos G, Sdrakas L, Tsianas N, Dalekos GN. HCV-RNA qualitative assay based on transcription mediated amplification improves the detection of hepatitis C virus infection in patients on hemodialysis: results from five hemodialysis units in central Greece. *J Clin Virol* 2005; **34**: 81-85
19 **Garinis G**, Spanakis N, Theodorou V, Gorgoulis V, Manolis E, Karameris A, Valis D. Comparison of the enzyme-linked immunosorbant assay III, recombinant immunoblot third generation assay, and polymerase chain reaction method in the detection of hepatitis C virus infection in haemodialysis patients. *J Clin Lab Anal* 1999; **13**: 122-125
20 **Sypsa V**, Psichogiou M, Katsoulidou A, Skoutelis G, Moutafis S, Hadjiconstantinou V, Kakavas J, Kalapothaki V, Boletis J, Hatzakis A. Incidence and patterns of hepatitis C virus seroconversion in a cohort of hemodialysis patients. *Am J Kidney Dis* 2005; **45**: 334-343
21 **Lombardi M**, Cerrai T, Geatti S, Negroni S, Pertusini L, Pegoraro M, Di Lullo G. Results of a national epidemiological investigation of HCV infection in dialysis patients. *EDTNA ERCA J* 1999; **25**: 38-42
22 **Petrosillo N**, Gilli P, Serraino D, Dentico P, Mele A, Ragni P, Puro V, Casalino C, Ippolito G. Prevalence of infected patients and understaffing have a role in hepatitis C virus transmission in dialysis. *Am J Kidney Dis* 2001; **37**: 1004-1010
23 **Covic A**, Iancu L, Apetrei C, Scripcaru D, Volovat C, Mititiuc I, Covic M. Hepatitis virus infection in haemodialysis patients from Moldavia. *Nephrol Dial Transplant* 1999; **14**: 40-45
24 **Schneeberger PM**, Keur I, van Loon AM, Mortier D, de Coul KO, van Haperen AV, Sanna R, van Der Heijden TG, van Den Hoven H, van Hamersvelt HW, Quint W, van Doorn LJ. The prevalence and incidence of hepatitis C virus infections among dialysis patients in the Netherlands: a nationwide prospective study. *J Infect Dis* 2000; **182**: 1291-1299
25 **Almroth G**, Ekermo B, Månsson AS, Svensson G, Widell A. Detection and prevention of hepatitis C in dialysis patients and renal transplant recipients. A long-term follow up (1989-January 1997). *J Intern Med* 2002; **251**: 119-128
26 **Kelley VA**, Everett-Kitchens J, Brannon LE, Connor K, Martinez EJ, Pearson TC, Nolte FS. Lack of seronegative hepatitis C virus infections in patients with chronic renal failure. *Transplantation* 2002; **74**: 1473-1475
27 **Kalantar-Zadeh K**, Miller LG, Daar ES. Diagnostic discordance for hepatitis C virus infection in hemodialysis patients. *Am J Kidney Dis* 2005; **46**: 290-300
28 **Saab S**, Martin P, Brezina M, Gitnick G, Yee HF Jr. Serum alanine aminotransferase in hepatitis c screening of patients on hemodialysis. *Am J Kidney Dis* 2001; **37**: 308-315

29 **Kalantar-Zadeh K**, Kilpatrick RD, McAllister CJ, Miller LG, Daar ES, Gjertson DW, Kopple JD, Greenland S. Hepatitis C virus and death risk in hemodialysis patients. *J Am Soc Nephrol* 2007; **18**: 1584-1593

30 **Sivapalasingam S**, Malak SF, Sullivan JF, Lorch J, Sepkowitz KA. High prevalence of hepatitis C infection among patients receiving hemodialysis at an urban dialysis center. *Infect Control Hosp Epidemiol* 2002; **23**: 319-324

31 **Wreghitt TG**. Blood-borne virus infections in dialysis units--a review. *Rev Med Virol* 1999; **9**: 101-109

32 **Daw MA**, Elkaber MA, Drah AM, Werfalli MM, Mihat AA, Siala IM. Prevalence of hepatitis C virus antibodies among different populations of relative and attributable risk. *Saudi Med J* 2002; **23**: 1356-1360

33 **El-Amin HH**, Osman EM, Mekki MO, Abderlraheem MB, Ismail MO, Yousif MEA, Abass A, Elhaj HS, Ammar HK. Hepatitis C virus infection in hemodialysis patients in Sudan:Two centers report. *Saudi J Kidney Dis Transplant* 2007; **18**: 101-106

34 **Bouzgarrou N**, Fodha I, Othman SB, Achour A, Grattard F, Trabelsi A, Pozzetto B. Evaluation of a total core antigen assay for the diagnosis of hepatitis C virus infection in hemodialysis patients. *J Med Virol* 2005; **77**: 502-508

35 **Ayed K**, Gorgi Y, Ben Abdallah T, Aouadi H, Jendoubi-Ayed S, Sfar I, Makni H. Hepatitis C virus infection in hemodialysis patients from Tunisia: national survey by serologic and molecular methods. *Transplant Proc* 2003; **35**: 2573-2575

36 **Albuquerque AC**, Coêlho MR, Lopes EP, Lemos MF, Moreira RC. Prevalence and risk factors of hepatitis C virus infection in hemodialysis patients from one center in Recife, Brazil. *Mem Inst Oswaldo Cruz* 2005; **100**: 467-470

37 **Lopes EP**, Gouveia EC, Albuquerque AC, Sette LH, Mello LA, Moreira RC, Coelho MR. Determination of the cut-off value of serum alanine aminotransferase in patients undergoing hemodialysis, to identify biochemical activity in patients with hepatitis C viremia. *J Clin Virol* 2006; **35**: 298-302

38 **Mello Lde A**, de Melo-Junior MR, de Albuquerque AC, Coelho MR. [Hepatitis C serum prevalence in hemodialyzed patients] *Rev Soc Bras Med Trop* 2007; **40**: 290-294

39 **Carneiro MA**, Martins RM, Teles SA, Silva SA, Lopes CL, Cardoso DD, Vanderborght BO, Yoshida CF. Hepatitis C prevalence and risk factors in hemodialysis patients in Central Brazil: a survey by polymerase chain reaction and serological methods. *Mem Inst Oswaldo Cruz* 2001; **96**: 765-769

40 **Méndez-Sánchez N**, Motola-Kuba D, Chavez-Tapia NC, Bahena J, Correa-Rotter R, Uribe M. Prevalence of hepatitis C virus infection among hemodialysis patients at a tertiary-care hospital in Mexico City, Mexico. *J Clin Microbiol* 2004; **42**: 4321-4322

41 **Sanchez JL**, Sjogren MH, Callahan JD, Watts DM, Lucas C, Abdel-Hamid M, Constantine NT, Hyams KC, Hinostroza S, Figueroa-Barrios R, Cuthie JC. Hepatitis C in Peru: risk factors for infection, potential iatrogenic transmission, and genotype distribution. *Am J Trop Med Hyg* 2000; **63**: 242-248

42 **López-Navedo PJ**, Lebrón-Rivera R, González-Trápaga J, Weber-Acevedo J, Lefevre-Ramos E, Flores-de Hostos E, Jaume-Anselmi F, Ramírez-Rivera J. Prevalence of hepatitis C virus infection at three hemodialysis units in the western region of Puerto Rico. *Bol Asoc Med P R* 1999; **91**: 100-102

43 **Alavian SM**, Einollahi B, Hajarizadeh B, Bakhtiari S, Nafar M, Ahrabi S. Prevalence of hepatitis C virus infection and related risk factors among Iranian haemodialysis patients. *Nephrology* (Carlton) 2003; **8**: 256-260

44 **Yang CS**, Chang HH, Chou CC, Peng SJ. Isolation effectively prevents the transmission of hepatitis C virus in the hemodialysis unit. *J Formos Med Assoc* 2003; **102**: 79-85

45 **Gallego E**, López A, Pérez J, Llamas F, Lorenzo I, López E, Illescas ML, Andrés E, Olivas E, Gómez-Roldan C. Effect of isolation measures on the incidence and prevalence of hepatitis C virus infection in hemodialysis. *Nephron Clin Pract* 2006; **104**: c1-c6

46 **Carneiro MA**, Teles SA, Dias MA, Ferreira RC, Naghettine AV, Silva SA, Lampe E, Yoshida CF, Martins RM. Decline of hepatitis C infection in hemodialysis patients in Central Brazil: a ten years of surveillance. *Mem Inst Oswaldo Cruz* 2005; **100**: 345-349

47 **Barril G**, Traver JA. Decrease in the hepatitis C virus (HCV) prevalence in hemodialysis patients in Spain: effect of time, initiating HCV prevalence studies and adoption of isolation measures. *Antiviral Res* 2003; **60**: 129-134

48 **Shamshirsaz AA**, Kamgar M, Bekheirnia MR, Ayazi F, Hashemi SR, Bouzari N, Habibzadeh MR, Pourzahedgilani N, Broumand V, Shamshirsaz AH, Moradi M, Borghei M, Haghighi NN, Broumand B. The role of hemodialysis machines dedication in reducing Hepatitis C transmission in the dialysis setting in Iran: a multicenter prospective interventional study. *BMC Nephrol* 2004; **5**: 13

49 **Saxena AK**, Panhotra BR, Sundaram DS, Naguib M, Venkateshappa CK, Uzzaman W, Mulhim KA. Impact of dedicated space, dialysis equipment, and nursing staff on the transmission of hepatitis C virus in a hemodialysis unit of the middle east. *Am J Infect Control* 2003; **31**: 26-33

50 **Valtuille R**, Moretto H, Lef L, Rendo P, Fernández JL. Decline of high hepatitis C virus prevalence in a hemodialysis unit with no isolation measures during a 6-year follow-up. *Clin Nephrol* 2002; **57**: 371-375

51 **Aucella F**, Vigilante M, Valente GL, Stallone C. Systematic monitor disinfection is effective in limiting HCV spread in hemodialysis. *Blood Purif* 2000; **18**: 110-114

52 **dos Santos JP**, Loureiro A, Cendoroglo Neto M, Pereira BJ. Impact of dialysis room and reuse strategies on the incidence of hepatitis C virus infection in haemodialysis units. *Nephrol Dial Transplant* 1996; **11**: 2017-2022

53 **Izopet J**, Sandres-Sauné K, Kamar N, Salama G, Dubois M, Pasquier C, Rostaing L. Incidence of HCV infection in French hemodialysis units: a prospective study. *J Med Virol* 2005; **77**: 70-76

54 **Hmaied F**, Ben Mamou M, Saune-Sandres K, Rostaing L, Slim A, Arrouji Z, Ben Redjeb S, Izopet J. Hepatitis C virus infection among dialysis patients in Tunisia: incidence and molecular evidence for nosocomial transmission. *J Med Virol* 2006; **78**: 185-191

55 **Lombardi M**, Cerrai T, Geatti S, Negroni S, Pertusini L, Pegoraro M, Di Lullo G. Results of a national epidemiological investigation on HCV infection among dialysis patients. (Survey by the Italian Branch of EDTNA/ERCA). *J Nephrol* 1999; **12**: 322-327

56 **Fabrizi F**, de Vecchi AF, Como G, Lunghi G, Martin P. De novo HCV infection among dialysis patients: a prospective study by HCV core antigen ELISA assay. *Aliment Pharmacol Ther* 2005; **21**: 861-869

57 **Fabrizi F**, Martin P, Dixit V, Brezina M, Russell J, Conrad A, Schmid P, Gerosa S, Gitnick G. Detection of de novo hepatitis C virus infection by polymerase chain reaction in hemodialysis patients. *Am J Nephrol* 1999; **19**: 383-388

58 **Kumagai J**, Komiya Y, Tanaka J, Katayama K, Tatsukawa Y, Yorioka N, Miyakawa Y, Yoshizawa H. Hepatitis C virus infection in 2,744 hemodialysis patients followed regularly at nine centers in Hiroshima during November 1999 through February 2003. *J Med Virol* 2005; **76**: 498-502

59 **Furusyo N**, Hayashi J, Kakuda K, Ariyama I, Kanamoto-Tanaka Y, Shimizu C, Etoh Y, Shigematsu M, Kashiwagi S. Acute hepatitis C among Japanese hemodialysis patients: a prospective 9-year study. *Am J Gastroenterol* 2001; **96**: 1592-1600

60 **Fissell RB**, Bragg-Gresham JL, Woods JD, Jadoul M, Gillespie B, Hedderwick SA, Rayner HC, Greenwood RN, Akiba T, Young EW. Patterns of hepatitis C prevalence and seroconversion in hemodialysis units from three continents:

the DOPPS. *Kidney Int* 2004; **65**: 2335-2342
61 **Moreira R**, Pinho JR, Fares J, Oba IT, Cardoso MR, Saraceni CP, Granato C. Prospective study of hepatitis C virus infection in hemodialysis patients by monthly analysis of HCV RNA and antibodies. *Can J Microbiol* 2003; **49**: 503-507
62 **Santos MA**, Souto FJ. Infection by the hepatitis C virus in chronic renal failure patients undergoing hemodialysis in Mato Grosso state, central Brazil: a cohort study. *BMC Public Health* 2007; **7**: 32
63 **Vladutiu DS**, Cosa A, Neamtu A, State D, Braila M, Gherman M, Patiu IM, Dulau-Florea I. Infections with hepatitis B and C viruses in patients on maintenance dialysis in Romania and in former communist countries: yellow spots on a blank map? *J Viral Hepat* 2000; **7**: 313-319
64 **Rutkowski B**. Changing pattern of end-stage renal disease in central and eastern Europe. *Nephrol Dial Transplant* 2000; **15**: 156-160
65 **Zeldis JB**, Depner TA, Kuramoto IK, Gish RG, Holland PV. The prevalence of hepatitis C virus antibodies among hemodialysis patients. *Ann Intern Med* 1990; **112**: 958-960
66 **Santana GO**, Cotrim HP, Mota E, Paraná R, Santana NP, Lyra L. [Antibodies to hepatitis C virus in patients undergoing hemodialysis in Salvador, BA, Brazil] *Arq Gastroenterol* 2001; **38**: 24-31
67 **Hruby Z**, Sliwiński J, Molin I, Zalewska M, Knysz B, Czyz W, Steciwko A, Bogucki J, Gładysz A. High prevalence of antibodies to hepatitis C virus in three haemodialysis centres in south-western Poland. *Nephrol Dial Transplant* 1993; **8**: 740-743
68 **Medeiros MT**, Lima JM, Lima JW, Campos Hde H, Medeiros MM, Coelho Filho JM. [Prevalence and associated factors to hepatitis C in hemodialysis patients in Brazil] *Rev Saude Publica* 2004; **38**: 187-193
69 **Amiri ZM**, Shakib AJ, Toorchi M. Seroprevalence of hepatitis C and risk factors in haemodialysis patients in Guilan, Islamic Republic of Iran. *East Mediterr Health J* 2005; **11**: 372-376
70 **Al-Shohaib SS**, Abd-Elaal MA, Zawawi TH, Abbas FM, Shaheen FA, Amoah E. The prevalence of hepatitis C virus antibodies among hemodialysis patients in Jeddah area, Saudi Arabia. *Saudi Med J* 2003; **2**: S125
71 **Ben Othman S**, Bouzgarrou N, Achour A, Bourlet T, Pozzetto B, Trabelsi A. [High prevalence and incidence of hepatitis C virus infections among dialysis patients in the East-Centre of Tunisia] *Pathol Biol* (Paris) 2004; **52**: 323-327
72 **Shaheen FA**, Huraib SO, Al-Rashed R, AlDrees A, Arif M, Al-Jeffry M, Tashkandy MA, Safwat M. Prevalence of hepatitis C antibodies among hemodialysis patients in Jeddah area, Saudi Arabia. *Saudi Med J* 2003, **2**: S125–S126
73 **Othman SB**, Trabelsi A, Monnet A, Bouzgarrou N, Grattard F, Beyou A, Bourlet T, Pozzetto B. Evaluation of a prototype HCV NS5b assay for typing strains of hepatitis C virus isolated from Tunisian haemodialysis patients. *J Virol Methods* 2004; **119**: 177-181
74 **López-Alcorocho JM**, Barril G, Ortiz-Movilla N, Traver JA, Bartolomé J, Sanz P, Selgas R, Carreño V. Prevalence of hepatitis B, hepatitis C, GB virus C/hepatitis G and TT viruses in predialysis and hemodialysis patients. *J Med Virol* 2001; **63**: 103-107
75 **Hardy NM**, Sandroni S, Danielson S, Wilson WJ. Antibody to hepatitis C virus increases with time on hemodialysis. *Clin Nephrol* 1992; **38**: 44-48
76 Hepatitis C virus antibodies in haemodialysis patients. *Lancet* 1990; **335**: 1409-1410
77 **Ahmetagić S**, Hantalasević L, Tihić N, Jusufović E, Stojić V. [Hepatitis C virus infection in hemodialysis patients in General Hospital Gracanica] *Med Arh* 2006; **60**: 298-300
78 **Ansar MM**, Kooloobandi A. Prevalence of hepatitis C virus infection in thalassemia and haemodialysis patients in north Iran-Rasht. *J Viral Hepat* 2002; **9**: 390-392
79 **Bdour S**. Hepatitis C virus infection in Jordanian haemodialysis units: serological diagnosis and genotyping. *J Med Microbiol* 2002; **51**: 700-704
80 **Hussein MM**, Mooij JM, Hegazy MS, Bamaga MS. The impact of polymerase chain reaction assays for the detection of hepatitis C virus infection in a hemodialysis unit. *Saudi J Kidney Dis Transpl* 2007; **18**: 107-113
81 **Sullivan DG**, Kim SS, Wilson JJ, Stehman-Breen C, Gretch DR. Investigating hepatitis C virus heterogeneity in a high prevalence setting using heteroduplex tracking analysis. *J Virol Methods* 2001; **96**: 5-16
82 **Iwasaki Y**, Esumi M, Hosokawa N, Yanai M, Kawano K. Occasional infection of hepatitis C virus occurring in haemodialysis units identified by serial monitoring of the virus infection. *J Hosp Infect* 2000; **45**: 54-61
83 **Kondili LA**, Genovese D, Argentini C, Chionne P, Toscani P, Fabro R, Cocconi R, Rapicetta M. Nosocomial transmission in simultaneous outbreaks of hepatitis C and B virus infections in a hemodialysis center. *Eur J Clin Microbiol Infect Dis* 2006; **25**: 527-531
84 **Delarocque-Astagneau E**, Baffoy N, Thiers V, Simon N, de Valk H, Laperche S, Couroucé AM, Astagneau P, Buisson C, Desenclos JC. Outbreak of hepatitis C virus infection in a hemodialysis unit: potential transmission by the hemodialysis machine? *Infect Control Hosp Epidemiol* 2002; **23**: 328-334
85 **Savey A**, Simon F, Izopet J, Lepoutre A, Fabry J, Desenclos JC. A large nosocomial outbreak of hepatitis C virus infections at a hemodialysis center. *Infect Control Hosp Epidemiol* 2005; **26**: 752-760
86 **Alfurayh O**, Sabeel A, Al Ahdal MN, Almeshari K, Kessie G, Hamid M, Dela Cruz DM. Hand contamination with hepatitis C virus in staff looking after hepatitis C-positive hemodialysis patients. *Am J Nephrol* 2000; **20**: 103-106
87 **Harmankaya O**, Cetin B, Obek A, Seber E. Low prevalence of hepatitis C virus infection in hemodialysis units: effect of isolation? *Ren Fail* 2002; **24**: 639-644
88 **Taskapan H**, Oymak O, Dogukan A, Utas C. Patient to patient transmission of hepatitis C virus in hemodialysis units. *Clin Nephrol* 2001; **55**: 477-481
89 **Gilli P**, Soffritti S, De Paoli Vitali E, Bedani PL. Prevention of hepatitis C virus in dialysis units. *Nephron* 1995; **70**: 301-306
90 **Abacioglu YH**, Bacaksiz F, Bahar IH, Simmonds P. Molecular evidence of nosocomial transmission of hepatitis C virus in a haemodialysis unit. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 182-186
91 **Irish DN**, Blake C, Christophers J, Craske JE, Burnapp L, Abbs IC, MacMahon EM, Muir P, Banatvala JE, Simmonds P. Identification of hepatitis C virus seroconversion resulting from nosocomial transmission on a haemodialysis unit: implications for infection control and laboratory screening. *J Med Virol* 1999; **59**: 135-140
92 **Kokubo S**, Horii T, Yonekawa O, Ozawa N, Mukaide M. A phylogenetic-tree analysis elucidating nosocomial transmission of hepatitis C virus in a haemodialysis unit. *J Viral Hepat* 2002; **9**: 450-454
93 **Olmer M**, Bouchouareb D, Zandotti C, de Micco P, de Lamballerie X. Transmission of the hepatitis C virus in an hemodialysis unit: evidence for nosocomial infection. *Clin Nephrol* 1997; **47**: 263-270
94 **Sánchez-Tapias JM**. Nosocomial transmission of hepatitis C virus. *J Hepatol* 1999; **31** Suppl 1: 107-12
95 **Rahnavardi M**, Hosseini Moghaddam SM, Alavian SM. Hepatitis C in hemodialysis patients: current global magnitude, natural history, diagnostic difficulties, and preventive measures. *Am J Nephrol* 2008; **28**: 628-640
96 Recommendations for preventing transmission of infections among chronic hemodialysis patients. *MMWR Recomm Rep* 2001; **50**: 1-43
97 **Flett A**, Teo M, Mah YI, Mortlock F, Choo BB, Challinor SP,

Woods HF. Low seroconversion for hepatitis C virus (HCV) antibody achieved by universal precautions alone. *EDTNA ERCA J* 1998; **24**: 40-42

98 **Jadoul M**. Transmission routes of HCV infection in dialysis. *Nephrol Dial Transplant* 1996; **11** Suppl 4: 36-38

99 **Natov SN**, Pereira BJ. Hepatitis C in dialysis patients. *Adv Ren Replace Ther* 1996; **3**: 275-283

100 **Arenas Jiménez MD**, Sánchez Paya J, González C, Rivera F, Enríquez R. Isolation of HCV patient is efficient in reducing the annual incidence of HCV infection, but is it really necessary? *Nephrol Dial Transplant* 1999; **14**: 1337-1339

101 **Djordjević V**, Stojanović K, Stojanović M, Stefanović V. Prevention of nosocomial transmission of hepatitis C infection in a hemodialysis unit. A prospective study. *Int J Artif Organs* 2000; **23**: 181-188

102 **Saab S**. Hepatitis C virus transmission in the hemodialysis community. *Am J Kidney Dis* 2001; **37**: 1052-1055

103 **Saab S**, Brezina M, Gitnick G, Martin P, Yee HF Jr. Hepatitis C screening strategies in hemodialysis patients. *Am J Kidney Dis* 2001; **38**: 91-97

104 **Pol S**, Romeo R, Zins B, Driss F, Lebkiri B, Carnot F, Berthelot P, Bréchot C. Hepatitis C virus RNA in anti-HCV positive hemodialyzed patients: significance and therapeutic implications. *Kidney Int* 1993; **44**: 1097-1100

105 **Alavian SM**. Hepatitis C, Chronic Renal Failure, Control Is Possible! *Hep Mon* 2006; **6**: 51-52

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

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.647

World J Gastroenterol 2009 February 14; 15(6): 647
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, Associate Professor, *Series Editor*

Editorial statement

Salvatore Gruttadauria, Bruno G Gridelli

In the following four articles, we will provide an overview of the current clinical work in different areas of liver transplantation.

For many decades, this transplantation has been the treatment choice for patients suffering from chronic and acute liver diseases.

Understanding of the complexity of this procedure can be read only through a multidisciplinary approach.

Liver transplantation has become a clinical reality thanks to the pioneer Thomas E Starzl, MD, PhD, who at the University of Colorado was one of the first to test cyclosporine in humans. Considered the father of liver transplantation, he performed the world's first liver transplant at the University of Colorado in 1963. Upon his arrival in Pittsburgh in 1981, when the university's liver transplant program began, Dr. Starzl continued research on the drug, which was approved by U.S. Food and Drug Administration (FDA) in November 1983.

Now, after many thousands of liver transplants have been successfully accomplished worldwide, the main problems to be solved remain the chronic shortage of organs and the need to investigate alternative and less aggressive forms of therapy, for the cure of end-stage liver disease.

The authors would like to thank Domenico Biondo for his help in editing these papers.

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.648

World J Gastroenterol 2009 February 14; 15(6): 648-674
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, Associate Professor, *Series Editor*

Pediatric liver transplantation

Marco Spada, Silvia Riva, Giuseppe Maggiore, Davide Cintorino, Bruno Gridelli

Marco Spada, Silvia Riva, Davide Cintorino, Bruno Gridelli, Istituto Mediterraneo per i Trapianti e Terapie ad alta specializzazione-IsMeTT, University of Pittsburgh Medical Center, 90127 Palermo, Italy
Giuseppe Maggiore, Department of Reproductive Medicine and Child Development, University of Pisa, 56100 Pisa, Italy
Author contributions: Spada M and Gridelli B were the principal authors of the paper, and wrote the following sections: introduction, prioritization, the transplant operation, early post-operative period, managing immunosuppressive therapy, late allograft dysfunction, outcome following transplantation; edited the final manuscript; Riva S and Maggiore G wrote the following sections: indications for liver transplantation, contraindications to liver transplantation, evaluation of the transplant candidate, infections, post-transplant lymphoproliferative disorders; Cintorino D was involved in much of the data acquisition and participated in the writing of the surgical sections of the manuscript; all authors gave their final approval for the paper.
Correspondence to: Marco Spada, MD, PhD, Istituto Mediterraneo per i Trapianti e Terapie ad alta specializzazione-IsMeTT, Via E. Tricomi 1, 90127 Palermo, Italy. mspada@ismett.edu
Telephone: +39-91-2192111 Fax: +39-91-2192400
Received: July 4, 2008 Revised: December 3, 2008
Accepted: December 10, 2008
Published online: February 14, 2009

Abstract

In previous decades, pediatric liver transplantation has become a state-of-the-art operation with excellent success and limited mortality. Graft and patient survival have continued to improve as a result of improvements in medical, surgical and anesthetic management, organ availability, immunosuppression, and identification and treatment of postoperative complications. The utilization of split-liver grafts and living-related donors has provided more organs for pediatric patients. Newer immunosuppression regimens, including induction therapy, have had a significant impact on graft and patient survival. Future developments of pediatric liver transplantation will deal with long-term follow-up, with prevention of immunosuppression-related complications and promotion of as normal growth as possible. This review describes the state-of-the-art in pediatric liver transplantation.

Key words: Pediatric liver transplantation; Indications; Surgical techniques; Complications

Peer reviewer: Michael Torbenson, MD, Associate Professor of Pathology, Room B314 1503 E Jefferson (Bond Street Building), The Johns Hopkins University School of Medicine, Baltimore, MD 21231, United States

Spada M, Riva S, Maggiore G, Cintorino D, Gridelli B. Pediatric liver transplantation. *World J Gastroenterol* 2009; 15(6): 648-674 Available from: URL: http://www.wjgnet.com/1007-9327/15/648.asp DOI: http://dx.doi.org/10.3748/wjg.15.648

INTRODUCTION

Liver transplantation has been very successful in treating children with end-stage liver disease, and offers the opportunity for a long healthy life. Organ scarcity, which is the main limitation to the full exploitation of transplantation, is being overcome thanks to innovative surgical techniques, and all children in need, even the youngest, today have the chance of being transplanted, with almost no waiting list mortality. Split-liver and living-donor transplantation have contributed to reversing a situation in which, during the 1980s and 90s, children had greater waiting list mortality compared to that of adult patients.

Several years ago, the main focus of care of children with end-stage liver disease was to find a liver transplant, but today, the main interest is in long-term follow-up, with prevention of immunosuppression-related complications and promotion of as normal growth as possible. The history of pediatric liver transplantation has clearly shown that success is dependent on strict and integrated collaboration between referring pediatricians, pediatric transplant hepatologists, transplant surgeons, nurses, transplant coordinators, psychologists and social workers. Everybody involved has the task of bringing a cure to a population of pediatric patients who present some of the most challenging clinical problems in modern medicine.

INDICATIONS FOR LIVER TRANSPLANTATION

The main indications for liver transplantation in the pediatric population are as follows: (1) Extra-hepatic cholestasis: biliary atresia. (2) Intra-hepatic

cholestasis: sclerosing cholangitis; Alagille's syndrome; non-syndromic paucity of intrahepatic bile ducts; and progressive familial intrahepatic cholestasis. (3) Metabolic diseases: Wilson's disease; α_1-antitrypsin deficiency; Crigler-Najjar syndrome; inborn error of bile acid metabolism; tyrosinemia; disorders of the urea cycle; organic acidemia; acid lipase defect; oxaluria type Ⅰ; and disorders of carbohydrate metabolism. (4) Acute liver failure. (5) Others: primary liver tumor and cystic fibrosis.

Cholestatic liver diseases

Typically, the child referred to a liver transplant center is a small baby with cholestatic liver disease. Out of 1187 children transplanted in North America between 1995 and May 2002, 33.5% were ⩽ 12 mo old at the time of transplantation, 55.6% had cholestatic disease, and 41.6% had biliary atresia. Of the children transplanted at < 1 year of age, 65.6% had biliary atresia[1]. Most of these children have undergone a Kasai procedure that failed to re-establish effective biliary flow, which caused rapid evolution to secondary biliary cirrhosis. When intrahepatic cholestatic diseases (Alagille's syndrome, progressive familial intrahepatic cholestasis, and sclerosing cholangitis) or sclerosing cholangitis are diagnosed, liver transplantation is indicated to eliminate severely debilitating symptoms, such as pruritus. Children affected by these diseases are also at high risk for the development of liver cancer[2].

Metabolic diseases

Metabolic diseases are the second most common indication for liver transplantation[3]. Metabolic diseases can be divided in two groups on the basis of the presence or absence of structural damage of the liver. To the first group belong α_1-antitripsin deficiency, tyrosinemia and Wilson's disease, which have the potential to progress to end-stage liver failure, liver cancer (Figure 1) and acute liver failure, while diseases such as Crigler-Najjar syndrome type Ⅰ and ornithine transcarbamylase (OTC) deficiency belong to the second group. In primary hyperoxaluria type Ⅰ, liver and kidney transplantation is considered when irreversible kidney damage from oxalic acid accumulation has developed. Different transplantation timings have been tested, combined liver and kidney transplantation (simultaneous or sequential) and pre-emptive liver transplantation (before end-stage renal failure occurs)[4,5]. Liver transplantation has been suggested recently for the treatment of organic acidemia (propionic aciduria, methylmalonic aciduria). In patients affected by these diseases, liver transplantation does not correct the enzyme deficiency in other organs beside the liver. Although quality of life is generally improved, patients remain at risk of severe extrahepatic disease complications[6-8]. Liver cirrhosis with severe portal hypertension develops in an about 25% of the patients affected by cystic fibrosis. Liver transplantation should be considered before the development of end-stage liver failure and when pulmonary function is still preserved (FEV_1 > 50%).

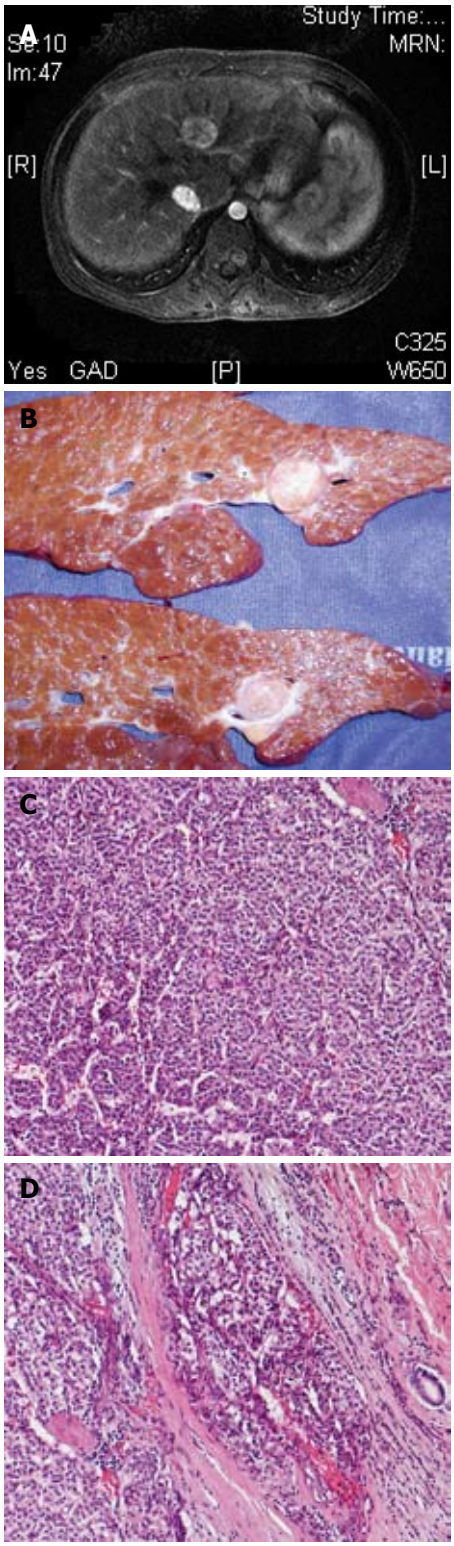


Figure 1 Adolescent affected by tyrosinemia who developed hepatocellular carcinoma, despite 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione therapy. A: Magnetic resonance imaging displays a 26-mm lesion. B: After liver transplantation, the resected liver showed multiple nodules in the left lobe. C: Histological sections from the nodule revealed hepatocellular carcinoma. D: Microvascular invasion.

Acute liver failure

Acute liver failure is a rare event in children; recovery without transplantation occurs in 15%-20% of patients with severe hepatic encephalopathy. A prospective study from the Pediatric Acute Liver Failure Study Group

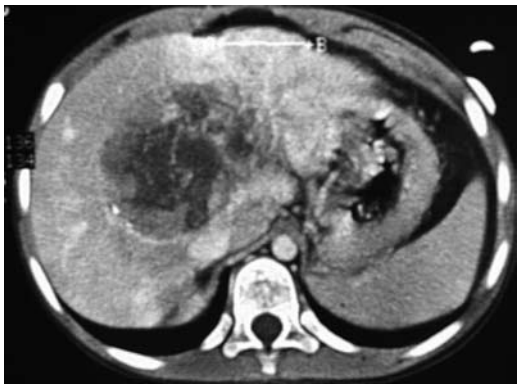


Figure 2 Non-resectable hepatoblastoma.

has indicated that in 49% of patients (54% of children aged 1 year), the cause of acute liver failure cannot be determined and that total bilirubin ⩾ 5 mg/dL, international normalized ratio (INR) ⩾ 2.55 and hepatic encephalopathy are risk factors predictive of death or liver transplantation[9]. In a large retrospective United Network for Organ Sharing (UNOS) data analysis, it has been shown that 5-year patient and graft survivals of children with acute liver failure are significantly lower than the survival of children transplanted for biliary atresia (73% and 59% *vs* 89% and 78%, respectively)[10].

Liver tumors

Hepatoblastoma is the most common liver tumor in children and, when non-resectable, should be treated with total hepatectomy and liver transplantation (Figure 2). Children with hepatoblastoma should first be treated with chemotherapy and then be evaluated for resection or transplantation[11]. Hepatocellular carcinoma in children is rare and is often secondary to congenital liver disease. The development of hepatocellular carcinoma has been reported in biliary atresia, Alagille's syndrome, progressive intrahepatic cholestasis (recently also hepatoblastoma has been reported in a child with this condition). In children with tyrosinemia, there is a 33% incidence of hepatocellular carcinoma before 2 years of age that seems to be reduced if not eliminated by 2-(2-nitro-4-3 trifluoromethylbenzoyl)-1,3-cyclohexanedione (NBTC) therapy.

CONTRAINDICATIONS TO LIVER TRANSPLANTATION

Current contraindications to liver transplantation in children are: (1) non-resectable extrahepatic malignant tumor; (2) concomitant end-stage organ failure that cannot be corrected by a combined transplant; (3) uncontrolled sepsis; and (4) irreversible serious neurological damage. Whereas in adults there are limitations to access to liver transplantation waiting lists for patients with primary liver tumors, in children, the approach is much more liberal and the indication should be discussed on a case by case analysis with pediatric oncologists.

EVALUATION OF THE TRANSPLANT CANDIDATE

The primary goal of the evaluation process is to identify appropriate candidates for liver transplantation and to establish a pre-transplantation plan. The following steps are usually considered: (1) confirm the indication for transplantation; (2) determine the severity of the disease; (3) consider alternative treatments to transplantation; (4) exclude contraindications to transplantation; (5) identify active infections and assess the immunological status of the child; (6) rule out cardiac malformations that might need to be corrected before transplantation; (7) establish a pre-transplant therapeutic plan: immunizations, when possible, nutritional support to optimize growth, dental care, prevention or treatment of drug-induced side effects (e.g. osteopenia secondary to prolonged steroid intake); (8) inform parents, and the patient if possible, on the transplantation procedure and on the post-transplantation period in order to motivate and prepare them to accept and deal with all issues and possible complications of the procedure; and (9) evaluate social status and logistic issues.

PRIORITIZATION

In the early 1980s, waiting time and severity of illness expressed by patient location (home, hospital, ICU) were the primary factors used to stratify patients. Later on, it was shown that waiting time had no relationship to mortality, except for urgent acute liver failure patients, and therefore, that an allocation policy based on objective medical criteria was needed. Based on data derived from the Studies of Pediatric Liver Transplantation research group, a pediatric end-stage liver disease score (PELD) was created, using bilirubin, INR, serum albumin, age > 1 year, and growth failure to predict waiting list mortality[12]. Additional PELD points are awarded for specific risk factors not taken into account in the PELD equation, such as hepatopulmonary syndrome, metabolic diseases, and liver tumors. The adoption of the PELD score in the USA has improved the access and accountability of the allocation system. However, the PELD score has not proven to be a successful predictor of outcome following transplantation[13,14].

THE TRANSPLANT OPERATION

The first liver transplant was performed by Thomas Starzl, in 1963, on a 2-year-old child affected by biliary atresia[15]. The patient died in the operating room of uncontrolled hemorrhage. After this first case, and up to the early 1980s, the only technical option for pediatric liver transplantation was to transplant the whole liver of a donor with a weight as close as possible to that of the recipient. Given the low number of pediatric donors, up to 50% of the children on the waiting list would die before they could receive a transplant[16]. The development of techniques that allow surgeons to transplant portions of livers from adult donors has

completely changed the fate of liver transplantation in pediatric patients.

Whole-liver transplantation

The procedure of whole-liver procurement in pediatric donors can be performed exactly as in adults, applying a technique that is a combination of the initial procurement technique described by Starzl *et al*[17], and the most recently described rapid flush technique[18,19]. Whole-liver pediatric transplantation can be performed with two different techniques: the classic technique with inferior vena cava replacement, and the piggyback technique[20] with preservation of the native inferior vena cava. The present authors routinely use the classic technique in the vast majority of whole liver transplants. Veno-venous bypass is generally not used in pediatric liver transplantation, given that patients generally tolerate explantation well, provided that volume replacement has been adequate. Adopted techniques are almost identical to the ones used in adults recipients. In cases in which the liver is encased in adhesions, as it is in biliary atresia, we recommend that surgeons first approach the hepatic hilum from the right posterolateral aspect, identifying the Roux-en-Y jejunal limb, which is transected with a linear stapler or between ligatures. This allows better exposure and dissection of the hilum. If the portal vein is small and sclerotic, it has to be dissected proximally to the confluence of the splenic and superior mesenteric vein, dividing the coronary vein of the stomach. The portal vein anastomosis will then be performed by means of a donor interposition vein graft. In difficult dissections, the vena cava can be clamped above and below the liver before completing the mobilization of the liver itself.

Several methods of arterial reconstruction have been proposed. It is our preference to anastomose the small arterial vessels encountered in pediatric whole liver transplantation in an end-to-end manner by using the magnification loops (3.5 ×) and interrupted or running 8-0 polypropylene sutures. We generally do not use the branch patch technique, and in the case of aberrant arterial anatomy, the supraceliac aorta is the inflow vessel of choice. The use of arterial conduits anastomosed to the infrarenal aorta is avoided if possible.

In theory, biliary tract continuity can be restored through direct anastomosis between the new liver's hepatic duct and the recipient's common bile duct. However, the most common type of biliary reconstruction adopted in pediatric patients is hepaticojejunostomy. In biliary atresia patients, the reconstruction uses the previous Roux-en-Y limb of the hepatic portoenterostomy, if suitable; otherwise a 40-cm Roux-en-Y jejunal limb is created. The authors' attitude is not to use a T tube, because no randomized trial so far has demonstrated any advantages in using it, and there are often biliary leaks when the T tube is pulled.

Occasionally in children, abdominal-wall closure may be impossible because of the large size of the transplanted liver. This may be remedied by creating a silo on the abdominal wall such that a temporary closure can be made[21].

Reduced-size liver transplantation

This procedure was first described by Bismuth *et al*[22] and consists in the procurement of the whole liver from an adult cadaver donor, which is reduced in its size on the back-table. In the original description, a right hepatectomy was performed on the back-table: the right lobe of the liver was discharged, while the left lobe (Couinaud liver segments 1 to 4), including the vena cava, was transplanted in a child. This technique of parenchymal reduction, very seldom used today, allows surgeons to overcome differences in size between the donor and the recipient of up to four or five times[23,24].

Following these first experiences, a more aggressive reduction that allows transplanting the liver from donors with a body weight up to 12 times the recipient's was introduced. On the back-table, the graft undergoes an extended right hepatectomy, including segment 4 and the caudate lobe. The resulting left lateral segment graft comprises segments 2 and 3, without the vena cava. The graft is transplanted retaining the recipient's vena cava, anastomosing the graft left hepatic vein to the recipient's vena cava.

Reduced-size liver transplantation shows outcomes in line, if not superior, to whole-liver transplantation, and has become an essential part of the technical expertise of pediatric transplant centers[25-30] (Table 1). The development of this technique has led to almost total elimination of child mortality on the waiting list, through the utilization of an adult liver cadaver donor. Its main limitation is that it withdraws organs from the larger adult recipient pool. For this reason, after the development of living-related and split-liver transplantation, reduced-size live transplantation is used increasingly less, and should not be considered an option anymore for pediatric liver transplantation.

Living-related liver transplantation

The first description of the procedure in which segments 2 and 3 were procured from a living donor (the mother), and transplanted in a child affected by biliary duct atresia, dates back to 1988[31,32]. Living-related liver transplants soon came to account for a substantial number of pediatric cases performed in many centers throughout the world, and the only possibility for liver transplants in countries where cadaveric organ procurement was not allowed until just a few years ago[33].

Living-donor procurement involves performing a left lobectomy during which segments 2 and 3 are separated from the remaining liver, and dissecting the parenchyma along a section running to the right of the round ligament. After the parenchyma dissection, the left branch of the portal vein, the hepatic artery, and the left suprahepatic vein are quickly clamped and dissected, and the left lobe perfused on the back-table. The recipient's procedure is similar to the one described for the transplant of segments 2 and 3 from a cadaver donor, except for the fact that the arterial anastomosis can be performed only in the left branch of the hepatic artery. The branch is small and usually anastomosed directly to the recipient's hepatic artery using the operative

Table 1 Series of pediatric reduced-size liver transplantation

Series	Period	*n*	Survival (%)		ReTX (%)	Complications (%)			
			Patient	Organ		HAT	PVT	BC	PNF
Broelsch *et al*[25]	1984-1987	9	44	33	11	0	0	11	11
Otte *et al*[26]	1984-1988	42	68	54	28	7	0	NA	5
Bismuth *et al*[22]	1984-1988	14	50	44	14	7	7	14	7
Houssin *et al*[27]	1986-1989	40	75	73	-	5	5	5	5
Kalayoglu *et al*[28]	1988-1989	12	83	67	25	16	8	0	0
Esquivel *et al*[29]	1988-1990	20	81	75	12	0	3	5	0
Langnas *et al*[30]	1988-1991	29	68	65	3	7	0	20	10

ReTX: Retransplantation; HAT: Hepatic artery thrombosis; PVT: Portal vein thrombosis; BC: Biliary complication; PNF: Primary non-function; NA: Not available.

Table 2 Series of pediatric living-related liver transplantation

Series	Period	*n*	Survival (%)		ReTX (%)	Complications (%)			
			Patient	Organ		HAT	PVT	BC	PNF
Tanaka *et al*[33]	1990-1992	37	E 90 U 57	E 90 U 57	0	U 14	E 3	E 10	0
Emond *et al*[34]	1991-1992	18	94	84	16	11	6	16	0
Broelsch *et al*[35]	1991	20	85	75	20	25	20	35	0
Malagò *et al*[36]	1991-1994	36	72	72	8	2.8	3	25	-
Otte *et al*[37]	1993-1995	30	97	93	-			20	
Haberal *et al*[38]	1990-1997	19	58	58	0	5	0	0	0
Darwish *et al*[39]	1993-2002	100	94	92	3	1	14	27	0

E: Elective cases; U: Urgent cases; ReTX: Retransplantation; HAT: Hepatic artery thrombosis; PVT: Portal vein thrombosis; BC: Biliary complication; PNF: Primary non-function.

microscope.

Living-related liver transplantation has been widely debated with regard to the ethics of performing major surgery on a healthy person. The validity of this procedure is broadly recognized, and over 1200 cases have been performed worldwide, with a donor mortality and morbidity of approximately 0.2% and 10%, respectively. Morbidity relates mainly to biliary fistulas, incisional hernias, and bleeding. In the majority of cases, living-related transplants register an excellent outcome for pediatric recipients, thanks to the possibility of performing the transplant before the child's clinical condition deteriorates. Centers with most experience in this area report survival rates between 80 and 90% after 1 year[34-39] (Table 2).

Split-liver transplantation

Split-liver transplantation, as described originally by Pichlmayr, involves procuring a whole liver from a cadaver donor and dividing it into two sections along the round ligament, leaving the vascular structures for the two portions of hepatic parenchyma intact[40]. In this way, two partial organs are obtained from a single liver: the left lateral segment (segments 2 and 3), which can be transplanted in a child, and the extended right liver (segments 1 and 4-8), which can be transplanted into an adult. This procedure involves a much longer ischemia time, which, at the beginning of its adoption, led to unsatisfactory results, with a high incidence of primary dysfunction and technical complications[41-55] (Table 3). In 1994, Rogiers described a technical variation in the split-liver technique, derived from the living-related transplant experience that consisted in dividing the liver *in situ* during the procurement procedure[56]. The technique has shown outcomes comparable to those obtained with conventional techniques[57-62] (Table 4).

The donor operation

A section of the liver is made along the falciform ligament to obtain a left graft, composed of segments 2 and 3, including the left hepatic vein, the left branch of the portal vein, and the left branch of the hepatic artery, along with the common hepatic artery and the celiac tripod, and a right graft, composed of segments 1 and 4 to 8, including the vena cava, the right branch of the hepatic artery, and the portal vein along with the origin of the mesenteric and splenic veins (Figure 3).

At the beginning of the split procedure, the hepatogastric ligament is inspected to detect an accessory left hepatic artery originating from the left gastric artery, which must be preserved. When this vessel is not detected, the ligament is sectioned. The common hepatic artery is then identified and dissected from the gastroduodenal artery up to its division into the right and left hepatic arteries. The left hepatic artery is then encircled (Figure 4A). If present, branches for the fourth segment originating from the left hepatic artery should be identified and divided. The base of the round ligament is exposed by dividing the small bridge of parenchyma that connects the lower portion of segment 4 to the left lateral section of the liver. The round ligament is dissected and completely mobilized with isolation and division of its venous connections to the fourth segment. Once the round ligament is dissected, the extrahepatic portion of the left branch of the portal vein can be identified just below the left hepatic artery.

Table 3 Series of *ex situ* split-liver transplantation

Series	Year	ADU (*n*)	PED (*n*)	Urgent (%)	Patient survival (%)		Graft survival (%)		Complications (%)			
					ADU	PED	ADU	PED	HAT	PVT	BC	PNF
Pichlmayr *et al*[40]	1989	2	0	0	50	-	50	-	0	0	0	0
Bismuth *et al*[41]	1989	2	0	100	0	-	0	-	0	0	0	0
Otte *et al*[42]	1990	1	3	75	0	66	0	66	0	0	0	0
Emond *et al*[16]	1990	5	13	38	40	63	40	53	6	6	27	24
Broelsch *et al*[24]	1990	4	21	40	25	66	20	48	NA	NA	27	NA
Langnas *et al*[30]	1992	1	9	73	NA	NA	NA	NA	7	0	20	17
Houssin *et al*[43]	1993	6	10	50	83	70	83	60	13	25	25	0
Otte *et al*[44]	1994	11	18	27	NA	NA	NA	NA	10	0	17	10
Kalayoglu *et al*[45]	1996	5	7	8	100	85	80	71	8	0	17	0
Rogiers *et al*[46]	1996	5	7	44	57	100	42	100	15	0	15	0
Azoulay *et al*[47]	1996	26	1	14	80	100	76	100	15	0	22	4
Dunn *et al*[48]	1997	0	12	50		75		66	0	0	0	0
Rela *et al*[49]	1998	15	26	12	93	89	93	84	3	0	15	0
Mirza *et al*[50]	1998	10	14	58	80	78	NA	NA	8	0	8	16
Chardot *et al*[51]	1999	0	15	31	-	66	-	62	12	19	25	0
Reyes *et al*[52]	2000	13	12	66	69	66	61	50	12	0	8	NA
Deshpande *et al*[53]	2002	0	80	20	-	89	-	86	5	1	9	0
Noujaim *et al*[54]	2003	24	36	25	NA	NA	NA	NA	3	0	20	3
Oswari *et al*[55]	2005	0	30	13	-	70	-	67	2	5	7	NA

ADU: Adults; PED: Children.

Table 4 Series of *in situ* split-liver transplantation

Series	Year	ADU (*n*)	PED (*n*)	Urgent (%)	Patient survival (%)		Graft survival (%)		Complications (%)			
					ADU	PED	ADU	PED	HAT	PVT	BC	PNF
Rogiers *et al*[56]	1996	7	7	35	100	85	85	71	0	0	0	0
Goss *et al*[57]	1997	14	12	58	85	100	78	91	0	0	14	11
Busuttil *et al*[58]	1999	NA	NA	66	85	96	86	75	3	1	3	8
Ghobrial *et al*[59]	2000	51	51	49	83	78	NA	NA	2	2	NA	8
Reyes *et al*[52]	2000	NA	NA	NA	93	100	79	83	3	0	3	7
Spada *et al*[60]	2000	36	35	25	84	85	79	76	5	10	28	2
Gridelli *et al*[61]	2003	0	90	28	-	90	-	80	7	6	33	1
Yersiz *et al*[62]	2003	57	104	-	78	75	69	64	13	11	19	26

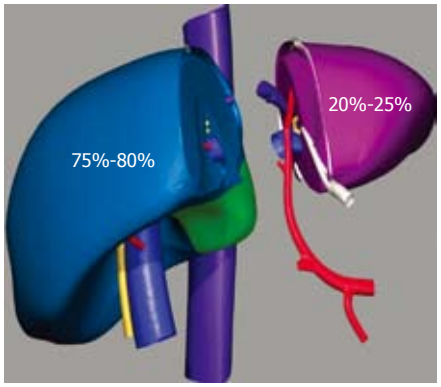


Figure 3 Spit liver allows for the procurement of two separate grafts of different size. A section of the liver is made along the falciform ligament and divides the left lateral segment from the extended right liver. The left graft, composed of segments 2 and 3, and representing 20%-25% of the total liver volume, includes the left hepatic vein, the left branch of the portal vein, and the left branch of the hepatic artery, along with the common hepatic artery and the celiac tripod. The right graft composed of segments 1 and 4-8, and representing 75%-80% of the total liver volume, includes the vena cava, the right branch of the hepatic artery, and the portal vein.

This vein must be carefully dissected and encircled (Figure 4B). The left lateral section is rotated laterally on the right side and the ligamentum venosum is dissected up to left lateral hepatic vein, which can be isolated and encircled (Figure 4C). The bile ducts of the left lateral segment are included in the porta hepatis and should not be dissected. On the contrary, the porta hepatis must be encircled and divided sharply (Figure 4D).

The section of the parenchyma can now be performed along the falciform ligament (Figure 4E). It is helpful when identifying the plane of the dissection to pass the cotton tape, which encircles the left hepatic vein on the posterior surface of the liver in the fossa of the ductus venosus, laterally to the left branch of the hepatic artery and of the portal vein (Figure 4F and G). Pulling up on this tape, the dissection of the parenchyma is usually easy. At this point, the procedure continues as a standard donor operation with heparinization, cannulation and cross-clamping of the aorta, perfusion, and cooling of the abdominal cavity. The left hepatic vein is then sectioned close to the vena cava. Care must be taken to identify a distal bifurcation of this vein. A double left hepatic vein significantly increases the technical difficulty of the implantation of the graft. In this case, the vessel should be removed with a cuff of vena cava to allow a single vascular anastomosis with

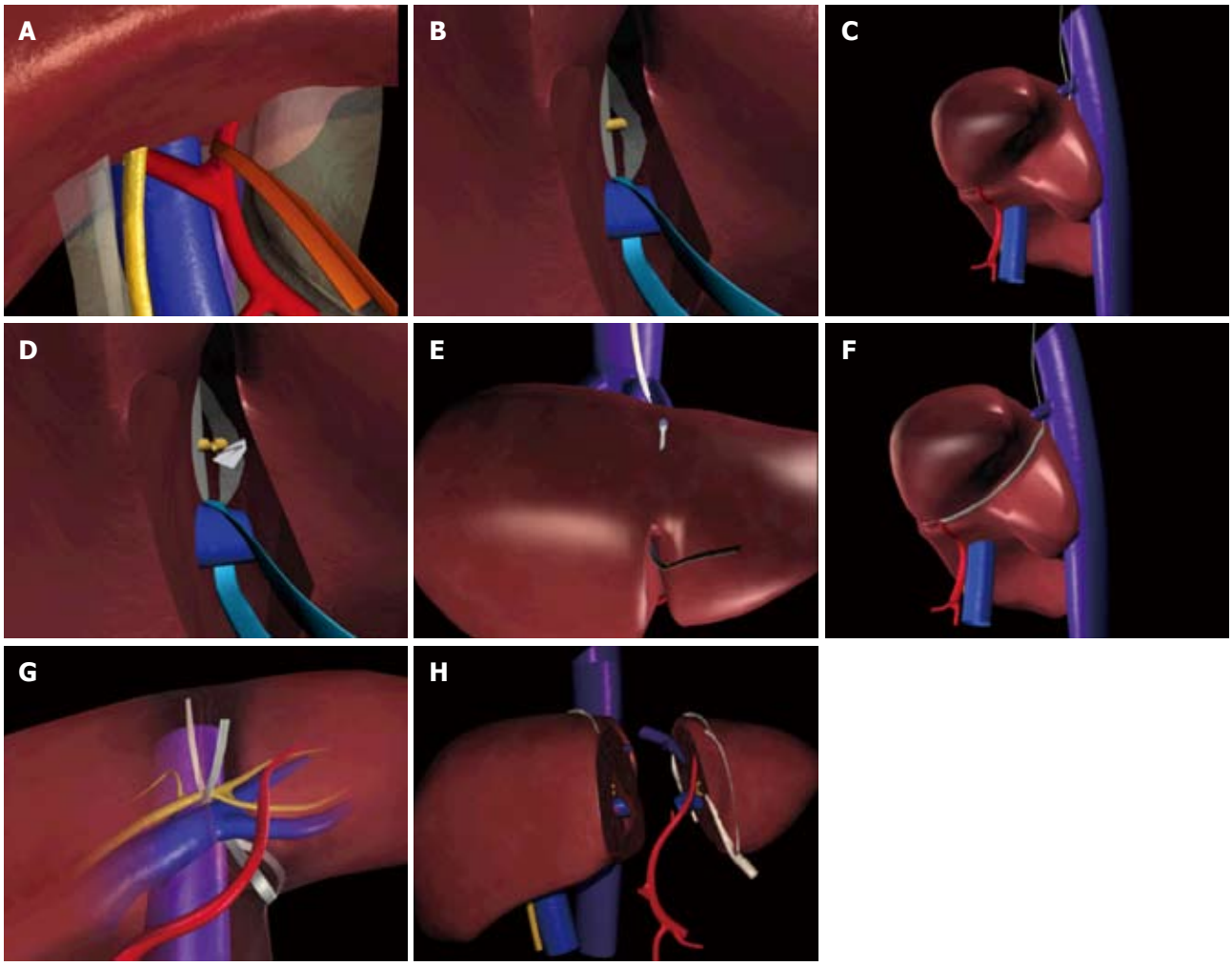


Figure 4 Main phases of split liver procurement. A: Dissection of the hepatogastric ligament and encircling of the left hepatic artery; B: Identification and encircling of the extrahepatic portion of the left branch of the portal vein; C: Isolation and encircling of the left hepatic vein; D: Division with a scalpel of the porta hepatis containing the bile duct(s) of the left lateral segment; E: Section of the parenchyma started along the falciform ligament; F: Identification of the plane of parenchymal dissection by passing the cotton tape, which encircled the left hepatic vein, on the posterior surface of the liver in the fossa of the ductus venosus; G: Laterally to the left branch of the hepatic artery and of the portal vein; H: The two partial grafts at the end of the procedure.

the recipient vena cava. The left branch of the portal vein is sectioned close to the parenchyma. The right hepatic artery is sectioned close to its origin, and the hepatic artery is dissected up to the celiac trunk, which is removed along with an aortic cuff.

The recipient operation

Recipient hepatectomy is performed, as previously described for whole-liver transplantation, with the piggy-back technique[63]. Implantation of the left lateral segment is substantially different from a whole-sized graft. Assuring an adequate venous outflow requires a careful technique of anastomosis between the left hepatic vein of the graft and the inferior vena cava of the recipient and a proper positioning of the graft itself, which is rotated clockwise 45° on a transversal plane and slightly on a frontal plane. The final position of the cut surface of the parenchyma, including the new hilum of the graft, is high and posterior, so that the portal vein and hepatic artery have a course that is curved and longer than usual.

The outflow anastomosis is end-to-side between the left hepatic vein of the graft and the inferior vena cava of the recipient, with the triangulation technique described by Emond *et al*[64]. The bridge between the ostia of the right and middle hepatic veins is cut to obtain a single opening. The ostium of the left hepatic vein may be treated in the same fashion, to obtain a further enlargement of the opening, or suture-closed. The opening is then enlarged by cutting the anterior face of the vena cava to obtain a wide reversed triangular orifice. The cuff of the left hepatic vein of the graft is trimmed as short as possible, to avoid kinking. Three 5/0 vascular monofilament sutures are placed, taking the three corners of the graft and recipient orifices (Figure 5). The graft is then placed in the hepatic fossa of the recipient and the triangular anastomosis performed with three running sutures.

The second anastomosis is the portal one, performed in an end-to-end fashion with running sutures of 6/0 or 7/0 vascular monofilament. Both the length and the section of the vessels are crucial. As already mentioned, the length should be sufficient for the vessel to make a gentle curve that reaches the hilum of the graft; as for

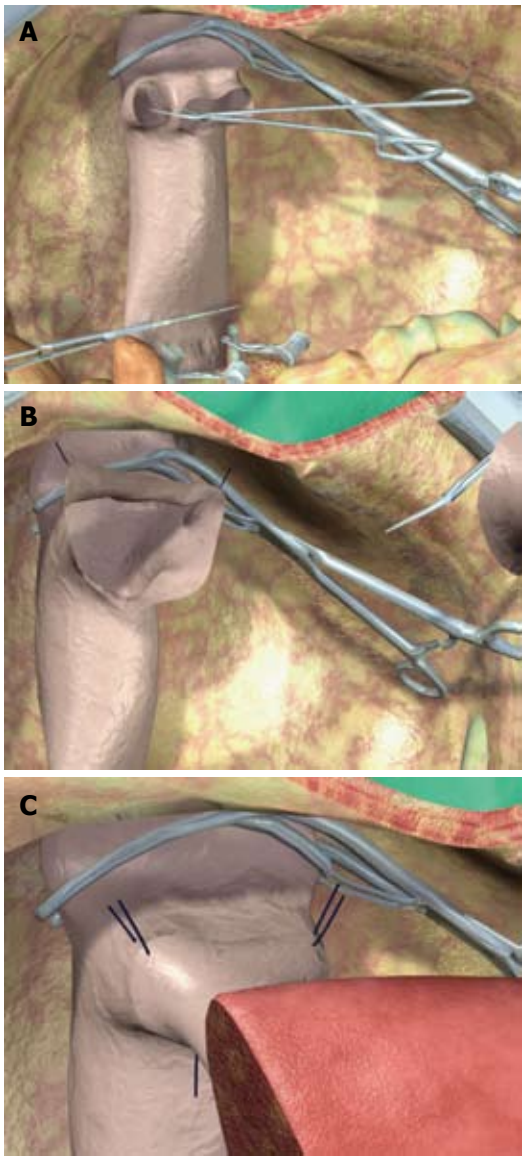


Figure 5 Anastomosis between the left hepatic vein of the graft and the inferior vena cava of the recipient, performed with the triangulation technique. A: The bridge between the ostia of the right, middle, and left hepatic veins is cut to obtain a single opening; B: The opening is further enlarged by cutting the anterior face of the vena cava to obtain a wide triangular orifice; C: Three 5/0 vascular monofilament sutures are placed, taking the three corners of the graft and recipient orifices.

the section, the limiting factor is the size of the graft cuff. In the majority of cases, the recipient's vessel matches this size rather well. If not, it can be cut at its bifurcation, to obtain a branch patch. In case of real hypoplasia of the recipient's portal vein, the confluence of the mesenteric and splenic vein can be clamped, the vessel sectioned at this level and a venous graft from the donor (usually the splenic or the external iliac vein) interposed between the confluence and the portal vein of the new liver. After completion of the anastomosis the graft is reperfused.

The arterial anastomosis comes next. The arterial axis of the graft usually includes the proper and common hepatic artery, in continuity with the celiac artery, and a patch of the aorta. The level of the anastomosis is chosen at any place along the recipient's arterial axis, and the two vessels are trimmed to obtain a similar section and an adequate length, according to what has already been stated concerning the portal vein. The anastomosis is performed end-to-end with a running suture of 7/0 or 8/0 vascular monofilament. If the recipient's arterial axis is deemed inadequate, the aorta can be clamped at the origin of the celiac artery or just below the renal arteries, and an end-to-side anastomosis can be performed at one of these sites. In the latter case, the interposition of an arterial graft from the donor, usually represented by an iliac artery, may be necessary.

The final stage is biliary reconstruction, which is always a hepaticojejunostomy with a Roux-en-Y loop. The bile duct of the graft may be single or double, although in the latter case two different anastomoses are performed (Figure 6).

Childhood hepatic malignancies have been considered a contraindication to the use of split-liver transplantation, since the need for the retention of the recipient's inferior vena cava potentially precludes obtaining a tumor-free margin[65]. A technical variation, which has allowed us and others to successfully use left lateral segment grafts to transplant children affected by hepatic malignancies, involves the replacement of the recipient's inferior vena cava using an iliac vein graft from the donor[66]. On the back-table, a wide V-shaped opening on the wall of the common iliac vein graft from the donor is made. The left hepatic vein of the left lateral segment graft is anastomosed end-to-side to the V-shaped opening on the common iliac vein with two 5/0 polypropylene running sutures (Figure 7). On the recipient, a total hepatectomy is usually performed using the standard technique of removing the liver together with the retrohepatic vena cava. At this point, the left lateral segment graft with the iliac vein graft is anastomosed to the suprahepatic vena cava in an end-to-end fashion with a 4/0 polypropylene running suture. The inferior edge of the iliac graft is then anastomosed to the infrahepatic vena cava with a 5/0 polypropylene running suture.

Donor selection

The following factors must be considered when a donor is evaluated for a specific patient.

Dimensional matching: The selection of a graft with an adequate parenchymal mass is critical to success. The minimal hepatic mass necessary for recovery is not clearly established, and its calculation must take into account the temporary loss of hepatocytes caused by the donor's injury or treatment, as well as preservation injury, acute rejection, or technical problems. Several formulas have been proposed to estimate adult and pediatric normal liver volume[67-72].

Considering that preservation injury is greater in organs from deceased donors, the hepatic mass of a graft procured from a cadaver donor should be greater than the calculated mass necessary using a living-donor liver segment. In the authors' experience, a donor weight range 20%-30% above or below that of the recipient is

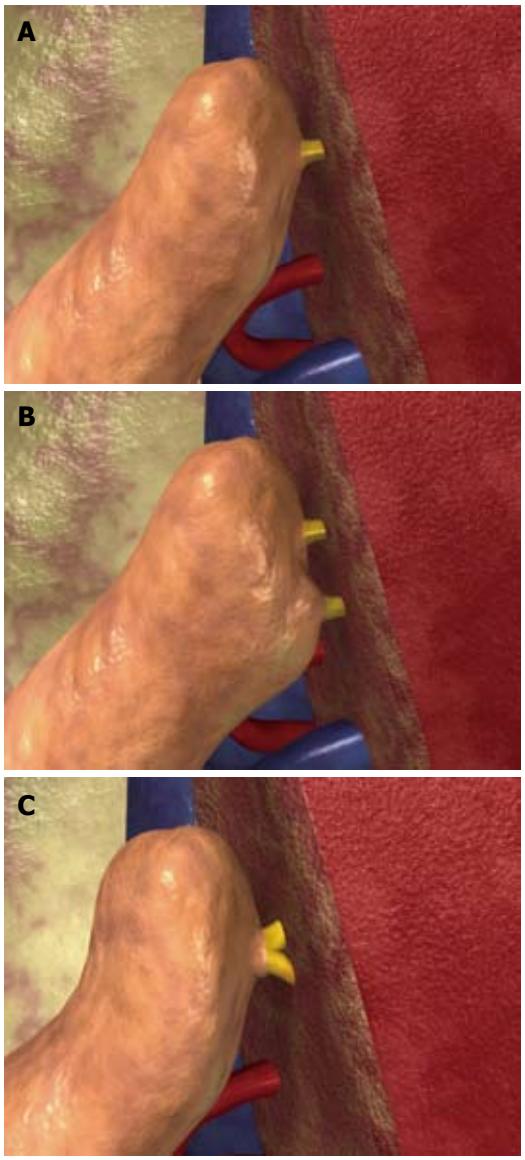


Figure 6 Biliary reconstruction performed by means of hepatico-jejunostomy. The bile duct of the graft can be single or double, although in the latter case, two different anastomoses are performed (B) or, if the two ducts are closed sufficiently, a common orifice can be created and anastomosed to the bowel loop (C).

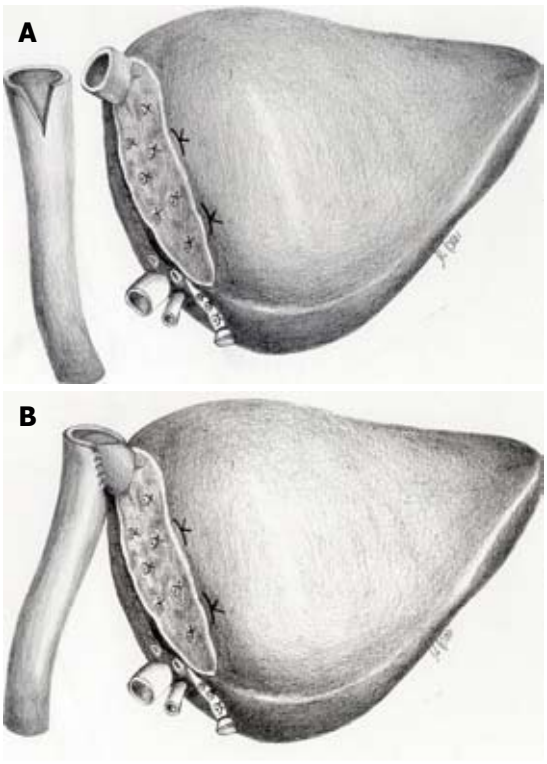


Figure 7 Use of left lateral segment grafts to transplant children affected by hepatic malignancies, with replacement of the recipient's inferior vena cava using an iliac vein graft from the donor. On the back table a wide V-shaped opening on the wall of the common iliac vein graft from the donor is made (A), and the left hepatic vein of the left lateral segment graft is anastomosed end-to-side to the V-shaped opening on the common iliac vein (B).

ideal for whole-organ donors, however these values can be extended down to 50% below and 100% above, taking into consideration body habitus and factors that would increase recipient abdominal size, such as ascites and hepatosplenomegaly. When selecting donors of partial grafts, a graft fraction of 1%-3% of the recipient body mass is optimum, while a graft-to-recipient weight ratio < 0.7 is usually associated with inferior overall allograft and patient survival. In the authors' experience, a liver is procured and transplanted as a whole graft when the donor-to-recipient body weight ratio is ⩽ 2. When the donor-to-recipient body weight ratio is between 2 and 12, the graft is considered for split liver[60,61].

Donor characteristics: Donor-organ suitability is assessed by evaluating clinical information and by biochemical tests. Particular attention is paid to donor age, intensive care hospitalization time, infections, hemodynamic stability. Biochemical tests do not serve as good benchmarks of functional capability, even if severe electrolyte disturbances and deteriorating trends identify increased risk. In questionable cases, biopsy of the donor liver at the time of organ harvest or during evaluation of live donors is helpful to identify pre-existing liver disease or steatosis. Quite extended criteria can be used in donors of whole allografts, especially when ischemic time is limited, without compromising the outcome. On the contrary, restricted selection criteria have been proposed when split-liver transplantation is considered. Commonly accepted donor selection criteria for split-liver procurement are: (1) age 15-50 years; (2) weight > 40 kg; (3) no past history of liver dysfunction/damage; (4) liver function tests within 2-5-fold of normal values; (5) normal macroscopic appearance of the graft; and (6) hemodynamic stability[73]. Nevertheless, the authors have adopted a liberal policy of liver splitting. The decision of whether or not to split a graft is based mainly on recipient, rather than on donor, criteria. Children requiring re-transplantation or who have fulminant hepatic failure are not excluded. Donor evaluation does not require special or additional invasive or non-invasive tests. Using these extended criteria for donor selection, we have been able to transplant all the children in need with no mortality on the waiting list and good overall patient and graft survival rates[60,61].

No consistent data exist on the effect of donor age on the long-term results of pediatric liver transplantation. Data from multicenter registries have shown that pediatric patients receiving livers from pediatric-age donors have significantly better graft survival compared to those receiving livers from donors aged > 18 years[74,75]. These data strongly support the primary use of pediatric donors for pediatric recipients, but are not to be considered a contraindication to the use of adult donors in pediatric transplantation. The limited availability of pediatric donor organs does not allow us to satisfy the need of an increased waiting list population. Moreover, the results obtained using adult donors are biased by the policy to use older donors only in high-risk urgent cases. For split-liver transplantation, the authors used donors over the age of 50 years without affecting the 3-year patient and graft survival[76]. In addition, pediatric donors can be safely used for split-liver procurement and transplantation: left lateral segment is transplanted in a small child, while the extended right lobe can be used in larger children, adolescents or adults[77,78].

Living-donor selection: In living-donor transplantation, the evaluation and selection of a donor, usually a parent or first-degree relative is performed on the assumption that donor safety can be assured and that the donor's liver function is normal. Donors should be 18-55 years of age, and have an ABO-compatible blood type. Following a satisfactory medical and psychological examination by physicians who are not directly involved with the transplantation program, vascular imaging is performed to assess the hepatic arterial anatomy. Donor safety has been excellent in all living donor series.

EARLY POSTOPERATIVE PERIOD

The early postoperative period consists of managing problems related to technical complications and to the prevention, diagnosis, and treatment of acute rejection and infection episodes. Postoperative complications usually present with a combination of cholestasis, rising hepatocellular enzyme levels, and variable fever, lethargy and anorexia. This non-specific symptom complex requires specific diagnostic evaluation before establishing treatment, and empiric therapy may result in misdiagnosis, morbidity and mortality.

Primary non-function

The lack of graft functional recovery can be seen in the first hours following transplantation, with high lactate levels, increased prothrombin time and partial thromboplastin time, and failure of the patient to wake despite sedation suspension. This extremely serious complication must be treated aggressively and immediately by infusing prostaglandin E_1, adopting the necessary measures to prevent a brain edema (mannitol infusion, hyperventilation), and addressing the effects of the liver failure by infusing plasma and glucose. If the signs of lack of functional recovery persist for more than a few hours, the patient needs a new transplant as soon as possible. Lesser degrees of allograft dysfunction occur more frequently but are usually reversible. The status of the donor liver contributes significantly to the potential for primary non-function because of ischemic injury secondary to anemia, hypotension, hypoxia, or direct tissue injury. A possible cause of primary non-function is hyperacute rejection, a rare phenomenon characterized by rapid intraparenchymal vascular thrombosis, mediated by pre-formed antibodies that bind to the vascular endothelium and trigger the complement system. Antibodies are generally directed against protein alloantigens such as foreign MHC molecules or less differentiated alloantigens expressed on endothelial cells.

Vascular complications

The hepatic artery anastomosis carries the highest risk of thrombosis (5%-18%) and leads to massive graft necrosis in cases of early onset. Hepatic artery thrombosis occurs in children three to four times more frequently than in adult transplant patients, and occurs most often within the first 30 d after transplantation and in small babies transplanted with whole livers[62,79]. When hepatic artery thrombosis is identified early (Figure 8), reconstruction can be attempted to avoid allograft necrosis[80]. When allograft failure develops, urgent re-transplantation is the only option. Late thromboses (occurring some weeks after the transplant) can manifest with biliary complications (stenosis or dehiscence of the biliary anastomosis, intrahepatic bilomas) or sepsis. Rarely, allograft necrosis occurs. Stenosis of the hepatic artery usually occurs at the anastomosis and in many cases may progress to complete thrombosis. Clinical manifestations include cholestasis or graft failure caused by diminution in hepatic blood flow. Non-invasive diagnosis relies on Doppler ultrasound with calculation of resistive indices and systolic acceleration time. Treatment modalities include revision of the anastomosis or balloon angioplasty (Figure 9).

A typical complication of a left lateral segment graft is stenosis at the level of the anastomosis between the left hepatic vein of the graft and the native vena cava, which in the worst cases can lead to acute Budd-Chiari syndrome. However, since the introduction of the triangulation technique, this complication has become quite rare[68]. When present, outflow venous obstruction can be treated by cavography and balloon angioplasty (Figure 10).

Finally, portal vein thrombosis occurs in 5%-10% of recipients. It is more frequent in children transplanted for biliary atresia, because of pre-existing portal vein hypoplasia, which requires replacing the entire portal vein down to the confluence of the superior mesenteric vein with the splenic vein to avoid low-flow-related thrombosis. Early thrombosis following transplantation, detected by ultrasound screening, requires immediate anastomotic revision and thrombectomy[81]. Later thrombosis is usually detected by decreased platelet counts and increasing spleen size or gastrointestinal bleeding (Figure 11). Interventional radiographic stent

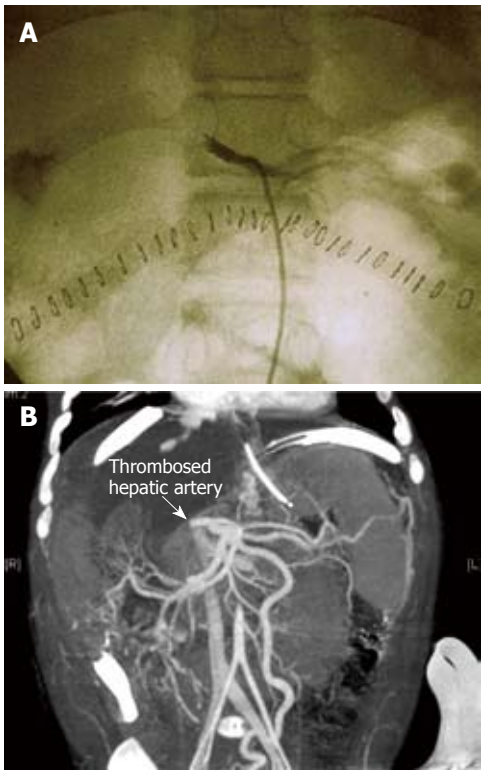


Figure 8 Selective celiac angiography showing early hepatic artery thrombosis after left lateral segment transplantation. A: Conventional angiography is the gold standard for radiographic diagnosis of hepatic artery thrombosis. B: Nowadays, the sensitivity of multiphase, multislice computed tomographic angiography with multidetector reconstruction approaches that of conventional angiography.

placement or balloon dilation has been successful in patients who have portal anastomotic stenosis but is less successful when complete thrombosis has occurred[82]. Portal venous shunting may be needed in patients who have progressive portal hypertensive complications.

Biliary complications

Biliary complications occur in approximately 10%-30% of pediatric liver transplant recipients, depending on the type of allograft used[62,83-85]. In the early postoperative period, the presence of bile-like fluid in the abdominal drainage is strongly suggestive of a bile leak. Ultrasound evidence of intrahepatic biliary ducts dilatation, elevated alkaline phosphatase and γ-glutamyl transferase (GT), and/or recurrent cholangitis suggest anastomotic or intrahepatic biliary stricture or small bowel obstruction at or distal to the Roux-en-Y anastomosis. Sometimes, non-specifically elevated liver function tests may be caused by a biliary stricture; in these cases a liver biopsy showing biliary duct proliferation and portal tract enlargement may help in differential diagnosis (Figure 12). Complications after duct-to-duct biliary reconstruction can be treated by dilation and internal stenting. With recurrent stenosis or persistent postoperative leak, Roux-en-Y choledochojejunostomy is the preferred treatment. In small children and in all patients transplanted for biliary atresia or with a partial graft, Roux-en-Y choledochojejunostomy is the reconstruction method of choice. In these patients,

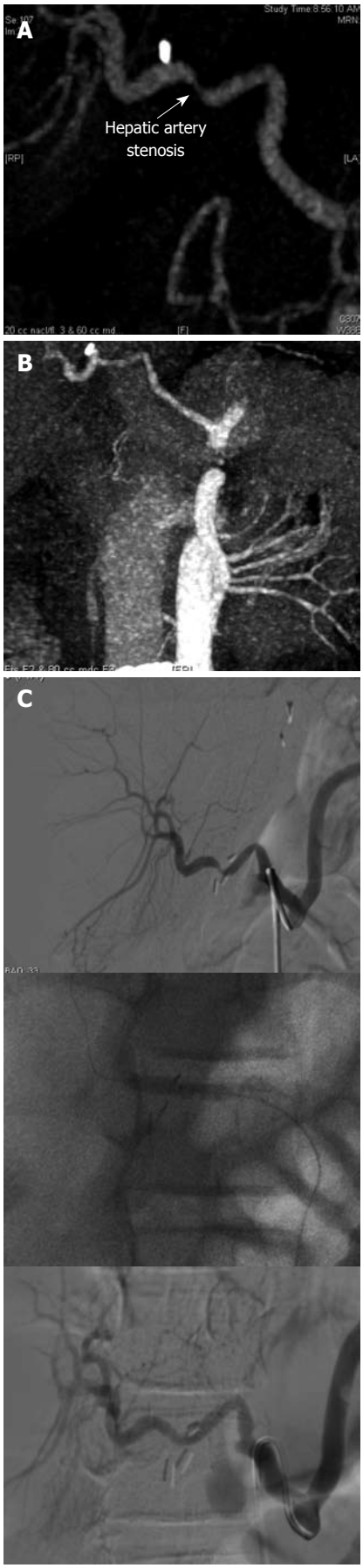


Figure 9 A case of hepatic artery stenosis. Reconstructed computed tomographic angiography demonstrating severe hepatic artery stenosis in an extended right graft recipient (A), and complete resolution of the stenosis 6 mo later (B), after stenosis treatment by early interventionally guided balloon angioplasty (C).

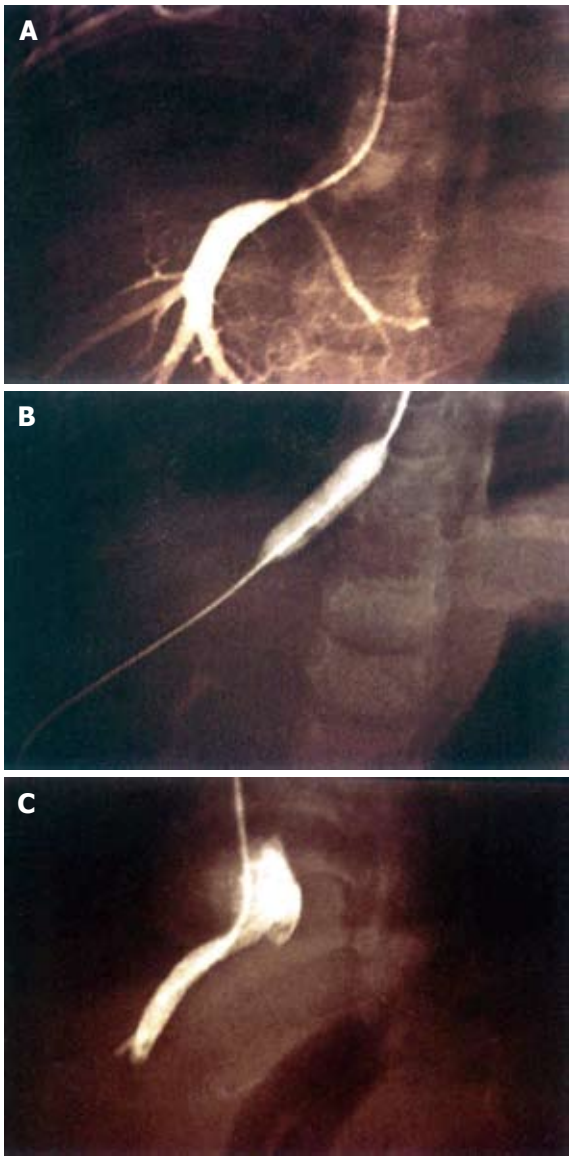


Figure 10 Venogram of hepatic venous outflow obstruction after left lateral segment split-liver transplantation. Venogram demonstrates a stenosis at the left hepatic vein anastomosis (A). Balloon angioplasty is performed (B), with resolution of the stenosis (C).

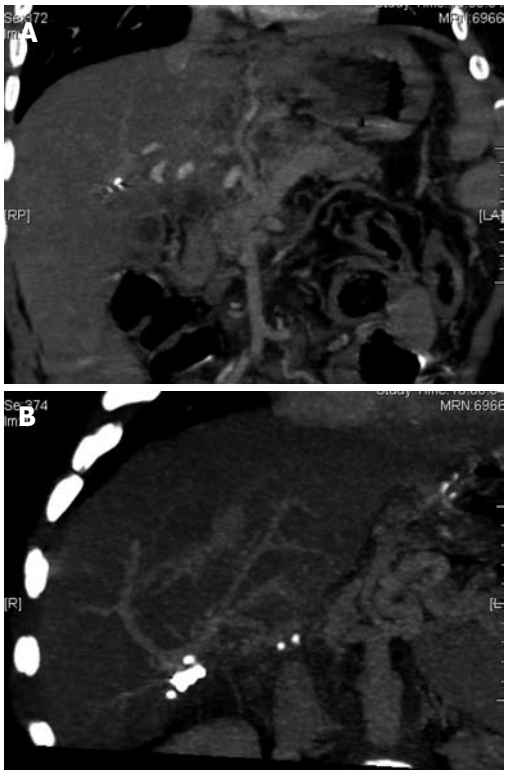


Figure 11 Portal vein thrombosis. Computed tomographic angiography with evidence of portal vein thrombosis and cavernomatous degeneration with collateral drainage through the left gastric vein (A), and evidence of intrahepatic portal flux restoration (B).

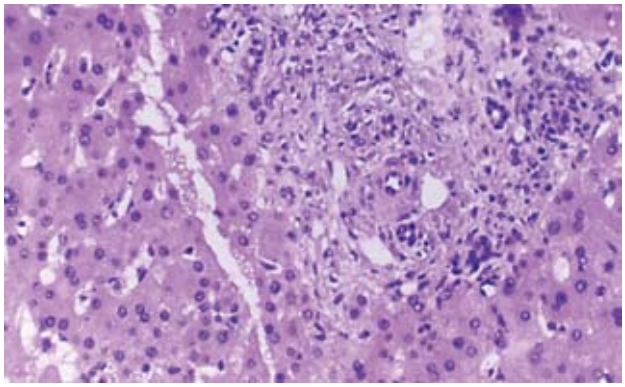


Figure 12 Liver biopsy performed in a left lateral segment recipient because of non-specifically elevated liver function tests. Histology shows biliary duct proliferation and portal tract enlargement suggestive of mechanic cholestasis.

dilatation and stenting are performed by percutaneous transhepatic cholangiography (Figure 13). The presence of multiple bile ducts has a documented increased risk for biliary complications[86].

Reoperation and re-transplantation

Early second-look reoperation is commonly used in several centers for the best diagnosis and treatment of bile leakage, hemorrhage, bowel injury secondary to multiple intra-abdominal adhesions, and sepsis. Infants and small children who have had only initial skin closure require secondary laparotomy for musculofascial closure in 5-7 d[87].

The overall incidence of re-transplantation ranges from 8% to 29%. The incidence of re-transplantation is similar for whole-organ allografts and partial allografts. The majority of re-transplantations result from acute allograft damage caused by either hepatic artery thrombosis or primary non-function; chronic rejection and biliary complications are uncommon causes. When re-transplantation for acute organ failure is undertaken in a timely manner, patient survival exceeds 80%. When re-transplantation is performed after prolonged immunosuppression for chronic allograft failure, often complicated by multiorgan insufficiency, the survival is only 50%.

Acute rejection

About 20%-50% of patients develop at least one episode of acute rejection in the first weeks after liver transplantation. The clinical picture of rejection

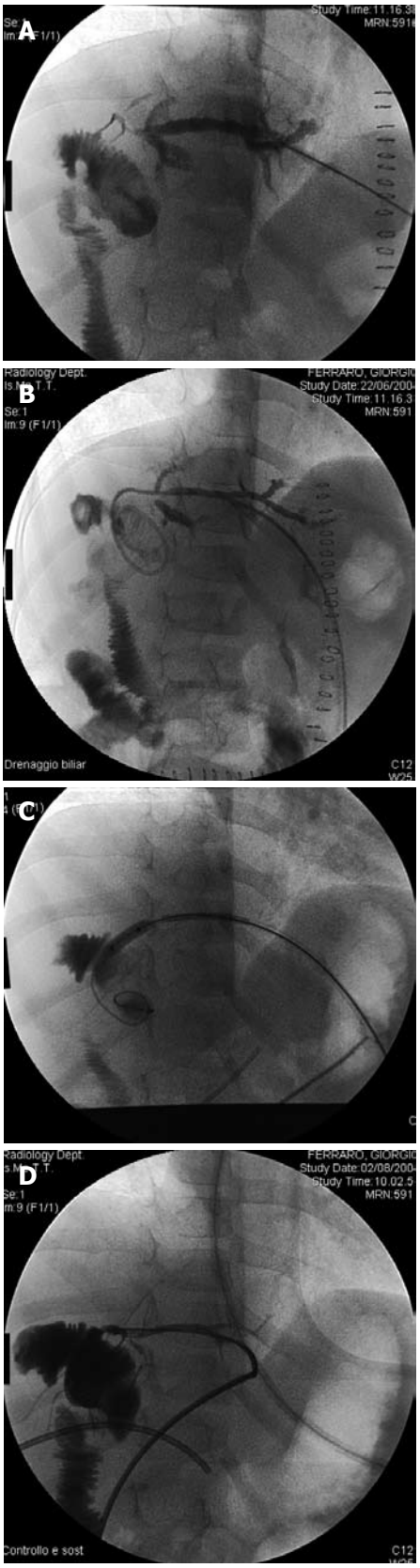


Figure 13 A case of biliary stenosis. Percutaneous transhepatic cholangiography performed in a left lateral segment recipient demonstrating intrahepatic biliary tree dilatation with stenosis of the hepaticojejunostomy (A), balloon bilioplasty (B), and transanastomotic percutaneous transhepatic biliary drainage positioning (C). Resolution of the stenosis after three sessions of bilioplasty (D).

includes fever, irritability, malaise, leucocytosis, often with eosinophilia, and increased γ-GT, bilirubin,

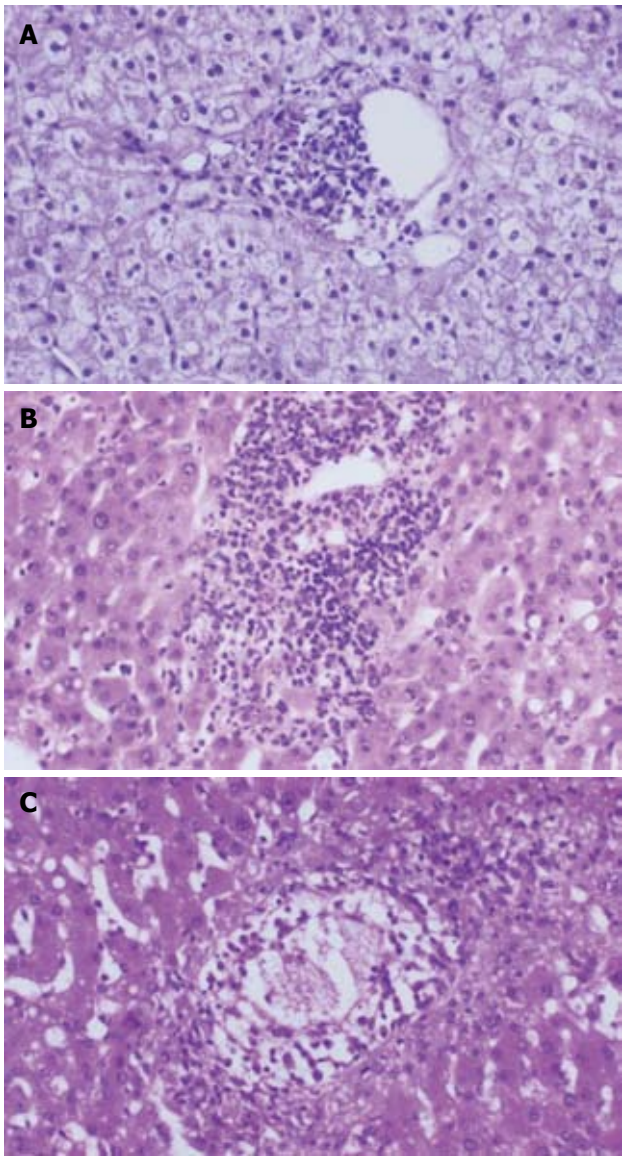


Figure 14 Acute cellular rejection: histopathological findings and grading. A: Mild acute cellular rejection, portal tracts are mildly expanded because of a predominantly mononuclear, but mixed portal inflammation. Rejection infiltrate is composed of blastic and small lymphocytes, eosinophils, macrophages, and occasional plasma cells. Lymphocytes are also present inside the basement membrane of the small bile ducts and in the subendothelial space of small portal vein branches. B: Moderate acute cellular rejection, all the portal tracts are markedly expanded by a predominantly mononuclear, but mixed inflammation. Centrilobular inflammation and hepatocyte necrosis and dropout are absent. C: evere acute cellular rejection, severe expansion of the portal tracts because of inflammation with focal portal-to-portal bridging; perivenular inflammation with hepatocyte necrosis and dropout; inflammation and damage to small bile ducts.

and transaminases. A liver biopsy is required to confirm rejection. Acute rejection is characterized by the histological triad of endothelialitis, portal triad lymphocyte infiltration with bile duct injury, and hepatic parenchymal cell damage[88] (Figure 14). Severity of acute rejection is scored according to the Banff scheme, which includes the descriptive grades indeterminate, mild, moderate, and severe, and a semi-quantitative rejection activity index (RAI) scoring on a scale from 0 to 3 the prevalence and severity of portal inflammation, bile duct damage, and subendothelial inflammation[89] (Tables 5

Table 5 Banff grading of acute liver allograft rejection

Assessment	Criteria	RAI
Indeterminate	Portal inflammatory infiltrate that fails to meet criteria for the diagnosis of acute rejection	1-2
Mild	Rejection infiltrate in a minority of the triads that is generally mild and confined within the portal spaces	3-4
Moderate	Rejection infiltrate expanding most or all of the triads	5-6
Severe	As above for moderate, with spillover into the periportal areas and moderate to severe perivenular inflammation that extends into the hepatic perenchyma and is associated with perivenular hepatocyte necrosis	> 6

Table 6 Rejection activity index (RAI)

Category	Criteria	Score
Portal inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils, and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile duct inflammation damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as an increased nuclear-to-cytoplasmatic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity, and cytoplasmatic vacuolization of the epithelium	2
	As above for the 2nd criterion, with most or all of the ducts showing degenerative changes or focal luminal disruption	3
Venous endothelial inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority, of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for the 2nd criterion, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

and 6).

The primary treatment of rejection is a short course of high-dose steroids. Bolus doses administered over a 3-6-day period with a rapid taper to baseline therapy are successful in the majority of cases. When refractory or recurrent rejection occurs, conversion from cyclosporine to tacrolimus, or antilymphocyte therapy using the monoclonal antibody, ornithine-ketoacid transaminase orthoclone, have been successfully used[90,91].

INFECTIONS

Immunosuppressive drugs used to prevent rejection inhibit activation of T lymphocytes, medullar cell proliferation and macrophage function, therefore creating an optimal environment for the development of infections. Infectious complications now represent the most common source of morbidity and mortality after transplantation.

Bacterial infections occur in the immediate post-transplantation period and are most often caused by Gram-negative enteric organisms, enterococci, or staphylococci. Sepsis originating at sites of invasive monitoring lines can be minimized by replacing or removing all of the intraoperative lines soon after transplantation. The use of prophylactic antibacterial antibiotics is discontinued as soon as possible to avoid the development of resistant organisms.

Fungal infection is a potential problem in the early post-transplantation period. To prevent fungal infection, aggressive protocols for pre-transplantation prophylaxis have been proposed[92]. Fungal infection most often occurs in high-risk patients requiring multiple operative procedures, re-transplantation, hemodialysis or continuous hemofiltration, pre-transplant chemotherapy, and multiple antibiotic courses. The authors use antifungal postoperative prophylaxis with liposomal amphotericin B only in high-risk patients undergoing liver transplantation.

Early and severe viral infections are caused by viruses of the herpes family, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus[93]. The risk of developing either CMV or EBV infection is influenced by the preoperative serological status of the transplant donor and recipient[94,95]. Seronegative recipients receiving seropositive donor organs are at greatest risk. Various prophylactic protocols, including intravenous IgG and hyperimmune anti-CMV IgG, associated with acyclovir or ganciclovir have been used to decrease the incidence of symptomatic CMV and EBV infection, although seroconversion in naive recipients inevitably occurs[94,96]. The suspicion of CMV infection is suggested by the presence of fever, leukopenia, maculopapular rash and hepatocellular abnormalities, respiratory insufficiency, or gastrointestinal hemorrhage. Hepatic biopsy or endoscopic biopsy of colonic or gastroduodenal sites allows early diagnosis with immunohistochemical recognition. Nowadays, the availability of specific antiviral drugs like ganciclovir, foscarnet and more recently valaciclovir, have radically modified the prognosis of CMV infection. At the start of the 1990s, the concept of pre-symptomatic therapy

was introduced as a strategy to prevent the incidence of CMV-related disease, based on the principle of not administering antiviral medications up to the point when these will have maximum effect, and monitoring CMV antigenemia (pp65) or viremia (CMV DNA)[97,98].

Herpes simplex virus infections, similar to those seen in non-transplant patients, require treatment with acyclovir when diagnosed.

EBV infection represents a potential risk for the pediatric transplant recipient. EBV infection has a variable clinical picture including a mononucleosis-like syndrome, hepatitis-simulating rejection, extranodal lymphoproliferative infiltration, peritonsillar or lymph node enlargement, or encephalopathy. Monitoring of EBV blood viral load by quantitative polymerase chain reaction (PCR) is the best predictor of risk. When evidence of active infection exists, an acute reduction in immunosuppression is mandatory. The authors recommend monthly EBV-DNA PCR counts and more frequent monitoring in case of increasing viral load levels. As a result of the lack of a standardized EBV DNA count methodology, no common cutoff exists. In the authors' experience, more than 500 genomes/10^5 peripheral blood leukocytes identify patients who benefit from reduction in primary immunosuppression[99]. Antiviral therapy with ganciclovir and CMV-IgG is also used, although no definitive data support their use[100,101].

Other post-transplantation infectious complications include adenovirus hepatitis, varicella, and enterovirus-induced gastroenteritis. *Pneumocystis carinii* infection has been nearly eliminated by the prophylactic administration of sulfisoxazole and trimethoprim or aerosolized pentamidine.

MANAGING IMMUNOSUPPRESSIVE THERAPY

The immune system recognizes the graft as foreign and begins a destructive immune response mediated principally by the T lymphocytes. In order to avoid destruction of the graft, immunosuppressive drugs must be administered. Progress in transplant surgery in the last 20 years has been characterized in large part by the introduction of calcineurin inhibitors that today represent the keystone of most immunosuppressive protocols[102,103]. In the last decade, new drugs that selectively target various cellular activation pathways have been proposed and used. The following are the most commonly used drugs in pediatric liver recipients.

Corticosteroids

Corticosteroids were the first drugs to be used to control rejection and are still an essential element of the immunosuppressive regimen; they are effective in both the prevention and treatment of graft rejection. They act through intracellular receptors expressed in all cells of the body. Their immunosuppressive action mechanism, not fully clarified yet, is linked to the suppression of antibody production; inhibition of synthesis of cytokines such as interleukin-2 (IL-2) and interferon-γ; reduction in the proliferation of helper and suppressor T cells, cytotoxic T cells, and B cells; and the migration and activity of neutrophils.

Long-term clinical experience with steroid use has documented a host of adverse effects. Over-immunosuppression is associated with increased incidence of bacterial, fungal and viral infections. In addition, patients taking steroids carry an increased risk for developing malignancies, especially lymphomas and skin cancers[104]. Detrimental metabolic effects of steroids are wide ranging and are of particular concern for the pediatric transplant patient[105-107]. In terms of hospital costs, the calculated 10-year cumulative expense for steroid-related complications in adult kidney recipients has been shown to be 5300 $ per patient per year[108]. Efforts are underway to develop immunotherapy regimens in which steroids can be withdrawn early, or not used at all.

The experience of steroid weaning after pediatric liver transplantation was summarized in 2000 by Reding[109]. There are a total of nine recent studies, not all of which were non-randomized and uncontrolled. Steroid treatment could be successfully stopped in 21%-100% of the transplanted patients. The risk of rejection was not significantly increased, and varied from 7% to 29%. Chronic rejection did not seem to be increased[110-118] (Table 7). The conclusions of this review are the following: (1) weaning of steroids after pediatric liver transplanatation is safe and, most of the time, beneficial; and (2) in many patients, calcineurin inhibitor monotherapy can be achieved, suggesting that the next step could be the adoption of steroid-free immunosuppressive protocols.

In a non-randomized study, Reding *et al*[119] compared pediatric liver transplantation under steroid-free immunosuppression in children who received combined tacrolimus and antibody to the IL-2 receptor of T cells (basiliximab), with matched historical recipients taking tacrolimus and steroids. Twelve-month rejection-free survival was similar in the steroid-free group compared with the corticosteroid group. The authors performed the first prospective, controlled, randomized study designed for children undergoing liver transplantation to test the possibility of avoiding the use of corticosteroids under baseline tacrolimus immunosuppression plus basiliximab induction, which confirmed no harmful effect of steroid avoidance on graft acceptance[120].

Corticosteroid withdrawal or avoidance can be difficult in patients with autoimmune hepatitis, primary biliary cirrhosis, or primary sclerosing cholangitis. In these patients it might be desirable to include steroids in the immunosuppressive protocol as a principle, although definitive and convincing data are not available.

Calcineurin inhibitors

Cyclosporine and tacrolimus are classified as calcineurin inhibitors because they inhibit T-cell responses and bind to intracellular proteins called immunophilins.

Table 7 Literature review of immunosuppressive protocol with steroid weaning after pediatric liver transplantation

Author	Year	Patients (*n*)	Protocol	Weaning (%)		Graft loss	Rejection (%)	
				Performed	Success		Acute	Chronic
Margarit *et al*[110]	1989	18	CsA+Aza	83	61	13%	27	13
Andrews *et al*[111]	1994	119	CsA+Aza[1]	44	67	No	13	No
Dunn *et al*[112]	1994	73	CsA+Aza	51	76	4%	7	4
McDiarmid *et al*[113]	1995	13	CsA+Aza			No	No	No
McKee *et al*[114]	1997	29	TAC	83	71		29	
Martin *et al*[115]	1998	55	CsA+Aza	44	76	No	11	No
Reding *et al*[109,116]	2000	375	CsA (*n* = 23)		21	No	No	No
			CsA-ME (*n* = 24)			No	No	No
			TAC (*n* = 31)			No	10	No
Atkison *et al*[117]	2002	94	CsA+Aza[2]	71	91		21	
Toyoki *et al*[118]	2004	8	TAC	100	100	No	13	No

CsA: Cyclosporine; CsA-ME: Cyclosporine microemulsion; Aza: Azathioprine; TAC: Tacrolimus. [1]In 53% of the weaned children; [2]Some patients received antilymphocyte globulin or OKT3 induction.

The immunophilin-drug complex competitively binds to and inhibits the phosphatase activity of calcineurin. Calcineurin inhibition indirectly blocks the transcription of cytokines, particularly IL-2, which regulate the proliferative T-cell response[121]. Calcineurin inhibitors have similar side-effect profiles, which include dose-dependent nephrotoxicity, neurotoxicity, and hypertension. Most adverse effects are reversible after dose reduction or discontinuation of the drug[122,123]. Tacrolimus has not been associated with cosmetic adverse effects such as hypertrichosis and gingival hyperplasia observed in cyclosporine-immunosuppressed children. Moreover, tacrolimus is associated with less hyperlipidemia and a lower adverse cardiovascular risk profile than cyclosporine[124], but with slightly more *de novo* diabetes and gastrointestinal symptoms[125]. In some studies, tacrolimus has been described to cause a higher incidence of post-transplant lymphoproliferative disease[126,127], but this has not been confirmed in other authors' experiences[128]. Hypertrophic cardiomyopathy has been reported with prolonged use of tacrolimus at unusually high levels[129].

Calcineurin inhibitors are mainly absorbed from the small intestine and are metabolized in the liver and small intestine by the cytochrome P4503A enzyme system[130]. The majority of their metabolites are excreted in bile[131]. The most important interactions are with enzymes or drugs that induce or inhibit the cytochrome P4053A, which results in reduced or increased calcineurin inhibitors levels.

Tacrolimus or cyclosporine usually represents the primary drug of most immunosuppressive regimens. Over the last 10 years, the use of tacrolimus has increased, being nowadays preferred to cyclosporine[132]. Tacrolimus and cyclosporine have been compared in large multicenter trials that showed similar 1-year patient and graft survival, with a significantly reduced incidence of acute rejection as well as steroid-resistant rejection in children treated with tacrolimus. Moreover, tacrolimus is superior to cyclosporine for the treatment of rejection episodes that may resolve when patients are switched from cyclosporine to tacrolimus therapy[97,133].

Table 8 Desired trough concentrations of calcineurin inhibitors after pediatric liver transplantation

Time post-transplant (mo)	Target level (mg/L)	
	Cyclosporine	Tacrolimus
0-3	200-250	10-15
4-12	150-200	8-10
> 12	50-100	5-8

Cyclosporine: The microemulsion form of cyclosporine, Neoral, is the formulation mainly used, which has replaced the original formulation Sandimmune because of its greater and more consistent bioavailability. Pharmacokinetics features of cyclosporine that are to be considered in children are the following: (1) cyclosporine bioavailability correlates with age, being lower in younger patients; and (2) cyclosporine is metabolized in children at a higher rate than adults, and appears to be inversely related to age[134]. The type of biliary anastomosis (e.g. Roux-en-Y biliary anastomosis for biliary atresia) and concomitant disease (e.g. cystic fibrosis) may affect absorption and bioavailability[135,136]. The recommended starting dose of Neoral is 5 mg/kg twice daily, which should be administered orally within the first 12 h of abdominal closure. Intravenous cyclosporine can be administered at a dose of 2 mg/kg per day in two divided doses by continuous infusion over 2-6 h in case of poor absorption or inadequate trough concentrations. After the first administration, the dose is adjusted in order to keep trough concentrations within a recommended target range (Table 8). Trough levels are poor predictors of rejection episodes or outcome of graft recipients[137], therefore, drug concentration in blood drawn 2 h post-dose has been proposed recently to be a superior estimate of the subsequent 12 h cyclosporine exposure[138,139].

Tacrolimus: The recommended tacrolimus starting dose is 0.05-0.1 mg/kg, administered orally within the first 12 h after abdominal closure. Subsequently, doses are adjusted in order to maintain trough concentrations

Table 9 Use of sirolimus in primary immunosuppressive regimens in liver transplantation

Author	Immunosuppression	No. of patients	Survival (%)		Acute rejection (%)	Follow-up (mo)
			Patient	Graft		
McAlister *et al*[153]	TRL, SRL, STER[1]	32	92		3	8
McAlister *et al*[154]	TRL, SRL, STER[1]	56	93	91	14	23
Peltekian *et al*[155]	TRL, SRL, STER[1]	42	93	90	10	14
Pridöhl *et al*[156]	TRL, SRL, STER	22	91	78	14	14
Sindhi *et al*[157]	TRL, early SRL, STER	6			17	15
	TRL, late SRL, ATG	9			33[2]	3

ATG: Antithymoglobulin; SRL: Sirolimus; STER: Corticosteroids; TRL: Tacrolimus; [1]Corticosteroids withdrawal 3 mo after transplantation; [2]Rejection episodes observed before sirolimus was introduced in the immunosuppressive regimen.

within a recommended target range (Table 8). The trough level is widely accepted for routine tacrolimus drug level monitoring. Large inter- and intra-individual differences in pharmacokinetics exist. The elimination half-life of tacrolimus in children is 50% of that in adults, and clearance is correspondingly two to four times faster[140,141]. Therefore, children require higher doses to achieve similar tacrolimus concentrations.

Mycophenolate mofetil

The active metabolite of mycophenolate mofetil, mycophenolic acid, is a selective inhibitor of the enzyme inosine monophosphate dehydrogenase, which is essential for the *de novo* pathway of purine synthesis[142]. Inhibition of the *de novo* pathway results in the depletion of guanosine nucleotides and arrested lymphocytes replication because they are unable to use the alternative pathway for nucleotide production[143].

Mycophenolate mofetil has been used successfully as an alternative immunosuppressive agent in patients with chronic rejection, refractory rejection, or severe calcineurin inhibitor toxicity[144,145]. Mycophenolate mofetil has also been used in calcineurin-inhibitor and corticosteroid-sparing immunosuppressive protocols, without increasing the risk of rejection[146,147]. The suggested dose for pediatric liver transplant recipients is 15 mg/kg twice daily[148]. Pharmacokinetic studies showed large inter-individual variations in mycophenolic acid parameters[149,150], which indicates the need for therapeutic drug monitoring and individualized dosing. The most relevant adverse effects of mycophenolate mofetil are dose-dependent gastrointestinal symptoms and bone marrow suppression[147,151]. Acyclovir and ganciclovir increase mycophenolic acid efficacy, whereas cholestyramine, oral antibiotics, antacids, cyclosporine, and high tacrolimus concentrations reduce its concentration[148-150].

Sirolimus

Sirolimus (rapamycin) is a macrolide antibiotic with potent immunosuppressive properties that acts by blocking T-cell activation by way of IL-2R post-receptor signal transduction[152]. Sirolimus has been used in small, uncontrolled studies in liver transplant recipients (Table 9) and reduces rate of acute rejection, when used in combination with calcineurin inhibitors, even at low doses, or facilitates early steroid withdrawal, while maintaining low rates of acute rejection[153-157].

Sirolimus has also been used as rescue treatment in chronic rejection and calcineurin inhibitor toxicity[157-159], whereas attempts to use sirolimus as a single primary immunosuppressive agent have resulted in a high rate of acute rejection[160]. Sirolimus has not yet been approved by the US Food and Drug Administration for use in liver transplantation. One trial to evaluate sirolimus in liver transplant recipients was halted because of an increased incidence of hepatic artery thrombosis. In contrast, other studies have not confirmed this finding[154,161,162], and a possible benefit of sirolimus in the prevention of coronary artery restenosis after percutaneous coronary revascularization has been described[163]. Sirolimus has shown antineoplastic activity, inhibiting angiogenesis in malignant tissue through reduction of vascular endothelial growth factor secretion, which may provide a specific indication for using of the drug in patients transplanted for primary liver malignancy[164].

Sirolimus drug interactions are similar to those of calcineurin inhibitors. It has a long half-life (40-86 h) and intra- and inter-individual variation[152,165]. Therefore, daily sirolimus monitoring is not necessary and monitoring trough level twice weekly for the first month and weekly for the next month is recommended, targeting a 5-15 mg/L range. Sirolimus levels increase during simultaneous administration of cyclosporine[166]. The most relevant dose-related side effects of sirolimus are hyperlipidemia, thrombocytopenia and leukopenia[153,157].

IL-2 receptor antibodies

T cells involved in acute rejection act by exposing activation markers such as the IL-2 receptors. Therefore, anti-IL-2 receptor therapy appears to be a promising option for specific immunosuppression. IL-2 receptor antibodies have been used primarily in children as induction agents in double or triple immunosuppression protocols. Preliminary experience in pediatric liver recipients is encouraging: pooled data from the available papers from the literature encompassed 79 patients treated with daclizumab, 165 with basiliximab, and 209 no-induction controls; incidence of acute rejection was lower in the induction groups[119,120,167-172] (Table 10).

A multicenter trial studied basiliximab pharmacokinetics and pharmacodynamics in children. It demonstrated that to achieve efficacious results, pediatric patients less than 35 kg in weight should receive two intravenous 10-mg

Table 10 Use of IL-2 receptor antibodies in primary immunosuppressive regimens in pediatric liver transplantation

Author	Immunosuppression	No. of patients	Survival (%)		Acute rejection (%)	Follow-up (mo)
			Patient	Graft		
Asensio *et al*[167]	TRL, STER	21	80	80	63	12
	TRL, STER, BAS	34	80	80	30	
Strassburg *et al*[168]	TRL, STER	12			42	28
	CSA, STER, AZA	9			66	
	CSA, STER	12			42	
	CSA, STER, BAS	21			33	
Heffron *et al*[169]	TRL, MMF, STER	20	85	88	50	24
	TRL,[2] MMF, DAC, STER	61	93	73	15	
Reding *et al*[119]	TRL, STER	20			50	12
	TRL, BAS, MMF[1]	20			25	
Ganschow *et al*[170,171]	CSA, STER	54	94		54	28-52
	CSA, STER, BAS	54	98		17	
Schuller *et al*[172]	TRL, MMF, STER	12			66	14
	TRL, MMF, DAC, STER	18			0	6
Spada *et al*[120]	TRL, STER	36	91	86	32	24
	TRL, BAS	36	87	80	12	

CSA: Cyclosporine; DAC: Daclizumab. [1]Mycophenolate mofetil was given in the first 9 patients. [2]Tacrolimus was given starting from postoperative day 7.

doses, and those weighing ⩾ 35 kg should receive two 20-mg doses of basiliximab. The first dose should be given within 6 h after organ reperfusion, and the second on day 4 after transplantation. A supplemental dose may be considered for patients with a large volume of drained ascitic fluid relative to body size[173]. For daclizumab, various different dosing regimens have been used[169,174]. A dual regimen of 1 mg/kg on days 0 and 4 provides receptor saturation for up to 21 d.

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS (PTLDS)

PTLDs are a heterogeneous group of diseases, ranging from benign lymphatic hyperplasia to lymphomas. PTLD is the most frequent tumor in children following transplantation, and occurs in the majority of the cases within the first 2 years after transplantation[175]. Late forms have usually an aggressive clinical course and severe prognosis. The development of PTLD in pediatric liver transplant recipients is favored by the intensity of the immunosuppression, its lifetime duration, and the absence of prior exposure to EBV infection in 60%-80% of patients. Risk factors for PTLD development are: (1) high total immunosuppression load; (2) EBV-naive recipients; and (3) the intensity of active viral load[176,177]. No single immunosuppressive agent has been directly related to PTLD. An important pathogenic feature favoring PTLD development is EBV infection.

Treatment of PTLD is based on the immunological cell typing and clinical presentation. Documented PTLD requires an immediate decrease or withdrawal of immunosuppression, taking into account the increased risk of organ rejection[100,101]. If a tumor expresses the B-cell marker CD20, the anti-CD20 monoclonal antibody rituximab has been successfully used. In some studies, the combination of cyclophosphamide, prednisone and rituximab has shown a response rate of 100%, with minimal toxicity[178,179]. Patients who have aggressive monoclonal malignancies have poor prognosis even with immunosuppressive reduction, acyclovir, surgery, and conventional chemotherapy or radiation therapy. Recently, autologous EBV-specific cytotoxic T-lymphocytes have proved effective in enhancing EBV-specific immune responses and reducing viral load in organ transplant recipients with active infection, and have been successfully used as first-line treatment of EBV-related PTLD[180].

LATE LIVER ALLOGRAFT DYSFUNCTION

There are several potential causes of late liver allograft dysfunction and differential diagnosis can be difficult because of overlapping clinical, serological and histopathological features. Recurrence of the native liver diseases after transplantation is a less significant problem in the pediatric population in comparison to adults. Recurrent infections and immune-based diseases are the most difficult diagnostic challenges. Most late causes of liver allograft dysfunction are detected because of abnormalities in routinely monitored liver tests; clinical signs and symptoms are much less common. When signs or symptoms do occur, liver biopsy is indicated. Common causes of late dysfunction in the pediatric population are shown in Table 11.

Late-onset acute rejection

Late-onset acute rejection may show slightly different features than typical acute rejection episodes seen early after transplantation, and is commonly characterized by: (1) predominantly mononuclear portal inflammation; (2) venous subendothelial inflammation of portal or central veins or perivenular inflammation; and (3) bile duct inflammation and damage. Late-onset acute rejection can

Table 11 Common causes of late dysfunction in the pediatric population

	Incidence at 5 yr (%)	Risk factors
Acute rejection	Variable (< 30)	Inadequate immunosuppression Treatment with immune activating drugs (e.g. interferon) History of autoimmune liver disease
Chronic rejection	-3	Inadequate immunosuppression Treatment with immune-activating drugs (e.g. interferon) Refractory acute rejection Chronic rejection in a previous failed allograft
Recurrent AIH	-30	Suboptimal immunosuppression AIH type I Severe inflammation in native liver HLA DR3 or DR4
De novo AIH	< 5	
Recurrent PBC	20-30	Tacrolimus as baseline immunosuppression Living-related donor Steroid and other immunosuppression withdrawal
Recurrent PSC	20-30	Male sex; donor-recipient gender mismatch Intact colon at time of transplantation
Idiopathic post-transplant hepatitis	5-60	

AIH: Autoimmune hepatitis; PBC: Primary biliary cirrhosis; PSC: Primary sclerosing cholangitis.

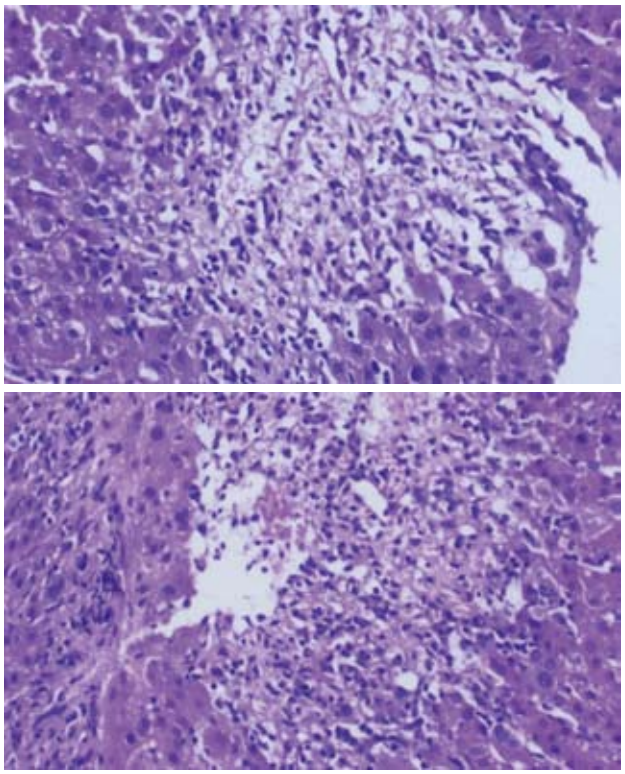


Figure 15 Histological findings in chronic rejection: Little portal inflammation in conjunction with bile duct loss affecting > 50% of the portal tracts and moderate or severe perivenular fibrosis.

also manifest as so-called central perivenulitis [181-183], or may resemble chronic hepatitis[184,185]. Mild cases may resolve spontaneously[183], but more severe forms warrant more aggressive treatment.

Chronic rejection

Chronic rejection develops in 5%-10% of transplanted patients. The primary clinical manifestation is progressive cholestasis. This course can occur within weeks from transplantation or later, and can be asymptomatic or follow persistent and/or unresponsive acute rejection and/or inadequate immunosuppression. Two clinical forms have been described[186]. In the first, named vanishing bile duct syndrome, the biliary epithelium is primarily injured with changes ranging from senescence (early stage) to severe ductopenia in at least 50% of the portal tracts (late stage)[187]. This form can be successfully treated by conversion from cyclosporine to tacrolimus immunosuppression protocols. Re-transplantation is necessary in non-responding children. The second subtype is characterized by the development of progressive ischemic injury to bile ducts and hepatocytes, which causes ductopenia and ischemic necrosis with fibrosis (Figure 15). In this setting, the diagnosis is rarely based on histology alone, because arteries with pathognomonic changes are rarely present in needle biopsy specimens. Bile duct injury and ductopenia, however, can be caused by biliary strictures, hepatic artery pathology, adverse drug reactions, and CMV. Selective hepatic angiography showing pruning of the intrahepatic arteries with poor peripheral filling and segmental narrowing supports a diagnosis of chronic rejection[188,189]. This form nearly always requires retransplantation.

Recurrent and new-onset or de novo autoimmune hepatitis

Theoretically all forms of autoimmune hepatitis after transplantation can be classified as rejection[190-192]. No conventional clinical tests differentiate an autoimmune response from rejection. The diagnosis of autoimmune hepatitis is established by a combination of serological, molecular biological and histopathological findings. Non-organ-specific autoantibodies are a requisite for the diagnosis, and they typically include smooth muscle antibodies (SMAs), antinuclear antibodies (ANAs), and antibodies to liver kidney microsome (anti-LKM)[193]. Minimal diagnostic criteria for recurrent or *de novo* au-

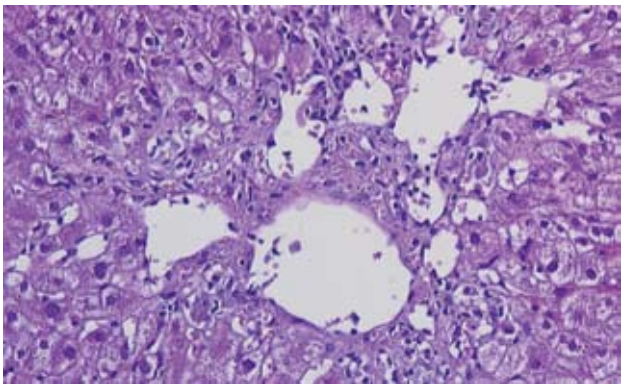


Figure 16 Histological appearance of recurrent or new-onset autoimmune hepatitis characterized by moderate portal inflammation, prominent interface activity, relative sparing of the bile ducts, and perivenular accumulation of inflammation.

toimmune hepatitis in an allograft are: (1) interface hepatitis with portal lymphocytic infiltrates (Figure 16); (2) presence of ANA, SMA or anti-LKM; (3) hypergammaglobulinemia; and (4) exclusion of virus-induced or drug-related hepatitis and late acute or chronic rejection. Most adult recipients respond to an increase in immunosuppression, whereas pediatric recipients often require the use of second-line immunosuppressive drugs (azathioprine, mycophenolate mofetil). A cautious approach to withdrawal of immunosuppression is warranted in all patients transplanted for autoimmune hepatitis, and the consequences of recurrent disease within the graft will require prolonged follow-up. A recent study, evaluating protocol liver biopsies performed in asymptomatic children 1, 5 and 10 years after transplantation, documented that chronic hepatitis is a common finding in children after liver transplantation, and is associated with a high risk of developing progressive fibrosis, which leads to cirrhosis, and with the presence of autoantibodies[194].

Idiopathic post-transplant hepatitis

Chronic hepatitis that cannot be ascribed to a particular cause is defined as idiopathic post-transplant hepatitis. Cases presenting with central perivenulitis probably represent centrilobular-based acute rejection or autoimmune hepatitis, if autoantibodies are also present[185], because allograft dysfunction usually responds to increased immunosuppression[185,195]. Some cases may represent a form of rejection with features of chronic hepatitis[195]. A diagnosis of idiopathic post-transplant hepatitis does not usually require treatment with increased immunosuppression. However, as some cases do show progressive fibrosis, the management of those with moderate to marked activity needs to be clarified.

Primary sclerosing cholangitis

Recurrent primary sclerosing cholangitis is nearly identical to that seen in native livers[196,197]. Most patients with suspected recurrent disease are asymptomatic after transplantation. An accurate diagnosis of primary sclerosing cholangitis recurrence requires well-defined cholangiographic and histological criteria. Other disorders that can produce biliary strictures after transplantation should be excluded. Graft with primary sclerosing cholangitis recurrence shows biliary strictures, acute and chronic pericholangitis, and centrilobular hepatocanalicular cholestasis periductal fibrosis[198].

Table 12 UNOS pediatric liver Kaplan-Meier patient and graft survival rates for transplants performed between 1997 and 2004

Recipient age (yr)	Patient survival (yr)			Graft survival (yr)		
	1	3	5	1	3	5
< 1	89	82	78	81	70	63
1-5	86	80	77	78	71	67
6-10	91	86	86	84	76	75
11-17	93	87	81	87	77	67

One-year survival based on 2002-2004 transplants, 3-year survival based on 1999-2002 transplants, 5-year survival based on 1997-2000 transplants.

OUTCOME FOLLOWING TRANSPLANTATION

The overall results following liver transplantation are rewarding. The European Liver Transplantation Registry (ELTR) reports liver transplantation activity in Europe, and represents 5895 children transplanted between 1988 and 2005. Overall 1-year patient and graft survival was 84% and 73%, respectively, in patients older than 2 years at the time of transplantation, and 81% and 71%, respectively, in children < 2 years of age. Ten-year patient and graft survival rates for the same age groups were 75% and 61%, and 74% and 60%, respectively. Similarly, UNOS reported survival rates of the 9064 pediatric patients transplanted between 1997 and 2004. One-, 3- and 5-year patient and graft survival rates stratified according to recipient age at the time of transplant are reported in Table 12. Overall 1-year patient and allograft survival reported to the Studies of Pediatric Liver Transplantation (SPLIT) registry, representing 1611 patients, reached 88% and 82%, respectively, while these were 83% and 74%, respectively, 4 years after transplantation. Specific factors influencing early survival include age, diagnosis, severity of illness, and possibly allograft type[199].

Age

Survival for infants < 1 year of age or weighing < 10 kg has been reported to be between 65% and 80% overall, an improvement over the previously reported rates of 50%-60%[200]. Experienced programs have described even better patient survival rates at 3 mo[201]. Improved survival in these recipients results from technical innovations, better graft preparation and avoidance of life- and graft-threatening complications such as hepatic artery thrombosis and primary non-function.

Diagnosis

Survival after transplantation is similar in patients who have cholestatic and metabolic liver disease. Early survival rates are worse for patients who have acute liver failure[9,202,203] and liver tumors[11], but their long-term survival rates are similar to those of other recipients. Asso-

ciated multiorgan failure and a limited organ-acquisition time frame influence this result. Similar decreased survival trends are seen in patients who have a PELD score > 20, in status 1 recipients, and in patients whose PELD scores deteriorate significantly before transplantation[204].

Graft type

Donor factors influencing patient and graft survival include a donor age < 6 mo or > 50 years, even if some studies have demonstrated that elderly donors can be used safely[76]. The impact on the outcome of graft type (whole, reduced, split, or living-donor) is less clear. In the SPLIT registry, recipients of whole organs had better patient and graft survival than recipients of reduced, split, or living-donor allografts[205]. The US Scientific Registry of Transplant Patients database review has reported significantly lower risk of graft failure for patients aged < 2 years who received living-donor grafts compared to whole- and split-liver recipients. Older recipients showed a higher risk of graft loss and mortality after living-donor transplantation[206]. These conflicting results may have been influenced by the diverse experience accumulated in the transplant centers. Reports of whole-organ, living-donor, and split-liver outcomes from experienced centers showed no difference in patient and graft survival, and in biliary and vascular complications[53,60-62,207,208]. Successful transplantation of very small recipients with monosegments has been reported[209]. Overall, the best results can be achieved at centers that have extensive experience with all age groups and allograft types, allowing transplantation according to the needs of the recipient. The most important prognostic factor is the severity of the patient's illness at the time of transplantation[210]. The good survival rates obtained in patients receiving living-donor transplantation are positively influenced by the possibility to schedule transplantation before the development of life-threatening complications or severe malnutrition[211]. Children with acute liver failure, PELD > 20, and severe growth retardation have significantly lower overall survival than other groups. Previous major surgery influences the incidence of complications, especially bowel perforation, but do not negatively impact overall patient or graft survival. Long-term survival is mainly influenced by the consequences of prolonged immunosuppression such as infection, PTLD, renal insufficiency, hypertension, diabetes mellitus, and coronary artery disease[212].

REFERENCES

1 **McDiarmid SV**, Anand R, Lindblad AS. Studies of Pediatric Liver Transplantation: 2002 update. An overview of demographics, indications, timing, and immunosuppressive practices in pediatric liver transplantation in the United States and Canada. *Pediatr Transplant* 2004; **8**: 284-294

2 **Kaufman SS**, Wood RP, Shaw BW Jr, Markin RS, Gridelli B, Vanderhoof JA. Hepatocarcinoma in a child with the Alagille syndrome. *Am J Dis Child* 1987; **141**: 698-700

3 **Kayler LK**, Rasmussen CS, Dykstra DM, Punch JD, Rudich SM, Magee JC, Maraschio MA, Arenas JD, Campbell DA Jr, Merion RM. Liver transplantation in children with metabolic disorders in the United States. *Am J Transplant* 2003; **3**: 334-339

4 **Kemper MJ**. The role of preemptive liver transplantation in primary hyperoxaluria type 1. *Urol Res* 2005; **33**: 376-379

5 **Kemper MJ**. Concurrent or sequential liver and kidney transplantation in children with primary hyperoxaluria type 1? *Pediatr Transplant* 2005; **9**: 693-696

6 **Barshes NR**, Vanatta JM, Patel AJ, Carter BA, O'Mahony CA, Karpen SJ, Goss JA. Evaluation and management of patients with propionic acidemia undergoing liver transplantation: a comprehensive review. *Pediatr Transplant* 2006; **10**: 773-781

7 **Kasahara M**, Horikawa R, Tagawa M, Uemoto S, Yokoyama S, Shibata Y, Kawano T, Kuroda T, Honna T, Tanaka K, Saeki M. Current role of liver transplantation for methylmalonic acidemia: a review of the literature. *Pediatr Transplant* 2006; **10**: 943-947

8 **Wendel U**, Saudubray JM, Bodner A, Schadewaldt P. Liver transplantation in maple syrup urine disease. *Eur J Pediatr* 1999; **158** Suppl 2: S60-S64

9 **Squires RH Jr**, Shneider BL, Bucuvalas J, Alonso E, Sokol RJ, Narkewicz MR, Dhawan A, Rosenthal P, Rodriguez-Baez N, Murray KF, Horslen S, Martin MG, Lopez MJ, Soriano H, McGuire BM, Jonas MM, Yazigi N, Shepherd RW, Schwarz K, Lobritto S, Thomas DW, Lavine JE, Karpen S, Ng V, Kelly D, Simonds N, Hynan LS. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr* 2006; **148**: 652-658

10 **Futagawa Y**, Terasaki PI. An analysis of the OPTN/UNOS Liver Transplant Registry. *Clin Transpl* 2004; 315-329

11 **Otte JB**, de Ville de Goyet J, Reding R. Liver transplantation for hepatoblastoma: indications and contraindications in the modern era. *Pediatr Transplant* 2005; **9**: 557-565

12 **Freeman RB Jr**, Wiesner RH, Roberts JP, McDiarmid S, Dykstra DM, Merion RM. Improving liver allocation: MELD and PELD. *Am J Transplant* 2004; **4** Suppl 9: 114-131

13 **McDiarmid SV**, Merion RM, Dykstra DM, Harper AM. Selection of pediatric candidates under the PELD system. *Liver Transpl* 2004; **10**: S23-S30

14 **Bourdeaux C**, Tri TT, Gras J, Sokal E, Otte JB, de Ville de Goyet J, Reding R. PELD score and posttransplant outcome in pediatric liver transplantation: a retrospective study of 100 recipients. *Transplantation* 2005; **79**: 1273-1276

15 **The donors and the organs**. In: The Puzzle people: Memories of a transplant surgeon. Starzl TE editor. Pittsburgh: University of Pittsburgh Press, 1992

16 **Emond JC**, Whitington PF, Thistlethwaite JR, Cherqui D, Alonso EA, Woodle IS, Vogelbach P, Busse-Henry SM, Zucker AR, Broelsch CE. Transplantation of two patients with one liver. Analysis of a preliminary experience with 'split-liver' grafting. *Ann Surg* 1990; **212**: 14-22

17 **Starzl TE**, Hakala TR, Shaw BW Jr, Hardesty RL, Rosenthal TJ, Griffith BP, Iwatsuki S, Bahnson HT. A flexible procedure for multiple cadaveric organ procurement. *Surg Gynecol Obstet* 1984; **158**: 223-230

18 **Starzl TE**, Miller C, Broznick B, Makowka L. An improved technique for multiple organ harvesting. *Surg Gynecol Obstet* 1987; **165**: 343-348

19 **Nakazato PZ**, Concepcion W, Bry W, Limm W, Tokunaga Y, Itasaka H, Feduska N, Esquivel CO, Collins GM. Total abdominal evisceration: an en bloc technique for abdominal organ harvesting. *Surgery* 1992; **111**: 37-47

20 **Tzakis A**, Todo S, Starzl TE. Orthotopic liver transplantation with preservation of the inferior vena cava. *Ann Surg* 1989; **210**: 649-652

21 **Jones WT**, Ratner I, Abrahamian G, Washburn WK, Esterl R, Neigut D, Halff G. Use of a silastic silo for closure of the abdominal wall in a pediatric patient receiving a cadaveric split liver. *J Pediatr Surg* 2003; **38**: E20-E22

22 **Bismuth H**, Houssin D. Reduced-sized orthotopic liver graft in hepatic transplantation in children. *Surgery* 1984; **95**: 367-370

23 **Broelsch CE**, Emond JC, Thistlethwaite JR, Whitington PF,

Zucker AR, Baker AL, Aran PF, Rouch DA, Lichtor JL. Liver transplantation, including the concept of reduced-size liver transplants in children. *Ann Surg* 1988; **208**: 410-420
24 **Broelsch CE**, Emond JC, Whitington PF, Thistlethwaite JR, Baker AL, Lichtor JL. Application of reduced-size liver transplants as split grafts, auxiliary orthotopic grafts, and living related segmental transplants. *Ann Surg* 1990; **212**: 368-375; discussion 375-377
25 **Broelsch CE**, Emond JC, Thistlethwaite JR, Rouch DA, Whitington PF, Lichtor JL. Liver transplantation with reduced-size donor organs. *Transplantation* 1988; **45**: 519-524
26 **Otte JB**, de Ville de Goyet J, Sokal E, Alberti D, Moulin D, de Hemptinne B, Veyckemans F, van Obbergh L, Carlier M, Clapuyt P. Size reduction of the donor liver is a safe way to alleviate the shortage of size-matched organs in pediatric liver transplantation. *Ann Surg* 1990; **211**: 146-157
27 **Houssin D**, Soubrane O, Boillot O, Dousset B, Ozier Y, Devictor D, Bernard O, Chapuis Y. Orthotopic liver transplantation with a reduced-size graft: an ideal compromise in pediatrics? *Surgery* 1992; **111**: 532-542
28 **Kalayoglu M**, D'Alessandro AM, Sollinger HW, Hoffman RM, Pirsch JD, Belzer FO. Experience with reduced-size liver transplantation. *Surg Gynecol Obstet* 1990; **171**: 139-147
29 **Esquivel CO**, Nakazato P, Cox K, Concepcion W, Berquist W, Russell TR. The impact of liver reductions in pediatric liver transplantation. *Arch Surg* 1991; **126**: 1278-1285; discussion 1285-1286
30 **Langnas AN**, Marujo WC, Inagaki M, Stratta RJ, Wood RP, Shaw BW Jr. The results of reduced-size liver transplantation, including split livers, in patients with end-stage liver disease. *Transplantation* 1992; **53**: 387-391
31 **Raia S**, Nery JR, Mies S. Liver transplantation from live donors. *Lancet* 1989; **2**: 497
32 **Strong RW**, Lynch SV, Ong TH, Matsunami H, Koido Y, Balderson GA. Successful liver transplantation from a living donor to her son. *N Engl J Med* 1990; **322**: 1505-1507
33 **Tanaka K**, Uemoto S, Tokunaga Y, Fujita S, Sano K, Yamamoto E, Sugano M, Awane M, Yamaoka Y, Kumada K. Living related liver transplantation in children. *Am J Surg* 1994; **168**: 41-48
34 **Emond JC**, Heffron TG, Kortz EO, Gonzalez-Vallina R, Contis JC, Black DD, Whitington PF. Improved results of living-related liver transplantation with routine application in a pediatric program. *Transplantation* 1993; **55**: 835-840
35 **Broelsch CE**, Whitington PF, Emond JC, Heffron TG, Thistlethwaite JR, Stevens L, Piper J, Whitington SH, Lichtor JL. Liver transplantation in children from living related donors. Surgical techniques and results. *Ann Surg* 1991; **214**: 428-437; discussion 437-439
36 **Malagó M**, Rogiers X, Burdelski M, Broelsch CE. Living related liver transplantation: 36 cases at the University of Hamburg. *Transplant Proc* 1994; **26**: 3620-3621
37 **Otte JB**, de Ville de Goyet J, Reding R, Sokal E, Lerut J, Vanormelingen P, Janssen M. Living related donor liver transplantation in children: the Brussels experience. *Transplant Proc* 1996; **28**: 2378-2379
38 **Haberal M**, Bilgin N, Büyükpamukçu N, Karakayali H, Moray G, Arslan G. Living-related partial liver transplantation in pediatric patients. *Transplant Proc* 1998; **30**: 706-707
39 **Darwish AA**, Bourdeaux C, Kader HA, Janssen M, Sokal E, Lerut J, Ciccarelli O, Veyckemans F, Otte JB, de Goyet Jde V, Reding R. Pediatric liver transplantation using left hepatic segments from living related donors: surgical experience in 100 recipients at Saint-Luc University Clinics. *Pediatr Transplant* 2006; **10**: 345-353
40 **Pichlmayr R**, Ringe B, Gubernatis G, Hauss J, Bunzendahl H. [Transplantation of a donor liver to 2 recipients (splitting transplantation)--a new method in the further development of segmental liver transplantation] *Langenbecks Arch Chir* 1988; **373**: 127-130
41 **Bismuth H**, Morino M, Castaing D, Gillon MC, Descorps Declere A, Saliba F, Samuel D. Emergency orthotopic liver transplantation in two patients using one donor liver. *Br J Surg* 1989; **76**: 722-724
42 **Otte JB**, de Ville de Goyet J, Alberti D, Balladur P, de Hemptinne B. The concept and technique of the split liver in clinical transplantation. *Surgery* 1990; **107**: 605-612
43 **Houssin D**, Boillot O, Soubrane O, Couinaud C, Pitre J, Ozier Y, Devictor D, Bernard O, Chapuis Y. Controlled liver splitting for transplantation in two recipients: technique, results and perspectives. *Br J Surg* 1993; **80**: 75-80
44 **Otte JB**. Is it right to develop living related liver transplantation? Do reduced and split livers not suffice to cover the needs? *Transpl Int* 1995; **8**: 69-73
45 **Kalayoglu M**, D'Alessandro AM, Knechtle SJ, Hoffmann RM, Pirsch JD, Judd RH, Armbrust M, Spaith E, Pilli G, Young CJ, Geffner SR, Odorico JS, Sollinger HW, Belzer FO. Preliminary experience with split liver transplantation. *J Am Coll Surg* 1996; **182**: 381-387
46 **Rogiers X**, Malagó M, Gawad KA, Kuhlencordt R, Fröschle G, Sturm E, Sterneck M, Pothmann W, Schulte am Esch J, Burdelski M, Broelsch C. One year of experience with extended application and modified techniques of split liver transplantation. *Transplantation* 1996; **61**: 1059-1061
47 **Azoulay D**, Astarcioglu I, Bismuth H, Castaing D, Majno P, Adam R, Johann M. Split-liver transplantation. The Paul Brousse policy. *Ann Surg* 1996; **224**: 737-746; discussion 746-748
48 **Dunn SP**, Haynes JH, Nicolette LA, Falkenstein K, Pierson A, Billmire DF, Vinocur CD, Weintraub W. Split liver transplantation benefits the recipient of the 'leftover liver'. *J Pediatr Surg* 1997; **32**: 252-254; discussion 254-255
49 **Rela M**, Vougas V, Muiesan P, Vilca-Melendez H, Smyrniotis V, Gibbs P, Karani J, Williams R, Heaton N. Split liver transplantation: King's College Hospital experience. *Ann Surg* 1998; **227**: 282-288
50 **Mirza DF**, Achilleos O, Pirenne J, Buckels JA, McMaster P, Mayer AD. Encouraging results of split-liver transplantation. *Br J Surg* 1998; **85**: 494-497
51 **Chardot C**, Branchereau S, de Dreuzy O, Dubuisson C, Le Pommelet C, Waguet J, Vellutini G, Gauthier F, Valayer J. Paediatric liver transplantation with a split graft: experience at Bicêtre. *Eur J Pediatr Surg* 1999; **9**: 146-152
52 **Reyes J**, Gerber D, Mazariegos GV, Casavilla A, Sindhi R, Bueno J, Madariaga J, Fung JJ. Split-liver transplantation: a comparison of ex vivo and in situ techniques. *J Pediatr Surg* 2000; **35**: 283-289; discussion 289-290
53 **Deshpande RR**, Bowles MJ, Vilca-Melendez H, Srinivasan P, Girlanda R, Dhawan A, Mieli-Vergani G, Muiesan P, Heaton ND, Rela M. Results of split liver transplantation in children. *Ann Surg* 2002; **236**: 248-253
54 **Noujaim HM**, Gunson B, Mayer DA, Mirza DF, Buckels JA, Candinas D, McMaster P, de Ville de Goyet J. Worth continuing doing ex situ liver graft splitting? A single-center analysis. *Am J Transplant* 2003; **3**: 318-323
55 **Oswari H**, Lynch SV, Fawcett J, Strong RW, Ee LC. Outcomes of split versus reduced-size grafts in pediatric liver transplantation. *J Gastroenterol Hepatol* 2005; **20**: 1850-1854
56 **Rogiers X**, Malagó M, Gawad K, Jauch KW, Olausson M, Knoefel WT, Gundlach M, Bassas A, Fischer L, Sterneck M, Burdelski M, Broelsch CE. In situ splitting of cadaveric livers. The ultimate expansion of a limited donor pool. *Ann Surg* 1996; **224**: 331-339; discussion 339-341
57 **Goss JA**, Yersiz H, Shackleton CR, Seu P, Smith CV, Markowitz JS, Farmer DG, Ghobrial RM, Markmann JF, Arnaout WS, Imagawa DK, Colquhoun SD, Fraiman MH, McDiarmid SV, Busuttil RW. In situ splitting of the cadaveric liver for transplantation. *Transplantation* 1997; **64**: 871-877
58 **Busuttil RW**, Goss JA. Split liver transplantation. *Ann Surg* 1999; **229**: 313-321
59 **Ghobrial RM**, Yersiz H, Farmer DG, Amersi F, Goss J, Chen

P, Dawson S, Lerner S, Nissen N, Imagawa D, Colquhoun S, Arnout W, McDiarmid SV, Busuttil RW. Predictors of survival after In vivo split liver transplantation: analysis of 110 consecutive patients. *Ann Surg* 2000; **232**: 312-323

60 **Spada M**, Gridelli B, Colledan M, Segalin A, Lucianetti A, Petz W, Riva S, Torre G. Extensive use of split liver for pediatric liver transplantation: a single-center experience. *Liver Transpl* 2000; **6**: 415-428

61 **Gridelli B**, Spada M, Petz W, Bertani A, Lucianetti A, Colledan M, Altobelli M, Alberti D, Guizzetti M, Riva S, Melzi ML, Stroppa P, Torre G. Split-liver transplantation eliminates the need for living-donor liver transplantation in children with end-stage cholestatic liver disease. *Transplantation* 2003; **75**: 1197-1203

62 **Yersiz H**, Renz JF, Farmer DG, Hisatake GM, McDiarmid SV, Busuttil RW. One hundred in situ split-liver transplantations: a single-center experience. *Ann Surg* 2003; **238**: 496-505; discussion 506-507

63 **Rogiers X**, Malagó M, Habib N, Broelsch CE. An easy technique for inferior vena cava control in pediatric liver transplantation. *J Am Coll Surg* 1996; **182**: 555-556

64 **Emond JC**, Heffron TG, Whitington PF, Broelsch CE. Reconstruction of the hepatic vein in reduced size hepatic transplantation. *Surg Gynecol Obstet* 1993; **176**: 11-17

65 **Chardot C**, Saint Martin C, Gilles A, Brichard B, Janssen M, Sokal E, Clapuyt P, Lerut J, Reding R, Otte JB. Living-related liver transplantation and vena cava reconstruction after total hepatectomy including the vena cava for hepatoblastoma. *Transplantation* 2002; **73**: 90-92

66 **Corno V**, Colledan M, Segalin A, Lucianetti A, Spada M, Gridelli B. Recostrction of inferior vena cava in pediatric liver trasplantation for malignancy. *Liver Trasplant Surg* 1999; **5**: 170

67 **Urata K**, Kawasaki S, Matsunami H, Hashikura Y, Ikegami T, Ishizone S, Momose Y, Komiyama A, Makuuchi M. Calculation of child and adult standard liver volume for liver transplantation. *Hepatology* 1995; **21**: 1317-1321

68 **Heinemann A**, Wischhusen F, Püschel K, Rogiers X. Standard liver volume in the Caucasian population. Liver *Transpl Surg* 1999; **5**: 366-368

69 **Noda T**, Todani T, Watanabe Y, Yamamoto S. Liver volume in children measured by computed tomography. *Pediatr Radiol* 1997; **27**: 250-252

70 **Yoshizumi T**, Gondolesi GE, Bodian CA, Jeon H, Schwartz ME, Fishbein TM, Miller CM, Emre S. A simple new formula to assess liver weight. *Transplant Proc* 2003; **35**: 1415-1420

71 **Vauthey JN**, Abdalla EK, Doherty DA, Gertsch P, Fenstermacher MJ, Loyer EM, Lerut J, Materne R, Wang X, Encarnacion A, Herron D, Mathey C, Ferrari G, Charnsangavej C, Do KA, Denys A. Body surface area and body weight predict total liver volume in Western adults. *Liver Transpl* 2002; **8**: 233-240

72 **DeLand FH**, North WA. Relationship between liver size and body size. *Radiology* 1968; **91**: 1195-1198

73 **Emond JC**, Freeman RB Jr, Renz JF, Yersiz H, Rogiers X, Busuttil RW. Optimizing the use of donated cadaver livers: analysis and policy development to increase the application of split-liver transplantation. *Liver Transpl* 2002; **8**: 863-872

74 **McDiarmid SV**, Davies DB, Edwards EB. Improved graft survival of pediatric liver recipients transplanted with pediatric-aged liver donors. *Transplantation* 2000; **70**: 1283-1291

75 **Adam R**, Cailliez V, Majno P, Karam V, McMaster P, Caine RY, O'Grady J, Pichlmayr R, Neuhaus P, Otte JB, Hoeckerstedt K, Bismuth H. Normalised intrinsic mortality risk in liver transplantation: European Liver Transplant Registry study. *Lancet* 2000; **356**: 621-627

76 **Petz W**, Spada M, Sonzogni A, Colledan M, Segalin A, Lucianetti A, Bertani A, Guizzetti M, Peloni G, Gridelli B. Pediatric split liver transplantation using elderly donors. *Transplant Proc* 2001; **33**: 1361-1363

77 **Cescon M**, Spada M, Colledan M, Andorno E, Valente U, Rossi G, Reggiani P, Grazi GL, Tisone G, Majno P, Rogiers X, Santamaria ML, Baccarani U, Ettorre GM, Cillo U, Rossi M, Scalamogna M, Gridelli B. Split-liver transplantation with pediatric donors: a multicenter experience. *Transplantation* 2005; **79**: 1148-1153

78 **Cescon M**, Spada M, Colledan M, Torre G, Andorno E, Valente U, Rossi G, Reggiani P, Cillo U, Baccarani U, Grazi GL, Tisone G, Filipponi F, Rossi M, Ettorre GM, Salizzoni M, Cuomo O, De Feo T, Gridelli B. Feasibility and limits of split liver transplantation from pediatric donors: an italian multicenter experience. *Ann Surg* 2006; **244**: 805-814

79 **Renz JF**, Yersiz H, Reichert PR, Hisatake GM, Farmer DG, Emond JC, Busuttil RW. Split-liver transplantation: a review. *Am J Transplant* 2003; **3**: 1323-1335

80 **Langnas AN**, Marujo W, Stratta RJ, Wood RP, Li SJ, Shaw BW. Hepatic allograft rescue following arterial thrombosis. Role of urgent revascularization. *Transplantation* 1991; **51**: 86-90

81 **Corno V**, Torri E, Bertani A, Guizzetti M, Lucianetti A, Maldini G, Pinelli D, Zambelli M, Aluffi A, Alberti D, Spada M, Gridelli B, Torre G, Colledan M. Early portal vein thrombosis after pediatric split liver transplantation with left lateral segment graft. *Transplant Proc* 2005; **37**: 1141-1142

82 **Ueda M**, Egawa H, Ogawa K, Uryuhara K, Fujimoto Y, Kasahara M, Ogura Y, Kozaki K, Takada Y, Tanaka K. Portal vein complications in the long-term course after pediatric living donor liver transplantation. *Transplant Proc* 2005; **37**: 1138-1140

83 **Heffron TG**, Emond JC, Whitington PF, Thistlethwaite JR Jr, Stevens L, Piper J, Whitington S, Broelsch CE. Biliary complications in pediatric liver transplantation. A comparison of reduced-size and whole grafts. *Transplantation* 1992; **53**: 391-395

84 **Peclet MH**, Ryckman FC, Pedersen SH, Dittrich VS, Heubi JE, Farrell M, Balistreri WF, Ziegler MM. The spectrum of bile duct complications in pediatric liver transplantation. *J Pediatr Surg* 1994; **29**: 214-219; discussion 219-220

85 **Sunku B**, Salvalaggio PR, Donaldson JS, Rigsby CK, Neighbors K, Superina RA, Alonso EM. Outcomes and risk factors for failure of radiologic treatment of biliary strictures in pediatric liver transplantation recipients. *Liver Transpl* 2006; **12**: 821-826

86 **Salvalaggio PR**, Whitington PF, Alonso EM, Superina RA. Presence of multiple bile ducts in the liver graft increases the incidence of biliary complications in pediatric liver transplantation. *Liver Transpl* 2005; **11**: 161-166

87 **Seaman DS**, Newell KA, Piper JB, Bruce DS, Woodle ES, Cronin DC 2nd, Alonso EM, Whitington PF, Thistlethwaite JR, Millis JM. Use of polytetrafluoroethylene patch for temporary wound closure after pediatric liver transplantation. *Transplantation* 1996; **62**: 1034-1036

88 **Terminology for hepatic allograft rejection. International Working Party**. *Hepatology* 1995; **22**: 648-654

89 **Banff schema for grading liver allograft rejection: an international consensus document**. *Hepatology* 1997; **25**: 658-663

90 **Ryckman FC**, Schroeder TJ, Pedersen SH, Fisher RA, Farrell MK, Heubi JE, Ziegler MM, Balistreri WF. The use of monoclonal antibody immunosuppressive therapy in pediatric renal and liver transplantation. *Clin Transplant* 1991; **5**: 186-190

91 **Spada M**, Corno V, Colledan M, Segalin A, Lucianetti A, Torre G, Riva S, Sonzogni A, Petz W, Gridelli B. Rejection and tacrolimus conversion therapy in paediatric liver transplantation. *Transpl Int* 2000; **13** Suppl 1: S341-S344

92 **Wiesner RH**, Hermans PE, Rakela J, Washington JA 2nd, Perkins JD, DiCecco S, Krom R. Selective bowel decontamination to decrease gram-negative aerobic bacterial and Candida colonization and prevent infection after orthotopic liver transplantation. *Transplantation* 1988; **45**: 570-574

93 **Singh N**, Carrigan DR, Gayowski T, Marino IR. Human

herpesvirus-6 infection in liver transplant recipients: documentation of pathogenicity. *Transplantation* 1997; **64**: 674-678
94 **Patel R**, Snydman DR, Rubin RH, Ho M, Pescovitz M, Martin M, Paya CV. Cytomegalovirus prophylaxis in solid organ transplant recipients. *Transplantation* 1996; **61**: 1279-1289
95 **Fox AS**, Tolpin MD, Baker AL, Broelsch CE, Whittington PF, Jackson T, Thistlethwaite JR, Stuart FP. Seropositivity in liver transplant recipients as a predictor of cytomegalovirus disease. *J Infect Dis* 1988; **157**: 383-385
96 **Darenkov IA**, Marcarelli MA, Basadonna GP, Friedman AL, Lorber KM, Howe JG, Crouch J, Bia MJ, Kliger AS, Lorber MI. Reduced incidence of Epstein-Barr virus-associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. *Transplantation* 1997; **64**: 848-852
97 **Manez R**, Kusne S, Rinaldo C, Aguado JM, St George K, Grossi P, Frye B, Fung JJ, Ehrlich GD. Time to detection of cytomegalovirus (CMV) DNA in blood leukocytes is a predictor for the development of CMV disease in CMV-seronegative recipients of allografts from CMV-seropositive donors following liver transplantation. *J Infect Dis* 1996; **173**: 1072-1076
98 **Kusne S**, Grossi P, Irish W, St George K, Rinaldo C, Rakela J, Fung J. Cytomegalovirus PP65 antigenemia monitoring as a guide for preemptive therapy: a cost effective strategy for prevention of cytomegalovirus disease in adult liver transplant recipients. *Transplantation* 1999; **68**: 1125-1131
99 **Spada M**, Guizzetti M, Petz W, Colledan M, Segalin A, Lucianetti A, Bertani A, Peloni G, Sonzogni A, Alberti D, Riva S, Melzi M, Gridelli B. Circulating EBV-DNA in the monitoring of EBV infection in pediatric liver transplant recipients. *Transplant Proc* 2001; **33**: 1835-1837
100 **Holmes RD**, Orban-Eller K, Karrer FR, Rowe DT, Narkewicz MR, Sokol RJ. Response of elevated Epstein-Barr virus DNA levels to therapeutic changes in pediatric liver transplant patients: 56-month follow up and outcome. *Transplantation* 2002; **74**: 367-372
101 **Holmes RD**, Sokol RJ. Epstein-Barr virus and post-transplant lymphoproliferative disease. *Pediatr Transplant* 2002; **6**: 456-464
102 **Starzl TE**, Klintmalm GB, Porter KA, Iwatsuki S, Schröter GP. Liver transplantation with use of cyclosporin a and prednisone. *N Engl J Med* 1981; **305**: 266-269
103 **Starzl TE**, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A. FK 506 for liver, kidney, and pancreas transplantation. *Lancet* 1989; **2**: 1000-1004
104 **Fryer JP**, Granger DK, Leventhal JR, Gillingham K, Najarian JS, Matas AJ. Steroid-related complications in the cyclosporine era. *Clin Transplant* 1994; **8**: 224-229
105 **Viner RM**, Forton JT, Cole TJ, Clark IH, Noble-Jamieson G, Barnes ND. Growth of long-term survivors of liver transplantation. *Arch Dis Child* 1999; **80**: 235-240
106 **Bartosh SM**, Thomas SE, Sutton MM, Brady LM, Whitington PF. Linear growth after pediatric liver transplantation. *J Pediatr* 1999; **135**: 624-631
107 **Hyams JS**, Carey DE. Corticosteroids and growth. *J Pediatr* 1988; **113**: 249-254
108 **Veenstra DL**, Best JH, Hornberger J, Sullivan SD, Hricik DE. Incidence and long-term cost of steroid-related side effects after renal transplantation. *Am J Kidney Dis* 1999; **33**: 829-839
109 **Reding R**. Steroid withdrawal in liver transplantation: benefits, risks, and unanswered questions. *Transplantation* 2000; **70**: 405-410
110 **Margarit C**, Martínez Ibañez V, Tormo R, Infante D, Iglesias H. Maintenance immunosuppression without steroids in pediatric liver transplantation. *Transplant Proc* 1989; **21**: 2230-2231
111 **Andrews WS**, Shimaoka S, Sommerauer J, Moore P, Hudgins P. Steroid withdrawal after pediatric liver transplantation. *Transplant Proc* 1994; **26**: 159-160
112 **Dunn SP**, Falkenstein K, Lawrence JP, Meyers R, Vinocur CD, Billmire DF, Weintraub WH. Monotherapy with cyclosporine for chronic immunosuppression in pediatric liver transplant recipients. *Transplantation* 1994; **57**: 544-547
113 **McDiarmid SV**, Farmer DA, Goldstein LI, Martin P, Vargas J, Tipton JR, Simmons F, Busuttil RW. A randomized prospective trial of steroid withdrawal after liver transplantation. *Transplantation* 1995; **60**: 1443-1450
114 **McKee M**, Mattei P, Schwarz K, Wise B, Colombani P. Steroid withdrawal in tacrolimus (FK506)-treated pediatric liver transplant recipients. *J Pediatr Surg* 1997; **32**: 973-975
115 **Martin SR**, Paradis K, Alvarez F. Cyclosporine monotherapy in long-term pediatric liver transplant recipients. *Transplant Proc* 1998; **30**: 1424-1426
116 **Diem HV**, Sokal EM, Janssen M, Otte JB, Reding R. Steroid withdrawal after pediatric liver transplantation: a long-term follow-up study in 109 recipients. *Transplantation* 2003; **75**: 1664-1670
117 **Atkison PR**, Ross BC, Williams S, Howard J, Sommerauer J, Quan D, Wall W. Long-term results of pediatric liver transplantation in a combined pediatric and adult transplant program. *CMAJ* 2002; **166**: 1663-1671
118 **Toyoki Y**, Hakamada K, Narumi S, Totsuka E, Nara M, Ono H, Ishizawa Y, Sasaki M. Primary immunosuppression regimen of rapid steroid withdrawal after living related liver transplantation: a single-center experience. *Transplant Proc* 2004; **36**: 2279-2281
119 **Reding R**, Gras J, Sokal E, Otte JB, Davies HF. Steroid-free liver transplantation in children. *Lancet* 2003; **362**: 2068-2070
120 **Spada M**, Petz W, Bertani A, Riva S, Sonzogni A, Giovannelli M, Torri E, Torre G, Colledan M, Gridelli B. Randomized trial of basiliximab induction versus steroid therapy in pediatric liver allograft recipients under tacrolimus immunosuppression. *Am J Transplant* 2006; **6**: 1913-1921
121 **Liu J**, Farmer JD Jr, Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and FKBP-FK506 complexes. *Cell* 1991; **66**: 807-815
122 **Staatz CE**, Taylor PJ, Lynch SV, Tett SE. A pharmacodynamic investigation of tacrolimus in pediatric liver transplantation. *Liver Transpl* 2004; **10**: 506-512
123 **Aw MM**, Samaroo B, Baker AJ, Verma A, Rela M, Heaton ND, Mieli-Vergani G, Dhawan A. Calcineurin-inhibitor related nephrotoxicity- reversibility in paediatric liver transplant recipients. *Transplantation* 2001; **72**: 746-749
124 **Manzarbeitia C**, Reich DJ, Rothstein KD, Braitman LE, Levin S, Munoz SJ. Tacrolimus conversion improves hyperlipidemic states in stable liver transplant recipients. *Liver Transpl* 2001; **7**: 93-99
125 **Van Thiel DH**, Iqbal M, Jain A, Fung J, Todo S, Starzl TE. Gastrointestinal and metabolic problems associated with immunosuppression with either CyA or FK 506 in liver transplantation. *Transplant Proc* 1990; **22**: 37-40
126 **Cox KL**, Lawrence-Miyasaki LS, Garcia-Kennedy R, Lennette ET, Martinez OM, Krams SM, Berquist WE, So SK, Esquivel CO. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. *Transplantation* 1995; **59**: 524-529
127 **Younes BS**, McDiarmid SV, Martin MG, Vargas JH, Goss JA, Busuttil RW, Ament ME. The effect of immunosuppression on posttransplant lymphoproliferative disease in pediatric liver transplant patients. *Transplantation* 2000; **70**: 94-99
128 **Jain A**, Mazariegos G, Kashyap R, Green M, Gronsky C, Starzl TE, Fung J, Reyes J. Comparative long-term evaluation of tacrolimus and cyclosporine in pediatric liver transplantation. *Transplantation* 2000; **70**: 617-625
129 **Atkison P**, Joubert G, Barron A, Grant D, Paradis K, Seidman E, Wall W, Rosenberg H, Howard J, Williams S. Hypertrophic cardiomyopathy associated with tacrolimus in paediatric transplant patients. *Lancet* 1995; **345**: 894-896
130 **Drewe J**, Beglinger C, Kissel T. The absorption site of

cyclosporin in the human gastrointestinal tract. *Br J Clin Pharmacol* 1992; **33**: 39-43
131 **Faulds D**, Goa KL, Benfield P. Cyclosporin. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in immunoregulatory disorders. *Drugs* 1993; **45**: 953-1040
132 **Martin SR**, Atkison P, Anand R, Lindblad AS. Studies of Pediatric Liver Transplantation 2002: patient and graft survival and rejection in pediatric recipients of a first liver transplant in the United States and Canada. *Pediatr Transplant* 2004; **8**: 273-283
133 **Millis JM**, Cronin DC, Newell KA, Bruce DS, Woodle ES, Grewal HP, Loss GE, Lissoos T, Conjeevaram H, Schiano T, O'Laughlin R, Charette J, McNaughton M, Baker AL, Thistlethwaite JR Jr. Successful use of tacrolimus for initial rejection episodes after liver transplantation. *Transplant Proc* 1998; **30**: 1407-1408
134 **Cooney GF**, Habucky K, Hoppu K. Cyclosporin pharmacokinetics in paediatric transplant recipients. *Clin Pharmacokinet* 1997; **32**: 481-495
135 **Trull AK**, Tan KK, Tan L, Alexander GJ, Jamieson NV. Absorption of cyclosporin from conventional and new microemulsion oral formulations in liver transplant recipients with external biliary diversion. *Br J Clin Pharmacol* 1995; **39**: 627-631
136 **Pescovitz MD**, Puente JG, Jindal RM, Fitzgerald J, Chong SK, Milgrom ML, Leapman SB, Filo RS. Improved absorption of cyclosporine for microemulsion in a pediatric liver transplant recipient with cystic fibrosis. *Transplantation* 1996; **61**: 331-333
137 **Lindholm A**, Kahan BD. Influence of cyclosporine pharmacokinetics, trough concentrations, and AUC monitoring on outcome after kidney transplantation. *Clin Pharmacol Ther* 1993; **54**: 205-218
138 **Cantarovich M**, Barkun JS, Tchervenkov JI, Besner JG, Aspeslet L, Metrakos P. Comparison of neoral dose monitoring with cyclosporine through levels versus 2-hr postdose levels in stable liver transplant patients. *Transplantation* 1998; **66**: 1621-1627
139 **Grant D**, Kneteman N, Tchervenkov J, Roy A, Murphy G, Tan A, Hendricks L, Guilbault N, Levy G. Peak cyclosporine levels (Cmax) correlate with freedom from liver graft rejection: results of a prospective, randomized comparison of neoral and sandimmune for liver transplantation (NOF-8). *Transplantation* 1999; **67**: 1133-1137
140 **McDiarmid SV**, Colonna JO 2nd, Shaked A, Vargas J, Ament ME, Busuttil RW. Differences in oral FK506 dose requirements between adult and pediatric liver transplant patients. *Transplantation* 1993; **55**: 1328-1332
141 **McDiarmid SV**. The use of tacrolimus in pediatric liver transplantation. *J Pediatr Gastroenterol Nutr* 1998; **26**: 90-102
142 **Sollinger HW**. Mycophenolate mofetil. *Kidney Int Suppl* 1995; **52**: S14-S17
143 **Dayton JS**, Lindsten T, Thompson CB, Mitchell BS. Effects of human T lymphocyte activation on inosine monophosphate dehydrogenase expression. *J Immunol* 1994; **152**: 984-991
144 **Chardot C**, Nicoluzzi JE, Janssen M, Sokal E, Lerut J, Otte JB, Reding R. Use of mycophenolate mofetil as rescue therapy after pediatric liver transplantation. *Transplantation* 2001; **71**: 224-229
145 **Evans HM**, McKiernan PJ, Kelly DA. Mycophenolate mofetil for renal dysfunction after pediatric liver transplantation. *Transplantation* 2005; **79**: 1575-1580
146 **Stegall MD**, Wachs ME, Everson G, Steinberg T, Bilir B, Shrestha R, Karrer F, Kam I. Prednisone withdrawal 14 days after liver transplantation with mycophenolate: a prospective trial of cyclosporine and tacrolimus. *Transplantation* 1997; **64**: 1755-1760
147 **Eckhoff DE**, McGuire BM, Frenette LR, Contreras JL, Hudson SL, Bynon JS. Tacrolimus (FK506) and mycophenolate mofetil combination therapy versus tacrolimus in adult liver transplantation. *Transplantation* 1998; **65**: 180-187
148 **Aw MM**, Brown NW, Itsuka T, Gonde CE, Adams JE, Heaton ND, Tredger JM, Mieli-Vergani G, Dhawan A. Mycophenolic acid pharmacokinetics in pediatric liver transplant recipients. *Liver Transpl* 2003; **9**: 383-388
149 **Brown NW**, Aw MM, Mieli-Vergani G, Dhawan A, Tredger JM. Mycophenolic acid and mycophenolic acid glucuronide pharmacokinetics in pediatric liver transplant recipients: effect of cyclosporine and tacrolimus comedication. *Ther Drug Monit* 2002; **24**: 598-606
150 **Tredger JM**, Brown NW, Adams J, Gonde CE, Dhawan A, Rela M, Heaton N. Monitoring mycophenolate in liver transplant recipients: toward a therapeutic range. *Liver Transpl* 2004; **10**: 492-502
151 **Fulton B**, Markham A. Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. *Drugs* 1996; **51**: 278-298
152 **Napoli KL**, Taylor PJ. From beach to bedside: history of the development of sirolimus. *Ther Drug Monit* 2001; **23**: 559-586
153 **McAlister VC**, Gao Z, Peltekian K, Domingues J, Mahalati K, MacDonald AS. Sirolimus-tacrolimus combination immunosuppression. *Lancet* 2000; **355**: 376-377
154 **McAlister VC**, Peltekian KM, Malatjalian DA, Colohan S, MacDonald S, Bitter-Suermann H, MacDonald AS. Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. *Liver Transpl* 2001; **7**: 701-708
155 **Peltekian K**, McAlister VC, Colohan S, Gao Z, Salazar AB, Bitter-Suermann H, MacDonald AS. De novo use of low-dose tacrolimus and sirolimus in liver transplantation. *Transplant Proc* 2001; **33**: 1341
156 **Pridöhl O**, Heinemann K, Hartwig T, Witzigmann H, Lamesch P, Fangmann J, Berr F, Hauss J, Kohlhaw K. Low-dose immunosuppression with FK 506 and sirolimus after liver transplantation: 1-year results. *Transplant Proc* 2001; **33**: 3229-3231
157 **Sindhi R**, Ganjoo J, McGhee W, Mazariegos G, Reyes J. Preliminary immunosuppression withdrawal strategies with sirolimus in children with liver transplants. *Transplant Proc* 2002; **34**: 1972-1973
158 **Cotterell AH**, Fisher RA, King AL, Gehr TW, Dawson S, Sterling RK, Stravitz RT, Luketic VA, Sanyal AJ, Shiffman ML, Posner MP. Calcineurin inhibitor-induced chronic nephrotoxicity in liver transplant patients is reversible using rapamycin as the primary immunosuppressive agent. *Clin Transplant* 2002; **16** Suppl 7: 49-51
159 **Neff GW**, Montalbano M, Slapak-Green G, Berney T, Bejarano PA, Joshi A, Icardi M, Nery J, Seigo N, Levi D, Weppler D, Pappas P, Ruiz J, Schiff ER, Tzakis AG. A retrospective review of sirolimus (Rapamune) therapy in orthotopic liver transplant recipients diagnosed with chronic rejection. *Liver Transpl* 2003; **9**: 477-483
160 **Watson CJ**, Friend PJ, Jamieson NV, Frick TW, Alexander G, Gimson AE, Calne R. Sirolimus: a potent new immunosuppressant for liver transplantation. *Transplantation* 1999; **67**: 505-509
161 **MacDonald AS**. A worldwide, phase III, randomized, controlled, safety and efficacy study of a sirolimus/cyclosporine regimen for prevention of acute rejection in recipients of primary mismatched renal allografts. *Transplantation* 2001; **71**: 271-280
162 **Dunkelberg JC**, Trotter JF, Wachs M, Bak T, Kugelmas M, Steinberg T, Everson GT, Kam I. Sirolimus as primary immunosuppression in liver transplantation is not associated with hepatic artery or wound complications. *Liver Transpl* 2003; **9**: 463-468
163 **Morice MC**, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schuler G, Barragan P, Guagliumi G, Molnàr F, Falotico R. A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. *N Engl J Med* 2002; **346**: 1773-1780
164 **Groth CG**, Bäckman L, Morales JM, Calne R, Kreis H,

Lang P, Touraine JL, Claesson K, Campistol JM, Durand D, Wramner L, Brattström C, Charpentier B. Sirolimus (rapamycin)-based therapy in human renal transplantation: similar efficacy and different toxicity compared with cyclosporine. Sirolimus European Renal Transplant Study Group. *Transplantation* 1999; **67**: 1036-1042

165 **Kahan BD**, Napoli KL, Kelly PA, Podbielski J, Hussein I, Urbauer DL, Katz SH, Van Buren CT. Therapeutic drug monitoring of sirolimus: correlations with efficacy and toxicity. *Clin Transplant* 2000; **14**: 97-109

166 **Kaplan B**, Meier-Kriesche HU, Napoli KL, Kahan BD. The effects of relative timing of sirolimus and cyclosporine microemulsion formulation coadministration on the pharmacokinetics of each agent. *Clin Pharmacol Ther* 1998; **63**: 48-53

167 **Asensio M**, Margarit C, Chavez R, Ortega J, Charco R, Iglesias J. Induction with basiliximab reduces acute rejection in pediatric liver transplant patients treated with tacrolimus and steroids. *Transplant Proc* 2002; **34**: 1970-1971

168 **Strassburg A**, Pfister ED, Arning A, Nashan B, Ehrich JH, Melter M. Basiliximab reduces acute liver allograft rejection in pediatric patients. *Transplant Proc* 2002; **34**: 2374-2375

169 **Heffron TG**, Pillen T, Smallwood GA, Welch D, Oakley B, Romero R. Pediatric liver transplantation with daclizumab induction. *Transplantation* 2003; **75**: 2040-2043

170 **Ganschow R**, Broering DC, Stuerenburg I, Rogiers X, Hellwege HH, Burdelski M. First experience with basiliximab in pediatric liver graft recipients. *Pediatr Transplant* 2001; **5**: 353-358

171 **Ganschow R**, Grabhorn E, Schulz A, Von Hugo A, Rogiers X, Burdelski M. Long-term results of basiliximab induction immunosuppression in pediatric liver transplant recipients. *Pediatr Transplant* 2005; **9**: 741-745

172 **Schuller S**, Wiederkehr JC, Coelho-Lemos IM, Avilla SG, Schultz C. Daclizumab induction therapy associated with tacrolimus-MMF has better outcome compared with tacrolimus-MMF alone in pediatric living donor liver transplantation. *Transplant Proc* 2005; **37**: 1151-1152

173 **Kovarik JM**, Gridelli BG, Martin S, Rodeck B, Melter M, Dunn SP, Merion RM, Tzakis AG, Alonso E, Bucuvalas J, Sharp H, Gerbeau C, Chodoff L, Korn A, Hall M. Basiliximab in pediatric liver transplantation: a pharmacokinetic-derived dosing algorithm. *Pediatr Transplant* 2002; **6**: 224-230

174 **Eckhoff DE**, McGuire B, Sellers M, Contreras J, Frenette L, Young C, Hudson S, Bynon JS. The safety and efficacy of a two-dose daclizumab (zenapax) induction therapy in liver transplant recipients. *Transplantation* 2000; **69**: 1867-1872

175 **Smets F**, Sokal EM. Lymphoproliferation in children after liver transplantation. *J Pediatr Gastroenterol Nutr* 2002; **34**: 499-505

176 **Guthery SL**, Heubi JE, Bucuvalas JC, Gross TG, Ryckman FC, Alonso MH, Balistreri WF, Hornung RW. Determination of risk factors for Epstein-Barr virus-associated posttransplant lymphoproliferative disorder in pediatric liver transplant recipients using objective case ascertainment. *Transplantation* 2003; **75**: 987-993

177 **Penn I**. Post-transplant malignancy: the role of immunosuppression. *Drug Saf* 2000; **23**: 101-113

178 **Orjuela M**, Gross TG, Cheung YK, Alobeid B, Morris E, Cairo MS. A pilot study of chemoimmunotherapy (cyclophosphamide, prednisone, and rituximab) in patients with post-transplant lymphoproliferative disorder following solid organ transplantation. *Clin Cancer Res* 2003; **9**: 3945S-3952S

179 **Gross TG**. Low-dose chemotherapy for children with post-transplant lymphoproliferative disease. *Recent Results Cancer Res* 2002; **159**: 96-103

180 **Comoli P**, Ginevri F, Maccario R, Frasson C, Valente U, Basso S, Labirio M, Huang GC, Verrina E, Baldanti F, Perfumo F, Locatelli F. Successful in vitro priming of EBV-specific CD8+ T cells endowed with strong cytotoxic function from T cells of EBV-seronegative children. *Am J Transplant* 2006; **6**: 2169-2176

181 **Demetris AJ**, Fung JJ, Todo S, McCauley J, Jain A, Takaya S, Alessiani M, Abu-Elmagd K, Van Thiel DH, Starzl TE. Conversion of liver allograft recipients from cyclosporine to FK506 immunosuppressive therapy--a clinicopathologic study of 96 patients. *Transplantation* 1992; **53**: 1056-1062

182 **Tsamandas AC**, Jain AB, Felekouras ES, Fung JJ, Demetris AJ, Lee RG. Central venulitis in the allograft liver: a clinicopathologic study. *Transplantation* 1997; **64**: 252-257

183 **Krasinskas AM**, Ruchelli ED, Rand EB, Chittams JL, Furth EE. Central venulitis in pediatric liver allografts. *Hepatology* 2001; **33**: 1141-1147

184 **Pappo O**, Ramos H, Starzl TE, Fung JJ, Demetris AJ. Structural integrity and identification of causes of liver allograft dysfunction occurring more than 5 years after transplantation. *Am J Surg Pathol* 1995; **19**: 192-206

185 **Hübscher SG**. Recurrent autoimmune hepatitis after liver transplantation: diagnostic criteria, risk factors, and outcome. *Liver Transpl* 2001; **7**: 285-291

186 **Freese DK**, Snover DC, Sharp HL, Gross CR, Savick SK, Payne WD. Chronic rejection after liver transplantation: a study of clinical, histopathological and immunological features. *Hepatology* 1991; **13**: 882-891

187 **Demetris A**, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. Update of the International Banff Schema for Liver Allograft Rejection: working recommendations for the histopathologic staging and reporting of chronic rejection. An International Panel. *Hepatology* 2000; **31**: 792-799

188 **White RM**, Zajko AB, Demetris AJ, Bron KM, Dekker A, Starzl TE. Liver transplant rejection: angiographic findings in 35 patients. *AJR Am J Roentgenol* 1987; **148**: 1095-1098

189 **Devlin J**, Page AC, O'Grady J, Portmann B, Karani J, Williams R. Angiographically determined arteriopathy in liver graft dysfunction and survival. *J Hepatol* 1993; **18**: 68-73

190 **Kerkar N**, Hadzić N, Davies ET, Portmann B, Donaldson PT, Rela M, Heaton ND, Vergani D, Mieli-Vergani G. De-novo autoimmune hepatitis after liver transplantation. *Lancet* 1998; **351**: 409-413

191 **Aguilera I**, Wichmann I, Sousa JM, Bernardos A, Franco E, García-Lozano JR, Núñez-Roldán A. Antibodies against glutathione S-transferase T1 (GSTT1) in patients with de novo immune hepatitis following liver transplantation. *Clin Exp Immunol* 2001; **126**: 535-539

192 **Czaja AJ**. Autoimmune hepatitis after liver transplantation and other lessons of self-intolerance. *Liver Transpl* 2002; **8**: 505-513

193 **Czaja AJ**, Freese DK. Diagnosis and treatment of autoimmune hepatitis. *Hepatology* 2002; **36**: 479-497

194 **Evans HM**, Kelly DA, McKiernan PJ, Hübscher S. Progressive histological damage in liver allografts following pediatric liver transplantation. *Hepatology* 2006; **43**: 1109-1117

195 **Kemnitz J**, Gubernatis G, Bunzendahl H, Ringe B, Pichlmayr R, Georgii A. Criteria for the histopathological classification of liver allograft rejection and their clinical relevance. *Transplant Proc* 1989; **21**: 2208-2210

196 **Hubscher SG**, Elias E, Buckels JA, Mayer AD, McMaster P, Neuberger JM. Primary biliary cirrhosis. Histological evidence of disease recurrence after liver transplantation. *J Hepatol* 1993; **18**: 173-184

197 **Neuberger J**, Portmann B, Calne R, Williams R. Recurrence of autoimmune chronic active hepatitis following orthotopic liver grafting. *Transplantation* 1984; **37**: 363-365

198 **Sebagh M**, Yilmaz F, Karam V, Falissard B, Roche B, Azoulay D, Samuel D, Guettier C. The histologic pattern of "biliary tract pathology" is accurate for the diagnosis of biliary complications. *Am J Surg Pathol* 2005; **29**: 318-323

199 **Studies of Pediatric Liver Transplantation (SPLIT) Annual Report**. Rockville (MD): SPLIT, 2004: 1-27
200 **Sokal EM**, Veyckemans F, de Ville de Goyet J, Moulin D, Van Hoorebeeck N, Alberti D, Buts JP, Rahier J, Van Obbergh L, Clapuyt P. Liver transplantation in children less than 1 year of age. *J Pediatr* 1990; **117**: 205-210
201 **Lucianetti A**, Guizzetti M, Bertani A, Corno V, Maldini G, Pinelli D, Aluffi A, Codazzi D, Spotti A, Spada M, Gridelli B, Torre G, Colledan M. Liver transplantation in children weighting less than 6 kg: the Bergamo experience. *Transplant Proc* 2005; **37**: 1143-1145
202 **Pinelli D**, Spada M, Lucianetti A, Riva S, Guizzetti M, Giovanelli M, Maldini G, Corno V, Sonzogni V, Vedovati S, Bertani A, Zambelli M, Gridelli B, Colledan M. Transplantation for acute liver failure in children. *Transplant Proc* 2005; **37**: 1146-1148
203 **Baliga P**, Alvarez S, Lindblad A, Zeng L. Posttransplant survival in pediatric fulminant hepatic failure: the SPLIT experience. *Liver Transpl* 2004; **10**: 1364-1371
204 **Bourdeaux C**, Tri TT, Gras J, Sokal E, Otte JB, de Ville de Goyet J, Reding R. PELD score and posttransplant outcome in pediatric liver transplantation: a retrospective study of 100 recipients. *Transplantation* 2005; **79**: 1273-1276
205 **Lozanov J**, Millis JM, Anand R. Surgical outcomes in primary pediatric liver transplantation: split database report. *Am J Transplant* 2005; **5**: 525
206 **Roberts JP**, Hulbert-Shearon TE, Merion RM, Wolfe RA, Port FK. Influence of graft type on outcomes after pediatric liver transplantation. *Am J Transplant* 2004; **4**: 373-377
207 **Kim JS**, Broering DC, Tustas RY, Fischer L, Ganschow R, Burdelski M, Rogiers X. Split liver transplantation: past, present and future. *Pediatr Transplant* 2004; **8**: 644-648
208 **Busuttil RW**, Farmer DG, Yersiz H, Hiatt JR, McDiarmid SV, Goldstein LI, Saab S, Han S, Durazo F, Weaver M, Cao C, Chen T, Lipshutz GS, Holt C, Gordon S, Gornbein J, Amersi F, Ghobrial RM. Analysis of long-term outcomes of 3200 liver transplantations over two decades: a single-center experience. *Ann Surg* 2005; **241**: 905-916; discussion 916-918
209 **Enne M**, Pacheco-Moreira L, Balbi E, Cerqueira A, Santalucia G, Martinho JM. Liver transplantation with monosegments. Technical aspects and outcome: a meta-analysis. *Liver Transpl* 2005; **11**: 564-569
210 **Bilik R**, Greig P, Langer B, Superina RA. Survival after reduced-size liver transplantation is dependent on pretransplant status. *J Pediatr Surg* 1993; **28**: 1307-1311
211 **Austin MT**, Feurer ID, Chari RS, Gorden DL, Wright JK, Pinson CW. Survival after pediatric liver transplantation: why does living donation offer an advantage? *Arch Surg* 2005; **140**: 465-470; discussion 470-471
212 **Ryckman FC**, Alonso MH, Bucuvalas JC, Balistreri WF. Long-term survival after liver transplantation. *J Pediatr Surg* 1999; **34**: 845-849; discussion 849-850

S- Editor Li JL **L- Editor** Kerr C **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.675

World J Gastroenterol 2009 February 14; 15(6): 675-683
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, Associate Professor, *Series Editor*

Imaging in liver transplantation

Settimo Caruso, Roberto Miraglia, Luigi Maruzzelli, Salvatore Gruttadauria, Angelo Luca, Bruno Gridelli

Settimo Caruso, Roberto Miraglia, Luigi Maruzzelli, Angelo Luca, Department of Diagnostic and Interventional Radiology, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center Italy, Via Tricomi 1, Palermo 90127, Italy
Salvatore Gruttadauria, Bruno Gridelli, Department of Transplantation Surgery, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center Italy, Via Tricomi 1, Palermo 90127, Italy
Author contributions: Caruso S wrote the paper; Caruso S, Miraglia R, Maruzzelli L, Gruttadauria S, Luca A, Gridelli B contributed equally to this work.
Correspondence to: Settimo Caruso, Department of Diagnostic and Interventional Radiology, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center, Palermo 90127, Italy. secaruso@ismett.edu
Telephone: +39-91-2192111 Fax: +39-91-2192344
Received: July 4, 2008 Revised: September 12, 2008
Accepted: September 19, 2008
Published online: February 14, 2009

Abstract

The aim of this study was to illustrate the role of non-invasive imaging tools such as ultrasonography, multi-detector row computed tomography, and magnetic resonance imaging in the evaluation of pediatric and adult liver recipients and potential liver donors, and in the detection of potential complications arising from liver transplantation.

Key words: Complications; Liver donor; Liver transplantation; Magnetic resonance; Multi detector computed tomography

Peer reviewers: James F Trotter, MD, Associate Professor, University of Colorado, Division of Gastroenterology, 4200 E. 9th Avenue, b-154, Denver, CO 80262, United States; Chao-Long Chen, Professor, Department of Surgery, Chang Gung Memorial Hospital, Kaohsiung Medical Center, 123, Tapei Rd, Niao Sung Hsiang, Kaohsiung Hsien 83305, Taiwan, China

Caruso S, Miraglia R, Maruzzelli L, Gruttadauria S, Luca A, Gridelli B. Imaging in liver transplantation. *World J Gastroenterol* 2009; 15(6): 675-683 Available from: URL: http://www.wjgnet.com/1007-9327/15/675.asp DOI: http://dx.doi.org/10.3748/wjg.15.675

INTRODUCTION

In liver transplantation (LT) candidates, the goal of imaging is to evaluate the intra- and extra-hepatic anatomy, identify conditions that can complicate LT, and stage the neoplastic disease.

Preoperative assessment of potential living liver donors requires the evaluation of liver parenchyma to identify steatosis or lesions; the accurate evaluation of intra and extrahepatic biliary and vascular anatomy to identify congenital variants and, overall, to detect the dominant arterial branch to segment 4 to prevent accidental removal at surgery; and an accurate estimation of the volume of both liver lobes to exclude complications related to graft volume [small-for-size grafts or large-for-size grafts, characterized by a graft-to-recipient weight ratio (GRWR) less than 1%, and more than 3%, respectively].

In the post-transplant period, the goal of imaging is to identify vascular and biliary complications. The long-term follow-up also allows clinicians to identify recurrence of the primary disease and/or detect disease related to long-term immunosuppression.

In the pediatric recipient a wide spectrum of diffuse and focal diseases are indications for LT. Biliary atresia represents at least 50% of all pediatric transplantation, while the most common hepatic malignancy leading to LT is hepatoblastoma[1-5]. Other indications for LT in pediatric patients are Alagille syndrome, cystic fibrosis, tyrosinemia type 1 (associated with a high risk of hepatocellular carcinoma (HCC) development), Wilson's disease, Langerhans' cell histiocytosis, HCC, infantile hepatic hemangioendothelioma type Ⅱ (IHE), and hemangiomatosis[5-10].

In the adult recipient the most common indication for LT is still hepatitis-related liver cirrhosis with or without HCC. HCC is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. In adults it typically occurs within the cirrhotic liver. Non-alcoholic post-hepatitic cirrhosis is the most common association, but any condition that causes cirrhosis may potentially lead to HCC, including conditions such as inborn errors of metabolism. Exposure to chemical carcinogens can also cause the development of HCC. LT based on the Milan criteria is considered the optimal treatment for HCC, especially in patients with underlying chronic liver disease, because it offers a potential cure for both HCC and the underlying

liver disease. The prognosis of cirrhotic patients depends on the occurrence and progression of HCC. α-feto protein (AFP) alone shows low predictivity and sensitivity for the screening of HCC, and so imaging plays a key role in the surveillance of the cirrhotic patient[11-14].

Other focal diseases that require LT in adult patients are metastasis of neuroendocrine tumors, adenomatosis, giant angiomas, hepatic epitheloid hemangioendothelioma, and cholangiocarcinoma in selected cases, while the most common diffuse liver diseases in adults that require LT are primary biliary cirrhosis, primary sclerosing cholangitis, polycystic liver disease, Caroli's disease and Budd-Chiari Syndrome[15]. Fulminant hepatic failure can occur in both pediatric and adult patients[16].

RADIOLOGICAL ASSESSMENT OF LIVER TRANSPLANT RECIPIENTS

Ultrasonography (US)

US is usually the first imaging modality in the evaluation of a transplant candidate, independent of the disease, because it is easy to perform, widely available, relatively inexpensive, and is cost effective. US can detect morphological changes in the liver, hepatic focal lesions (cystic or solid) or abdominal masses, and signs of portal hypertension such as hypersplenism, perihepatic or perisplenic varices, and ascites.

US plays a specific role in the diagnosis of biliary atresia and in the screening of HCC in cirrhotic patients[17-20].

In biliary atresia, US may show the absence, or reduction in size, of the gallbladder and the presence of a triangular cord (TC) sign (thickness of 4 mm or more of the anterior wall of the right portal vein, seen near the portal bifurcation). The identification of a TC sign results in a sensitivity of 80%, a specificity of 98%, a positive predictive value of 94%, a negative predictive value of 94%, and an accuracy of 94% for diagnosing biliary atresia[17].

US is the most common examination for the screening of HCC in cirrhotic patients, usually performed at either 3-, 6- or 12-mo intervals, although the sensitivity and specificity reported in the literature show a wide heterogeneity, ranging from 58% to 89%, and from 75% to 94%, respectively[18-20].

On gray-scale US, HCC is predominantly hypoechoic and sometimes isoechoic, with a thin hypoechoic halo corresponding to the tumor capsule. In diffuse HCC, there is subtle disruption of the normal echo pattern, with anechoic areas due to necrosis. Color Doppler and power Doppler modes permit a real-time evaluation of the hemodynamics in liver tumors. There are, however, many limitations that can affect the assessment of tumor hemodynamics[21].

For the diagnosis of HCC, contrast-enhanced US (CEUS) is recommended by the European Association for the Study of the Liver (EASL) as the modality for evaluation of the vascularity of hepatic nodules in cirrhotic patients. Two dynamic imaging studies that show arterial hypervascularity and washout in the portal venous phase for diagnosis of HCC ranging from 1 cm to 2 cm in diameter are required. For a mass greater than 2 cm, the coincident findings of characteristic arterial vascularization that is seen on at least two imaging techniques, or hypervascularity in one imaging technique associated with washout in the portal venous and/or delayed phase, may be used to confidently establish the diagnosis without biopsy[22-26].

However, there is currently no indication for the use of microbubble contrast agents to increase the detection rate of HCC in patients undergoing US surveillance. In fact, with CEUS one can only observe the perfusion in selected lesions identified with other imaging modalities, and not in the whole liver[23-25].

US also shows a high sensitivity and specificity for excluding portal vein thrombosis (PVT). In patients with hypervascular tumors such as HCC, it is important to establish the nature of the thrombus because tumoral vascular invasion worsens prognosis and may result in exclusion from the LT program. The presence of pulsatile arterial signals inside the thrombus at color Doppler ultrasound (CDUS) is reported to be a highly sensitive and specific sign of malignant PVT[27]. CEUS, using sulfur hexafluoride microbubbles (SonoVue, Bracco SpA), seems to increase sensitivity (88%) and accuracy (92.5%) when distinguishing between benign and malignant PVT[28].

Multi-detector row computed tomography (MDCT)

In liver recipients, MDCT provides important information about liver morphology (normal or cirrhotic), intrahepatic and extrahepatic malignancy, venous benign and/or malignant thrombosis, patency of main portal vein, portosystemic collateral due to portal hypertension (spleno-renal spontaneous shunt, gastroesophageal and/or paracaval varices, and paraumbilical and caput medusae), celiac stenosis, splenic artery aneurysm, congenital arterial variants, patency, and anomalies of the inferior vena cava[29]. These findings may influence the decision to transplant, or the surgical planning of arterial and venous reconstruction. In addition, combined arterial, portal venous, and delayed-phase imaging improves the sensitivity of MDCT in detecting hypervascular neoplasms such as HCC or neuroendocrine metastases, and can also detect other tumors that enhance in a delayed phase, such as cholangi-ocarcinoma[30-33].

In pediatric candidates, the studies are usually performed with and without isosmolar or lower osmolar contrast media intravenous (c.m.i.v.) injection (Iodixanol 320 mgI/mL, Optiray 320, respectively) at a dose of 1.5 mL/kg, and at a rate that depends on the age of the patient (0.5-4 mL/s). When needed, the patient is anesthetized with intravenous propofol (0.5-1 mg/kg), without intubation. Images of the liver are acquired in the cranium-caudal direction, with slice thickness 1.25 mm or 0.625 mm, collimation 2.5 mm and table

speed 7.5 mm per gantry rotation. Usually only three phases are acquired: unenhanced phase, arterial phase, and portal venous phase. Postprocessing of the dataset offers a variety of advanced three-dimensional models of the hepatic artery and vein using multi-planar reconstruction (MPR), maximum intensity projection (MIP), and volume rendering (VR) reconstructions. The volume of the liver is usually calculated, using dedicated software, in pediatric recipients as a guide in the donor-to-dimensional matching[34,35].

In adult candidates, MDCT studies are usually performed with and without iodinated c.m.i.v. with a dose ranging from 1.5 mL/kg to 1.8 mL/kg of body weight, at a rate of 4-5 mL/s. Images of the liver are acquired in the cranium-caudal direction, during a single breath-hold acquisition, with slice thickness 1.25 mm or 0.625 mm, collimation 2.5 mm and table speed 7.5 mm per gantry rotation. A triple or quadruple-phase protocol is used: unenhanced phase, arterial phase, and portal venous phase, without and with late phase, respectively. Before the study, patients receive 500 mL of water as an oral contrast agent. Bolus tracking or test bolus technique (10 mL of contrast material at 5 mL/s) is used to calculate the correct time of the arterial phase. The portal venous phase and late phase acquisitions are generally obtained after 60 s and 180 s from the beginning of contrast injection, respectively. MIP and MPR reconstructions are usually made[30-33].

Magnetic resonance imaging (MRI)

MRI is a non-invasive and sensitive technique that is devoid of ionizing radiation. For this reason, MRI is the preferred modality in the assessment of pediatric recipients. MRI examinations are usually performed using a head coil for small infants, or body coils for larger children. All images are acquired in the axial plane in breath-hold, or with suspended respiration if under general anesthesia. If necessary, contrast medium is injected. Using Gadobenato dimeglumina 0.1 mL/kg (Bracco, SpA) it is possible to obtain information about perfusion of the liver, changes in the parenchyma and the vasculature related to cirrhosis and portal hypertension (PVT, varices, ascites), and to detect vascular congenital anomalies. In addition, the multiphasic contrast enhancement study can detect malignancy in the liver, and locoregional involvement.

Mangafodipir trisodium (MnDPDP, Teslascan, GE) is a contrast agent composed of a water-soluble chelate complex salt that is between a paramagnetic manganese (Mn^{2+}) ion and the ligand dipyridoxyl diphosphate, a vitamin B6 analogue; 50%-60% of the contrast administered is excreted through the gastrointestinal tract. For this reason, Teslascan has recently been used for the early diagnosis of biliary atresia, based on the absence of the bowel excretion of contrast material[36].

In adult recipients, especially in cirrhotic patients, MRI plays a role in detecting and differentiating HCC from other regenerative or dysplastic nodules, because it is more sensitive than multiphasic contrast-enhanced MDCT. However, it is still unclear whether MRI is more sensitive than MDCT in detecting HCC[19,20,26].

MR cholangio-pancreatography (MRCP) using single shot fast spin echo (SSFSE) single and multisection, parallel and radial acquisition can well depict disease (such as sclerosing cholangitis, Caroli's disease) of the biliary tree in adults, while in pediatric recipients, it is limited by the small caliber of the duct, thus rarely visible in neonates. In biliary atresia, it can help to demonstrate the absence of the gallbladder.

RADIOLOGICAL ASSESSMENT OF POTENTIAL LIVER DONORS

US

US is usually the first imaging modality for the evaluation of potential donors because it can identify hepatic lesions, obtain important information on the anatomy of the great vessels, such as hepatic veins and portal system, and evaluate the presence of steatosis. Due to a lack of accepted methods for quantification of steatosis on imaging, in many hospitals a biopsy is incorporated in the work-up, while in other centers, a biopsy is performed only in cases of suspicion based on clinical or imaging grounds[37].

MDCT

MDCT is the most important tool in the assessment of potential donors. MDCT can precisely depict congenital variants, if present, that can influence the surgical technique, identify focal lesions (hemangiomas, focal nodular hyperplasia, adenomas) or diffuse liver diseases (steatosis, hemocromatosis), and calculate the volume of the two liver lobes.

Congenital arterial variants are frequent, and are found in approximately 45% of donors. The identification of the dominant arterial branch to segment 4 is very important because its integrity is indispensable for the regeneration of the residual left hemiliver. This artery usually arises from the left hepatic artery (LHA), while in 25% of cases it arises from the right hepatic artery (RHA) or from both the LHA and RHA (Figure 1).

Anatomical variants of the portal system occur in 20% of the donor population; although the anomalies are not a contraindication to surgery, they must be known because they may require multiple portal anastomoses during the implantation of the right lobe into the recipient (Figure 2).

Identifying the hepatic venous anatomy is a fundamental step because it determines the hepatectomy plane that runs 1 cm to the right of the middle hepatic vein (MHV). Both accessory hepatic veins of the right inferior lobe (68% of the donor population), and large branching veins (> 5 mm) draining into the MHV from the right lobe require separate anastomosis to prevent venous congestion in the graft[37-41] (Figure 3).

Accurate volume of both liver lobes needs to be estimated to ensure that the hepatic mass is adequate for both liver donor and recipient.

MDCT scan studies are performed with and without

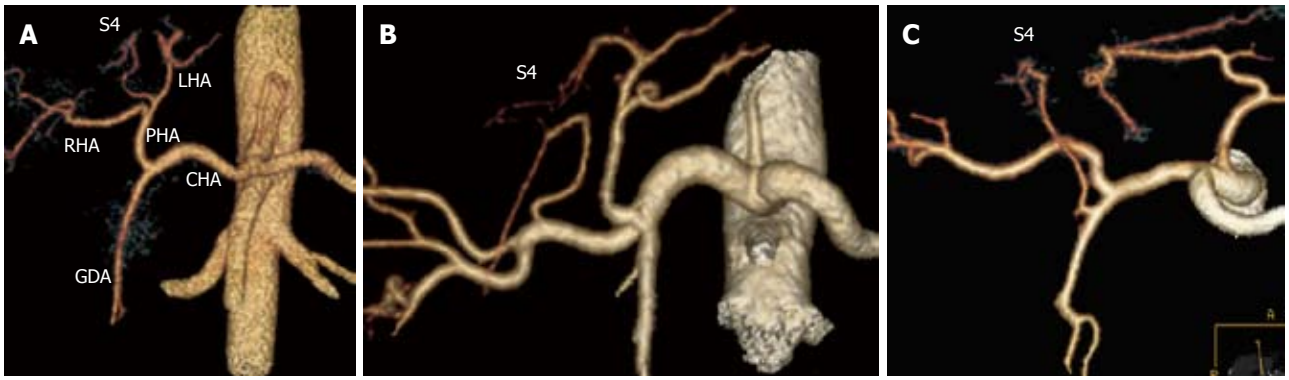


Figure 1 MDCT. VR reconstruction images. A: 35-year-old male, potential living liver donor. Normal anatomy of hepatic artery. CHA: Common hepatic artery; GDA: Gastroduodenal artery; PHA: Proper hepatic artery; LHA: Left hepatic artery; RHA: Right hepatic artery; S4: Artery to segment 4; B: 29-year-old female, potential living liver donor. Early bifurcation of hepatic artery. Two large arterial branches to segment 4 arising from LHA and RHA; C: 25-year-old male, potential living liver donor. LHA arising from left gastric artery. The artery to segment 4 arising from the gastroduodenal artery.

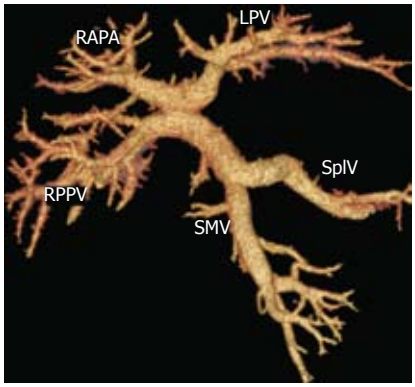


Figure 2 Thirty-two-year-old male, potential living liver donor. VR reconstruction shows a right anterior branch arising from left portal branch. SMV: Superior mesenteric vein; SplV: Splenic vein; LPV: Left portal vein; RAPD: Right anterior portal vein; RPPV: Right posterior portal vein.

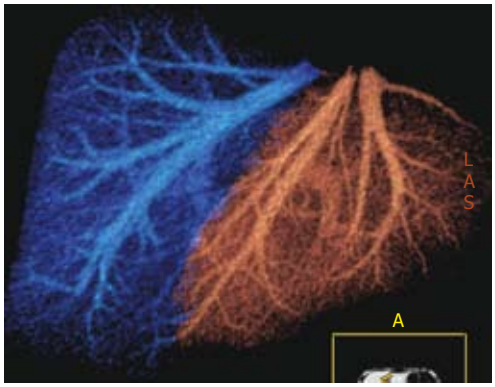


Figure 3 Forty-one-year-old male, potential living liver donor. VR reconstruction shows normal anatomy of hepatic veins. The right lobe and right hepatic vein are blue, the left lobe and the MHV and left hepatic veins are red. A cut-plane runs 1 cm to the right of the MHV.

iodinated c.m.i.v. at a dose ranging from 1.5 mL/kg to 1.8 mL/kg of body weight, and at a rate of 4-5 mL/s. Images of the liver are acquired in the cranium-caudal direction, during a single breath-hold acquisition, with slice thickness 1.25 mm or 0.625 mm, collimation 2.5 mm and table speed 7.5 mm per gantry rotation. Before the study, patients receive 500 mL of water as an oral contrast agent. Usually, a triple-phase protocol is used: unenhanced phase, arterial phase, and portal venous phase. Bolus tracking or test bolus technique (10 mL of contrast material at 4/5 mL per second) is used to calculate the correct time of the arterial phase. The peak enhancement plus 2 s is deemed as the start of the arterial acquisition to depict the arterial system. The portal venous phase is generally taken 70 s after the contrast agent has been injected to determine the exact delineation of the portal and hepatic veins. MIP and VR image reconstruction of the artery and portal venous system are usually created in the post-processing stage. The portal-venous acquisition is used for the volumetric evaluation, using dedicated software, in the postprocessing of the right and left lobe[37-39].

Some authors have proposed an all-in-one protocol to depict the biliary system, using a biliary contrast agent (Biliscopin; Schering, Berlin, Germany). However, a high incidence of adverse reactions to the biliary contrast agent, ranging from mild and self-resolving to severe systemic adverse reactions (shock-syndrome and death), has been observed[38].

Magnetic resonance cholangio-pancreatography (MRCP)

MRCP is currently considered the primary imaging tool for biliary anatomy evaluation in potential living liver donors. In fact, it is performed with new generation units equipped with high performance gradient and phased-array coils, allowing for high quality heavily T2-weighted images with increased spatial resolution in a few seconds or with respiratory triggering, eliminating most motion-related artifacts.

Only 57% of donors have a conventional biliary anatomy (Figure 4). Although the congenital variants of biliary anatomy do not represent a contraindication to liver donation, they must be identified before surgery to prevent ligation of major branches of the right lobe in the recipient and/or of the liver lobe in the donor. Multiple biliary anastomoses during the implantation of the right lobe into the recipient can be required to avoid atrophy due to biliary obstruction.

Improvements in hepatocyte-specific contrast

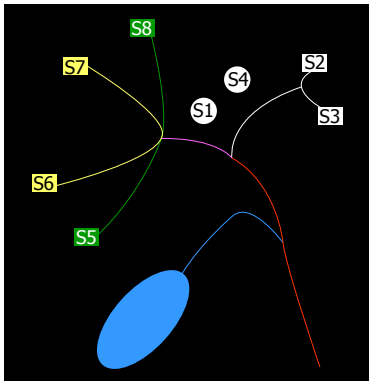


Figure 4 Normal anatomy of biliary drainage: Right posterior duct (RPD) and right anterior duct (RAD) drain, respectively, S6-S7 and S8-S5; right hepatic duct (RHD) is formed by confluence of RPD and RAD. Left hepatic duct (LHD) drains S2-S3. S1-S4 can be drained by LHD or by RHD. The common hepatic duct (CHD) arises from the confluence of RHD and LHD.

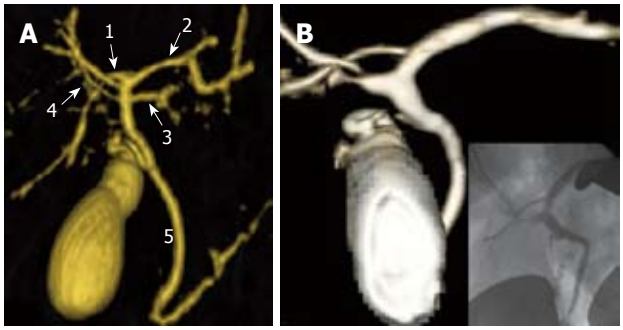


Figure 5 MRCP. VR reconstruction images. A: Twenty-two-year-old male, potential living liver donor. VR image using MRCP acquisition shows congenital anomalies of biliary drainage: RPD (1) draining in LHD (2), accessory LHD (3) and accessory RPD (4) draining in MHD (5); B: 24-year-old female, potential living liver donor. VR image after Teslascan injection shows congenital anomalies of biliary drainage: RPD draining in LHD. The finding is confirmed with intraoperative cholangiogram.

agents with biliary excretion (mangafodipir trisodium and gadobenate dimeglumine) seem to have increased the accuracy of MRI in depicting the biliary system[41,42] (Figure 5).

Some studies propose MRI as a single imaging modality for the preoperative assessment of potential donors to depict the arterial, portal and venous anatomy using MR-angiography with 3D sequence after the administration of extracellular c.m.i.v. However, MR-angiography can rarely depict the artery supplying segment 4[40,43].

RADIOLOGICAL ASSESSMENT OF POST-TRANSPLANT COMPLICATIONS

US

US and CDUS are the most important tools in the follow-up of LT patients because they show high sensitivity and specificity in detecting vascular complications.

During transplantation, CDUS is usually performed to detect the intraparenchymal flows (arterial, portal and venous), and to evaluate the velocity of flow and

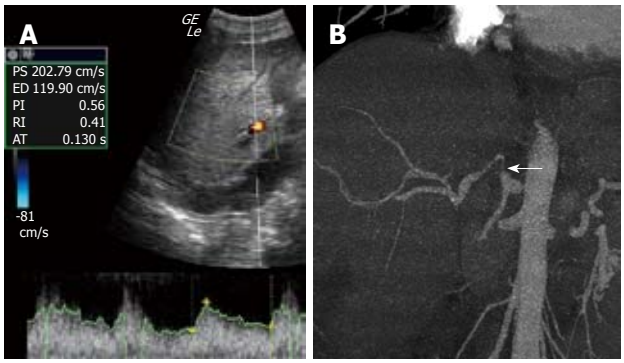


Figure 6 Fifteen-year-old male underwent orthotopic LT (OLT) for biliary atresia. A: Ten days after OLT, CDUS shows a pathologic resistance index (< 0.5) and pathologic acceleration time (> 0.08), strongly suspicious for stenosis; B: AngioMDCT confirms a stenosis of the hepatic artery after the anastomosis (arrow).

waveform to detect very early complications such as hyperacute hepatic artery or PVT[44].

After LT, CDUS is usually performed once a day during the first week, and once a week in the following 2 mo, and is key in the suspicion or identification of vascular or biliary complications.

Hepatic artery thrombosis (HAT) occurs more frequently in pediatric recipients (9%-42%) than in adult recipients (4%-12%). It frequently leads to graft failure, due to biliary wall necrosis with bilomas, biliary leakage, and hepatic infarction[45].

CDUS is able to identify up to 92% of cases of HAT, demonstrating the absence of flow in the common hepatic artery and in the intrahepatic branches. In younger subjects, in the event of complete hepatic artery thrombosis, intrahepatic flow can be sustained by small collateral neoformed vessels from the superior mesenteric artery. In these cases, the flow has a *tardus parvus* waveform. In adults, the formation of collateral vessels is almost never sufficient to prevent ischemic biliary complications. Ultrasound findings can be false positive if the hepatic artery is small or stenotic, if the flow is very slow, or if there is coexistent systemic hypotension. If US does not show an arterial flow, administration of contrast media (microbubble) can help to improve the flow visualization in the HA, differentiating between thrombosis and a patent artery in patients without HA flow on conventional Doppler US[46-49].

Hepatic artery stenosis is reported in 5%-10% of transplant recipients and can be anastomotic (in 70% of cases), perianastomotic or intrahepatic. It is most frequently caused by an error in surgical technique or by arterial damage during explantation. Near the stenosis, the Doppler ultrasound shows a focal velocity greater than 2 mL/s, and turbulence; more distally, it detects a *tardus parvus* arterial waveform with a resistance index lower than 0.5 and a systolic acceleration time (between the end of the diastole and the first systolic peak) greater than 0.08 s[46-49] (Figures 6 and 7).

Post-transplant PVT is extremely rare in adults. In children, particularly with biliary atresia, post-transplant PVT, although not usual, is not rare. The underlying

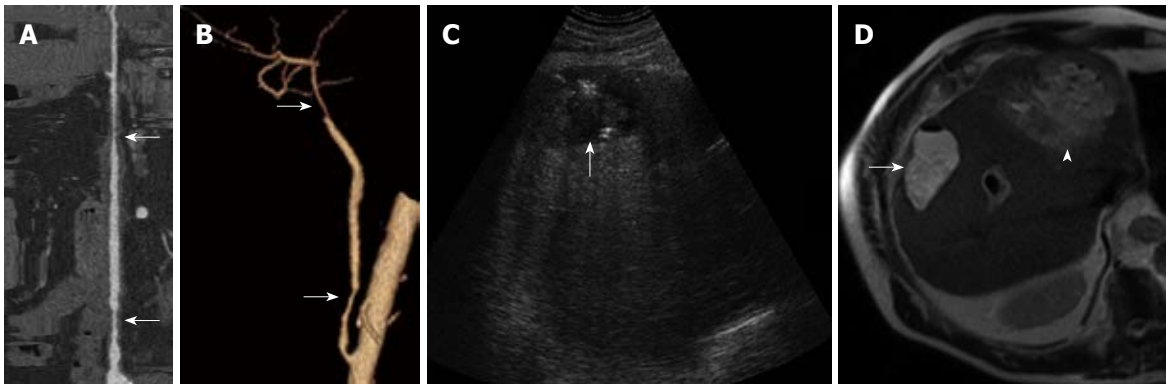


Figure 7 Fifty-four-year-old male underwent living related liver transplant (LRLT) for HCC in HCV-related cirrhosis. A and B: 3 mo after LRLT, a lumen stripe reconstruction (A) and VR reconstruction (B) show irregularities (arrow) of aortohepatic by-pass. Six months after LRLT, the patient was admitted to hospital with fever; C: US shows a hypoechoic and inhomogeneous lesion in the right lobe (arrow); D: MR T2W images show a hyperintense lesion, confirming an abscess in the right lobe (arrow). The left lobe appears inhomogeneously and diffusely hyperintense, showing a large abscess (head of arrow).

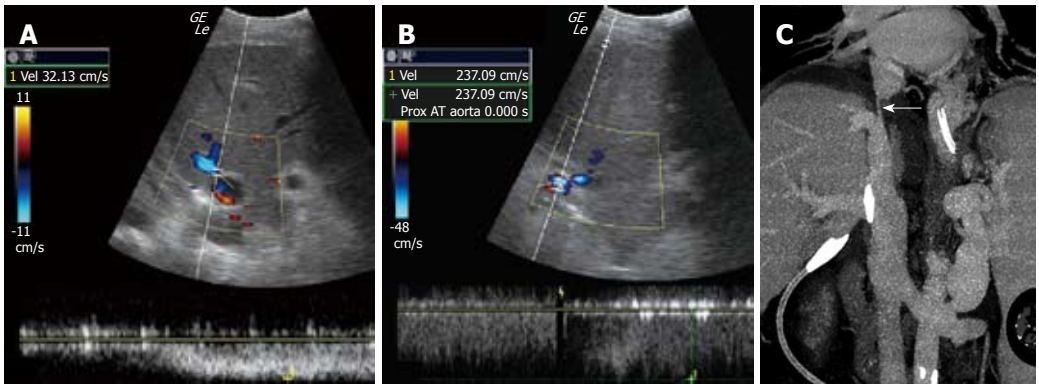


Figure 8 Fifty-one-year-old male underwent OLT for HCC in HCV-related cirrhosis. A and B: Eight days after OLT, CDUS shows a high velocity gradient between the subhepatic (A) and suprahepatic (B) segments of the inferior vena cava, with a velocity of 32 cm/s and 237 cm/s, respectively, strongly suspicious for stenosis; C: MDCT with MPR image confirms stenosis of the suprahepatic segment of the inferior vena cava (arrow).

problems in small children are not only due to smaller caliber vessels but also due to hypoplastic and sclerotic vessels brought about by pre-transplant recurrent cholangitis[50]. Color Doppler ultrasound shows the absence of flow in the portal vein, and whether it is anechogenic (recent thrombosis) or echogenic (old thrombosis). If the thrombosis is recent, it can be treated with local thrombolysis and mechanical thrombectomy.

Portal vein stenosis is more frequent in partial liver transplants than in whole liver transplants. The suspicion of stenosis arises if color Doppler shows a turbulent flow with focal aliasing in the stenotic tract, and a velocity gradient of > 4-fold[46-49].

Post-liver transplant stenosis of the inferior vena cava is rare and generally secondary to technical issues. CDUS shows an increased trans-anastomotic velocity gradient (> 4 times) and the loss of the tracing's normal phasicity[44,46,49] (Figure 8).

In partial liver transplants, hepatic vein stenosis is a frequent complication. CDUS of the hepatic veins reveals a slow (< 10 cm/s) and monophasic flow[44,46,49].

All the vascular complications described, when suspected on US and CDUS, need confirmation with contrast-enhanced MDCT, contrast-enhanced MR or with angiography.

US shows low diagnostic accuracy in identifying biliary complications, particularly in early anastomotic stenosis without a significant intrahepatic biliary duct dilatation. US is, however, an accurate tool for evaluating necrosis, bilomas, or abscess of the graft.

MDCT

MDCT angiography is the best option for confirming the ultrasonographic suspicion of early and late vascular complications (HAT, main portal vein or inferior vena cava (IVC) stenosis or thrombosis)[51]. In addition, it permits a good assessment of liver parenchyma and other abdominal organs, and the evaluation of bilomas (Figure 9), bleeding, abdominal or hepatic abscesses (Figure 7), adrenal infarction, and intestinal perforation or obstruction. MDCT can identify biliary duct dilatation, even if the anastomosis is not easy to depict.

MDCT also plays a key role in detecting late complications, such as recurrence of the primary disease, post-transplant lymphoproliferative disease (PTLD), Kaposi's sarcoma or other malignancies related to long-term immunosuppression.

MRI

MRCP after LT is the modality of choice for the

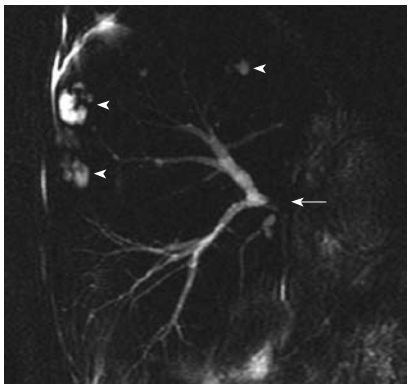


Figure 9 Sixty-one-year-old male underwent OLT for primary biliary cirrhosis, with bilio-enteric anastomosis. MRCP shows a severe anastomotic stricture (arrow) and multiple intrahepatic biliary leakages (head of arrows).

diagnosis and management of biliary complications, and shows a sensitivity ranging from 87.5% to 95.3%, a positive predictive value ranging from 92.3% to 97.6%, and an accuracy ranging from 90.4% to 95.2%[52-54]. The T2W images easily identify fluid collection (bilomas, bile leakage, biliary duct dilatation) that appear strongly hyperintense, while MRCP using SSFSE single and multisection, parallel and radial acquisition can finely depict filling defects, anastomotic or non-anastomotic stenosis, or irregularities of the biliary duct.

Bile duct complications in the various series vary from 7% to 50%. In partial liver transplants, biliary complications are more frequent because the diameters of the bile ducts to be anastomosed are smaller, and multiple biliary anastomoses are often necessary. Most of these complications occur within the first 3 mo, even though biliary stenoses and gallstones can occur months and years after transplantation.

Bile extravasation has an incidence ranging from 5% to 19%, and may occur in the T-tube insertion site, in the region of the anastomosis, or intrahepatically.

Intrahepatic bile leakage (biloma) entails the suspicion of bile duct necrosis secondary to HAT. In these cases, a new transplant is almost always necessary. However, the percutaneous drainage of a biloma can prevent sepsis and increase the likelihood of graft survival. In partial liver transplants, bile can also leak from a large bile duct damaged at the time of liver "splitting," or from the surface of the resection margin, which exposes thousands of small bile ducts (Figure 9).

Stenoses can be classified as anastomotic or non-anastomotic. Anastomotic stenoses are the result of postoperative fibroses or of errors associated with surgical technique (Figure 9). Non-anastomotic stenoses can be intra- or extrahepatic, single or multiple, and are often due to ischemic damage. In these cases, it is necessary to assess the patency of the hepatic artery. Rarely are they due to prolonged graft ischemia time, chronic rejection or cytomegalovirus infections. In transplants related to sclerosing cholangitis, intrahepatic stenosis can indicate disease recurrence.

Rare causes of bile duct obstruction are the dislocation/obstruction of the T-tube, biliary sludge, gallstones or an excessive choledochus length after the choledochocholedochostomy. The mucocele of the residual cystic duct can cause bile duct stenosis resulting from extrinsic compression.

Enhancement with mangafodipir trisodium improves the performance of MRCP for the detection and exclusion of biliary abnormalities after orthotopic LT[55].

CONCLUSION

Imaging plays a primary role in LT. It is used in the assessment of the recipient, the assessment of the potential living liver donor, and the detection of early and late complications. US, MDCT and MRI have different roles, depending on accuracy, in depicting the different goal in each period of the orthotopic LT.

REFERENCES

1 **Hasegawa T**, Kimura T, Sasaki T, Okada A. Living-related liver transplantation for biliary atresia associated with polysplenia syndrome. *Pediatr Transplant* 2002; **6**: 78-81
2 **Meyers RL**. Tumors of the liver in children. *Surg Oncol* 2007; **16**: 195-203
3 **Avila LF**, Luis AL, Hernandez F, Garcia Miguel P, Jara P, Andres AM, Lopez Santamaria M, Tovar JA. Liver transplantation for malignant tumours in children. *Eur J Pediatr Surg* 2006; **16**: 411-414
4 **Emre S**, McKenna GJ. Liver tumors in children. *Pediatr Transplant* 2004; **8**: 632-638
5 **Sevmis S**, Karakayali H, Ozcay F, Canan O, Bilezikci B, Torgay A, Haberal M. Liver transplantation for hepatocellular carcinoma in children. *Pediatr Transplant* 2008; **12**: 52-56
6 **Colombo C**, Battezzati PM, Crosignani A, Morabito A, Costantini D, Padoan R, Giunta A. Liver disease in cystic fibrosis: A prospective study on incidence, risk factors, and outcome. *Hepatology* 2002; **36**: 1374-1382
7 **Kvittingen EA**. Tyrosinaemia--treatment and outcome. *J Inherit Metab Dis* 1995; **18**: 375-379
8 **Schilsky ML**. Diagnosis and treatment of Wilson's disease. *Pediatr Transplant* 2002; **6**: 15-19
9 **Zandi P**, Panis Y, Debray D, Bernard O, Houssin D. Pediatric liver transplantation for Langerhans' cell histiocytosis. *Hepatology* 1995; **21**: 129-133
10 **Mehrabi A**, Kashfi A, Fonouni H, Schemmer P, Schmied BM, Hallscheidt P, Schirmacher P, Weitz J, Friess H, Buchler MW, Schmidt J. Primary malignant hepatic epithelioid hemangioendothelioma: a comprehensive review of the literature with emphasis on the surgical therapy. *Cancer* 2006; **107**: 2108-2121
11 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236
12 **Mazzaferro V**, Chun YS, Poon RT, Schwartz ME, Yao FY, Marsh JW, Bhoori S, Lee SG. Liver transplantation for hepatocellular carcinoma. *Ann Surg Oncol* 2008; **15**: 1001-1007
13 **Taketomi A**, Soejima Y, Yoshizumi T, Uchiyama H, Yamashita Y, Maehara Y. Liver transplantation for hepatocellular carcinoma. *J Hepatobiliary Pancreat Surg* 2008; **15**: 124-130
14 **Ishizaki Y**, Kawasaki S. The evolution of liver transplantation for hepatocellular carcinoma (past, present, and future). *J Gastroenterol* 2008; **43**: 18-26
15 **Seaberg EC**, Belle SH, Beringer KC, Schivins JL, Detre KM. Liver transplantation in the United States from 1987-1998: updated results from the Pitt-UNOS Liver Transplant Registry. *Clin Transpl* 1998; 17-37

16 **Mondragon R**, Mieli-Vergani G, Heaton ND, Mowat AP, Vougas V, Williams R, Tan KC. Liver transplantation for fulminant liver failure in children. *Transpl Int* 1992; **5** Suppl 1: S206-S208

17 **Lee HJ**, Lee SM, Park WH, Choi SO. Objective criteria of triangular cord sign in biliary atresia on US scans. *Radiology* 2003; **229**: 395-400

18 **Chalasani N**, Horlander JC Sr, Said A, Hoen H, Kopecky KK, Stockberger SM Jr, Manam R, Kwo PY, Lumeng L. Screening for hepatocellular carcinoma in patients with advanced cirrhosis. *Am J Gastroenterol* 1999; **94**: 2988-2993

19 **Gambarin-Gelwan M**, Wolf DC, Shapiro R, Schwartz ME, Min AD. Sensitivity of commonly available screening tests in detecting hepatocellular carcinoma in cirrhotic patients undergoing liver transplantation. *Am J Gastroenterol* 2000; **95**: 1535-1538

20 **Teefey SA**, Hildeboldt CC, Dehdashti F, Siegel BA, Peters MG, Heiken JP, Brown JJ, McFarland EG, Middleton WD, Balfe DM, Ritter JH. Detection of primary hepatic malignancy in liver transplant candidates: prospective comparison of CT, MR imaging, US, and PET. *Radiology* 2003; **226**: 533-542

21 **Gaiani S**, Volpe L, Piscaglia F, Bolondi L. Vascularity of liver tumours and recent advances in doppler ultrasound. *J Hepatol* 2001; **34**: 474-482

22 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430

23 **Maruyama H**, Yoshikawa M, Yokosuka O. Current role of ultrasound for the management of hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1710-1719

24 **Lencioni R**, Piscaglia F, Bolondi L. Contrast-enhanced ultrasound in the diagnosis of hepatocellular carcinoma. *J Hepatol* 2008; **48**: 848-857

25 **Dai Y**, Chen MH, Fan ZH, Yan K, Yin SS, Zhang XP. Diagnosis of small hepatic nodules detected by surveillance ultrasound in patients with cirrhosis: Comparison between contrast-enhanced ultrasound and contrast-enhanced helical computed tomography. *Hepatol Res* 2008; **38**: 281-290

26 **Taouli B**, Krinsky GA. Diagnostic imaging of hepatocellular carcinoma in patients with cirrhosis before liver transplantation. *Liver Transpl* 2006; **12**: S1-S7

27 **Dodd GD 3rd**, Memel DS, Baron RL, Eichner L, Santiguida LA. Portal vein thrombosis in patients with cirrhosis: does sonographic detection of intrathrombus flow allow differentiation of benign and malignant thrombus? *AJR Am J Roentgenol* 1995; **165**: 573-577

28 **Tarantino L**, Francica G, Sordelli I, Esposito F, Giorgio A, Sorrentino P, de Stefano G, Di Sarno A, Ferraioli G, Sperlongano P. Diagnosis of benign and malignant portal vein thrombosis in cirrhotic patients with hepatocellular carcinoma: color Doppler US, contrast-enhanced US, and fine-needle biopsy. *Abdom Imaging* 2006; **31**: 537-544

29 **Pannu HK**, Maley WR, Fishman EK. Liver transplantation: preoperative CT evaluation. *Radiographics* 2001; **21**: S133-S146

30 **Kanematsu M**, Oliver JH 3rd, Carr B, Baron RL. Hepatocellular carcinoma: the role of helical biphasic contrast-enhanced CT versus CT during arterial portography. *Radiology* 1997; **205**: 75-80

31 **Iannaccone R**, Laghi A, Catalano C, Rossi P, Mangiapane F, Murakami T, Hori M, Piacentini F, Nofroni I, Passariello R. Hepatocellular carcinoma: role of unenhanced and delayed phase multi-detector row helical CT in patients with cirrhosis. *Radiology* 2005; **234**: 460-467

32 **Ronzoni A**, Artioli D, Scardina R, Battistig L, Minola E, Sironi S, Vanzulli A. Role of MDCT in the diagnosis of hepatocellular carcinoma in patients with cirrhosis undergoing orthotopic liver transplantation. *AJR Am J Roentgenol* 2007; **189**: 792-798

33 **Zhao H**, Zhou KR, Yan FH. Role of multiphase scans by multirow-detector helical CT in detecting small hepatocellular carcinoma. *World J Gastroenterol* 2003; **9**: 2198-2201

34 **Cheng YF**, Chen CL, Jawan B, Huang TL, Chen TY, Chen YS, Wang CC, de Villa V, Wang SH, Wah CK, Chiang YC, Eng HL, Lee TY, Goto S. Multislice computed tomography angiography in pediatric liver transplantation. *Transplantation* 2003; **76**: 353-357

35 **Ravindra KV**, Guthrie JA, Woodley H, Davison S, McClean P, Prasad KR, Stringer MD. Preoperative vascular imaging in pediatric liver transplantation. *J Pediatr Surg* 2005; **40**: 643-647

36 **Ryeom HK**, Choe BH, Kim JY, Kwon S, Ko CW, Kim HM, Lee SB, Kang DS. Biliary atresia: feasibility of mangafodipir trisodium-enhanced MR cholangiography for evaluation. *Radiology* 2005; **235**: 250-258

37 **Low G**, Wiebe E, Walji AH, Bigam DL. Imaging evaluation of potential donors in living-donor liver transplantation. *Clin Radiol* 2008; **63**: 136-145

38 **Schroeder T**, Radtke A, Kuehl H, Debatin JF, Malago M, Ruehm SG. Evaluation of living liver donors with an all-inclusive 3D multi-detector row CT protocol. *Radiology* 2006; **238**: 900-910

39 **Chen WH**, Xin W, Wang J, Huang QJ, Sun YF, Xu Q, Yu SN. Multi-slice spiral CT angiography in evaluating donors of living-related liver transplantation. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 364-369

40 **Makuuchi M**, Sugawara Y. Technical progress in living donor liver transplantation for adults. *HPB* (Oxford) 2004; **6**: 95-98

41 **Ayuso JR**, Ayuso C, Bombuy E, De Juan C, Llovet JM, De Caralt TM, Sanchez M, Pages M, Bruix J, Garcia-Valdecasas JC. Preoperative evaluation of biliary anatomy in adult live liver donors with volumetric mangafodipir trisodium enhanced magnetic resonance cholangiography. *Liver Transpl* 2004; **10**: 1391-1397

42 **Sirvanci M**, Duran C, Ozturk E, Balci D, Dayangac M, Onat L, Yuzer Y, Tokat Y, Killi R. The value of magnetic resonance cholangiography in the preoperative assessment of living liver donors. *Clin Imaging* 2007; **31**: 401-405

43 **Cheng YF**, Chen CL, Huang TL, Chen TY, Lee TY, Chen YS, Wang CC, de Villa V, Goto S, Chiang YC, Eng HL, Jawan B, Cheung HK. Single imaging modality evaluation of living donors in liver transplantation: magnetic resonance imaging. *Transplantation* 2001; **72**: 1527-1533

44 **Choi JY**, Lee JY, Lee JM, Kim SH, Lee MW, Han JK, Choi BI. Routine intraoperative Doppler sonography in the evaluation of complications after living-related donor liver transplantation. *J Clin Ultrasound* 2007; **35**: 483-490

45 **Ametani F**, Itoh K, Shibata T, Maetani Y, Tanaka K, Konishi J. Spectrum of CT findings in pediatric patients after partial liver transplantation. *Radiographics* 2001; **21**: 53-63

46 **Hom BK**, Shrestha R, Palmer SL, Katz MD, Selby RR, Asatryan Z, Wells JK, Grant EG. Prospective evaluation of vascular complications after liver transplantation: comparison of conventional and microbubble contrast-enhanced US. *Radiology* 2006; **241**: 267-274

47 **Dodd GD 3rd**, Memel DS, Zajko AB, Baron RL, Santaguida LA. Hepatic artery stenosis and thrombosis in transplant recipients: Doppler diagnosis with resistive index and systolic acceleration time. *Radiology* 1994; **192**: 657-661

48 **Vit A**, De Candia A, Como G, Del Frate C, Marzio A, Bazzocchi M. Doppler evaluation of arterial complications of adult orthotopic liver transplantation. *J Clin Ultrasound* 2003; **31**: 339-345

49 **Tamsel S**, Demirpolat G, Killi R, Aydin U, Kilic M, Zeytunlu M, Parildar M, Oran I, Ucar H. Vascular complications after liver transplantation: evaluation with Doppler US. *Abdom Imaging* 2007; **32**: 339-347

50 **Chen CL**, Concejero A, Wang CC, Wang SH, Lin CC, Liu YW, Yong CC, Yang CH, Lin TS, Chiang YC, Jawan

B, Huang TL, Cheng YF, Eng HL. Living donor liver transplantation for biliary atresia: a single-center experience with first 100 cases. *Am J Transplant* 2006; **6**: 2672-2679

51 **Kayahan Ulu EM**, Coskun M, Ozbek O, Tutar NU, Ozturk A, Aytekin C, Haberal M. Accuracy of multidetector computed tomographic angiography for detecting hepatic artery complications after liver transplantation. *Transplant Proc* 2007; **39**: 3239-3244

52 **Linhares MM**, Gonzalez AM, Goldman SM, Coelho RD, Sato NY, Moura RM, Silva MH, Lanzoni VP, Salzedas A, Serra CB, Succi T, D'Ippolito G, Szejnfeld J, Trivino T. Magnetic resonance cholangiography in the diagnosis of biliary complications after orthotopic liver transplantation. *Transplant Proc* 2004; **36**: 947-948

53 **Aufort S**, Molina E, Assenat E, Rigole H, Bauret P, Calvet C, Navarro F, Fabre JM, Blanc P, Taourel P, Larrey D, Bruel JM, Pageaux GP, Gallix BP. [Value of MRCP for diagnosis of biliary complications after liver transplantation] *J Radiol* 2008; **89**: 221-227

54 **Valls C**, Alba E, Cruz M, Figueras J, Andia E, Sanchez A, Llado L, Serrano T. Biliary complications after liver transplantation: diagnosis with MR cholangiopancreatography. *AJR Am J Roentgenol* 2005; **184**: 812-820

55 **Bridges MD**, May GR, Harnois DM. Diagnosing biliary complications of orthotopic liver transplantation with mangafodipir trisodium-enhanced MR cholangiography: comparison with conventional MR cholangiography. *AJR Am J Roentgenol* 2004; **182**: 1497-1504

S- Editor Li DL **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.684

World J Gastroenterol 2009 February 14; 15(6): 684-693
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, Associate Professor, *Series Editor*

Interventional radiology procedures in adult patients who underwent liver transplantation

Roberto Miraglia, Luigi Maruzzelli, Settimo Caruso, Mariapina Milazzo, Gianluca Marrone, Giuseppe Mamone, Vincenzo Carollo, Salvatore Gruttadauria, Angelo Luca, Bruno Gridelli

Roberto Miraglia, Luigi Maruzzelli, Settimo Caruso, Mariapina Milazzo, Gianluca Marrone, Giuseppe Mamone, Vincenzo Carollo, Angelo Luca, Department of Diagnostic and Interventional Radiology, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center, Via Tricomi 1, Palermo 90127, Italy
Salvatore Gruttadauria, Bruno Gridelli, Department of Transplantation Surgery, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center, Via Tricomi 1, Palermo 90127, Italy
Author contributions: Miraglia R wrote the paper; Miraglia R, Maruzzelli L, Caruso S, Milazzo M, Marrone G, Mamone G, Carollo V, Gruttadauria S performed research; Luca A and Gridelli B reviewed the paper.
Correspondence to: Roberto Miraglia, Department of Diagnostic and Interventional Radiology, Mediterranean Institute for Transplantation and Advanced Specialized Therapies (IsMeTT), University of Pittsburgh Medical Center, Via Tricomi 1, Palermo 90127, Italy. rmiraglia@ismett.edu
Telephone: +39-91-2192111 Fax: +39-91-2192344
Received: July 4, 2008 Revised: November 24, 2008
Accepted: December 1, 2008
Published online: February 14, 2009

Abstract

Interventional radiology has acquired a key role in every liver transplantation (LT) program by treating the majority of vascular and non-vascular post-transplant complications, improving graft and patient survival and avoiding, in the majority of cases, surgical revision and/or re-transplantation. The aim of this paper is to review indications, technical consideration, results achievable and potential complications of interventional radiology procedures after deceased donor LT and living related adult LT.

Key words: Liver transplantation; Interventional radiology; Complication; Review; Liver

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

Miraglia R, Maruzzelli L, Caruso S, Milazzo M, Marrone G, Mamone G, Carollo V, Gruttadauria S, Luca A, Gridelli B. Interventional radiology procedures in adult patients who underwent liver transplantation. *World J Gastroenterol* 2009; 15(6): 684-693 Available from: URL: http://www.wjgnet.com/1007-9327/15/684.asp DOI: http://dx.doi.org/10.3748/wjg.15.684

INTRODUCTION

Liver transplantation (LT) in patients with end-stage liver diseases has become an accepted treatment. Advances in the field of percutaneous, radiological, minimally invasive techniques have increased the importance of interventional radiology in the management of patients after LT[1]. In this article, we discuss the possible applications of interventional radiology in the management of adult recipients after deceased donor LT or living related LT (LRLT), including diagnosis of graft disease, treatment of vascular complications and treatment of biliary complications. Techniques used, results and possible complications of interventional radiology procedures are described by reviewing our experience and other protocols present in literature.

DIAGNOSIS OF GRAFT DISEASE

Random liver biopsy is frequently requested after LT. Any alteration of liver function tests, not explained by diagnostic imaging, requires a liver biopsy to exclude rejection and/or other pathologies. Liver biopsy can be performed by a percutaneous approach (blind or ultrasound guided) or, in selected patients, with a transjugular approach. In our practice, in patients without ascites, percutaneous core liver biopsies are performed with an 18-Ga needle under ultrasound guide to avoid entering the bowel or other adjacent organs, and to avoid the perforation of main intrahepatic vascular structures. If coagulation defects are present (platelets < 50 000 mm^3 and/or prothrombin activity < 50%) patients receive infusion of platelets and/or fresh frozen plasma. No antibiotic prophylaxis is performed before the procedure. Complications are

infrequent, most of them being minor (pain, decrease in hematocrit value not necessitating treatment). Possible major complications of percutaneous liver biopsies are bleeding, emobilia, arterio-portal fistula and infections, and are reported in up to 3% of cases[2]. Controlled studies have shown that blind percutaneous biopsy carries a higher risk for major complications compared to ultrasound guided liver biopsy[3]. Although it has been reported by Little *et al*[2] that the presence of perihepatic ascites does not statistically affect the major or minor complications rate of image-guided percutaneous hepatic biopsy, in our center, the transjugular approach is preferred if perihepatic ascites are present. The transjugular approach is considered mandatory if a severe coagulopathy and/or massive amounts of perihepatic ascites are present. This technique reduces the risk of hemorrhage, because a biopsy specimen is acquired through the hepatic vein and any bleeding from the puncture site remains in the vascular space. In addition, if a clinical suspicion of portal hypertension is present, hepatic vein pressure gradient (HVPG) can be measured during the same procedure. The right internal jugular vein approach is usually preferred, but in selected cases, if the right jugular vein is not usable (thrombosis or difficult catheterization), the left internal jugular vein can be used[4]. In our experience, there are no major complications related to transjugular liver biopsies performed in adult liver transplant recipients. Despite transjugular liver biopsy being effective and safe for patients with contraindications to percutaneous liver biopsy, in patients with small liver or in patient with partial LT (from a living donor or deceased donor), subcapsular or intraperitoneal bleeding due to accidental perforation of the capsule is possible. Hemobilia, accidental puncture of kidney, transient dysrhythmias, and hematoma at the puncture site are the most common complications reported in other studies[5-7].

TREATMENT OF VASCULAR COMPLICATIONS

Vascular complications following LT are associated with high morbidity, graft lost and mortality rate. The majority of vascular complications develop within 3 mo of the transplant and possible complications should be considered in any LT patient with alteration of liver function tests. The clinical presentation of vascular complications is often indistinguishable from other post-transplantation complications (biliary complications, rejection, graft dysfunction, infections). Color Doppler ultrasonography (US), multidetector-row computed tomography (MDCT), and magnetic resonance are useful for diagnosis. Vascular complications can affect the hepatic artery, hepatic vein, portal vein and inferior vena cava (IVC).

Hepatic artery thrombosis (HAT)

HAT is a dramatic, potentially life-threatening, complication of LT occurring in 3%-9% of adult liver-transplanted patients[8,9]. Risk factors for HAT are considered to be surgical technique, small donor vessels, slow flow secondary to hepatic artery stenosis (HAS), ischemia-reperfusion injury, coagulation abnormalities, ABO blood group incompatible transplantation, use of aortic jumping graft and multiple rejection episodes. Ischemia caused by HAT results in severe biliary and parenchyma damage and is associated with high rates of graft loss and mortality. Urgent thrombectomy and revascularization or re-transplantation is currently considered the treatment of choice in case of early diagnosis of HAT[10]. If Doppler US and/or computed tomograghy (CT) suspicious of HAT are present, an arteriogram is usually performed to confirm the imaging finding and, in very early diagnosis of HAT, to try to restore the hepatic flow with selective thrombolytic therapy and eventual treatment of concomitant HAS with balloon angioplasty and/or stent placement. In our practice, selective catheterization of the hepatic artery stump is performed with a microcatheter and an infusion of thrombolytic drugs. In selected patients with concomitant steal syndrome from splenic and/or gastroduodenal arteries, percutaneous splenic and/or gastroduodenal artery embolization can be performed in the same session in order to increase the flow in the hepatic artery (Figure 1). Surgical thrombectomy and/or re-transplantation should be reserved for cases in which percutaneous techniques fail. Saad *et al*[11] reported successful in re-establishment of arterial

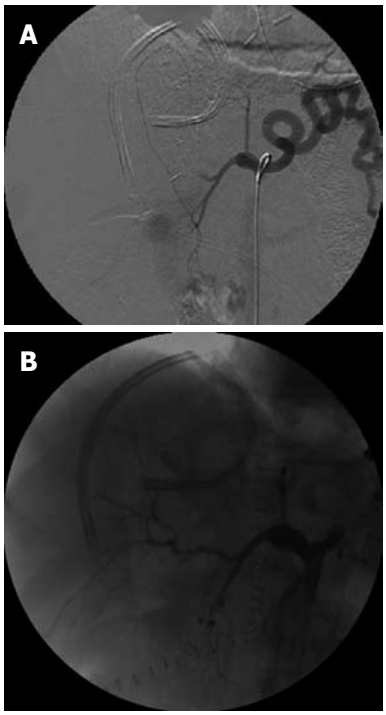


Figure 1 Status post right lobe LRLT in 18-year-old male. A: Celiac arteriogram showed acute HAT. Local thrombolysis with TPA was performed by placing a microcatheter in the hepatic artery stump; B: Celiac arteriogram after TPA infusion showed patent hepatic artery. In this patient, splenic artery embolization was performed by coils and PVA in order to increase the flow in hepatic artery. Although a good and early flow restoration was achieved, the patient underwent re-transplantation 2 d later for graft failure.

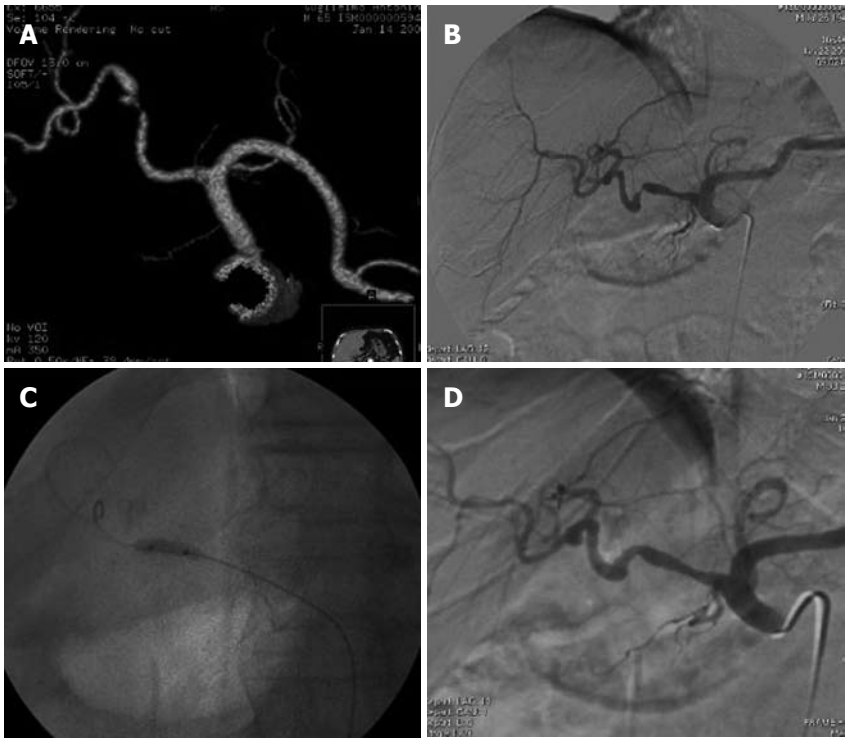


Figure 2 Status post deceased donor LT. Doppler US performed 5 mo after the transplant showed low intrahepatic resistive index 0.50 and prolonged systolic acceleration time 0.113 s, suspected for HAS. A: MDCT volume rendering 3D reconstruction showed a severe stenosis in the hepatic artery anastomosis; B: Digital subtraction angiography (DSA) celiac arteriogram confirmed the stenosis, trans-stenotic pressure gradient measured by a microcatheter was 50 mmHg; C: DSA percutaneous transluminal angioplasty performed by 4 mm diameter balloon catheter; D: DSA final arteriogram showed good patency of the arterial anastomosis with trans-stenotic pressure gradient reduced to 4 mmHg. Doppler US performed 4 mo later showed regular resistive index 0.70 and systolic acceleration time 0.100 s. Patient currently in good general condition and without biliary tree impairment after 6 mo of follow-up.

flow and uncovered underlying arterial anatomical defects in four out of five patients treated, but none were treated definitively by endoluminal procedures, due to the inability to resolve the underlying arterial stenosis, showing that the treatment of the underlying arterial defect is mandatory. Several cases of successful thrombolytic treatment of HAT in adult transplanted recipients are reported, usually if the diagnosis of HAT is performed a few hours after LT[12-15], but further analysis is needed to understand the correct timing of a possible thrombolysis and if endovascular procedures can be safely considered, a viable option to prevent re-transplantation after HAT. Possible complications of endovascular therapies are dissection and/or rupture of the hepatic artery during the arterial manipulation (requiring the placement of a covered stent) and bleeding from the arterial anastomosis[16].

Hepatic artery stenosis (HAS)

HAS is an insidious complication of LT occurring in approximately 5% of patients[17] leading to graft ischemia and possible hepatic artery occlusion as a result of slow flow with high incidence of morbidity and mortality due to hepatic insufficiency, biliary damage and possible sepsis. The majority of HAS arises at the anastomosis site and usually occurs within 3 mo after transplantation. The etiology of HAS can be due to small caliber of arteries or vascular clamp injury. Non-anastomotic stenosis can be present in cases of rejection or hepatic necrosis. The most common complication seen on cholangiography of recipients with HAS is non-anastomotic biliary strictures (BSs) seen in up to 49% of patients[18]. Early detection of HAS is fundamental because many of the stenoses are suitable for percutaneous treatment with angioplasty and/or stent placement or surgical revision, allowing good long-term graft function. In adult recipients with HAS that underwent percutaneous transluminal angioplasty, a 60%-80% patency rate at 1 year is reported[19]. Percutaneous transluminal angioplasty has also been reported to be an effective treatment of HAS after living donor LT, with a success rate of 94% and a complication rate of 6%, with possible HAS recurrence in 33% of patients[20]. In our practice, in a case of suspicious Doppler US or MDCT scan of HAS, a hepatic arteriogram is performed, from a transfemoral approach, with a 5F Cobra 2 or SOS catheter. A coaxial microcatheter is then advanced through the stenosis and the trans-stenotic pressure gradient measured. If a significant pressure gradient is present (> 10 mmHg) then an angioplasty is performed. Before angioplasty, 0.2 mg of nitroglycerine and 2000 UI of heparin are infused into the hepatic artery to reduce the risk of spasm or thrombosis. A 6F guiding catheter is advanced and a balloon catheter advanced over a 0.018 inch or 0.014 inch stiff wire. The diameter of the balloon used varies according to the diameter of the hepatic artery, ranging from 3 to 6 mm. Procedural success is determined by reduction or absence of the stenosis in a final arteriogram with significant reduction of the trans-stenotic pressure gradient (Figure 2). If a good patency is not restored, a metallic stent is deployed. The use of low-profile coronary stents in the treatment of HAS, as a first therapeutic approach, also showed good results with a 1-year patency rate of 45%-53%[21,22].

Portal vein stenosis (PVS)

PVS is a postoperative complication reported in 3% of patients after LT[23]. Clinical symptoms of hemodynamically significant PVS are related to portal

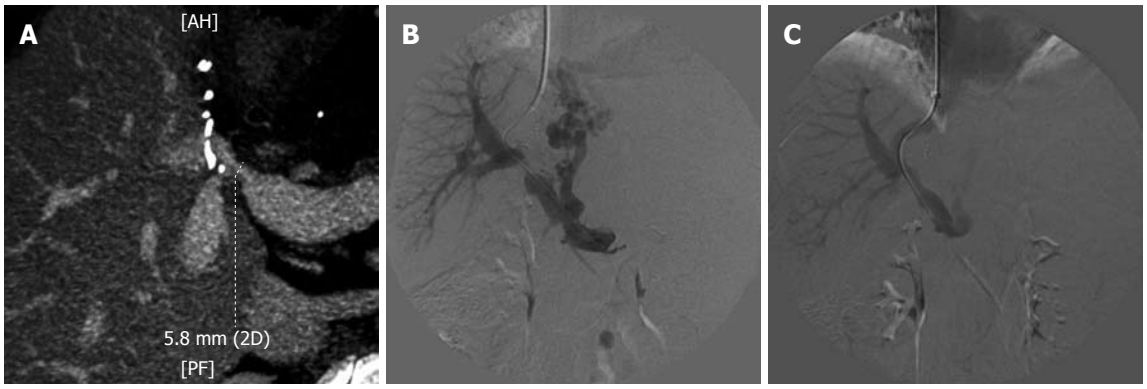


Figure 3 Status post right lobe LRLT in 60-year-old female. A: MDCT, MIP reconstruction showed a stenosis in portal vein anastomosis. The stenosis is very near the bifurcation of anterior and posterior branches; B: DSA, portogram performed from transjugular approach confirmed the PVS, 15 mmHg trans-stenotic pressure gradient was measured; note large patent coronary vein with filling gastro-oesophageal varices; C: DSA, final portogram performed after the deployement of a 10-mm diameter WallStent; note good filling of intrahepatic branches and no more evidence of the coronary vein, trans-stenotic pressure gradient reduced to 6 mmHg. Patient currently in good general condition with 1 year of follow-up.

hypertension and are bleeding from varices, splenomegaly and ascites. The portal vein is usually accessed by a transhepatic approach or by a transjugular approach. Percutaneous transhepatic angioplasty is considered an effective treatment and is usually considered as a first, non-surgical, therapeutic approach. Shibata *et al*[24] in a large series of patients, reported a success rate of 74% with a single session of balloon dilatation and a mean follow up of 24 mo. Recurrent stenoses were detected in 28% of patients and a maximum of three sessions of dilatation were necessary to resolve the stenosis. Funaki *et al*[25] reported metallic stent placement to treat recurrent and/or non-responsive, elastic stenosis with good long-term patency. Ko *et al*[26] reported a series of patients following living donor LT with early occurrence of PVS that were treated with transhepatic primary stent placement and showed good patency of the stents after a mean follow up of 66 mo in six out of nine patients. In the same paper, three post-procedural major complications were reported, two cases of hemoperitoneum and one case of intrahepatic pseudoaneurysm. In our practice, when a transhepatic approach is preferred, the procedure is performed under monitored anesthesia care. Transhepatic puncture of the portal vein is performed under ultrasound guide with a 21-Ga needle. An Accustik system (Boston Scientific) is advanced in the portal branch, over a nitinol wire, and then exchanged, over a 0.035 inch wire, for a 6F or 7F vascular sheath. The hemodynamic trans-stenotic pressure gradient measurement is performed using a 5F hydrophilic catheter. Before the dilatation, a bolus of heparin is administered (2000 UI) to reduce the risk of thrombosis during the balloon occlusion. The dilatation can be performed with balloon catheters up to 10 mm in diameter or more, according to the size of the vessel. Technical success is considered the resolution of the stenosis in a final portogram and a significant reduction of the trans-stenotic pressure gradient. In our practice, we embolize the transhepatic tracks with a coil to reduce the risk of bleeding, but in other series[25], transhepatic track embolization is not performed routinely without evidence of perihepatic bleeding. In patients with severe coagulopathy and/or ascites, the transjugular approach can be chosen, reducing the risk of bleeding[27,28]. The procedure is performed with monitored anesthesia care from the right internal jugular vein approach using the standard Colapinto set. Balloon dilatation and/or metallic stent placement are performed with the same technique as the transhepatic approach. Note that when the stenosis is very near the intrahepatic branches bifurcation, it is mandatory to use a non-covered metallic stent because the use of a covered stent, such as a Viatorr, would cause the occlusion of one branch (Figure 3).

IVC stenosis (IVCS)

IVC anastomosis stenosis after orthotopic LT is a rare complication occurring in approximately 1% of patients but more frequently in the superior anastomosis of IVC[29,30]. Clinical manifestations are usually refractory ascites and/or pleural effusion associated with renal insufficiency, lower extremities edema and alterations of liver function tests. IVCs is usually related to technical problems during surgery or fibrous scar development at the anastomosis, and concomitant stenosis at hepatic vein anastomosis can be present. For this reason, hepatic vein catheterization is recommended during the same procedure. Donor-recipient size mismatch can also be responsible for IVCS. Transluminal angioplasty is the first-choice treatment for this complication[31] (Figure 4). Trans-stenotic metallic stent deployment is reserved for resistant stenoses or those with elastic recoil not responsive to angioplasty[32]. A transfemoral approach is preferred for a diagnostic cavogram, as is trans-stenotic pressure gradient measurement. Filling of collateral peri-caval vessels is possible in case of hemodynamically significant stenosis. Balloon dilatation is performed with large-sized catheters. Due to the large size of the IVC, simultaneous inflation of multiple balloons has been described[1]. In our practice, during the procedure, radial or femoral artery pressure measurement is performed to continuously monitor changes in systemic hemodynamics during balloon dilatation, and consequent

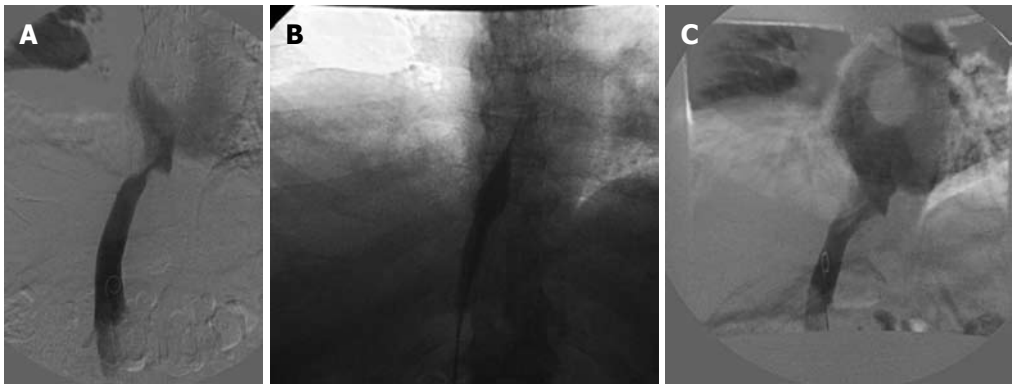


Figure 4 Status post deceased donor LT. Patient with refractory ascites in association with renal insufficiency and lower extremities edema 4 years after the transplant. A: DSA, cavogram showed a stenosis of IVC upper anastomosis, 12 mmHg trans-stenotic pressure gradient was measured. In the same session hepatic veins catheterization was performed showing no concomitant stenoses in hepatic vein anastomosis; B: DSA percutaneous transluminal angioplasty performed by 16-mm diameter balloon catheter; C: DSA final cavogram showed good patency of the caval anastomosis with trans-stenotic pressure gradient reduced to 2 mmHg. From 2001 to 2007, four other trans-luminal caval angioplasties were performed in the same patient. The patient is currently in good general condition after 15 mo and has avoided re-transplantation.

IVC occlusion, deflating the balloon before an excessive drop of systemic pressure. Repeat dilatations may be necessary for long-term patency. In patients with IVCS recurrence and severe renal insufficiency, trans-anastomotic pressure gradient measurement and balloon dilatation can be easily performed without injection of iodinate contrast.

Hepatic vein stenosis (HVS)

HVS, inducing outflow insufficiency, is a major postoperative complication of LT, especially in patients with partial liver graft transplantation producing graft failure with a reported incidence of 1%-4%[33-35]. Hepatic congestion can cause refractory ascites, refractory hydrothorax and alteration of liver function tests. HVS usually occurs at the anastomosis site; less frequent is the presence of an intrahepatic stenosis in the HV, likely due to previous venous injury during surgery or during previous percutaneous procedures, such as biopsy or biliary catheter placement. If a clinical and/or imaging suspicion of HVS is present, selective catheterization of all the HVs is mandatory to confirm the stenosis and measure the trans-stenotic pressure gradient (Figure 5). A pressure gradient greater than 3 mmHg between the HV and right atrium has been reported to be pathological[35]. Transjugular or transfemoral angioplasty or metallic stent placement is usually performed, as a first choice, to treat this complication[31,33-35]. In our experience, balloon dilatation is considered the preferred treatment choice because long-term patency of metallic stents is still unknown and metallic stent placement is reserved for persistent HVS not responsive to multiple angioplasties. Good technical and clinical success rates for percutaneous interventions are reported[33-36]. Long-term patency may require repeated interventions, especially if only trans-luminal angioplasty is performed. Better long-term patency results are reported in cases of stent deployment[36]. The primary percutaneous transhepatic approach for HVS treatment, has been reported[35] to have an easier negotiation through the stenosis, leading to shorter procedure time and ionizing radiation. In our experience, we use the percutaneous transhepatic approach only when the transjugular or the transfemoral approach fails. For the transhepatic approach, previous drainage of ascites and the embolization of the transhepatic tracks at the end of the procedure are, in our opinion, mandatory to reduce the risk of bleeding.

TREATMENT OF BILIARY COMPLICATIONS

Biliary complications occur in 10%-40% of patients after LT[37-40], with major incidence in patients with partial LT[41-42]. Complications include BSs, bile leakage (BL), biliary stones and bilomas. The majority of biliary complications develop during the first 3 mo, but strictures and stones may develop months or years after LT. The preferred methods for biliary tract reconstruction in LT are the duct-to-duct anastomosis between the donor and recipient common ducts and, less frequently, Roux-en-Y choledocojejunostomy. In right lobe split LT (from deceased or living related donor), a duct-to-duct anastomosis is usually performed between the donor right biliary duct and recipient bile duct, but due to possible anatomical variants of the donor biliary tree, two different biliary anastomoses, or less frequently three anastomoses, are performed with the recipient bile duct and the recipient cystic duct or with a Roux-en-Y limb, in up to 40% of patients[41,42]. Patients with multiple biliary reconstructions have a higher incidence of biliary complications[42]. Clinical presentation of biliary complications is often indistinguishable from other post-transplantation complications (vascular complications, rejection, graft dysfunction, infections). Ultrasonography is commonly used as screening test; however, due to the high rate of false-negative results, a negative test cannot exclude the presence of biliary complications.

Endoscopic retrograde cholangiopancreatography (ERCP) is usually the initial method of choice to

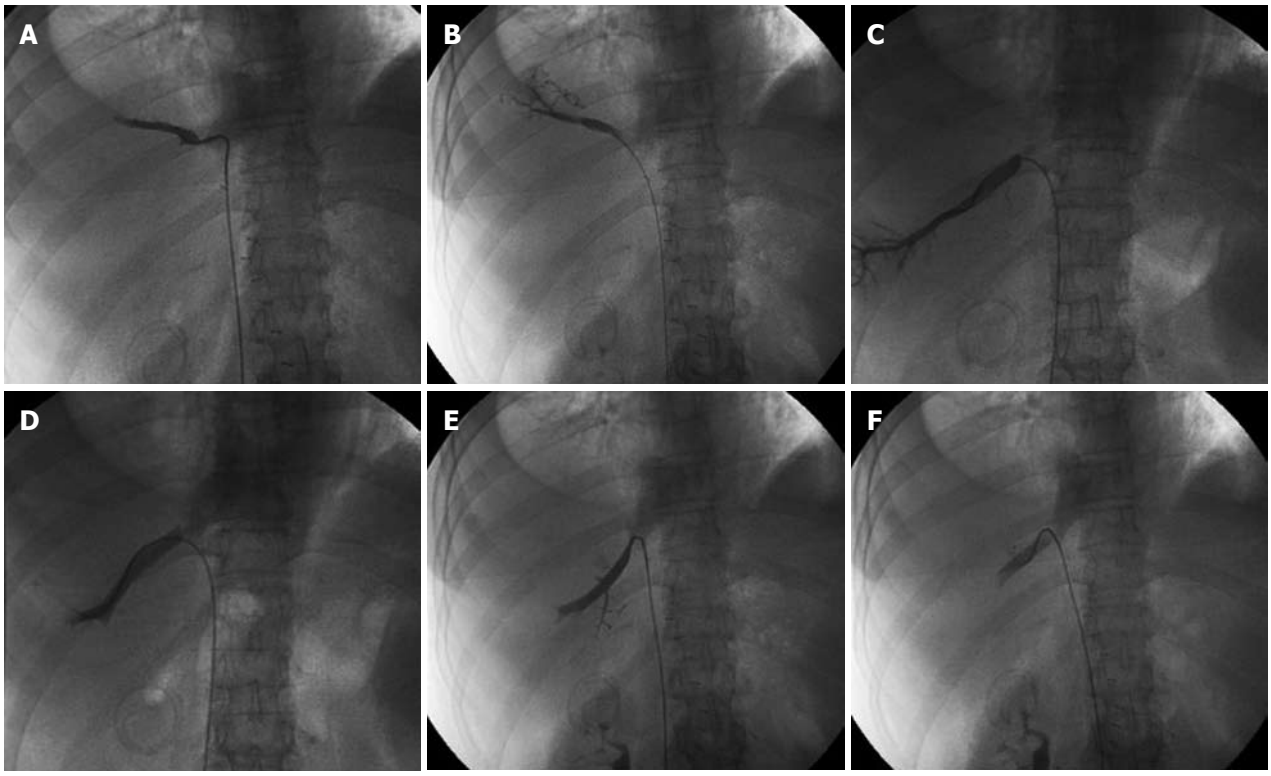


Figure 5 Status post deceased donor right lobe split LT in 65-year-old female. Three separates HV anastomoses were performed. One month after LT, patient developed refractory ascites and right pleural effusion with worsening of liver function tests. Doppler US was suspicious of HVs. A cavogram performed from the femoral approach showed widely patent IVC anastomosis. Selective catheterization of the three HVs (A, C and E) showed stenosis in the three anastomoses with trans-stenotic pressure gradient of 15, 20 and 16 mmHg, respectively. Balloon angioplasties were performed with balloon catheters ranging from 7 to 10 mm in diameter. Final venogram showed patent anastomoses (B, D and F). Trans-anastomotic pressure gradient reduced to 4, 2.5 and 8 mmHg. Eight months of follow-up without recurrence of refractory ascites and/or hydrothorax.

treat post LT biliary complications in patients with duct-to-duct anastomosis. Percutaneous transhepatic cholangiography (PTC) is the method to manage biliary complications in patients with choledochojejunostomy, in presence of intraepatic strictures and when endoscopic management fails.

Biliary strictures (BS)

BS is a common problem after LT with a reported incidence of 10%-35%[37-40]. Anastomotic BS is usually related to scar tissue and retraction at the suture site [41-42]. Intrahepatic BS is usually related to chronic rejection or arterial insufficiency due to HAS, thrombosis or ABO blood group incompatibility or infections. Single focal strictures and multiple/combined intrahepatic and anastomotic strictures can be present. Untreated BS is associated with high rate of morbidity and mortality. Endoscopic intervention is the preferred approach in patients with duct-to-duct anastomosis. PTC with biliary drainage placement and consequent percutaneous balloon dilatation is performed in patients with the Roux-en-Y reconstruction or in cases of endoscopic failure. In patients with partial LT, knowledge of the number of the anastomoses performed is mandatory before a possible percutaneous treatment. Possible complications of PTC are hemobilia, drop in hematocrit, intra or extrahepatic hematoma, fever with bacteremia, with a reported incidence of 3%-26% of cases[43,44]. Severe injury to intrahepatic arteries with massive hemobilia and possible formation of intrahepatic aneurysm is reported in 2% of cases. In those cases, emergency arterial embolization is required[43]. Suspicion of BS is based on one or more findings: clinical picture (fever, cholangitis), biochemistry (elevation of alkaline phosphatase, direct bilirubin, and transaminases), ultrasound and/or CT scan and/or MR (biliary duct dilatation), and liver biopsy (with histology consistent for cholestasis due to biliary obstruction). BS can also be present in cases of non-dilated biliary ducts. An ultrasound sensitivity of 38% in the detection of biliary obstruction has been reported in transplanted patients[45]. Better results, with sensitivity in detecting biliary obstructions ranging from 80% to 100%, are reported with the use of magnetic resonance cholangiography (MRCP)[46]. Percutaneous treatment of BS is considered safe and effective, avoiding in most cases the need for surgical revision of the anastomosis. Multiple treatments are often necessary. Long-term patency of percutaneous bilioplasty in adult recipients is reported from 50% to 60% at 5 years[47,48]. Prolonged cold ischemic and operative times, multiple or peripheral strictures and the presence of hepatic artery disease, predispose to treatment failure or a lower patency of anastomotic BS after balloon dilation[48,49]. The use of cutting balloon catheters or combined cutting and conventional balloon protocol has been proposed in patients with refractory anastomotic stricture[50,51].

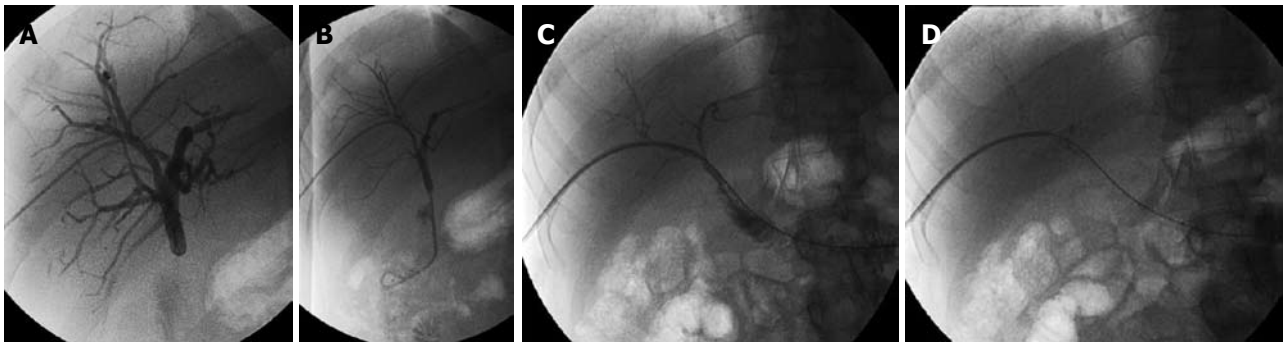


Figure 6 Status post deceased donor LT in 40-year-old man. Hepatico-jejunostomy was performed. A: PTC shows moderate biliary duct dilatation and subocclusive biliary anastomosis stricture; B: The stricture was crossed and trans-anastomotic. 6F Ring biliary catheter was placed; C: cholangiogram performed after four sessions of percutaneous bilioplasty shows no bile duct dilatation and patent anastomosis; D: Evidence of complete contrast transition from the bile ducts into the bowel loop within 2 min after the cholangiography. Three years of follow-up without clinical evidence of stricture recurrency.

Metallic stent deployment is reserved for patients who are refractory to repetitive balloon dilation of BS and who are poor surgical candidates[52]. In our practice, PTC is performed in an angiographic suite under monitored anesthesia care, with spontaneous respiration and additional local anesthesia. The patient is monitored continuously by electrocardiography, a pulse oximeter, and automatic blood pressure and pulse recordings. Intravenous antibiotic prophylaxis is administered before all procedures. Patients with coagulation defects (platelets < 50 000 mm^3 and/or prothrombin activity < 50%) receive infusions of platelets and/or fresh frozen plasma. PTC is generally performed through an intercostal approach, with an ultrasound- and fluoroscopy-guided 20-Ga needle inserted in a peripheral bile duct. If the cholangiography shows a stricture, the biliary tree is catheterized using an Accustick Introducer System (Boston Scientific, Natik, USA) over a Cope wire (Cook, Bjaeverskov, Denmark), the stricture crossed when possible with 0.035 or 0.038 inch hydrophilic guide wires, and a trans-anastomotic biliary catheter ranging from 6F to 8.5F with side holes placed above and below the stricture. The catheter is left to external gravity drainage for at least 1 d. If the patient has no fever and/or cholangitis the day after the procedure, the catheter is clamped for internal drainage. If, after the diagnostic cholangiogram, a guide wire cannot be passed through the stricture, an external drainage catheter is placed to allow for biliary decompression and to reduce the stricture's possible inflammatory component. A second attempt to cross the stricture is usually performed after 7 d. The first BS balloon dilatation session is never performed on the same day as the diagnostic cholangiogram, so as to reduce the risk of sepsis. It is generally performed after 1 wk, following a cholangiography performed with a 6F or 7F sheath and a balloon size ranging from 6 to 10 mm. Every dilatation session consists of three trans-anastomotic dilatations of 10 min each. Trans-anastomotic biliary catheters with sizes ranging from 6F to 14F according to the diameter of the anastomosis are placed after every dilatation session. An antibiotic infusion is re-administered 6 h after every procedure. At each dilatation session, the size of the balloon catheter is increased by 1 mm, up to a maximum diameter of 12 mm. Catheters are removed when the cholangiography performed through the sheath shows evidence of stricture resolution or of minimal residual stenosis of less than 20% of the expected lumen caliber. In all cases, catheters are removed only upon evidence of complete contrast transition from the bile ducts into the bowel loop within 3 min after the cholangiography (Figure 6), and if a significant reduction of cholestasis serum liver enzymes is achieved after bilioplasty. Our protocol envisages three BS dilatation sessions performed every 4-8 wk, followed by a cholangiographic evaluation 4 wk after the last session. If the stricture is resolved, the catheter is removed; if the BS persists, a supplementary balloon dilatation and follow up cholangiogram is performed, followed by potential catheter removal or balloon dilatation after 4-6 wk. In our experience, when a BS is crossed and a biliary catheter placed, percutaneous balloon dilatation gives good results, although multiple sessions over several months are necessary to obtain stricture resolution.

Bile leakage (BL)

Post-operative BL is a complication of LT that usually occurs within a few weeks from the transplant in 5%-20% of patients[37-39,53]. BL can arise from bile duct anastomosis of the resection margin in partial LT. Another possible site of leakage is the T-tube insertion in patients with choledocho-choledocho anastomosis. Small leakages usually close spontaneously, while large BLs are a serious complication and need to be treated because of possible associated complications such as fever, abdominal pain, fluid electrolyte depletion, fat malabsorption, possible sepsis or bleeding for hilar vascular erosion. The initial management should be non-operative. Percutaneous drainages, placed with a sonographic guide, are used to drain the bile collection. Endoscopic or percutaneous transhepatic management, with placement of large-size biliary catheters, allows achievement of good results in the treatment of large BL in adult patients, avoiding surgical repair in many cases[54-58]. In selected patients, when prior endoscopic or percutaneous transhepatic attempts to stent

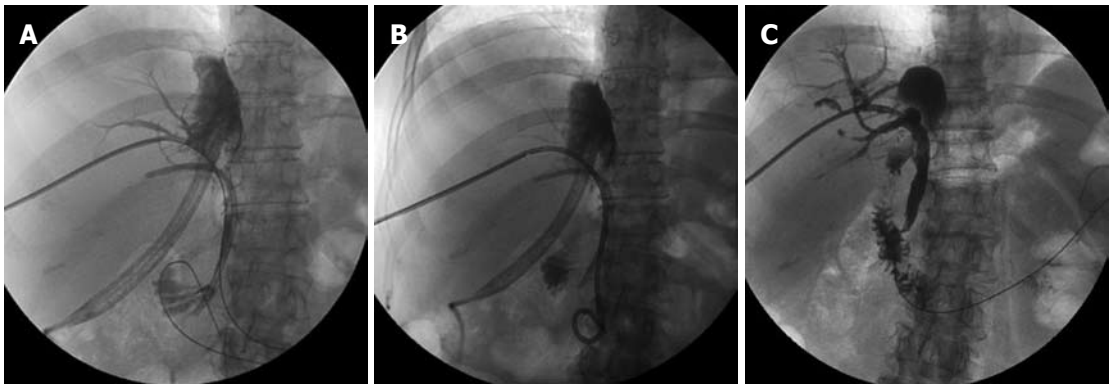


Figure 7 Status post LRLT (right lobe) in 56-year-old woman. Two separate biliary anastomoses were performed. Approximately 300 mL of bile was drained every day from the existing perihepatic drainage catheter (JP) placed during the transplantation. ERCP was performed, revealing a BL from the anastomotic region. An endoscopic stent was deployed in the posterior duct anastomosis but endoscopy failed to place a stent in the anterior duct anastomosis. A: PTC of the anterior duct showed no bile ducts dilatation and a BL arising from the anastomosis; B: 12F external internal catheter without side holes was placed in the leak region. The bile output from the JP progressively reduced and stopped a few days later. The JP and the endoscopic stent were removed 1 mo later; C: Final cholangiogram performed 4 mo later showed no BL and patent biliary anastomosis. The catheter was removed. The patient is still in good condition after 12 mo of follow-up.

the biliary tree have failed, the combined transhepatic-endoscopic approach (rendezvous technique) can be successfully used to place large-size biliary catheters[59-60]. When minimally invasive treatments fail, operative intervention is mandatory. In our practice, in patients with anastomotic BL who undergo percutaneous trans-hepatic treatment, we do not use biliary catheters with standard side holes, but we prefer to modify multipurpose large-size drainages by adding holes only in the intrahepatic bile ducts and in the distal bile duct, so as to reduce bile contact in the duct lesion to favor the repair process (Figure 7).

CONCLUSION

LT, in patients with end-stage liver diseases, has become an accepted treatment. Advances in the field of percutaneous, radiological, minimally invasive techniques have increased the importance of interventional radiology in the management of patients after LT. Interventional radiology procedures are used in the treatment of vascular and non-vascular complications, improving graft and patient survival and avoiding, in the majority of cases, surgical revision and/or re-transplantation.

REFERENCES

1 **Amesur NB**, Zajko AB. Interventional radiology in liver transplantation. *Liver Transpl* 2006; **12**: 330-351
2 **Little AF**, Ferris JV, Dodd GD 3rd, Baron RL. Image-guided percutaneous hepatic biopsy: effect of ascites on the complication rate. *Radiology* 1996; **199**: 79-83
3 **Al Knawy B**, Shiffman M. Percutaneous liver biopsy in clinical practice. *Liver Int* 2007; **27**: 1166-1173
4 **Yavuz K**, Geyik S, Barton RE, Petersen B, Lakin P, Keller FS, Kaufman JA. Transjugular liver biopsy via the left internal jugular vein. *J Vasc Interv Radiol* 2007; **18**: 237-241
5 **Little AF**, Zajko AB, Orons PD. Transjugular liver biopsy: a prospective study in 43 patients with the Quick-Core biopsy needle. *J Vasc Interv Radiol* 1996; **7**: 127-131
6 **Smith TP**, Presson TL, Heneghan MA, Ryan JM. Transjugular biopsy of the liver in pediatric and adult patients using an 18-gauge automated core biopsy needle: a retrospective review of 410 consecutive procedures. *AJR Am J Roentgenol* 2003; **180**: 167-172
7 **Bruzzi JF**, O'Connell MJ, Thakore H, O'Keane C, Crowe J, Murray JG. Transjugular liver biopsy: assessment of safety and efficacy of the Quick-Core biopsy needle. *Abdom Imaging* 2002; **27**: 711-715
8 **Silva MA**, Jambulingam PS, Gunson BK, Mayer D, Buckels JA, Mirza DF, Bramhall SR. Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl* 2006; **12**: 146-151
9 **Stange BJ**, Glanemann M, Nuessler NC, Settmacher U, Steinmuller T, Neuhaus P. Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl* 2003; **9**: 612-620
10 **Pinna AD**, Smith CV, Furukawa H, Starzl TE, Fung JJ. Urgent revascularization of liver allografts after early hepatic artery thrombosis. *Transplantation* 1996; **62**: 1584-1587
11 **Saad WE**, Davies MG, Saad NE, Westesson KE, Patel NC, Sahler LG, Lee DE, Kitanosono T, Sasson T, Waldman DL. Catheter thrombolysis of thrombosed hepatic arteries in liver transplant recipients: predictors of success and role of thrombolysis. *Vasc Endovascular Surg* 2007; **41**: 19-26
12 **Hidalgo EG**, Abad J, Cantarero JM, Fernandez R, Parga G, Jover JM, Manzanares J, Moreno E. High-dose intra-arterial urokinase for the treatment of hepatic artery thrombosis in liver transplantation. *Hepatogastroenterology* 1989; **36**: 529-532
13 **Figueras J**, Busquets J, Dominguez J, Sancho C, Casanovas-Taltavull T, Rafecas A, Fabregat J, Torras J, Jaurrieta E. Intra-arterial thrombolysis in the treatment of acute hepatic artery thrombosis after liver transplantation. *Transplantation* 1995; **59**: 1356-1357
14 **Kim BW**, Won JH, Lee BM, Ko BH, Wang HJ, Kim MW. Intraarterial thrombolytic treatment for hepatic artery thrombosis immediately after living donor liver transplantation. *Transplant Proc* 2006; **38**: 3128-3131
15 **Zhou J**, Fan J, Wang JH, Wu ZQ, Qiu SJ, Shen YH, Shi YH, Huang XW, Wang Z, Tang ZY, Wang YQ. Continuous transcatheter arterial thrombolysis for early hepatic artery thrombosis after liver transplantation. *Transplant Proc* 2005; **37**: 4426-4429
16 **Yamakado K**, Nakatsuka A, Takaki H, Usui M, Sakurai H, Isaji S, Uemoto S, Takeda K. Stent-graft for the management of hepatic artery rupture subsequent to transcatheter thrombolysis and angioplasty in a liver transplant recipient. *Cardiovasc Intervent Radiol* 2008; **31** Suppl 2: S104-S107
17 **Abbasoglu O**, Levy MF, Vodapally MS, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB. Hepatic artery

stenosis after liver transplantation--incidence, presentation, treatment, and long term outcome. *Transplantation* 1997; **63**: 250-255

18 **Orons PD**, Sheng R, Zajko AB. Hepatic artery stenosis in liver transplant recipients: prevalence and cholangiographic appearance of associated biliary complications. *AJR Am J Roentgenol* 1995; **165**: 1145-1149

19 **Saad WE**, Davies MG, Sahler L, Lee DE, Patel NC, Kitanosono T, Sasson T, Waldman DL. Hepatic artery stenosis in liver transplant recipients: primary treatment with percutaneous transluminal angioplasty. *J Vasc Interv Radiol* 2005; **16**: 795-805

20 **Kodama Y**, Sakuhara Y, Abo D, Shimamura T, Furukawa H, Todo S, Miyasaka K. Percutaneous transluminal angioplasty for hepatic artery stenosis after living donor liver transplantation. *Liver Transpl* 2006; **12**: 465-469

21 **Denys AL**, Qanadli SD, Durand F, Vilgrain V, Farges O, Belghiti J, Lacombe P, Menu Y. Feasibility and effectiveness of using coronary stents in the treatment of hepatic artery stenoses after orthotopic liver transplantation: preliminary report. *AJR Am J Roentgenol* 2002; **178**: 1175-1179

22 **Huang M**, Shan H, Jiang Z, Li Z, Zhu K, Guan S, Qian J, Chen G, Lu M, Yang Y. The use of coronary stent in hepatic artery stenosis after orthotopic liver transplantation. *Eur J Radiol* 2006; **60**: 425-430

23 **Lerut J**, Tzakis AG, Bron K, Gordon RD, Iwatsuki S, Esquivel CO, Makowka L, Todo S, Starzl TE. Complications of venous reconstruction in human orthotopic liver transplantation. *Ann Surg* 1987; **205**: 404-414

24 **Shibata T**, Itoh K, Kubo T, Maetani Y, Shibata T, Togashi K, Tanaka K. Percutaneous transhepatic balloon dilation of portal venous stenosis in patients with living donor liver transplantation. *Radiology* 2005; **235**: 1078-1083

25 **Funaki B**, Rosenblum JD, Leef JA, Zaleski GX, Farrell T, Lorenz J, Brady L. Percutaneous treatment of portal venous stenosis in children and adolescents with segmental hepatic transplants: long-term results. *Radiology* 2000; **215**: 147-151

26 **Ko GY**, Sung KB, Yoon HK, Lee S. Early posttransplantation portal vein stenosis following living donor liver transplantation: percutaneous transhepatic primary stent placement. *Liver Transpl* 2007; **13**: 530-536

27 **Glanemann M**, Settmacher U, Langrehr JM, Kling N, Hidajat N, Stange B, Staffa G, Bechstein WO, Neuhaus P. Portal vein angioplasty using a transjugular, intrahepatic approach for treatment of extrahepatic portal vein stenosis after liver transplantation. *Transpl Int* 2001; **14**: 48-51

28 **Gonzalez-Tutor A**, Abascal F, Cerezai L, Bustamante M. Transjugular approach to treat portal vein stenosis after liver transplantation--a case report. *Angiology* 2000; **51**: 511-514

29 **Brouwers MA**, de Jong KP, Peeters PM, Bijleveld CM, Klompmaker IJ, Slooff MJ. Inferior vena cava obstruction after orthotopic liver transplantation. *Clin Transplant* 1994; **8**: 19-22

30 **Glanemann M**, Settmacher U, Stange B, Haase R, Lopez-Haninnen E, Podrabsky P, Bechstein WO, Neuhaus P. Caval complications after orthotopic liver transplantation. *Transplant Proc* 2000; **32**: 539-540

31 **Zajko AB**, Sheng R, Bron K, Reyes J, Nour B, Tzakis A. Percutaneous transluminal angioplasty of venous anastomotic stenoses complicating liver transplantation: intermediate-term results. *J Vasc Interv Radiol* 1994; **5**: 121-126

32 **Borsa JJ**, Daly CP, Fontaine AB, Patel NH, Althaus SJ, Hoffer EK, Winter TC, Nghiem HV, McVicar JP. Treatment of inferior vena cava anastomotic stenoses with the Wallstent endoprosthesis after orthotopic liver transplantation. *J Vasc Interv Radiol* 1999; **10**: 17-22

33 **Darcy MD**. Management of venous outflow complications after liver transplantation. *Tech Vasc Interv Radiol* 2007; **10**: 240-245

34 **Ko GY**, Sung KB, Yoon HK, Kim JH, Song HY, Seo TS, Lee SG. Endovascular treatment of hepatic venous outflow obstruction after living-donor liver transplantation. *J Vasc Interv Radiol* 2002; **13**: 591-599

35 **Kubo T**, Shibata T, Itoh K, Maetani Y, Isoda H, Hiraoka M, Egawa H, Tanaka K, Togashi K. Outcome of percutaneous transhepatic venoplasty for hepatic venous outflow obstruction after living donor liver transplantation. *Radiology* 2006; **239**: 285-290

36 **Wang SL**, Sze DY, Busque S, Razavi MK, Kee ST, Frisoli JK, Dake MD. Treatment of hepatic venous outflow obstruction after piggyback liver transplantation. *Radiology* 2005; **236**: 352-359

37 **Egawa H**, Inomata Y, Uemoto S, Asonuma K, Kiuchi T, Fujita S, Hayashi M, Matamoros MA, Itou K, Tanaka K. Biliary anastomotic complications in 400 living related liver transplantations. *World J Surg* 2001; **25**: 1300-1307

38 **Testa G**, Malago M, Broelseh CE. Complications of biliary tract in liver transplantation. *World J Surg* 2001; **25**: 1296-1299

39 **Welling TH**, Heidt DG, Englesbe MJ, Magee JC, Sung RS, Campbell DA, Punch JD, Pelletier SJ. Biliary complications following liver transplantation in the model for end-stage liver disease era: effect of donor, recipient, and technical factors. *Liver Transpl* 2008; **14**: 73-80

40 **Rabkin JM**, Orloff SL, Reed MH, Wheeler LJ, Corless CL, Benner KG, Flora KD, Rosen HR, Olyaei AJ. Biliary tract complications of side-to-side without T tube versus end-to-end with or without T tube choledochocholedochostomy in liver transplant recipients. *Transplantation* 1998; **65**: 193-199

41 **Icoz G**, Kilic M, Zeytunlu M, Celebi A, Ersoz G, Killi R, Memis A, Karasu Z, Yuzer Y, Tokat Y. Biliary reconstructions and complications encountered in 50 consecutive right-lobe living donor liver transplantations. *Liver Transpl* 2003; **9**: 575-580

42 **Gondolesi GE**, Varotti G, Florman SS, Munoz L, Fishbein TM, Emre SH, Schwartz ME, Miller C. Biliary complications in 96 consecutive right lobe living donor transplant recipients. *Transplantation* 2004; **77**: 1842-1488

43 **L'Hermine C**, Ernst O, Delemazure O, Sergent G. Arterial complications of percutaneous transhepatic biliary drainage. *Cardiovasc Intervent Radiol* 1996; **19**: 160-164

44 **Hamlin JA**, Friedman M, Stein MG, Bray JF. Percutaneous biliary drainage: complications of 118 consecutive catheterizations. *Radiology* 1986; **158**: 199-202

45 **Kok T**, Van der Sluis A, Klein JP, Van der Jagt EJ, Peeters PM, Slooff MJ, Bijleveld CM, Haagsma EB. Ultrasound and cholangiography for the diagnosis of biliary complications after orthotopic liver transplantation: a comparative study. *J Clin Ultrasound* 1996; **24**: 103-115

46 **Kitazono MT**, Qayyum A, Yeh BM, Chard PS, Ostroff JW, Coakley FV. Magnetic resonance cholangiography of biliary strictures after liver transplantation: a prospective double-blind study. *J Magn Reson Imaging* 2007; **25:** 1168-1173

47 **Zajko AB**, Sheng R, Zetti GM, Madariaga JR, Bron KM. Transhepatic balloon dilation of biliary strictures in liver transplant patients: a 10-year experience. *J Vasc Interv Radiol* 1995; **6**: 79-83

48 **Sung RS**, Campbell DA Jr, Rudich SM, Punch JD, Shieck VL, Armstrong JM, Ford E, Sullivan P, Dasika NL, Magee JC. Long-term follow-up of percutaneous transhepatic balloon cholangioplasty in the management of biliary strictures after liver transplantation. *Transplantation* 2004; **77**: 110-115

49 **Saad WE**, Saad NE, Davies MG, Lee DE, Patel NC, Sahler LG, Kitanosono T, Sasson T, Waldman DL. Transhepatic balloon dilation of anastomotic biliary strictures in liver transplant recipients: the significance of a patent hepatic artery. *J Vasc Interv Radiol* 2005; **16**: 1221-1228

50 **Saad WE**, Davies MG, Saad NE, Waldman DL, Sahler LG, Lee DE, Kitanosono T, Sasson T, Patel NC. Transhepatic dilation of anastomotic biliary strictures in liver transplant recipients with use of a combined cutting and conventional

balloon protocol: technical safety and efficacy. *J Vasc Interv Radiol* 2006; **17**: 837-843
51 **Atar E**, Bachar GN, Bartal G, Mor E, Neyman H, Graif F, Belenky A. Use of peripheral cutting balloon in the management of resistant benign ureteral and biliary strictures. *J Vasc Interv Radiol* 2005; **16**: 241-245
52 **Petersen BD**, Maxfield SR, Ivancev K, Uchida BT, Rabkin JM, Rosch J. Biliary strictures in hepatic transplantation: treatment with self-expanding Z stents. *J Vasc Interv Radiol* 1996; **7**: 221-228
53 **Sheng R**, Sammon JK, Zajko AB, Campbell WL. Bile leak after hepatic transplantation: cholangiographic features, prevalence, and clinical outcome. *Radiology* 1994; **192**: 413-416
54 **Ernst O**, Sergent G, Mizrahi D, Delemazure O, L'Hermine C. Biliary leaks: treatment by means of percutaneous transhepatic biliary drainage. *Radiology* 1999; **211**: 345-348
55 **Cozzi G**, Severini A, Civelli E, Milella M, Pulvirenti A, Salvetti M, Romito R, Suman L, Chiaraviglio F, Mazzaferro V. Percutaneous transhepatic biliary drainage in the management of postsurgical biliary leaks in patients with nondilated intrahepatic bile ducts. *Cardiovasc Intervent Radiol* 2006; **29**: 380-388
56 **Liguory C**, Vitale GC, Lefebre JF, Bonnel D, Cornud F. Endoscopic treatment of postoperative biliary fistulae. *Surgery* 1991; **110**: 779-783; discussion 783-784
57 **Chang JM**, Lee JM, Suh KS, Yi NJ, Kim YT, Kim SH, Han JK, Choi BI. Biliary complications in living donor liver transplantation: imaging findings and the roles of interventional procedures. *Cardiovasc Intervent Radiol* 2005; **28**: 756-767
58 **Aytekin C**, Boyvat F, Harman A, Ozyer U, Sevmis S, Haberal M. Percutaneous management of anastomotic bile leaks following liver transplantation. *Diagn Interv Radiol* 2007; **13**: 101-104
59 **Aytekin C**, Boyvat F, Yimaz U, Harman A, Haberal M. Use of the rendezvous technique in the treatment of biliary anastomotic disruption in a liver transplant recipient. *Liver Transpl* 2006; **12**: 1423-1426
60 **Miraglia R**, Traina M, Maruzzelli L, Caruso S, Di Pisa M, Gruttadauria S, Luca A, Gridelli B. Usefulness of the "rendezvous" technique in living related right liver donors with postoperative biliary leakage from bile duct anastomosis. *Cardiovasc Intervent Radiol* 2008; **31**: 999-1002

S- Editor Li DL **L- Editor** Stewart GJ **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.694

World J Gastroenterol 2009 February 14; 15(6): 694-696
World Journal of Gastroenterology ISSN 1007-9327

TOPIC HIGHLIGHT

Salvatore Gruttadauria, MD, Associate Professor, *Series Editor*

Psychological evaluation and follow-up in liver transplantation

Josephine G Morana

Josephine G Morana, Department of Clinical Psychology, Mediterranean Institute for Transplantation and Advanced Therapies (ISMETT), University of Pittsburgh Medical Center, Via Tricomi 1, Palermo 90127, Italy
Author contributions: Morana JG wrote the manuscript.
Correspondence to: Dr. Josephine G Morana, Mediterranean Institute for Transplantation and Advanced Therapies (ISMETT), University of Pittsburgh Medical Center, Via Tricomi 1, Palermo 90127, Italy. jmorana@ismett.edu
Telephone: +39-91-2192111 Fax: +39-91-2192344
Received: July 4, 2008 Revised: October 12, 2008
Accepted: October 19, 2008
Published online: February 14, 2009

Abstract

An increasingly number of transplant centers have integrated a psychological assessment within their protocol for evaluation of patients being considered for transplantation. This paper highlights the psychological criteria for inclusion or exclusion for listing, briefly discusses the psychological dynamics of patients, and addresses possible psychotherapy and pharmacological therapy, before and after transplant.

Key words: Liver transplantation; Psychology; Contraindications; Cognitive behavior therapy

Peer reviewer: Justin Nguyen, MD, Mayo Clinic, 4500 San Pablo Road, Jacksonville 32224, United States

Morana JG. Psychological evaluation and follow-up in liver transplantation. *World J Gastroenterol* 2009; 15(6): 694-696 Available from: URL: http://www.wjgnet.com/1007-9327/15/694.asp DOI: http://dx.doi.org/10.3748/wjg.15.694

INTRODUCTION

Orthotopic liver transplantation (OLTx) is a major surgical procedure that can precipitate distress, anxiety and depression. The experience of the last few years of many transplantation centers has highlighted the importance of a thorough and routine psychological assessment before considering the patient as a possible candidate for listing[1]. The importance of identifying psychological/psychiatric, and/or possible psychosocial problems is necessary in order to eliminate or prevent the insurgence of possible psychological problems post-transplant. Most transplant centers have included an initial psychological evaluation in their work-up protocol, to evaluate the psychological strengths and possible liabilities of the patient who is being considered for an OLTx, so as to provide interventions such as: smoking cessation therapy, drug/alcohol rehabilitation, and improvement of compliance; that is, behavior that needs to be resolved before surgery, in order to reduce possible behavioral liabilities after transplantation[2].

The transplant itself has deep psychological implications, which may exist within the affective, social and interpersonal realm of the individual's personality. In the postoperative phase, there may be manifestations of adjustment disorders, psychopathological disturbances, problems with compliance, as well as non-adherence to the therapeutic plan[3]. To reiterate, it is therefore necessary to carry out an accurate evaluation of the psychological and personality profile of each individual being considered for listing for possible OLTx.

PSYCHOLOGICAL ASSESSMENT OF THE POTENTIAL CANDIDATE FOR TRANSPLANT

During the initial interview, the psychologist's main goal is to determine how much the candidate knows or is aware of his/her medical status, or better yet, whether he/she has accepted his/her medical condition. The communication of the necessity of a liver transplant automatically induces the patient to think that conventional therapies and/or less invasive surgery are no longer an option. In such a case, the patients' psychological-emotional reactions take a course of their own, for example, they start experiencing: (1) sense of despair; (2) concerns for his/her medical status and sense of imminent death; (3) reactive and/or correlated psychopathology.

During the course of the psychological evaluation, then, patients may find themsleves living two traumatic events (both real), at the same time: (1) sense of imminent death; or (2) rebirth through the transplant. During this phase, it has been observed that patients most often feel a sense of doubt, anxiety, ambivalence, fear and frustration,

which, if associated with a high level of psychological distress, can have consequences that can even lead to non-acceptance of the transplant. A careful psychological evaluation (cognitive, emotional and interpersonal) allows for an accurate course of psychotherapy[4].

The therapist must take into consideration those needs, deficits and assets that the patients possess in order to bring them step by step toward the final objective, which is the transplant. The specific, individualized treatment plan allows for an improvement of the quality of life (QOL) of the patient who will undergo a transplant and, specifically, during the postoperative period[4].

Ethically, the ability to give informed consent comprises three key elements: adequate information, adequate decision-making capacity, and freedom from coercion (President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, 1982)[5]. Therefore, to this end, it appears fundamental to the psychologist's evaluation, whose goal is to evaluate the degree of the patient's knowledge and understanding of the various items on the informed consent. To this extent, it is fundamental that the patient's ability for decision-making is evaluated. If there is a suspected inability to do this (because of mental retardation or social deficits, *etc*), then, it is imperative that further testing be done using standardized tools for the evaluation of IQ (e.g. Wechsler Adult Intelligence Scale-Revision). If mental deficiency is found, then it becomes a legal issue, that is, it is necessary to assign a legal guardian who can represent the patient, in order to protect his/her rights. In the absence of a cognitive defect, if it is evaluated that the patient has not fully understood the context of such a document because of difficulty in perceiving such information, or in the event that there is resistance in accepting such information, then it becomes imperative that the patients undergo a psycho-educational process, in order to induce them to adjust their non-functional behavior or lifestyle in accordance with the expectations of the transplant team. For example, patients might need to be educated on topics such as maintaining adequate personal hygiene, given that they will be treated with immunosuppressant medication for the rest of their lives.

Table 1 outlines the absolute and relative contraindications for transplant listing. Although each item needs detailed discussion, for the purpose of this paper, the discussion will focus on alcohol/drug addiction and psychopathology, which are the two contraindications that need the most active intervention of the psychologist. The candidacy of patients who have an addiction has varied within each transplant center; however, in recent years, there has been an attempt to formalize the criteria for such patients. In Italy, the Director of the National Transplant Institute assigned a group of psychologists and psychiatrists to work on the guidelines to be applied in transplant centers across the country. This group (GLI PSI TO), of which the present author is a member, debated and focused a lot of time and energy in determining the criteria for listing patients with addiction. The consensus was, also following the lead of guidelines set forth by the United Network for Organ Sharing, that patients may be considered for listing after 6-12 mo abstinence, and that they have to be active participants in a rehabilitation center (even as an out-patient). With such patients, during this period of abstinence at our center (Istituto Mediterraneo per Trapianti e Terapie ad alta Specializzazione; ISMETT), the treatment is two-fold: patients are sent to a rehabilitation center closest to their residence, where the main focus is the toxicological component of the problem; while at ISMETT, the psychologists work in full synergy with such centers in an attempt to give patient support, in order to access those possible psychosocial resources that are needed for a positive, favorable prognosis.

Table 1 Contraindications for OLTx

Absolute
Irriversible cognitive-neurological deficits
Active psychosis
Active addiction to drugs and/or alcohol
Relative
Personality disorder
History of psychiatric disorders
History of alcohol and/or drug addiction
Depression
Neurosis
History of use of psychotropic/neuroleptics
Limited family and social support
Limited ability to adhere to therapies
Inadequate motivation

With regard to psychopathology, it is important to note that it is not always a contraindication for transplantation *per se*. In fact, if patients manifest an active psychosis, not well compensated even with pharmacological therapy[6], it is obvious that this would be an absolute contraindication, especially since there is an absence of the necessary resources needed to undergo an OLTx. In other cases, however, such as in mood disorders and anxiety disorder, psychopharmacological therapy in conjunction with psychotherapy may ameliorate the disturbance to the point at which patients are placed in a condition in which they can reach a functional emotional, affective equilibrium that allows them to manage the eventual distress related to the transplant. Such patients, however, need constant support before, during and after transplantation. During the pre-transplantation phase, specifically for sensitivity to stress; in the post-transplant phase, most importantly because of immunosuppressant therapy that might precipitate mood swings, irritability, mania and anxiety. Psychotherapy and/or psychotherapy in conjunction with pharmacological treatment might be indicated during all the phases of the transplant process. Cognitive behavior therapy is the psychotherapeutic approach implemented at ISMETT, an approach which has been evaluated as being most beneficial with these patients, as they are individuals who tend to manifest traits such as depression, anxiety and phobia. Anxiety reduction techniques, autogenic training, systematic desensitization,

Table 2 Domains of the pre-transplant psychological evaluation

Informed consent
Personality profile
Psychopathology
Past/present psychiatric history
Effect of illness on daily life activities
Defense mechanism employed and coping skills
Motivation for surgery
Treatment compliance
Support from the family
Socioeconomic support (together with social worker's evaluation)
Awareness of information regarding the actual surgical event and future treatments
Use/abuse of alcohol and/or drugs (see paragraph on this topic)
QOL

relaxation techniques, guided imagery, pain management and hypnosis are techniques that might be implemented, and that normally bring more immediate results for the management of those symptoms already mentioned as those being manifested by patients during the transplant process (Table 2).

CONCLUSION

The role of the psychological assessment and monitoring during the pre- and post-transplant phases, as well as the ongoing follow-up intervention, is generally highly valued by organ transplant teams because of the significant health consequences of organ transplant failure. Identifying and reducing psychological risk factors can play an important role in overall long-term success of transplantation.

REFERENCES

1 **Widows MR**, Rodrigue JR. Clinical practice issues in solid organ transplantation. In: Llewelyn S, Kennedy P, eds. Handbook of Clinical Health Psychology. Oxford: John Wiley & Sons, 2003
2 **Everhart JE**, Beresford TP. Liver transplantation for alcoholic liver disease: a survey of transplantation programs in the United States. *Liver Transpl Surg* 1997; **3**: 220-226
3 **Dew MA**, Dunbar-Jacob J, Switzer GE, DiMartini AF, Stilley C, Kormos RL. Adherence to the medical regimen in organ transplantation. In: Rodriguez J, edtor. Biopsychosocial Perspectives on Transplantation. New York (NY): Kluwer/ Plenum Publishers, 2001: 93-124
4 **Dew MA**, Goycoolea JM, Switzer GE, Allen AS. Quality of life in organ transplantation: effects on adult recipients and their families. In: Trzepacz PT, DiMartini AF, eds. The transplant patient: Biological, Psychiatric and Ethical Issues in organ transplantation. Cambridge: Cambridge University Press, 2000: 67-145
5 **President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research**. Washington (DC): United States Government Printing Office, 1982
6 **Triffaux JM**, Wauthy J, Bertrand J, Limet R, Albert A, Ansseau M. Psychological evolution and assessment in patients undergoing orthotopic heart transplantation. *Eur Psychiatry* 2001; **16**: 180-185

S- Editor Li DL **L- Editor** Kerr C **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.697

World J Gastroenterol 2009 February 14; 15(6): 697-704
World Journal of Gastroenterology ISSN 1007-9327

N-cadherin knock-down decreases invasiveness of esophageal squamous cell carcinoma *in vitro*

Ke Li, Wei He, Na Lin, Xin Wang, Qing-Xia Fan

Ke Li, Wei He, Na Lin, Xin Wang, Qing-Xia Fan, Department of Oncology, the First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, Henan Province, China
Author contributions: Li K and Fan QX designed the research; Li K, He W, Lin N, Wang X and Fan QX performed the research; Li K and Fan QX analyzed data and wrote the paper.
Supported by The National Natural Science Foundation of China, 072102310054
Correspondence to: Qing-Xia Fan, Professor, Department of Oncology, the First Affiliated Hospital, Zhengzhou University, Zhengzhou 450052, Henan Province, China. fqx2243@yahoo.com.cn
Telephone: +86-371-66862243 Fax: +86-371-66862243
Received: October 31, 2008 Revised: December 11, 2008
Accepted: December 18, 2008
Published online: February 14, 2009

Abstract

AIM: To examine the expressions of N-cadherin and E-cadherin in specimens of 62 normal esophageal epithela, 31 adjacent atypical hyperplastic epithelia and 62 esophageal squamous cell carcinomas (ESCCs), and to investigate the roles of N-cadherin in the invasiveness of ESCC cell line EC9706 transfected by N-cadherin shRNA.

METHODS: PV immunohistochemistry was used to detect the expression pattern of N-cadherin and E-cadherin in specimens of 62 normal esophageal epithelia, 31 adjacent atypical hyperplastic epithelia and 62 ESCCs. The invasiveness of ESCC line EC9706 was determined by transwell assay after EC9706 was transfected by N-cadherin shRNA.

RESULTS: The positive rates of N-cadherin decreased in the carcinoma, adjacent atypical hyperplastic and normal esophageal tissues (75.8%, 61.3% and 29.0%, $P < 0.05$), respectively, while those of E-cadherin increased (40.3%, 71.0% and 95.2%, $P < 0.05$). The increased expression of N-cadherin and decreased expression of E-cadherin were related to invasion, differentiation, and lymph node metastasis ($P < 0.05$). The expression level of N-cadherin decreased in the N-cadherin knocked down cells, and the invasiveness of those cells decreased significantly as well. The number of cells which crossed the basement membrane filter decreased from 123.40 ± 8.23 to 49.60 ± 6.80 ($P < 0.05$).

CONCLUSION: E-cadherin and N-cadherin expression is correlated with the invasion and aggravation of ESCC. The down-regulation of N-cadherin lowers the invasiveness of EC9706 cell line.

Key words: Esophageal squamous cell carcinoma; RNAi; N-cadherin; EC9706

Peer reviewer: Naofumi Mukaida, MD, PhD, Chairperson and Professor, Division of Molecular Bioregulation, Cancer Research Institute, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-0934, Japan

Li K, He W, Lin N, Wang X, Fan QX. N-cadherin knock-down decreases invasiveness of esophageal squamous cell carcinoma *in vitro*. *World J Gastroenterol* 2009; 15(6): 697-704 Available from: URL: http://www.wjgnet.com/1007-9327/15/697.asp DOI: http://dx.doi.org/10.3748/wjg.15.697

INTRODUCTION

The malignancy of cancer cells depends largely on their proliferative, invasive and metastatic activities, and invasive and metastatic activities are most closely associated with cell-to-cell and cell-to-extracellular matrix adhesion. As a class of transmembrane proteins, cadherins play an important role in cell adhesion. Among the members of cadherin family, E-cadherin and N-cadherin have been extensively studied about their biological activities and associations with cancer cell invasion. It was reported that the invasion and metastasis were present in the esophageal squamous cell carcinoma (ESCC) with a low level of E-cadherin[1,2]. Recent studies on prostate cancer and breast cancer proved that the up-regulated N-cadherin plays an important role in cell progression and metastasis[3,4]. In addition, N-cadherin is involved in angiogenesis and tumor growth regulation, and contributes to the invasive morphology in squamous tumor cells, and stimulates migration, invasion and metastasis[5]. However, the association of E-cadherin and N-cadherin expression with the malignancy of the ESCC is unknown.

In the current study, we analyzed the expression of E-cadherin and N-cadherin in the ESCC tissues, adjacent atypical hyperplastic epithelium and normal esophageal

epithelium. The roles of N-cadherin in the invasiveness were investigated in ESCC cell line (EC9706) transfected by N-cadherin shRNA.

MATERIALS AND METHODS

Tissue samples

In this study, we randomly selected 62 esophageal cancer patients who underwent potentially curative surgery without preoperative chemotherapy or radiotherapy between February 26 and March 16, 2006 in Anyang Tumor Hospital, Henan, China. Among them, 36 were men and 26 were women, ranging from 38-75 years of age with a mean age of 52.6 years. Overall, 22 cases were poorly differentiated, 25 were moderately differentiated, and 15 were well differentiated. For the lymphatic node metastasis, 20 cases were positive and 42 cases were negative. Seven cases were classified as T_1 and T_2, and 55 as T_3 and T_4 in terms of T stages. Surgically removed specimens were routinely fixed in buffered formalin and embedded in paraffin blocks for clinical diagnosis and reclassification for this study. The normal esophageal squamous tissues were taken from mucosae 3 cm away from carcinomas, and the adjacent atypical hyperplastic epithelium 2 cm away from the carcinomas. The final pathological diagnosis was based on the result of histological examination.

Expression of E-cadherin and N-cadherin in ESCC

Immunohistochemical analysis for E-cadherin and N-cadherin was performed on 5-μm sections made from tissue microarray blocks. The Envision Plus detection system (Dako, Carpinteria, CA, USA) was used for the immunostaining. The sections were deparaffinized in xylene and then were microwaved in 10 mmol/L citrate buffer (pH 6.0) to unmask the epitopes. Endogenous peroxidase activity was blocked by incubation with 0.03% hydrogen peroxide in methanol for 5 min. Slides were incubated with mouse anti-human E-cadherin antibody (microwaving retrieval in citrate buffer at 1:100 concentration, Abcam) and mouse anti-human N-cadherin antibody (microwaving retrieval in low pH buffer at 1:100 concentration; Abcam), respectively. Then, polyperoxidase anti-mouse IgG (Beijing Zhongshan Golden Bridge Biotechnology Co. Ltd., China) was added for incubation for another 30 min followed by gentle rinsing with washing buffer for three times. Thereafter, the sections were stained for 5 min with 3,3-diaminobenzidine (DAB) (Beijing Zhongshan Golden Bridge Biotechnology), counterstained by hematoxylin, dehydrated, and mounted in Diatex. Expression of E-cadherin and N-cadherin in breast cancer was used as a positive control, while the same concentration of PBS was applied as a negative control for E-cadherin and N-cadherin.

Evaluation of staining for E-cadherin and N-cadherin

For both E-cadherin and N-cadherin, only membrane and cytoplasm staining was considered as positive. The intensity and percentage of immunostained carcinoma cells were all taken into consideration according to the previously published method with modification[6]. Briefly, the extent of positivity was scored as 0 when no positive cell was observed; 1 when the percentage of positive cells was < 30%; 2 when it was 30%-60%; and 3 when it was > 60%. The intensity was scored as 0 when no positive cells were identified; 1, weak; 2, moderate; and 3, strong staining. Multiplying the extent by intensity gave the following immunohistochemical staining grades as 0, 1, 2, 3, 4, 6 and 9. For statistical analyses, grades 0, 1 and 2 were considered as negatively stained, and grades > 2 were considered as positively stained.

Retroviral virus production and determination of viral titer

PT67 packaging cells were seeded onto a six-well plate at 1×10^5 cells per well and incubated for 24-48 h. The cells were allowed to grow to 60%-70% confluence and then rinsed with fresh DMEM medium. The control vector pEGFP-MSCVneo and recombinant retroviral vector pMSCVneo/N-cadherin plasmids (kindly provided by Dr. Ma Jie, Chinese Academy of Medical Sciences, China) (The former contained the enhanced green fluorescent protein and neomycin resistance genes, the latter contained the N-cadherin shRNA, U6-promotor, enhanced green fluorescent protein and neomycin resistance genes) were transfected into the packaging cell line PT67 by lipofectamine 2000 (Invitrogen Corp, UK) and incubated for 6 h following the manufacturer's instructions. The mixture was replaced with fresh medium to stop transfection and the transfected PT67 cells were further cultured with G418 (1000 mg/L) selecting medium for 2 wk. Viral supernatant of the drug-resistant clones was filtered through a 0.45-μm filter and condensed by ultracentrifugation at low temperature, then preserved at -80℃. The supernatant, from the fresh PT67 packaging cells containing recombinant retrovirus, was used to infect NIH3T3 cells to determine viral titer as previously described[7]. The highest titer clones were selected for further experiments.

Infection of EC9706 cells with viral supernatant

EC9706 cells were plated in a six-well plate at 5×10^4 cells/mL and cultured with 5% CO_2 at 37℃. Twenty-four hours later, the cells were exposed to 2 mL viral supernatant at a consecutive multiplicity of infection once for 12 h, in the presence of 8 μg/mL polybrene (Sigma). Subsequently, viral supernatant was replaced with fresh medium. After being incubated for another 24 h, the infected EC9706 cells were screened with G418 (600 mg/L) (Sigma) selecting medium for 2 wk. Drug-resistant clones were expanded with G418 (300 mg/L) (Sigma) selecting medium. Two days after culturing, the expression of EGFP in infected EC9706 cells was observed under fluorescence microscope.

Total RNA and protein isolation

Total RNA and protein isolation was performed using the Macherey-Nagel total RNA and protein isolation kit according to the user manual. About 5×10^6 EC9706

Table 1 Real-time PCR primers for N-cadherin, E-cadherin, MMP-9 and GAPDH

Target gene	Primers	Length of product (bp)
N-cadherin	Sense: 5'-GGTGGAGGAGAAGAAGACCAG-3' Antisense: 5'-GGCATCAGGCTCCACAGT-3'	72
E-cadherin	Sense: 5'-CCCGGGACAACGTTTATTAC-3' Antisense: 5'-GCTGGCTCAAGTCAAAGTCC-3'	72
MMP9	Sense: 5'-GAACCAATCTCACCGACAGG-3' Antisense: 5'-GCCACCCGAGTGTAACCATA-3'	67
GAPDH	Sense: 5'-AGCCACATCGCTCAGACA-3' Antisense: 5'-GCCCAATACGACCAAATCC-3'	66

cells transfected with N-cadherin RNAi, control vector, or the untreated cells were collected and lysed. Through the NucleoSpin RNA/Protein column, RNA and DNA were bound to the column and protein was contained in the flow-through. After digestion of DNA, total RNA was isolated by washing the column. Protein was isolated from the flow-through and incubated for 3 min at 98℃ for dissolving and denaturation, and stored at -20℃ until used. All of the preparation and handling steps of RNA took place in a laminar flow hood under RNase-free conditions.

cDNA synthesis

RNA quality and quantity were determined by absorbance readings at 260 and 280 nm with the Nano Drop (ND-1000) spectrophotometer. RNA integrity was tested by PCR amplification of the GAPDH gene. Reverse transcription of RNA was performed using Transcriptor First Strand cDNA Synthesis Kit (Roche). cDNA was synthesized from 5 μg total RNA isolated from EC9706 cells transfected with N-cadherin siRNA, control vector, or the untreated cells according to the manufacturer's handbook.

Primer/probe design

The primer pairs and hydrolysis probes for N-cadherin, E-cadherin, matrix metalloproteinase-9 (MMP-9) and GAPDH were designed by Universal ProbeLibrary Assay Design Center (Roche). All primer sequences listed in Table 1 were synthesized by Shanghai Sangon (China). The hydrolysis probes were designed and synthesized by Roche Diagnostics.

Real-time PCR

Real-time PCR was performed with the ABI Prism 7500 Sequence Detection System (ABI) in a total volume of 20 μL in glasscapillaries containing 2 μL of cDNA, 0.5 μmol/L of each primer, 0.1 μmol/L of hydrolysis probe and 4 μL of LightCycler TaqMan Master Mix (Roche Diagnostics). PCR reaction was initiated with a 12-min denaturation at 95℃ and terminated with a 30-s cooling step at 40℃. The cycling protocol consisted of denaturation at 95℃ for 10 s, annealing at 54℃ for 10 s, and extension at 72℃ for 10 s, and was cycled 45 times. Fluorescence detection was performed at the end of each extension step. The housekeeping genes GAPDH and DEPC-H_2O were set as internal control and negative control, respectively.

Western blot analysis

Twenty microliters of protein samples were separated on a 10% SDS-acrylamide gel (Bio-Rad) for 1 h at 150 V, and the proteins were transferred to nitrocellulose membrane (Whatman). After blocking in 5% fat-free milk, the membrane was treated with the dilution of the primary antibody overnight at 4℃ and the dilution of the secondary IgG-horseradish peroxidase (HRP) conjugated antibody for 1 h at room temperature. All dilutions were in PBS containing 5% Blotto (Santa Cruz) and 0.1% Tween-20. The stained membranes were visualized by enhanced chemiluminescence reaction using the ECL Plus (GE Healthcare). Western blot experiments were repeated at least three times on every sample, with similar results.

Transwell chamber migration assay

Matrigel-coated filter inserts with 8-μm pores that fit into 24-well invasion chambers were obtained from Becton Dickinson. EC9706 cells transfected with N-cadherin RNAi, control vector, or the untreated cells were detached from the tissue culture plates, washed, resuspended in conditioned medium (10^6 cells/mL), and then added to the upper compartment of the invasion chamber with or without plasmin (1.8 mg). Conditioned medium (500 μL) was added to the lower compartment of the invasion chamber. The Matrigel invasion chambers were incubated at 37℃ for 24 h in 5% CO_2. After incubation, the filter inserts were removed from the wells, and the cells on the upper side of the filter were removed using cotton swabs. The filters were fixed, mounted, and stained according to the manufacturer's instructions. The cells that invaded through the Matrigel were counted on the underside of the filter. Three to five invasion chambers were used for each experimental condition. The values obtained were calculated by averaging the total number of cells from three filters.

Statistical analysis

χ^2 test and Spearman rank correlation coefficient analysis were used to assess the univariate association between the immunohistochemical status and the clinicopathological characteristics. Results were expressed as mean ± SD. Statistical analysis was made using one way ANOVA or paired-samples *t* test of SPSS 11.0. $P < 0.05$ was considered statistically significant.

RESULTS

Immunoreactivity and clinicopathological correlations

For E-cadherin, positive immunostaining was observed on the membrane of cancer cells and the intercellular junctions. It was strongly expressed in the normal esophageal squamous tissues (95.2%), moderately expressed in the adjacent atypical hyperplastic epithelium (71.0%), and weakly expressed in the ESCC

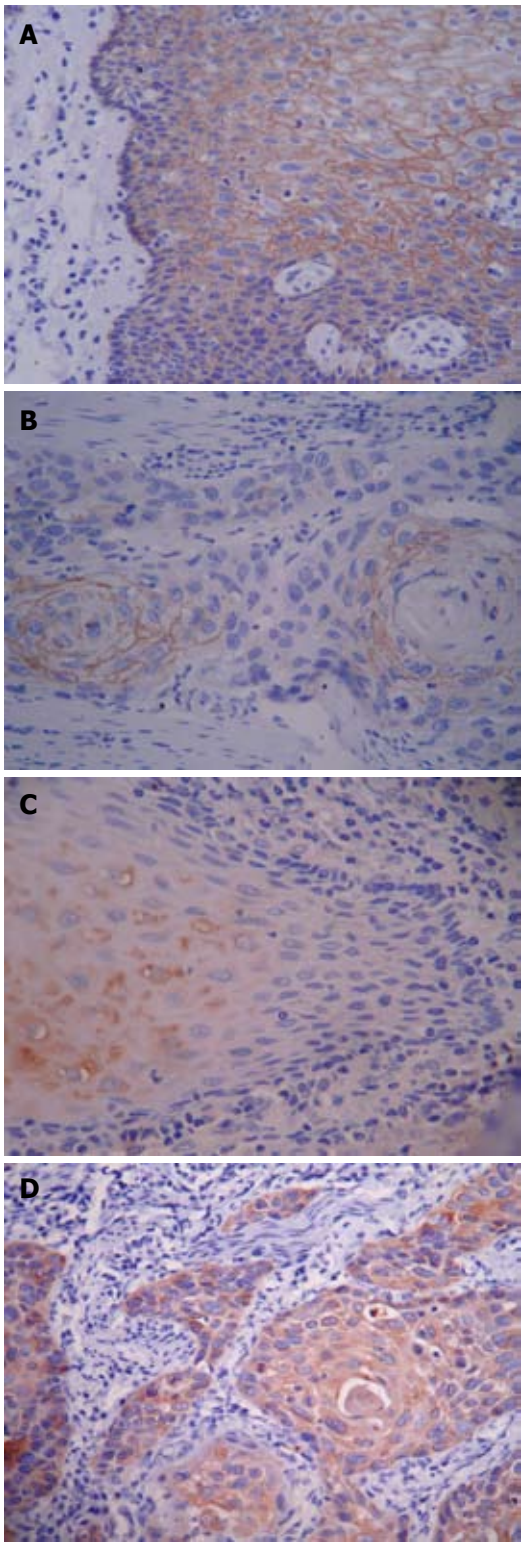


Figure 1 Expression of E-cadherin and N-cadherin in ESCC tissues and normal tissues. A: Strong expression (yellowish brown) of E-cadherin on the membrane of normal esophageal epithelial cells (PV, × 200); B: Weak expression (yellow) of E-cadherin on the membrane of ESCC cells (PV, × 200); C: Weak expression (yellow) of N-cadherin in the cytoplasm of normal esophageal epithelial cells (PV, × 200); D: Strong expression (yellowish brown) of N-cadherin in the cytoplasm of ESCC cells (PV, × 200).

tissues (40.3%) (Figure 1A, B and Table 2). Contrary to the E-cadherin, N-cadherin, which existed in the cytoplasm, was strongly expressed in the ESCC (75.8%)

Table 2 Correlations of E-cadherin, N-cadherin expression with clinicopathological features of ESCC

Items	*n*	E-cadherin			N-cadherin		
		Cases (%)	χ^2	*P*	Cases (%)	χ^2	*P*
Histological classification							
NEE	62	59 (95.2)			18 (29.0)		
AH	31	22 (71.0)	48.426	0.000	19 (61.3)	29.091	0.000
ESCC	62	25 (40.3)			47 (75.8)		
Histological grade							
I	15	11 (73.3)			8 (53.3)		
II	25	9 (36.0)	9.962	0.007	19 (76.0)	6.924	0.031
III	22	5 (22.7)			20 (90.9)		
Depth of invasion							
Not to serosa	7	6 (85.7)	4.797	0.029	2 (28.6)	6.916	0.009
To serosa	55	19 (34.5)			45 (81.8)		
Lymph node metastasis							
Yes	42	21 (50.0)	3.897	0.048	28 (66.7)	4.486	0.034
No	20	4 (20.0)			19 (95.0)		

NEE: Normal esophageal squamous tissues; AH: Adjacent atypical hyperplastic epithelium; ESCC: Esophageal squamous cell carcinoma tissues.

Table 3 Correlation between E-cadherin and N-cadherin protein expressions

	E-cadherin	N-cadherin (+)	N-cadherin (-)	γ	*P*
+	25	12	13	-0.534	0.000
–	37	35	2		

and moderately expressed in the adjacent atypical hyperplastic epithelium (61.3%), but weakly expressed in the normal esophageal squamous tissues (29.0%) (Figure 1C, D and Table 2). The correlations between the clinicopathological features and the expressions of E-cadherin and N-cadherin in the primary tumors are summarized in Table 2. Higher level of N-cadherin expression was significantly associated with higher histological grade, deeper invasion and more lymph node metastasis, while E-cadherin expression was associated with totally opposite sides.

Correlation between E-cadherin and N-cadherin protein expression

In order to know whether the expression of E-cadherin and N-cadherin in these tumors was associated, a crosstable analysis was performed (Table 3), which showed that their expression was significantly negatively correlated.

PT67 cells producing retrovirus

After G418 selection for 30 d, the stable colonies of pEGFP-MSCVneo plasmid (control vector) with viral titer 1×10^7 cfu/L and colonies of pMSCVneo/N-cadherin (RNAi vector) with viral titer 3×10^7 cfu/L were picked up to infect EC9706 cells (Figure 2A).

Establishment of transfected EC9706 cells

EC9706 cells were infected with the concentrated viral supernatant and selected with G418 as described above. After screening with G418 for 2 wk, the isolated G418-

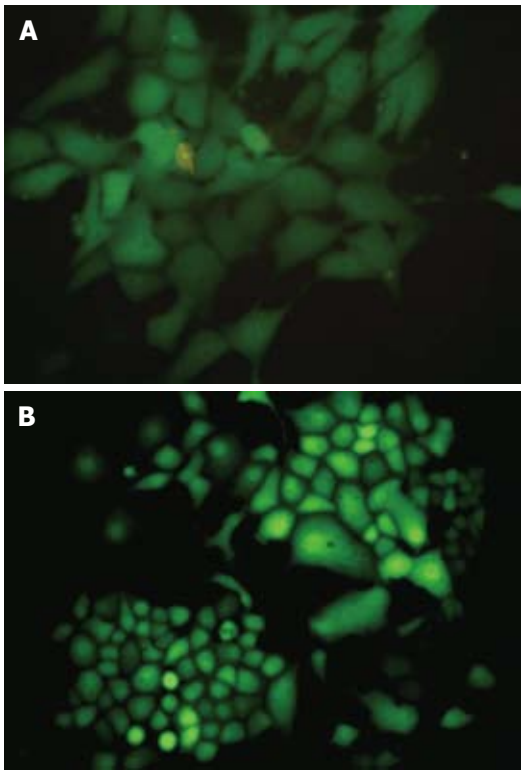


Figure 2 EGFP expression in PT67 cells and EC9706 cells after pMSCV-neo/N-cadherin transfection. A: Selected by 1000 mg/L G418 for 15 d and 300 mg/L G418 for 15 d, the PT-67 cells transfected with pMSCVneo/N-cadherin plasmid expressed EGFP stably. The figure was taken under fluorescence microscope (× 400, at 488 nm); B: Selected by 600 mg/L G418 for 14 d and 300 mg/L G418 for 10 d, the EC9706 cells infected with pMSCVneo/N-cadherin viral supernatant expressed EGFP stably. The figure was taken under fluorescence microscope (× 400, at 488 nm).

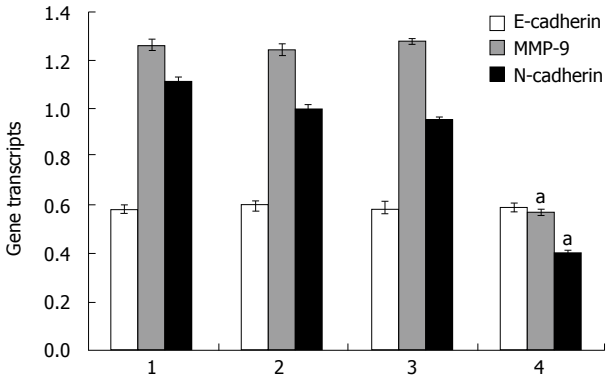


Figure 3 E-cadherin, N-cadherin and MMP-9 gene transcripts detected by real-time PCR. 1: Positive control; 2: Untreated EC9706 cells; 3: EC9706 cells with control vector; 4: EC9706 cells with N-cadherin RNAi. $^{a}P < 0.05$ *vs* 2 and 3.

resistant clones (Figure 2B) were transferred into larger culture vessels to expand for further experiments.

N-cadherin depletion affected expression of E-cadherin and MMP-9 by EC9706

To investigate the expression phenotypes of E-cadherin and MMP-9 when N-cadherin was down-regulated, real-time PCR and Western blotting were employed to examine the expressions of the N-cadherin, E-cadherin

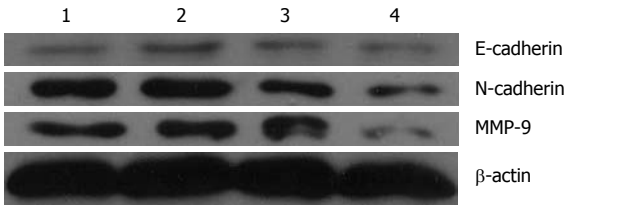


Figure 4 E-cadherin, N-cadherin and MMP-9 proteins detected by Western bloting. 1: Positive control; 2: Untreated EC9706 cells; 3: EC9706 cells with control vector; 4: EC9706 cells with N-cadherin RNAi. E-cadherin proteins were 0.247 ± 0.010, 0.252 ± 0.087, 0.249 ± 0.07 and 0.250 ± 0.006, respectively, from lane 1 to lane 4. $P > 0.05$. The N-cadherin proteins were 0.681 ± 0.003, 0.679 ± 0.004, 0.653 ± 0.009 and 0.342 ± 0.006, respectively, from lane 1 to lane 4. $P < 0.05$. The MMP-9 proteins were 0.624 ± 0.011, 0.628 ± 0.010, 0.623 ± 0.009 and 0.282 ± 0.010, respectively, from lane 1 to lane 4. $P < 0.05$.

and MMP-9 in ESCC cell line EC9706. Transfection of N-cadherin RNAi lowered N-cadherin mRNA level (0.397 ± 0.013) to less than 40%, compared with the untreated cells (1.000 ± 0.016) (Figure 3), and the N-cadherin protein (0.342 ± 0.006) to 50%, compared with the untreated cells (0.679 ± 0.004) (Figure 4).

The levels of MMP-9 mRNA and protein lowered by about 50% in the N-cadherin depleted cells compared with the untreated cells (Figures 3 and 4). The MMP-9 mRNA reduced from 1.241 ± 0.023 in the untreated cells to 0.566 ± 0.016 in N-cadherin RNAi cells, and the protein reduced from 0.628 ± 0.010 to 0.282 ± 0.010. However, it seems that N-cadherin shRNA did not affect the levels of E-cadherin mRNA and protein (Figures 3 and 4).

Knocking down N-cadherin decreased invasiveness of EC9706 in vitro

N-cadherin level was elevated with the malignancy in the ESCC tissues in a previous experiment. When N-cadherin was down-regulated, the migration of the cells was also reduced, compared with the untreated cells. Compared with the untreated EC9706 cells (Figure 5A) and the cells with control vector (Figure 5B), the N-cadherin negative-cells (Figure 5C) migration across the membranes decreased dramatically.

DISCUSSION

Cadherins are a class of type-1 transmembrane proteins. They are calcium dependent cell-cell adhesion glycoproteins. They play important roles in tissue formation and maintenance during embryonic development, and in the induction and maintenance of normal architecture and function in adult tissues. The most researched proteins of the cadherin family are E-cadherin and N-cadherin[8].

In the present research with ESCC, we found down-regulation of E-cadherin and increased N-cadherin. Normal E-cadherin expression contributes to the maintenance of epithelial integrity and polarized function[9,10]. Mutations in E-cadherin gene are correlated with gastric, breast, colorectal, thyroid and ovarian

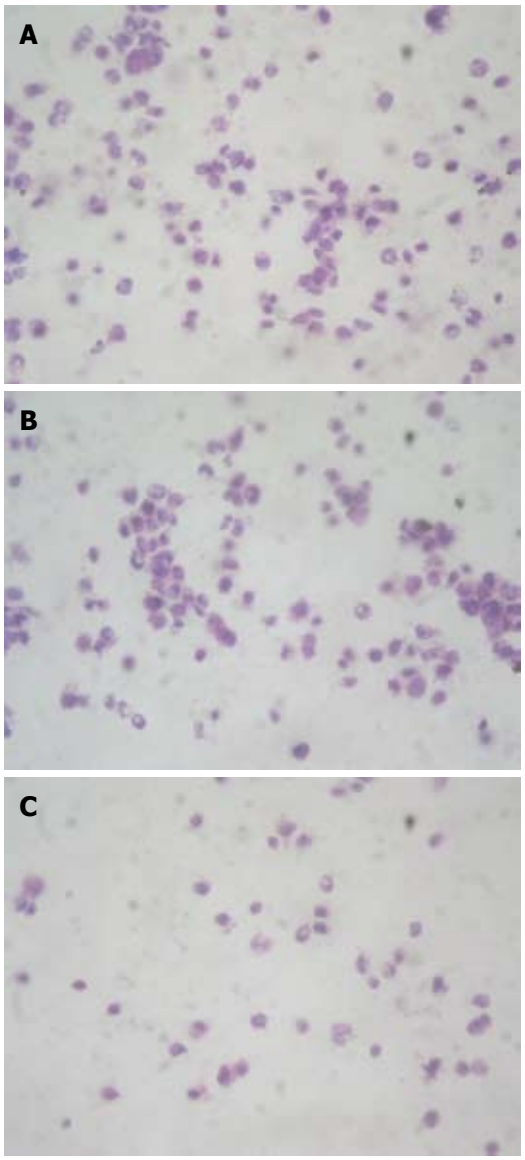


Figure 5 Migration assay in transwell chamber. A: Untreated EC9706 cells; B: EC9706 cells with control vector; C: EC9706 cells with N-cadherin RNAi. They were 123.40 ± 8.234, 126.00 ± 10.295 and 49.60 ± 6.804, respectively, from A to C. $P < 0.05$. (HE, × 200).

cancer[11,12]. Much lower levels were found to be present in the poorly differentiated lung cancer, indicating the worse prognosis[13]. Unlike E-cadherin, which is inversely correlated with invasiveness, N-cadherin may promote motility and invasion in carcinoma cells. N-cadherin has been shown to enhance cell migration during epithelial-mesenchymal transformation[14]. Aberrant N-cadherin expression was also found in breast carcinoma cells and prostate carcinoma cells[3,4]. In epithelial carcinoma, E-cadherin is down-regulated in most cases, sometimes accompanied by the up-regulation of another cadherin, for example, N-cadherin[15,16]. For the present research with ESCC, less E-cadherin and more N-cadherin were expressed in the ESCC tissue with deep invasion, poor differentiation and lymph node metastasis than with superfacial invasion, well differentiated and negative metastasis tissues. The N-cadherin expression was increased in the advanced ESCC tissues where E-cadherin was down-regulated, suggesting that they undergo a switch from E- to N-cadherin expression. The shift in expression from E- to N-cadherin and their mutually exclusive expression pattern in invasive tumor cell lines strongly reflect that the dedifferentiation from an epithelial to a mesenchymal phenotype was often associated with an increased invasive state[17]. The exact underlying mechanism has not been clear, but in many carcinomas, this "cadherin switch" was observed, especially in those where mild and non-progressive cells transformed into a more invasive phenotype[3,18,19]. Ras, Src, Rho, PI3K and Wnt signaling pathways were supposed to be involved in this switch[20-22].

We have proved that reduced E-cadherin and increased N-cadherin were present in advanced ESCC, and the following RNAi-mediated N-cadherin silence in EC9706 cell line further disclosed the correlation of E-cadherin and N-cadherin with ESCC progression. The down-regulation of N-cadherin did not change the expression of E-cadherin mRNA and its product. While N-cadherin and MMP-9 were reduced significantly in transcription level and translation level, less cells demonstrated invasiveness.

Local tumor invasion is characterized by at least two changes of function by the cancer cells. Firstly, these cells express higher levels of membrane-type and secreted proteolytic enzymes (e.g. the MMPs) in comparison with their normal epithelioid counterparts. Their contribution to invasion ranges from breakdown of the extracellular matrix, over-release of pro-invasive factors, to cleavage of cell-cell adhesion molecules[23]. Secondly, cancer cells are more motile than normal epithelial cells. Local tumor invasion is also made possible by disruption of epithelial cell junctions. E-cadherin, a part of the adherens junctions, plays an important role in maintaining the epithelioid cell organization and in preventing invasion[24]. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis[5]. Forced expression of N-cadherin in well-differentiated breast cell lines did not change their E-cadherin expression as indicated, but stimulated marked increases in cell migration and invasion[25]. The ability of N-cadherin-expressing EC9706 cells to adhere to N-cadherin-expressing endothelial sheets may facilitate their transit through the vasculature and improve their ability to form metastasis. The present results also confirmed that the N-cadherin expression down-regulation did not affect E-cadherin mRNA and protein levels, but the invasiveness of all the EC9706 cells was weakened as compared with the untreated EC9706 cells. It might be postulated that N-cadherin, rather than E-cadherin, plays an important role in the cancer progression and metastasis.

Less invasiveness was shown in the N-cadherin-negative tumor cells. Therefore, knocking down N-cadherin can weaken the aggressiveness of cancer cells, by the mechanism involving more than a change in cellular adhesion. MMPs were thought to predominantly degrade structural components of the extracellular matrix

(ECM), thereby facilitating cell migration. In addition to cleaving structural ECM components, collagen type Ⅳ and the cell-adhesive molecules are also MMP substrates, increasing the invasive behavior of cells. Type I collagen is the predominant constituent of the perivascular ECM, and as mentioned previously, a variety of MMPs are capable of degrading collagen, including interstitial collagenase and neutrophil collagenase. The metastatic cells thus use these proteases to invade basement membrane and its underlying connective tissues and then subsequently through the basement membrane of the small blood vessels and lymphatics[26-30]. With RT-PCR and Western blotting, the level of MMP-9 mRNA and protein in the RNAi-mediated N-cadherin silencing EC9706 cells was found to be reduced as compared with untreated cells. It was supposed that the lower invasiveness of EC9706 cells was the consequence of down-regulation of N-cadherin, by the mechanism of decreasing the MMP-9 expression. The MMP-9 reduction resulted in less degradation of ECM, and thereby, the cancer cells were less aggressive. But which signaling pathway was involved in the N-cadherin to MMP-9 should be studied in the future researches.

In this study, decreased E-cadherin expression and increased N-cadherin expression were found more frequently in advanced ESCC than in low grade ESCC, confirming that the down-regulation of E-cadherin expression and up-regulation of N-cadherin expression were closely associated with the infiltration, invasion and metastasis of ESCC. *In vitro* experiments also demonstrated that even the E-cadherin mRNA and protein did not change much in the N-cadherin knocking down EC9706 cells, but the invasiveness of cancer cells was dramatically reduced. The decreased MMP-9 mRNA and its product were observed in the N-cadherin-negative cells, the majority of which lost their ability of migration *in vitro*. It was supposed that the N-cadherin played a role of facilitating cell invasion by MMP-9. In summary, our data suggest that N-cadherin is an important factor in the invasiveness of esophageal squamous cell carcinoma and N-cadherin may serve as a potential molecular target for biotherapy of esophageal squamous cell carcinoma.

COMMENTS

Background

Among the members of cadherin family, E-cadherin and N-cadherin have been extensively studied for their biological activities and associations with cancer cell invasion. Latest research on prostate cancer and breast cancer has proved that the up-regulated N-cadherin plays even more important roles in cell progression and metastasis.

Research frontiers

The shift in expression from E- to N-cadherin and their mutually exclusive expression pattern in invasive tumors strongly reflects dedifferentiation from an epithelial to a mesenchymal phenotype, often associated with an increased invasive state. This "cadherin switch" has been observed, especially in those where mild and non-progressive cells transformed into more invasive phenotypes. Therefore, many studies have focused on the exact underlying mechanism involved in this cadherin switch.

Innovations and breakthroughs

In the current study, the expression of N-cadherin and E-cadherin was first examined in esophageal squamous cell carcinoma (ESCC) specimens and the results revealed that increased expression of N-cadherin and decreased expression of E-cadherin were related to invasion, differentiation, and lymph node metastasis, the roles of N-cadherin in the invasiveness of ESCC were first investigated in the EC9706 cell line transfected by retroviral-mediated N-cadherin RNAi and the results revealed that N-cadherin knock-down significantly decreased the invasiveness of EC9706 cells.

Application

This study has indicated that N-cadherin is an important factor in the invasiveness of ESCC and N-cadherin may serve as a potential molecular target for biotherapy of ESCC.

Terminology

Cadherins are a class of type-1 transmembrane proteins. They are calcium-dependent cell-cell adhesion glycoproteins. They play important roles in tissue formation and maintenance during embryonic development, and in the induction and maintenance of normal architecture and function in adult tissues.

Peer review

The authors examined the expression pattern of N-cadherin and E-cadherin. They demonstrated that N-cadherin is an important factor in the invasiveness of ESCC and it may serve as a potential molecular target for biotherapy of ESCC.

REFERENCES

1 **Usami Y**, Satake S, Nakayama F, Matsumoto M, Ohnuma K, Komori T, Semba S, Ito A, Yokozaki H. Snail-associated epithelial-mesenchymal transition promotes oesophageal squamous cell carcinoma motility and progression. *J Pathol* 2008; **215**: 330-339

2 **Nair KS**, Naidoo R, Chetty R. Microsatellite analysis of the APC gene and immunoexpression of E-cadherin, catenin, and tubulin in esophageal squamous cell carcinoma. *Hum Pathol* 2006; **37**: 125-134

3 **Jaggi M**, Nazemi T, Abrahams NA, Baker JJ, Galich A, Smith LM, Balaji KC. N-cadherin switching occurs in high Gleason grade prostate cancer. *Prostate* 2006; **66**: 193-199

4 **Nagi C**, Guttman M, Jaffer S, Qiao R, Keren R, Triana A, Li M, Godbold J, Bleiweiss IJ, Hazan RB. N-cadherin expression in breast cancer: correlation with an aggressive histologic variant--invasive micropapillary carcinoma. *Breast Cancer Res Treat* 2005; **94**: 225-235

5 **Derycke L**, Morbidelli L, Ziche M, De Wever O, Bracke M, Van Aken E. Soluble N-cadherin fragment promotes angiogenesis. *Clin Exp Metastasis* 2006; **23**: 187-201

6 **Xu JZ**, Yang WT. The criteria for judging the results of immunohistochemical method. *Zhonghua Zhongliu Zazhi* 1996; **6**: 229-231

7 **Kwon YJ**, Hung G, Anderson WF, Peng CA, Yu H. Determination of infectious retrovirus concentration from colony-forming assay with quantitative analysis. *J Virol* 2003; **77**: 5712-5720

8 **Christiansen JJ**, Rajasekaran AK. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. *Cancer Res* 2006; **66**: 8319-8326

9 **Larue L**, Ohsugi M, Hirchenhain J, Kemler R. E-cadherin null mutant embryos fail to form a trophectoderm epithelium. *Proc Natl Acad Sci USA* 1994; **91**: 8263-8267

10 **Nelson WJ**, Shore EM, Wang AZ, Hammerton RW. Identification of a membrane-cytoskeletal complex containing the cell adhesion molecule uvomorulin (E-cadherin), ankyrin, and fodrin in Madin-Darby canine kidney epithelial cells. *J Cell Biol* 1990; **110**: 349-357

11 **Bosch FX**, Andl C, Abel U, Kartenbeck J. E-cadherin is a selective and strongly dominant prognostic factor in squamous cell carcinoma: a comparison of E-cadherin with desmosomal components. *Int J Cancer* 2005; **114**: 779-790

12 **Bellovin DI**, Bates RC, Muzikansky A, Rimm DL, Mercurio AM. Altered localization of p120 catenin during epithelial to mesenchymal transition of colon carcinoma is prognostic for aggressive disease. *Cancer Res* 2005; **65**: 10938-10945

13 **Moersig W**, Horn S, Hilker M, Mayer E, Oelert H. Transfection of E-cadherin cDNA in human lung tumor

cells reduces invasive potential of tumors. *Thorac Cardiovasc Surg* 2002; **50**: 45-48
14 **Hazan RB**, Kang L, Whooley BP, Borgen PI. N-cadherin promotes adhesion between invasive breast cancer cells and the stroma. *Cell Adhes Commun* 1997; **4**: 399-411
15 **Hazan RB**, Phillips GR, Qiao RF, Norton L, Aaronson SA. Exogenous expression of N-cadherin in breast cancer cells induces cell migration, invasion, and metastasis. *J Cell Biol* 2000; **148**: 779-790
16 **Utsuki S**, Oka H, Sato Y, Tsutiya B, Kondo K, Tanizaki Y, Tanaka S, Fujii K. E, N-cadherins and beta-catenin expression in medulloblastoma and atypical teratoid/rhabdoid tumor. *Neurol Med Chir* (Tokyo) 2004; **44**: 402-406; discussion 407
17 **Hazan RB**, Qiao R, Keren R, Badano I, Suyama K. Cadherin switch in tumor progression. *Ann N Y Acad Sci* 2004; **1014**: 155-163
18 **Gravdal K**, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer. *Clin Cancer Res* 2007; **13**: 7003-7011
19 **Hotz B**, Arndt M, Dullat S, Bhargava S, Buhr HJ, Hotz HG. Epithelial to mesenchymal transition: expression of the regulators snail, slug, and twist in pancreatic cancer. *Clin Cancer Res* 2007; **13**: 4769-4776
20 **Nawshad A**, Lagamba D, Polad A, Hay ED. Transforming growth factor-beta signaling during epithelial-mesenchymal transformation: implications for embryogenesis and tumor metastasis. *Cells Tissues Organs* 2005; **179**: 11-23
21 **Alexander NR**, Tran NL, Rekapally H, Summers CE, Glackin C, Heimark RL. N-cadherin gene expression in prostate carcinoma is modulated by integrin-dependent nuclear translocation of Twist1. *Cancer Res* 2006; **66**: 3365-3369
22 **Gotzmann J**, Mikula M, Eger A, Schulte-Hermann R, Foisner R, Beug H, Mikulits W. Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. *Mutat Res* 2004; **566**: 9-20
23 **Stetler-Stevenson WG**, Yu AE. Proteases in invasion: matrix metalloproteinases. *Semin Cancer Biol* 2001; **11**: 143-152
24 **Behrens J**, Mareel MM, Van Roy FM, Birchmeier W. Dissecting tumor cell invasion: epithelial cells acquire invasive properties after the loss of uvomorulin-mediated cell-cell adhesion. *J Cell Biol* 1989; **108**: 2435-2447
25 **Nieman MT**, Prudoff RS, Johnson KR, Wheelock MJ. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. *J Cell Biol* 1999; **147**: 631-644
26 **Curran S**, Murray GI. Matrix metalloproteinases in tumour invasion and metastasis. *J Pathol* 1999; **189**: 300-308
27 **Rajapakse N**, Kim MM, Mendis E, Huang R, Kim SK. Carboxylated chitooligosaccharides (CCOS) inhibit MMP-9 expression in human fibrosarcoma cells via down-regulation of AP-1. *Biochim Biophys Acta* 2006; **1760**: 1780-1788
28 **Egeblad M**, Werb Z. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2002; **2**: 161-174
29 **Ortega N**, Behonick DJ, Werb Z. Matrix remodeling during endochondral ossification. *Trends Cell Biol* 2004; **14**: 86-93
30 **He Y**, Liu XD, Chen ZY, Zhu J, Xiong Y, Li K, Dong JH, Li X. Interaction between cancer cells and stromal fibroblasts is required for activation of the uPAR-uPA-MMP-2 cascade in pancreatic cancer metastasis. *Clin Cancer Res* 2007; **13**: 3115-3124

S- Editor Li LF **L-Editor** Ma JY **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.705

World J Gastroenterol 2009 February 14; 15(6): 705-712
World Journal of Gastroenterology ISSN 1007-9327

Epigenetics of proteasome inhibition in the liver of rats fed ethanol chronically

Joan Oliva, Jennifer Dedes, Jun Li, Samuel W French, Fawzia Bardag-Gorce

Joan Oliva, Jennifer Dedes, Jun Li, Samuel W French, Fawzia Bardag-Gorce, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90502, United States

Author contributions: Oliva J, Dedes J, and Li J performed the real-time PCR experiments, the microarray analysis, and the animal care and treatments, respectively; French SW provided the resources and facilities; Bardag-Gorce F designed the study, performed the nuclei and histone isolation, proteasome activity measurement, Western blot analysis, and wrote the manuscript.
Supported by The NIH/NIAAA grant 8116 and Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center seed grant 513217-00-00
Correspondence to: Fawzia Bardag-Gorce, PhD, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 W. Carson St. Torrance, CA 90502, United States. fgorce@labiomed.org
Telephone: +1-310-2221846 Fax: +1-310-2223614
Received: November 7, 2008 Revised: January 5, 2009
Accepted: January 12, 2009
Published online: February 14, 2009

Abstract

AIM: To examine the effects of ethanol-induced proteasome inhibition, and the effects of proteasome inhibition in the regulation of epigenetic mechanisms.

METHODS: Rats were fed ethanol for 1 mo using the Tsukamoto-French model and were compared to rats given the proteasome inhibitor PS-341 (Bortezomib, Velcade™) by intraperitoneal injection. Microarray analysis and real time PCR were performed and proteasome activity assays and Western blot analysis were performed using isolated nuclei.

RESULTS: Chronic ethanol feeding caused a significant inhibition of the ubiquitin proteasome pathway in the nucleus, which led to changes in the turnover of transcriptional factors, histone-modifying enzymes, and, therefore, affected epigenetic mechanisms. Chronic ethanol feeding was related to an increase in histone acetylation, and it is hypothesized that the proteasome proteolytic activity regulated histone modifications by controlling the stability of histone modifying enzymes, and, therefore, regulated the chromatin structure, allowing easy access to chromatin by RNA polymerase, and, thus, proper gene expression. Proteasome inhibition by PS-341 increased histone acetylation similar to chronic ethanol feeding. In addition, proteasome inhibition caused dramatic changes in hepatic remethylation reactions as there was a significant decrease in the enzymes responsible for the regeneration of S-adenosylmethionine, and, in particular, a significant decrease in the betaine-homocysteine methyltransferase enzyme. This suggested that hypomethylation was associated with proteasome inhibition, as indicated by the decrease in histone methylation.

CONCLUSION: The role of proteasome inhibition in regulating epigenetic mechanisms, and its link to liver injury in alcoholic liver disease, is thus a promising approach to study liver injury due to chronic ethanol consumption.

Key words: Alcohol liver injury; Betaine; Epigenetic mechanisms; Homocysteine methyltransferase; Proteasome inhibition; S-adenosylmethionine

Peer reviewer: Natalia A Osna, MD, PhD, Liver Study Unit, Research Service (151), VA Medical Center, 4101 Woolworth Avenue, Omaha NE 68105, United States

Oliva J, Dedes J, Li J, French SW, Bardag-Gorce F. Epigenetics of proteasome inhibition in the liver of rats fed ethanol chronically. *World J Gastroenterol* 2009; 15(6): 705-712 Available from: URL: http://www.wjgnet.com/1007-9327/15/705.asp DOI: http://dx.doi.org/10.3748/wjg.15.705

INTRODUCTION

A growing body of evidence indicates that specific histone modifications and modifying enzymes play essential roles in both global and tissue-specific chromatin organization[1]. Histone modifications, such as acetylation and methylation, play important roles in the regulation of gene expression, and impact many fundamental biological processes (i.e. in the cell cycle and cell proliferation). These modifications represent the inheritable epigenetic memory, which is transmitted with high fidelity to future cell generations.

Acetylation of histones is a major factor in

the regulation of chromatin remodeling and gene transcription. A sophisticated orchestra of proteins, such as histone acetyltransferases [HATs, gene activation (acetylation)], and histone deacetylases (HDACs), are required to regulate epigenetic mechanisms[2]. Acetylation of histone and non-histone protein is thus emerging as a central process in transcriptional activation, since nuclear HATs act as transcription co-activators, which have been shown to acetylate different transcriptions (i.e. p53, β-catenin, MyoD, SREBP-1)[3].

Alcohol consumption affects epigenetic mechanisms, and causes an increase in histone lysine acetylation, which has been associated with an increase in gene expression that may contribute to uncontrolled transcription[3]. These modifications are the results of changes in the activities of specific modifying enzymes that play essential roles in chromatin structure and specific gene expression. It has previously been shown that chronic ethanol feeding affects epigenetic mechanisms by causing an increase in the stability of histone-modifying enzymes, and thus, in histone modifications[4,5]. However, little is known about the mechanisms that control the life span of histone-modifying enzymes. For example, the consequence of proteasome inhibition in the nucleus, and its effects on epigenetic mechanisms, have not yet been investigated, and there is very little evidence on proteasome involvement in the turnover of the transcriptional factor and histone-modifying enzymes that regulate epigenetic mechanisms.

Our hypothesis is that proteasome inhibition, induced by ethanol feeding, is associated with histone modification, and is involved in the regulation of histone-modifying enzymes, such as the HAT p300.

The present study demonstrates the role of proteasome activity in epigenetic mechanisms, which significantly contribute to liver injury due to chronic ethanol feeding. Proteasome proteolytic activity regulates histone modifications by regulating the recruitment and stability of histone-modifying enzymes in the nucleus, and, therefore, regulates the chromatin structure, allowing easy access to chromatin by RNA polymerase and enhanced gene expression. The proteasome activity is also believed[6] to be critical for the expression of certain genes, such as those of the enzymes responsible for hepatic transmethylation reactions. In this study, microarray analysis showed up-regulation and down-regulation of a large number of genes, indicating that proteasome activity is essential for up-regulation, as well as down-regulation of specific gene expression. Proteasome inhibition caused a decrease in the gene expression of several enzymes involved in methionine metabolism, particularly betaine-homocysteine methyltransferase (BHMT), which was significantly down-regulated when the proteasome was inhibited[7]. These results indicated that DNA and histone methylation, which play important roles in the regulation of gene silencing, may be affected by proteasome inhibition, and, therefore, may impact many fundamental biological processes.

Previous reports have shown that gene expression changes were numerous at high levels of ethanol, when compared to their pair-fed controls. 1300 genes were changed[8-10]. Similarly, a preliminary microarray analysis of the livers of rats given the proteasome inhibitor PS-341, has shown marked changes in gene expression. Thus, a question arose: which mechanism is involved in this large number of gene expression changes? We believe that this mechanism is epigenetic in nature.

We believe that these modifications in gene expression are the result of a decrease in the activity of the proteasome in the nucleus, which would, for instance, increase the p300 HAT level and activity. Since p300 is responsible for a broad range of gene regulation, the activation of p300 acetylation in histones will increase and activate the expression of a large number of genes in a nonspecific and reversible manner.

The role of the proteasome in regulating histone methylation is also critical because the expression of several genes was changed when the proteasome was inhibited. We have previously demonstrated that proteasome inhibition causes a downregulation in the expression of several genes[8], particularly the gene involved in the remethylation pathway. The study of this remethylation, particularly, the reactional mechanism that regenerates S-adenosylmethionine (SAMe), the major methyl donor, is essential because this is the system that transfers the methyl group to DNA, histones and non-histone proteins *via* the methyltransferases, such as glycine N-methyltransferase (GNMT).

In this study, BHMT gene expression was markedly decreased by proteasome inhibition. BHMT is an essential enzyme in the remethylation pathway, and is involved in the recovery of SAMe. Betaine, a choline derivative which has been used clinically to treat, with some success, patients with methylenetetrahydrofolate reductase deficiency[11,12], acts as a substrate for BHMT, and serves as an alternative methyl donor for remethylation of homocysteine in the liver and kidney[13]. Therefore, betaine supplementation may cover the down-regulation of gene expression induced by proteasome inhibition, and correct the deregulation of hepatic transmethylation reactions due to the proteasome inhibition-induced decrease in BHMT activity.

MATERIALS AND METHODS

Animals

Male Wistar rats (Harleco, Hollister, CA, USA), weighing 250-300 g, were fed ethanol using the Tsukamoto-French intragastric model[14,15]. PS-341 was administered intraperitoneally, 24 h before sacrifice[16,17]. The rats were maintained according to the Guidelines of Animal Care, as described by the National Academy of Sciences and published by the National Institute of Health (1996).

Nuclei isolation

Histones were isolated from the nuclei, according to the method of Umlauf *et al*[18]. Liver tissues, frozen in isopentane and immersed in liquid nitrogen, were homogenized in a Dounce homogenizer with 10 strokes.

Homogenates were centrifuged for 10 min at 6000 × *g*. Pellets were resuspended, placed on ice for 10 min, and then centrifuged for 20 min at 9000 × *g* on a sucrose cushion. The pellets contained the nuclei. Histones were isolated from the nuclei, according to the method of Shechter *et al*[19]. Isolated nuclei were mixed with 0.2 mol/L H_2SO_4, and incubated on a rotator for 30 min at 4℃. Samples were spun in a microcentrifuge at 16 000 × *g*, for 10 min. Dissolved histones in the supernatant were precipitated with 33% TCA. After acetone wash, histones were dissolved in an appropriate buffer, and further analyses were carried out.

Proteasome chymotrypsin-like activity assay

Nuclei were isolated as mentioned above, and 1 μg of total protein was used. The reaction mixture contained 50 mmol/L Tris–HCl pH 8, 1 mmol/L DTT, and 40 μmol/L Suc-LLVY-AMC substrate for chymotrypsin-like activity. The mixture was incubated for 30 min at 37℃, and the reaction was then stopped by adding 100 μmol/L monochloroacetate and 30 mmol/L sodium acetate (pH 4.3). Fluorescence was determined by measuring the release of AMC (λ excitation: 370 nm, λ emission: 430 nm) using a Perkin Elmer LS 30 spectrofluorometer.

Western blot analysis

Proteins (50 μg) from isolated nuclei or isolated histones were separated by SDS-PAGE, and transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA) for 1 h in 25 mmol/L Tris-HCl (pH 8.3), 192 mmol/L glycine and 20% methanol. The membranes were stained using primary antibodies to the antigens. The appropriate species anti-polyclonal and anti-monoclonal HRP-conjugated antibodies were used as secondary antibodies. The membranes were subjected to chemiluminescence detection using luminal, according to the manufacturer's instructions (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

Microarray analysis

Fast frozen rat liver tissue was subjected to microarray analysis. Total liver RNAs were extracted with UltraspecTM RNA Isolation Systemic (Biotecx Laboratories, Houston, TX, USA), and cleaned with Rneasy columns (Qiagen, Valencia, CA, USA). Five micrograms of total RNA were used for preparing biotin-labeled cRNA. Labeled and fragmented cRNA was subsequently hybridized to Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA, USA). Labeling, hybridization, image scanning, and initial data analysis were performed at the Microarray Core at Los Angeles Biomedical Research Institute. Sample preparation and loading, hybridization, staining, and microarray data analysis were then performed[8].

Quantitative RT-PCR

Total liver RNAs were extracted with Trizol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA, USA). Synthesis of cDNAs was performed with 5 μg total RNA, and 50 ng random hexamer primers using SuperScriptIII RNase H-Reverse Transcriptase (Invitrogen). PCR primers were designed using the Primer Express software (Applied Biosystems, Foster City, CA, USA). The primers for rat p300 are shown in Table 1.

Table 1 List of primer sequences used in RT-PCR

p300	XM_576312	Forward	5GAGGTCACTGTTCGGGTTGTTC
p300	XM_576312	Reverse	5TGGTTCGATATGGAAAAGATTCTG
BHMT	NM_030850	Forward	5GGGCAGAAGGTCAATGAAGCT
BHMT	NM_030850	Reverse	5ACCAATGCATCCCCTTCGT

Quantitative PCR was achieved using the SYBR Green JumpStartTM (Applied Biosystems). Thermal cycling consisted of an initial step at 50℃ for 2 min, followed by a denaturation step at 95℃ for 10 min, and then 40 cycles at 95℃ for 15 s and 60℃ for 1 min. A single PCR product was confirmed with the heat dissociation protocol at the end of the PCR cycles. Each data point was repeated three times.

Sense and anti-sense: quantitative PCR was achieved using the SYBR Green JumpStart™ Tag ReadyMix (Sigma, St. Louis, MO, USA) on an ABI PRISM 7700 Sequence Detector System (Applied Biosystems). The thermal cycling consisted of an initial step at 50℃ for 2 min, followed by a denaturation step at 95℃ for 10 min, then 40 cycles at 95℃ for 15 s and 60℃ for 1 min. A single PCR product was confirmed with the heat dissociation protocol at the end of the PCR cycles. Quantitative values were obtained from the threshold PCR cycle number (Ct) at which point the increase in signal associated with an exponential growth for the PCR product was detected. The target mRNA abundance in each sample was normalized to its 18S level as $\Delta Ct = Ct_{\text{target gene}} - Ct_{18S}$. For each target gene, the highest ΔCt was assigned as ΔCt_{max}.

Statistical analysis

Data were obtained from at least three animals in each group. Bars represent mean ± SE. *P* values were determined by one-way ANOVA and Student-Newman Keuls for multiple group comparisons (Sigma-Stat software, San Francisco, CA, USA). $P \leqslant 0.05$ was used to establish significant differences. Correlation of data was done by linear regression analysis using Pearson's period momentum method.

RESULTS

Microarray analysis of liver samples from rats fed ethanol showed that a large number of genes (about 1300) were up-regulated and down-regulated due to chronic ethanol feeding[8]. Microarray analyses of liver samples from rats given PS-341 (Bortezomib, Velcade®) also showed dramatic changes in gene expression (about 2082 genes changed) affecting several functional pathways (Figure 1).

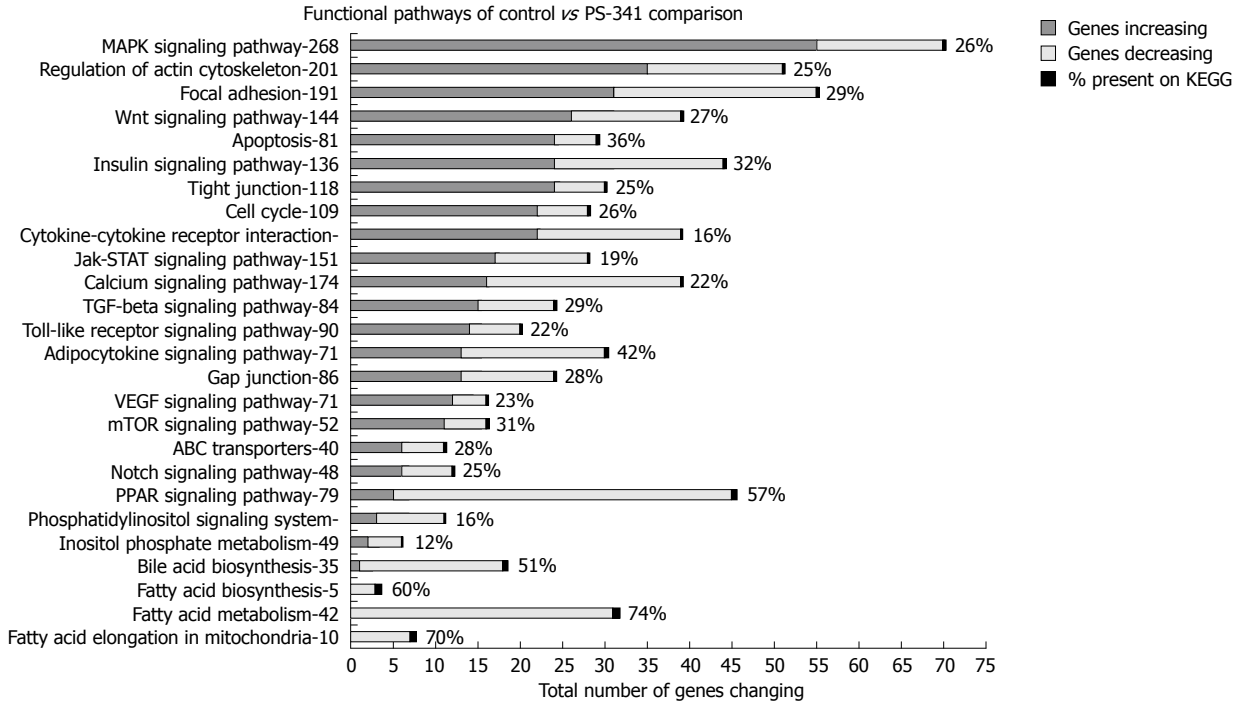


Figure 1 Kegg functional pathway changes in gene expression induced by proteasome inhibition. Both ethanol feeding and proteasome inhibition affected almost all pathways.

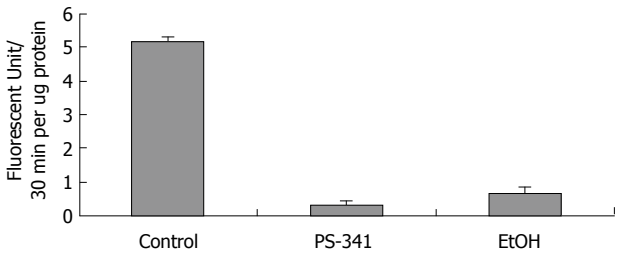


Figure 2 Nuclear proteasome chymotrypsin-like activity. 20S proteasome chymotrypsin-like activity was measured in isolated nuclei from the liver of rats fed ethanol chronically and from the liver of rats given PS-341.

The present study was based on the observation that the inhibition of proteasome, caused by chronic ethanol feeding, participated in the development of liver injury due to ethanol by altering the mechanisms through which normal epigenetic regulation occurs. The consequence of this was a marked change in the gene expression of several functional pathways in liver cells (Figure 1).

Data mining and gene specific pathway clustering showed that, similar to ethanol feeding, several transcriptional factors, such as cell cycle, histone modifying enzymes, and the remethylation pathway, were significantly changed by proteasome inhibition. Proteasome inhibition by PS-341 thus proved to be a powerful tool to investigate the role of proteasome activity in epigenetic mechanisms.

To verify the hypothesis that gene expression changes are regulated by nuclear proteasome activity, where inhibition is caused by chronic ethanol feeding[20], proteasome activity was measured in isolated nuclei from the liver of rats fed ethanol chronically, and from the liver of rats given PS-341. Figure 2 shows that chronic ethanol feeding caused a significant decrease in proteasome chymotrypsin-like activity in isolated liver nuclei.

To further investigate the role of proteasome activity in regulating epigenetic mechanisms, histone acetylation was analyzed in the liver of rats given PS-341, and compared to histone acetylation in the liver of rats fed ethanol chronically. Figure 3A shows that acetylated histone 3 lysine 9 (AcH3K9) was increased in the liver of rats fed ethanol, and that acetylated histone 3 lysine 27 (AcH3K27) was increased in the liver of rats given PS-341 (Figure 3B).

Increased acetylation was concomitant with an increase in the level of HAT p300, which was linked to significant proteasome inhibition shown in the liver of rats fed ethanol chronically (Figure 3D), and in the liver of rats given PS-341 (Figure 3C). P300 was increased in the ethanol-isolated nuclear extract, which confirmed a previous report[4]. It is now well established that ethanol feeding increases histone acetylation[4,5], which correlates with an increase in the acetyltransferase CBP/p300, and a decrease in Sirt1 activity[3,21]. Under our experimental conditions, Sirt1 gene expression and protein level were up-regulated[22], or showed no significant changes[4]. However, the activity of the enzyme may be decreased due to the low level of NAD+, a Sirt1 cofactor[23].

The increased level of histone acetylation substantiated the increase in p300 activity in the liver of rats fed ethanol. In addition, proteasome inhibition by PS-341 caused a significant increase in histone acetylation, which substantiated our hypothesis that

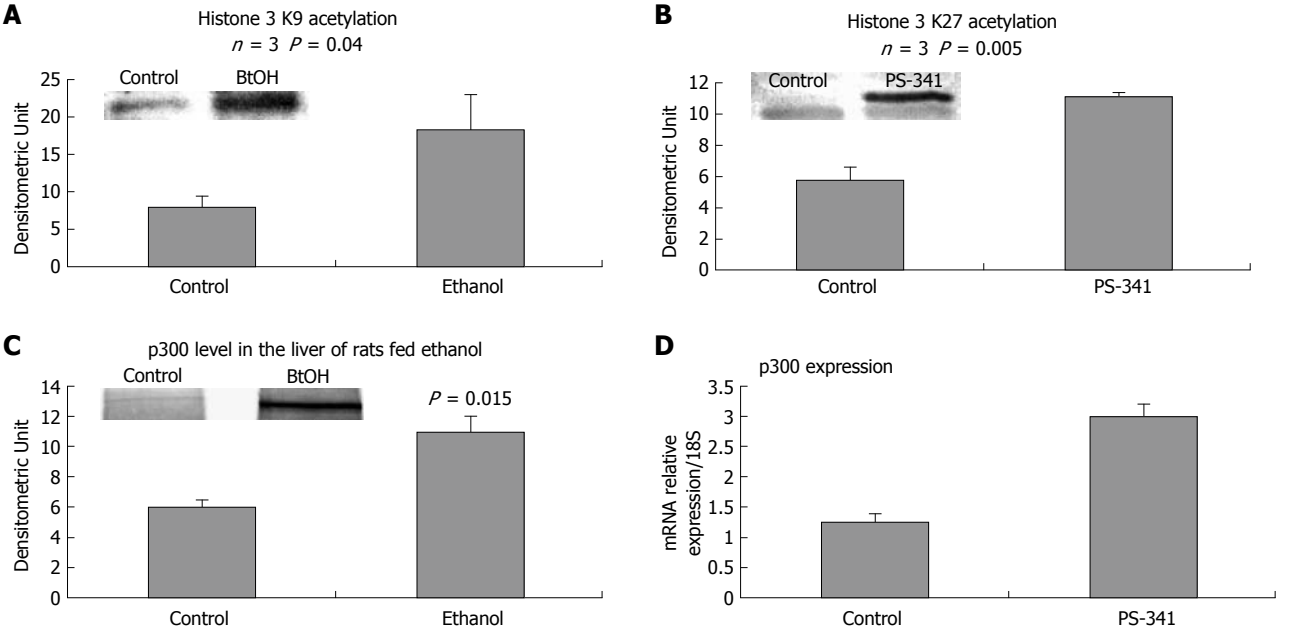


Figure 3 Role of proteasome inhibition in histone acetylation. A: Proteasome inhibition caused a significant increase in histone acetylation as shown in the liver nuclear extracts of rats fed ethanol and in the liver nuclear extracts of rats given PS-341(B). C: p300 protein level was increased in the nuclear extract from the livers of rats fed ethanol chronically, and in the liver nuclear extracts of rats given PS-341, as shown by real time PCR (D), $P = 0.003$. (mean ± SE, $n = 3$).

ethanol induced-histone acetylation is associated with proteasome inhibition. The level of p300 was increased in the nucleus when ethanol was fed chronically, and may also have accumulated due to proteasome proteolysis slowdown in the nucleus.

Proteasome activity has also been found to be involved in the regulation of remethylation enzyme gene expression, thus affecting DNA and histone methylation when it is inhibited. Microarray data mining showed a down-regulation in gene expression of the remethylation enzymes when the proteasome was inhibited (Figure 4A), especially BHMT, which reconstitutes methionine, the major element in the methylation pathway (Figure 4B). Mat1a, Adm (S-adenosylmethionine decarboxylase), and Ahcy (S-adenosylhomocysteine hydrolase) were also down-regulated by proteasome inhibition (Figure 4A), indicating the role of proteasome activity in the methionine-metabolizing enzymes system. Figure 4C shows that proteasome inhibition significantly decreased the protein level of BHMT in the liver of rats given PS-341, and, to a lower extent, in rats subjected to chronic ethanol feeding. These findings demonstrated the role of the proteasome in regulating gene expression in the remethylation pathway, and the role of proteasome activity in the cellular remethylation pathway. Western blot analysis showed a significant decrease in H3K9 dimethylation in the liver of rats given PS-341. A decrease in H3K9 dimethylation (Figure 4D) was also observed in ethanol-treated cells[24], suggesting similar effects of proteasome inhibition and ethanol feeding. These results showed for the first time the role of proteasome activity in regulating the mechanisms of cellular remethylation, and is a promising approach in chemotherapy regimens which use proteasome inhibitor as an anti tumor drug.

DISCUSSION

Several studies have shown that the inhibition of the ubiquitin-proteasome pathway is a pathobiological mechanism associated with the development of liver disease, especially alcoholic liver disease[25,26]. We believe that ethanol-induced inhibition of proteasome activity may play a significant role in the deregulation of epigenetic mechanisms and the mechanism related to liver injury in alcoholic liver disease (ALD).

Many cellular signaling pathways are controlled by selective proteolysis of key regulatory proteins via the ubiquitin-proteasome system[4,12,27]. Proteasome is involved in RNA polymerase Ⅱ degradation[28], which is a key step in controlling the transcriptional mechanism, preventing uncontrolled transcription that may occur when ethanol metabolism generates oxidative stress, which causes DNA damage[29]. Reports have shown that proteasome dysfunction leads to apoptotic death of hepatocytes and sensitization to tumor necrosis factor (TNF) cytotoxicity, leading to direct hepatic injury[25]. Although it is now evident that inhibition of cytoplasmic proteasome function is consistently shown in ALD models, the way in which proteasome dysfunction may enhance hepatotoxicity is not well defined. In addition, the effects of ethanol feeding on the activity of nuclear proteasome and the consequences of proteasome inhibition on changes in epigenetic mechanisms have not yet been demonstrated. Most importantly, our previous investigations have repeatedly shown that chronic ethanol feeding causes significant proteasome inhibition in the cell[30,31]. The ubiquitin-proteasome pathway is the cellular proteolytic pathway dedicated to controlling protein stability, and understanding the link between the ubiquitin-proteasome pathway and the histone-

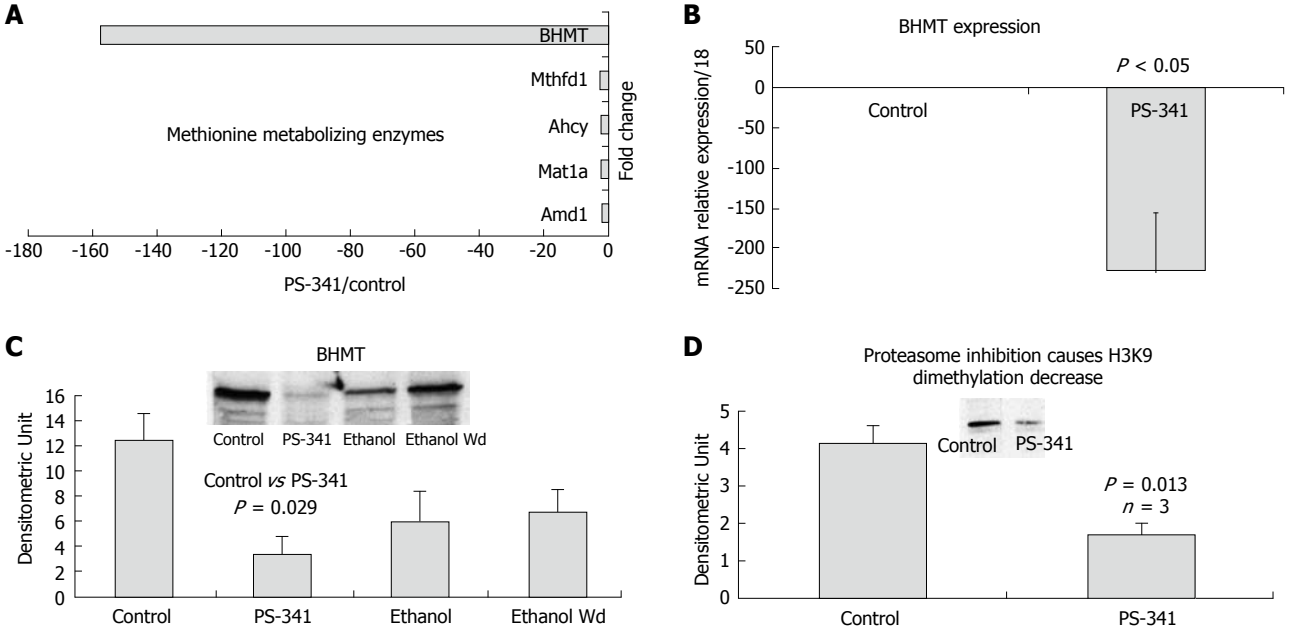


Figure 4 Role of proteasome inhibition in the remethylation pathway. A: Proteasome inhibition by PS-341 (PS) markedly decreased BHMT gene expression; B: Real time PCR analysis of BHMT expression; C: Western blot analysis of BHMT level in the liver of rats fed ethanol chronically and the liver of rats given PS-341. Note that the BHMT level was significantly reduced in the liver of rats treated with PS-341; D: Proteasome inhibition caused a decrease in histone methylation.

modifying machinery will define the link between proteasome proteolytic activity, epigenetic mechanisms, and the effect of toxic substances, such as ethanol and its metabolism generated end products, in the regulation and control of epigenetic mechanisms.

As predicted, our results showed an increase in acetylation of H3K9 in the liver nuclear extracts of rats fed ethanol chronically. This increase was associated with an increase in HAT p300, which confirmed a previous report[4]. In addition, p300 activation and histone acetylation were also obtained when proteasome activity was inhibited using PS-341, which supported the role of proteasome activity in regulating the stability of p300 in the nucleus and therefore supported the role of proteasome in regulating the acetylation mechanisms and thus gene expression. These results corroborate the findings of Marcu *et al*[32], which showed that p300 is a proteasome substrate, and that p300 is accumulated when the proteasome is inhibited.

The modification of histones mirrors the sophisticated protein machinery that controls gene expression and regulates transcription. Therefore, it would be naïve to suggest that, for instance, only H3K9 acetylation explained all the changes in the observed gene expression[11]. The balance between all histone lysine residue modifications accounted for the pattern of gene expression and further histone modifications are certainly involved in defining specific gene expression.

Histone methylation also plays a critical role in regulating gene expression and transcription. The cellular remethylation pathway is the major player in the methylation mechanism, because it produces the methyl donor SAMe. It is well known that chronic ethanol ingestion causes a serious deregulation of this pathway. Methionine adenosyltransferase 1-alpha, which is responsible for the conversion of methionine to SAMe, is decreased in the liver of rats fed ethanol[33]. Since SAMe is thus decreased, the level of DNA and protein methylation is decreased[34], and the level of homocysteine is increased[35]. The ratio SAMe/SAH (S-adenosylhomocysteine) is critical in the cell because it controls most of the methyltransferase activity. BHMT, which hydrolyzes betaine, helps remove SAH and homocysteine with subsequent regeneration of the methyl group and a reduction in the level of SAH. Our results show that BHMT was significantly down-regulated when the proteasome activity was inhibited, either by chronic ethanol feeding or by treatment with a proteasome inhibitor. In addition, there was a decrease in histone methylation, reflecting the role of proteasome activity in regulating the methylation mechanisms in the liver cell. Proteasome inhibition plays a critical role in regulating the gene expression of key enzymes in the remethylation pathway, such as BHMT.

COMMENTS

Background

Alcohol ingestion causes alterations in several cellular mechanisms, and leads to inflammation, apoptosis, and fibrosis. These phenomena are associated with significant changes in epigenetic mechanisms and with a subsequent liver cell memory. The ubiquitin-proteasome pathway is a vital cellular pathway which undergoes dysfunction due to chronic ethanol consumption.

Research frontiers

Although inhibition of proteasome function has been widely reported in models of alcoholic liver disease (ALD), why proteasome dysfunction may enhance hepatotoxicity is not well defined. In addition, there is no evidence of the effect of ethanol feeding on the activity of nuclear proteasome and the consequences of proteasome inhibition in epigenetic mechanisms and DNA repair.

Innovations and breakthroughs

The present study focused on the role of proteasome activity in gene expression and the effects of proteasome inhibition in changing epigenetic mechanisms.

The model used to study the consequences of proteasome inhibition due to chronic intragastric tube ethanol administration was proteasome inhibition by PS-341, a dipeptide boronic acid currently used in clinical trials as an anti tumor drug, and is associated with profound side effects. Inhibition of proteasome activity occurred in the nucleus of liver cells taken from rats fed ethanol chronically and from rats given PS-341. This inhibition caused changes in the turnover of transcriptional factors, histone modifying enzymes, and, therefore, affected epigenetic mechanisms. Histone acetylation was increased following both treatments and gene expression was changed. Identification of DNA and histone modifications was critical in regulating gene expression, especially genes involved in the cell cycle and apoptosis, and those involved in the metabolism of ethanol. In addition, proteasome inhibition has been shown to significantly affect the hepatic remethylation pathway. In particular, proteasome inhibition caused a decrease in gene expression of the enzyme betaine-homocysteine methyltransferase (BHMT), which is involved in the recovery of s-adenosylmethionine (SAMe). Histone methylation was also decreased when the proteasome was inhibited suggesting that hypomethylation was associated with the decrease in proteasome activity.

Applications

The phenomenon of hypomethylation is currently corrected by diet supplementation with a methyl donor (betaine) to redirect methionine/homocysteine metabolism toward recovery of methionine, and SAMe regeneration.

Terminology

Chronic ethanol feeding causes proteasome inhibition, which leads to cellular dysfunction including the accumulation of damaged proteins which form Mallory bodies in the liver of severe alcoholic patients, immune response deficiency, cell cycle deregulation and incorrect gene expression. The mechanism of incorrect gene expression was the focus of this study, particularly the consequences of ethanol induced-proteasome inhibition in the nucleus and the role played by proteasome in regulating epigenetic mechanisms.

Peer review

The impact of this study is great because when epigenetic mechanisms associated with proteasome inhibition are fully identified, a therapeutic approach will be initiated to counteract the ethanol induced-epigenetic changes, and prevent any cellular memory for future cell generations.

REFERENCES

1 **Lin W**, Dent SY. Functions of histone-modifying enzymes in development. *Curr Opin Genet Dev* 2006; **16**: 137-142
2 **Bronner C**, Chataigneau T, Schini-Kerth VB, Landry Y. The "Epigenetic Code Replication Machinery", ECREM: a promising drugable target of the epigenetic cell memory. *Curr Med Chem* 2007; **14**: 2629-2641
3 **You M**, Liang X, Ajmo JM, Ness GC. Involvement of mammalian sirtuin 1 in the action of ethanol in the liver. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G892-G898
4 **Bardag-Gorce F**, French BA, Joyce M, Baires M, Montgomery RO, Li J, French S. Histone acetyltransferase p300 modulates gene expression in an epigenetic manner at high blood alcohol levels. *Exp Mol Pathol* 2007; **82**: 197-202
5 **Kim JS**, Shukla SD. Acute in vivo effect of ethanol (binge drinking) on histone H3 modifications in rat tissues. *Alcohol Alcohol* 2006; **41**: 126-132
6 **Kinyamu HK**, Archer TK. Proteasome activity modulates chromatin modifications and RNA polymerase II phosphorylation to enhance glucocorticoid receptor-mediated transcription. *Mol Cell Biol* 2007; **27**: 4891-4904
7 **Dedes J**, Li J, Bardag-Gorce F. Chromatin remodeling is regulated by the ubiquitin proteasome pathway. *FASEB J* 2008; **22**: lb194
8 **Bardag-Gorce F**, French BA, Dedes J, Li J, French SW. Gene expression patterns of the liver in response to alcohol: in vivo and in vitro models compared. *Exp Mol Pathol* 2006; **80**: 241-251
9 **French BA**, Dedes J, Bardag-Gorce F, Li J, Wilson L, Fu P, Nan L, French SW. Microarray analysis of gene expression in the liver during the urinary ethanol cycle in rats fed ethanol intragastrically at a constant rate. *Exp Mol Pathol* 2005; **79**: 87-94
10 **Tsukamoto H**, French SW, Reidelberger RD, Largman C. Cyclical pattern of blood alcohol levels during continuous intragastric ethanol infusion in rats. *Alcohol Clin Exp Res* 1985; **9**: 31-37
11 **Margueron R**, Trojer P, Reinberg D. The key to development: interpreting the histone code? *Curr Opin Genet Dev* 2005; **15**: 163-176
12 **Bardag-Gorce F**, Oliva J, Villegas J, Fraley S, Amidi F, Li J, Dedes J, French B, French SW. Epigenetic mechanisms regulate Mallory Denk body formation in the livers of drug-primed mice. *Exp Mol Pathol* 2008; **84**: 113-121
13 **Fuchs SY**. The role of ubiquitin-proteasome pathway in oncogenic signaling. *Cancer Biol Ther* 2002; **1**: 337-341
14 **Li J**, Nguyen V, French BA, Parlow AF, Su GL, Fu P, Yuan QX, French SW. Mechanism of the alcohol cyclic pattern: role of the hypothalamic-pituitary-thyroid axis. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G118-G125
15 **French SW**. Intragastric ethanol infusion model for cellular and molecular studies of alcoholic liver disease. *J Biomed Sci* 2001; **8**: 20-27
16 **Bardag-Gorce F**, Li J, French BA, French SW. Ethanol withdrawal induced CYP2E1 degradation in vivo, blocked by proteasomal inhibitor PS-341. *Free Radic Biol Med* 2002; **32**: 17-21
17 **Bardag-Gorce F**, Francis T, Nan L, Li J, He Lue Y, French BA, French SW. Modifications in P62 occur due to proteasome inhibition in alcoholic liver disease. *Life Sci* 2005; **77**: 2594-2602
18 **Umlauf D**, Goto Y, Feil R. Site-specific analysis of histone methylation and acetylation. *Methods Mol Biol* 2004; **287**: 99-120
19 **Shechter D**, Dormann HL, Allis CD, Hake SB. Extraction, purification and analysis of histones. *Nat Protoc* 2007; **2**: 1445-1457
20 **Bardag-Gorce F**, French BA, Nan L, Song H, Nguyen SK, Yong H, Dede J, French SW. CYP2E1 induced by ethanol causes oxidative stress, proteasome inhibition and cytokeratin aggresome (Mallory body-like) formation. *Exp Mol Pathol* 2006; **81**: 191-201
21 **You M**, Cao Q, Liang X, Ajmo JM, Ness GC. Mammalian sirtuin 1 is involved in the protective action of dietary saturated fat against alcoholic fatty liver in mice. *J Nutr* 2008; **138**: 497-501
22 **Oliva J**, French BA, Li J, Bardag-Gorce F, Fu P, French SW. Sirt1 is involved in energy metabolism: the role of chronic ethanol feeding and resveratrol. *Exp Mol Pathol* 2008; **85**: 155-159
23 **Bardag-Gorce F**, French BA, Li J, Riley NE, Yuan QX, Valinluck V, Fu P, Ingelman-Sundberg M, Yoon S, French SW. The importance of cycling of blood alcohol levels in the pathogenesis of experimental alcoholic liver disease in rats. *Gastroenterology* 2002; **123**: 325-335
24 **Pal-Bhadra M**, Bhadra U, Jackson DE, Mamatha L, Park PH, Shukla SD. Distinct methylation patterns in histone H3 at Lys-4 and Lys-9 correlate with up- & down-regulation of genes by ethanol in hepatocytes. *Life Sci* 2007; **81**: 979-987
25 **Donohue TM Jr**. The ubiquitin-proteasome system and its role in ethanol-induced disorders. *Addict Biol* 2002; **7**: 15-28
26 **French SW**, Mayer RJ, Bardag-Gorce F, Ingelman-Sundberg M, Rouach H, Neve And E, Higashitsuji H. The ubiquitin-proteasome 26s pathway in liver cell protein turnover: effect of ethanol and drugs. *Alcohol Clin Exp Res* 2001; **25**: 225S-229S
27 **Starkova NN**, Koroleva EP, Rotanova TV. [Intracellular proteolysis: signals of selective protein degradation] *Bioorg Khim* 2000; **26**: 83-96
28 **Szutorisz H**, Georgiou A, Tora L, Dillon N. The proteasome restricts permissive transcription at tissue-specific gene loci in embryonic stem cells. *Cell* 2006; **127**: 1375-1388
29 **Ribar B**, Prakash L, Prakash S. Requirement of ELC1 for RNA polymerase II polyubiquitylation and degradation in response to DNA damage in Saccharomyces cerevisiae. *Mol*

Cell Biol 2006; **26**: 3999-4005

30 **Bardag-Gorce F**, Yuan QX, Li J, French BA, Fang C, Ingelman-Sundberg M, French SW. The effect of ethanol-induced cytochrome p4502E1 on the inhibition of proteasome activity by alcohol. *Biochem Biophys Res Commun* 2000; **279**: 23-29

31 **Bardag-Gorce F**, Venkatesh R, Li J, French BA, French SW. Hyperphosphorylation of rat liver proteasome subunits: the effects of ethanol and okadaic acid are compared. *Life Sci* 2004; **75**: 585-597

32 **Marcu MG**, Jung YJ, Lee S, Chung EJ, Lee MJ, Trepel J, Neckers L. Curcumin is an inhibitor of p300 histone acetylatransferase. *Med Chem* 2006; **2**: 169-174

33 **Song Z**, Zhou Z, Song M, Uriarte S, Chen T, Deaciuc I, McClain CJ. Alcohol-induced S-adenosylhomocysteine accumulation in the liver sensitizes to TNF hepatotoxicity: possible involvement of mitochondrial S-adenosylmethionine transport. *Biochem Pharmacol* 2007; **74**: 521-531

34 **Kharbanda KK**, Mailliard ME, Baldwin CR, Beckenhauer HC, Sorrell MF, Tuma DJ. Betaine attenuates alcoholic steatosis by restoring phosphatidylcholine generation via the phosphatidylethanolamine methyltransferase pathway. *J Hepatol* 2007; **46**: 314-321

35 **Purohit V**, Abdelmalek MF, Barve S, Benevenga NJ, Halsted CH, Kaplowitz N, Kharbanda KK, Liu QY, Lu SC, McClain CJ, Swanson C, Zakhari S. Role of S-adenosylmethionine, folate, and betaine in the treatment of alcoholic liver disease: summary of a symposium. *Am J Clin Nutr* 2007; **86**: 14-24

S- Editor Li LF **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.713

World J Gastroenterol 2009 February 14; 15(6): 713-716
World Journal of Gastroenterology ISSN 1007-9327

Systemic chemotherapy for hepatocellular carcinoma in non-cirrhotic liver: A retrospective study

Julien Edeline, Jean-Luc Raoul, Elodie Vauleon, Anne Guillygomac'h, Karim Boudjema, Eveline Boucher

Julien Edeline, Jean-Luc Raoul, Elodie Vauleon, Eveline Boucher, Department of Medical Oncology, Centre Eugène Marquis CS 44229, 35042 Rennes cedex, France
Anne Guillygomac'h, Department of Liver Disease, Pontchaillou University Hospital, 35033 Rennes cedex , France
Karim Boudjema, Department of Liver Surgery and Transplantation, Pontchaillou University Hospital, 35033 Rennes cedex, France
Jean-Luc Raoul, Elodie Vauleon, Karim Boudjema, European University in Brittany, 35042 Rennes cedex, France
Author contributions: Edeline J, Raoul JL and Boucher E wrote the paper; Edeline J and Vauleon E analyzed data; Raoul JL, Guillygomac'h A, Boudjema K and Boucher E collected and assembled the data.
Correspondence to: Jean-Luc Raoul, MD, PhD, Department of Medical Oncology, Centre E Marquis, 35042 Rennes Cedex, France. raoul@rennes.fnclcc.fr
Telephone: +33-299253172 Fax: +33-299253108
Received: October 14, 2008 Revised: December 4, 2008
Accepted: December 11, 2008
Published online: February 14, 2009

Abstract

AIM: To investigate the efficacy and toxicity of systemic chemotherapy in a retrospective study of patients with hepatocellular carcinoma (HCC) occurring in normal or fibrotic liver without cirrhosis.

METHODS: Twenty-four patients with metastatic or locally advanced HCC in a normal or a fibrotic liver were given systemic chemotherapy (epirubicin, cisplatin and 5-fluorouracil or epirubicin, cisplatin and capecitabine regimens). Tumor response, time to progression, survival, and toxicity were evaluated.

RESULTS: There were 7 women and 17 men, mean age 54 ± 10 years; 18 patients had a normal liver and 6 had a fibrotic liver (F1/F2 on biopsy). Mean tumor size was 14 cm, 5 patients had portal vein thrombosis and 7 had metastasis. Patients received a median of 4 chemotherapy sessions. Overall tolerance was good. There were 5 partial responses (objective response rate = 22%), and tumor control rate was 52%. Second line surgical resection was possible in two patients. Median survival was 11 mo, and 1- and 2-year overall survival rates were 50% ± 10% and 32% ± 11%, respectively.

CONCLUSION: In patients with HCC in a non-cirrhotic liver, chemotherapy was well tolerated and associated with an objective response rate of 22%, including two patients who underwent secondary surgical resection.

Key words: Antineoplastic protocols; Chemotherapy; Hepatocellular carcinoma

Peer reviewer: Saùl Villa-Trevio, MD, PhD, Departamento de Biologia Celular, Centro de Investigacion y de Estudios Avanzados del IPN (Cinvestav), Ave, IPN n° 2508, Col. San Pedro, Zacatenco, CP 07360, México, DF, Mexico

Edeline J, Raoul JL, Vauleon E, Guillygomac'h A, Boudjema K, Boucher E. Systemic chemotherapy for hepatocellular carcinoma in non-cirrhotic liver: A retrospective study. *World J Gastroenterol* 2009; 15(5): 713-716 Available from: URL: http://www.wjgnet.com/1007-9327/15/713.asp DOI: http://dx.doi.org/10.3748/wjg.15.713

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer, the fifth most common malignancy worldwide, and the third most common cause of cancer deaths[1]. Unfortunately, most patients are seen when the disease has reached a stage beyond curative treatment (surgery or percutaneous ablation), leaving palliative care as the only alternative. Based on the Barcelona-Clinic Liver Cancer (BCLC) staging classification[1] and treatment schedule, chemoembolization is the best option for intermediate stage patients, while for advanced stage patients, no standard treatment was established until 2007. Fortunately, after the positive results of the SHARP trial[2], a new treatment, sorafenib, was approved for advanced stage patients because of major improvements in overall survival and time to progression in comparison with placebo. In contrast to studies of most other malignancies, the efficacy of systemic chemotherapy has never been demonstrated in HCC. Despite the lack of demonstrated efficacy, doxorubicin is accepted by some physicians as a possible treatment for advanced HCC. In some recent trials using doxorubicin as a control arm, the investigational arms, a combination of platinum, doxorubicin, 5-fluorouracil and interferon in one trial[3] or nolatrexed[4], a novel thymidylate synthase inhibitor

in another trial, did not demonstrate any advantage or were associated with worse overall survival than doxorubicin alone. In one trial, this could have been partly due to toxicity, particularly because of hepatitis B virus reactivation[5]. Most likely, systemic chemotherapy lacks efficacy because of the frequently observed multidrug tumor resistance[6-8] (P-glycoprotein overexpression, p53 gene mutations), although greater drug toxicity related to the underlying cirrhosis might certainly have had an effect. In a search for clues to resolve this question, we retrospectively analyzed our records of cirrhosis-free HCC patients who received a standard chemotherapy regimen {ECF [epirubicin, cisplatin (CDDP) and 5-fluorouracil (5FU)][9] or ECC (epirubicin, CDDP and capecitabine)[10]}. Our goal was to determine whether chemotherapy was more effective in these patients than in HCC patients with cirrhosis.

MATERIALS AND METHODS

Between July 1999 and June 2006, we delivered standard chemotherapy regimens to 30 patients with HCC occurring in a non-cirrhotic liver. There was no indication for curative surgery or palliative treatment in these patients who had good performance status (ECOG PS 0 or 1), preserved liver function, normal blood cell counts (neutrophils > 15 000/mm^3, platelets > 100 × 10^9/L), normal renal function (serum creatinine < 110 μmol/L), and a measurable tumor target. Biopsy specimens, from the tumor and unaffected liver tissue, provided the diagnosis of HCC in non-cirrhotic liver in all 30 patients. None had a fibrolamellar cancer. The diagnosis of a normal liver was, however, less than certain in one patient since tumor cells had invaded most of the "normal liver" biopsy specimen. Five of the 30 patients had received a chemotherapy regimen other than ECF or ECC: capecitabine plus oxaliplatin (n = 3), and gemcitabine plus oxaliplatin (n = 2). Excluding these 6 patients, we thus retrospectively analyzed the records of 24 patients who received an ECF or ECC regimen for HCC occurring in a non-cirrhotic liver (normal liver or F1-F2 fibrosis); the ECF regimen was given from 1999 to 2002 and then we used the ECC regimen, which was much more convenient because it could be given orally.

The ECF treatment schedule was: epirubicin 60 mg/m^2 on day 1, CDDP 50 mg/m^2 on day 1, and 5FU 200 mg/m^2 administered in a continuous infusion from day 1 to day 21; in the ECC regimen, 5FU was replaced by capecitabine 1000 mg/m^2 twice a day from day 1 to day 14 followed by a 7-d off period. Courses were repeated every 21 d.

Tumor response was assessed with computed tomography performed before treatment onset and then every 9 wk (three chemotherapy courses). The tumor response (World Health Organization criteria) was considered as complete in the case of total disappearance of all tumors with normalization of α-fetoprotein (AFP) level, as partial for a decrease of tumor size less than 50%, and as stable disease with absence of progression and a decrease in tumor size less than 50%. Progression was an increase in tumor size greater than 25%. An objective response was defined by achievement of a complete or partial response. Patient tolerance was assessed using NCI-CTC AE version 2.0. In some patients with an excellent general status, second line therapy was proposed in the event of disease progression. Patient survival could be precisely evaluated for all patients and was calculated using the Kaplan-Meier method. The cause of death was recorded when available.

RESULTS

The ECF protocol was given to 10 patients, and the ECC protocol to 14. There was no difference in disease status between these two groups. The population was composed of 17 men and 7 women, mean age 54.3 ± 10.7 years (range: 23-77 years). The liver tumors were revealed in all patients by tumor-related complications (pain, fever, constitutional syndrome). The tumor developed in a non-cirrhotic liver in 18 patients and in a fibrotic liver in 6 (classified as F1 or F2). The etiological investigation revealed that 6 patients drank more than 4 units of alcohol per day, one had serological markers of hepatitis C, 2 had steatosis and were overweight, and one had genetic hemochromatosis treated by phlebotomy and was without iron liver overload on liver biopsy; no contributory factor was recognized in 14 patients. Among the 6 patients with minor fibrosis, 3 were drinkers, one had hepatitis C virus infection, one had genetic hemochromatosis and one had steatosis.

There was a unique tumor in 14 patients, 2-4 lesions in 6 and multiple or diffuse tumors in 4. Mean tumor size was huge: 14 ± 7 cm (range: 6-29 cm). Portal vein thrombosis was found in 5 patients and metastases in 7 (bones, lungs, lymph nodes). In the BCLC staging, all these patients could be classified as having an "advanced HCC", and in cases of unique tumor, tumor size was > 75% of the liver volume and surgery or transarterial chemoembolization could not be considered. Following Child-Pugh classification, and despite the absence of cirrhosis, all these patients were considered as Child class A and, using the CLIP system, 8 patients had 4 points, 10 had 3 points, 5 had 2 points and only one had 1 point (but this patient was metastatic).

None of these patients had previously received treatment for HCC. The patients received from one to 12 courses of the ECF/ECC regimen (median = 4). As the best response, 5 patients had a partial response (objective response rate 22%; 95% CI: 6-38%), 8 patients had stable disease (30%) and 11 had tumor progression; the disease control rate was then 52%. The median time to progression was 6 mo. At the cut-off date of March 15, 2007, 18 patients had died due to progression of their tumor and 6 patients were still alive; median survival time was 11 mo (range 3-90 mo). Actuarial overall survival rates (mean ± SD) at 6 mo, 1 year and 2 years were 71% ± 9%, 50% ± 10% and 32% ± 11%, respectively (Figure 1). The 5 patients with an objective response achieved prolonged survival: 3 died 13, 27 and 48 mo after treatment onset and 2 were still alive at the cut-off date. Surgical resection was undertaken in one

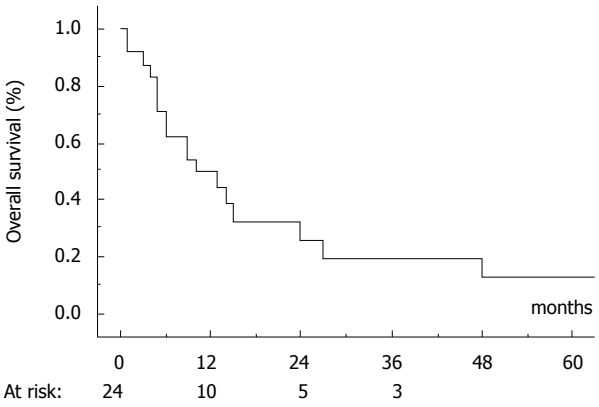


Figure 1 Overall survival in the population of 24 HCC patients with non-cirrhotic liver treated by an ECC/ECF regimen.

Table 1 Adverse effects in this series of 24 patients with HCC in non-cirrhotic liver treated by chemotherapy (ECC regimen)

Adverse effets	Grade 1-2	Grade 3-4 (%)
Alopecia	8	-
Neutropenia	4	4 (17)
Mucositis	4	0
Diarrhea	2	0
Renal failure	1	0
Asthenia	2	0
Hand-foot syndrome	5	0
Nausea, vomiting	12	2 (4)
Anemia	2	0
Coronary spasm	0	1

Percentages not given in this column

One patient died suddenly during the first course of chemotherapy.

patient 10 mo after treatment onset as a result of an objective response. This patient was alive at 90 mo with progression (the same chemotherapy was successfully resumed; she was subsequently treated with sorafenib). The other prolonged survivor had a 22-cm tumor; she underwent surgical resection when a minor response was observed after 9 courses of ECC. She was still alive and disease-free 36 mo after surgery. Initially, these 2 patients had a unique tumor involving more than 75% of the liver without portal vein thrombosis or extrahepatic metastases; both had an AFP level above 500 ng/mL; they were considered as CLIP 3.

Toxicity was graded using NCI-CTC criteria version 2.0. The main toxic effects are summarized in Table 1. Overall tolerance was good. Signs of grade 3-4 toxicity developed in 7 patients (29%), neutropenia in 4 (no cases of febrile neutropenia), gastrointestinal disorders (nausea, vomiting) in 2; one patient died suddenly after the first cycle and one presented with mild thoracic pain assumed to be related to 5FU. Among the other patients, 8 developed alopecia and renal function worsened in one, leading to discontinuation of the treatment.

DISCUSSION

Despite the lack of proof of efficacy, doxorubicin is commonly used as a first line therapy for HCC. Recent randomized studies with doxorubicin[3,4] have confirmed the minimal efficacy of this drug in HCC. Results obtained in phase Ⅱ studies with different regimens using new cytotoxic drugs have not been very impressive. Thus, systemic chemotherapy cannot be considered as the standard of care for HCC patients. This situation could be related to a combination of poor efficacy and increased toxicity. Poor efficacy might result from intrinsic resistance caused by overexpression of multidrug resistance genes observed in most tumors. Obviously the underlying liver cirrhosis increases the risk of severe adverse events as many chemotherapeutic drugs are metabolized or eliminated via the liver. Moreover severe complications are certainly more likely if a cytotoxicity-related side effect occurs on a cirrhotic liver. Certain causes of the underlying cirrhosis, e.g. hepatitis B virus infection[5], may be reactivated after chemotherapy-induced immunodepression, producing an additive toxic effect.

In this particular retrospective series of HCC developed in normal or fibrotic livers, we attempted to estimate the raw efficacy and toxicity of systemic chemotherapy, regardless of liver status. The objective response rate in these patients given the ECF/ECC regimen was 22%, with a disease control rate (objective response plus stable disease) of 52%. The median time to progression was 6 mo, that is to say, quite similar to that observed using sorafenib[2], In addition, despite the fact that most tumors were huge, the reduction in tumor size was sufficient to allow surgical resection in 2 patients having only one huge tumor. Toxicity was mild and most side effects were manageable; one patient died suddenly between two courses. These two regimens (ECF and ECC) are very similar: capecitabine is the oral form of 5FU and, in a randomized 2 × 2 study conducted in advanced esophagogastric cancers[11], these two regimens were demonstrated to be effective. Such results with objective response rates approaching 20% have been reported in some other phase Ⅱ studies[12-14], including patients with or without cirrhosis: a similar ECC regimen gave, in a Korean series of 29 patients, an objective response rate of 24%[15]. Similar findings have been reported with the PIAF regimen (combination of CDDP, doxorubicin, 5FU and interferon)[16], where, in a series of 50 patients, 26% had a partial response including 9 who underwent surgical resection, and in 4, there were no viable cancer cells on resected specimens. Unfortunately, this did not translate into any difference in overall survival versus doxorubicin alone in a randomized phase Ⅲ trial despite the fact that less than half of the patients had underlying cirrhosis (but 17% of the patients had Child B class cirrhosis). The response rate of 22% we have observed in this series is a little bit higher than that observed in a retrospective series of 21 patients we have previously published[17] (16 having underlying cirrhosis). Comparing the results of these two series, we suggest that efficacy was better in the non-cirrhotic group. In our first series of 21 HCC patients we observed 3 responders (objective response rate of 14.5%)

including one non-cirrhotic patient (among cirrhotic patients, 2/16 were responders); median survival was 10 mo and surgery was possible in one patient. Toxicity appeared to be more frequent in the whole population, as we described 18 cases of grade 3-4 toxicities versus 7 cases in the current 24 non-cirrhotic patients. This might suggest that an ECF/ECC chemotherapy regimen (particularly the ECC regimen which is more convenient, especially in cirrhotic patients) could be delivered to non-cirrhotic patients with a hope of the possibility of secondary resection.

As a result of the recent SHARP trial, which demonstrated the efficacy of sorafenib in advanced-stage HCC, sorafenib is now the new standard of care in that setting. Combinations of sorafenib plus doxorubicin[18] seemed to be well tolerated and have yielded promising results in a randomized phase Ⅱ trial. A combination of sorafenib with the ECC regimen deserves a phase Ⅱ trial particularly in patients with HCC in a non-cirrhotic liver.

COMMENTS

Background

Systemic chemotherapy is not usually considered as a standard treatment in hepatocellular carcinoma (HCC) developed in cirrhotic patients.

Research frontiers

New targeted agents, like sorafenib, are efficient in advanced HCC and could be associated with systemic chemotherapy.

Innovations and breakthroughs

When systemic chemotherapy is given to patients who develop HCC without liver cirrhosis, the tolerance and efficacy are better and can allow curative surgery in some cases.

Applications

In selected cases of HCC without liver cirrhosis, systemic chemotherapy can be safely given with promising results.

Peer review

The peer reviewer emphasized the differences between these two regimens; one (ECF) requires a continuous infusion of chemotherapeutic drug (5FU) while the other is given orally and is easier to use.

REFERENCES

1 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917

2 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390

3 **Yeo W**, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, Koh J, Mo FK, Yu SC, Chan AT, Hui P, Ma B, Lam KC, Ho WM, Wong HT, Tang A, Johnson PJ. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005; **97**: 1532-1538

4 **Gish RG**, Porta C, Lazar L, Ruff P, Feld R, Croitoru A, Feun L, Jeziorski K, Leighton J, Gallo J, Kennealey GT. Phase III randomized controlled trial comparing the survival of patients with unresectable hepatocellular carcinoma treated with nolatrexed or doxorubicin. *J Clin Oncol* 2007; **25**: 3069-3075

5 **Yeo W**, Lam KC, Zee B, Chan PS, Mo FK, Ho WM, Wong WL, Leung TW, Chan AT, Ma B, Mok TS, Johnson PJ. Hepatitis B reactivation in patients with hepatocellular carcinoma undergoing systemic chemotherapy. *Ann Oncol* 2004; **15**: 1661-1666

6 **Fardel O**, Loyer P, Lecureur V, Glaise D, Guillouzo A. Constitutive expression of functional P-glycoprotein in rat hepatoma cells. *Eur J Biochem* 1994; **219**: 521-528

7 **Chan KT**, Lung ML. Mutant p53 expression enhances drug resistance in a hepatocellular carcinoma cell line. *Cancer Chemother Pharmacol* 2004; **53**: 519-526

8 **Endo T**, Yoshikawa M, Ebara M, Kato K, Sunaga M, Fukuda H, Hayasaka A, Kondo F, Sugiura N, Saisho H. Immunohistochemical metallothionein expression in hepatocellular carcinoma: relation to tumor progression and chemoresistance to platinum agents. *J Gastroenterol* 2004; **39**: 1196-1201

9 **Bamias A**, Hill ME, Cunningham D, Norman AR, Ahmed FY, Webb A, Watson M, Hill AS, Nicolson MC, O'Brien ME, Evans TC, Nicolson V. Epirubicin, cisplatin, and protracted venous infusion of 5-fluorouracil for esophagogastric adenocarcinoma: response, toxicity, quality of life, and survival. *Cancer* 1996; **77**: 1978-1985

10 **Cho EK**, Lee WK, Im SA, Lee SN, Park SH, Bang SM, Park DK, Park YH, Shin DB, Lee JH. A phase II study of epirubicin, cisplatin and capecitabine combination chemotherapy in patients with metastatic or advanced gastric cancer. *Oncology* 2005; **68**: 333-340

11 **Cunningham D**, Starling N, Rao S, Iveson T, Nicolson M, Coxon F, Middleton G, Daniel F, Oates J, Norman AR. Capecitabine and oxaliplatin for advanced esophagogastric cancer. *N Engl J Med* 2008; **358**: 36-46

12 **Louafi S**, Boige V, Ducreux M, Bonyhay L, Mansourbakht T, de Baere T, Asnacios A, Hannoun L, Poynard T, Taieb J. Gemcitabine plus oxaliplatin (GEMOX) in patients with advanced hepatocellular carcinoma (HCC): results of a phase II study. *Cancer* 2007; **109**: 1384-1390

13 **Boige V**, Raoul JL, Pignon JP, Bouche O, Blanc JF, Dahan L, Jouve JL, Dupouy N, Ducreux M. Multicentre phase II trial of capecitabine plus oxaliplatin (XELOX) in patients with advanced hepatocellular carcinoma: FFCD 03-03 trial. *Br J Cancer* 2007; **97**: 862-867

14 **Pastorelli D**, Cartei G, Zustovich F, Marchese F, Artioli G, Zovato S, Binato S, Ceravolo R, Cingarlini S, Salmaso F, Mattiazzi M, Sanavio C, Farinati F, Zanus G, Cillo U. Gemcitabine and liposomal doxorubicin in biliary and hepatic carcinoma (HCC) chemotherapy: preliminary results and review of the literature. *Ann Oncol* 2006; **17** Suppl 5: v153-v157

15 **Park SH**, Lee Y, Han SH, Kwon SY, Kwon OS, Kim SS, Kim JH, Park YH, Lee JN, Bang SM, Cho EK, Shin DB, Lee JH. Systemic chemotherapy with doxorubicin, cisplatin and capecitabine for metastatic hepatocellular carcinoma. *BMC Cancer* 2006; **6**: 3

16 **Leung TW**, Patt YZ, Lau WY, Ho SK, Yu SC, Chan AT, Mok TS, Yeo W, Liew CT, Leung NW, Tang AM, Johnson PJ. Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. *Clin Cancer Res* 1999; **5**: 1676-1681

17 **Boucher E**, Corbinais S, Brissot P, Boudjema K, Raoul JL. Treatment of hepatocellular carcinoma (HCC) with systemic chemotherapy combining epirubicin, cisplatinum and infusional 5-fluorouracil (ECF regimen). *Cancer Chemother Pharmacol* 2002; **50**: 305-308

18 **Abou-Alfa GK**, Johnson P, Knox J, Lacava J, Leung T, Mori A. Preliminary results from a phase II, randomized, double-blind study of sorafenib plus doxorubicin versus placebo plus doxorubicin in patients with advanced hepatocellular carcinoma. ECCO 14, Abst 3500, Barcelona, 23-27 September 2007

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.717

World J Gastroenterol 2009 February 14; 15(6): 717-721
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Early recognition of abdominal compartment syndrome in patients with acute pancreatitis

Zilvinas Dambrauskas, Audrius Parseliunas, Antanas Gulbinas, Juozas Pundzius, Giedrius Barauskas

Zilvinas Dambrauskas, Antanas Gulbinas, Laboratory for Research of Digestive System, Institute for Biomedical Research and Department of Surgery, Kaunas University of Medicine, Eiveniu Str. 2, 50009 Kaunas, Lithuania
Giedrius Barauskas, Juozas Pundzius, Audrius Parseliunas, Department of Surgery, Kaunas University of Medicine, Eiveniu Str. 2, 50009 Kaunas, Lithuania
Author contributions: Dambrauskas Z, Barauskas G designed research; Pundzius J was involved in editing the manuscript; Dambrauskas Z and Parseliunas A performed research; Dambrauskas Z, Gulbinas A analyzed data and wrote the paper.
Correspondence to: Dr. Zilvinas Dambrauskas, Department of Surgery, Kaunas University of Medicine Hospital, Eiveniu Str. 2, 50009 Kaunas,
Lithuania. zilvinas.dambrauskas@gmail.com
Telephone: +370-686-69255 Fax: +370-37-327163
Received: November 4, 2008 Revised: January 7, 2009
Accepted: January 14, 2009
Published online: February 14, 2009

Abstract

AIM: To assess the value of widely used clinical scores in the early identification of acute pancreatitis (AP) patients who are likely to suffer from intra-abdominal hypertension (IAH) and abdominal compartment syndrome (ACS).

METHODS: Patients (n = 44) with AP recruited in this study were divided into two groups (ACS and non-ACS) according to intra-abdominal pressure (IAP) determined by indirect measurement using the transvesical route *via* Foley bladder catheter. On admission and at regular intervals, the severity of the AP and presence of organ dysfunction were assessed utilizing different multifactorial prognostic systems: Glasgow-Imrie score, Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE-Ⅱ) score, and Multiorgan Dysfunction Score (MODS). The diagnostic performance of scores predicting ACS development, cut-off values and specificity and sensitivity were established using receiver operating characteristic (ROC) curve analysis.

RESULTS: The incidence of ACS in our study population was 19.35%. IAP at admission in the ACS group was 22.0 (18.5-25.0) mmHg and 9.25 (3.0-12.4) mmHg in the non-ACS group (P < 0.01). Univariate statistical analysis revealed that patients in the ACS group had significantly higher multifactorial clinical scores (APACHE Ⅱ, Glasgow-Imrie and MODS) on admission and higher maximal scores during hospitalization (P < 0.01). ROC curve analysis revealed that APACHE Ⅱ, Glasgow-Imrie, and MODS are valuable tools for early prediction of ACS with high sensitivity and specificity, and that cut-off values are similar to those used for stratification of patients with severe acute pancreatitis (SAP).

CONCLUSION: IAH and ACS are rare findings in patients with mild AP. Based on the results of our study we recommend measuring the IAP in cases when patients present with SAP (APACHE Ⅱ > 7; MODS > 2 or Glasgow-Imrie score > 3).

Key words: Acute pancreatitis; Abdominal compartment syndrome; Intra-abdominal pressure; Intra-abdominal hypertension; Organ dysfunction

Peer reviewer: Rakesh Kumar Tandon, Professor, Pushpawati Singhania Research Institute for Liver, Renal and Digestive Diseases, Sheikh Sarai Phase Ⅱ, New Delhi 110017, India

Dambrauskas Z, Parseliunas A, Gulbinas A, Pundzius J, Barauskas G. Early recognition of abdominal compartment syndrome in patients with acute pancreatitis. *World J Gastroenterol* 2009; 15(6): 717-721 Available from: URL: http://www.wjgnet.com/1007-9327/15/717.asp DOI: http://dx.doi.org/10.3748/wjg.15.717

INTRODUCTION

Acute pancreatitis (AP) remains a disease with an unpredictable clinical course, and significant associated morbidity and mortality[1]. Recently, the elevated intra-abdominal pressure (IAP) following the onset of AP has attracted growing attention, because it is increasingly recognized as an important risk factor for mortality in the early phase of the disease[2-4]. It was shown that intra-abdominal hypertension (IAH) is associated with higher mortality and morbidity rates, and prolonged ICU stay, in comparison to other patients who had normal IAP[5-8]. IAH has been recognized as a cause of organ dysfunction in critically ill patients, including those suffering from severe acute pancreatitis (SAP)[9-12]. Abdominal compartment syndrome (ACS) is defined as an increase of IAP > 20 mmHg, which is associated with occurrence of a new organ failure. A previously

reported incidence of ACS among patients with SAP ranges from 23% to 56%[11,13-15]. The mechanisms involved in the development of IAH and ACS include increased capillary permeability, hypoalbuminemia and volume overload, which produce a large retroperitoneal and visceral edema[6,16].

It has been shown that early recognition and treatment of IAH and ACS result in a significant improvement in patient survival and decreased morbidity. Due to its simplicity and minimal cost, the standard for intermittent IAP measurement is *via* the urinary bladder with a maximal instillation volume of 25 mL sterile saline[17]. Compared with bladder pressure measurements, clinical abdominal assessment showed poor sensitivity (56%) and accuracy (77%) for identifying elevated IAP[18]. It was shown that the essential approach to diagnosis and management of ACS is a timely IAP measurement. It is still not clear whether early IAP measurement should be routine for all AP patients and which patients would benefit most from the IAP monitoring.

This study aimed to assess the value of Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ), Multiorgan Dysfunction Score (MODS) and Glasgow-Imrie clinical scores in early recognition of patients who are likely to suffer from IAH and ACS, and who would benefit from IAP monitoring and management. We also investigated the incidence of ACS, the role of its interventional management and clinical outcomes in patients with AP.

MATERIALS AND METHODS

Ethics

This work has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The Regional Ethics Committee approved the study (protocols no. BE-2-47 and P1-113/2005) and all patients provided written informed consent.

Study design and patient population

The study population included 44 patients with AP admitted to the Department of Surgery, Kaunas University of Medicine Hospital, from May 2007 to February 2008. General inclusion criteria were defined as follows: (1) a time interval between onset of typical abdominal symptoms and study inclusion of 72 h and less; (2) at least 3-fold elevated serum amylase or lipase levels; (3) no previous history of acute or chronic pancreatitis. On the first day of admission, the severity of the AP and presence of organ dysfunction were assessed utilizing three different multifactorial prognostic systems: Glasgow-Imrie score, APACHE-Ⅱ score, and MODS. Later, the severity of disease and clinical status were repeatedly reassessed using the same prognostic tools every 7 d, and when the deterioration of clinical condition occurred and after interventional treatment of ACS. The contrast-enhanced CT scan was performed on day 4 to 7 after the onset of disease to demonstrate the presence of pancreatic necrosis. According to the clinical course and clinical severity scores (APACHE Ⅱ > 7; Glasgow-Imrie > 2; MODS > 2; peak C-reactive protein value > 150 mg/L) patients were stratified into mild and severe AP groups. The data were prospectively recorded in a specially created database. All patients were treated according to our standard AP management protocol following the recent international guidelines.

Measurement of IAP and clinical assessment of patients

For IAP measurement, we used a standard two-way 16 Fr. Foley catheter inserted into the urinary bladder. The patient was placed in supine position. Twenty-five milliliters of 0.9% sterile NaCl were instilled and the catheter was connected to a tube from the urine collection bag. The pubic symphysis was considered level 0 and IAP was measured in cm H_2O, then recalculated in mmHg. IAP was measured every 24 h during a period of 3 d in all patients. For patients that developed IAH (IAP > 12 mmHg), the conservative treatment (according to the recommendations of international experts on IAH and ACS) was initiated and IAP was monitored every 12 h until the normal IAP was reached and sustained at least for 24 h. In cases when IAH > 18 mmHg was recorded, IAP was monitored every 4-6 h until IAP normalized or ACS developed. ACS was defined as an increase of IAP > 20 mmHg, which is associated with occurrence of a new organ failure[16,17].

Statistical analysis

Statistical analysis was performed using SPSS® for Windows release 16.0 (SPSS, Chicago, IL, USA). The quantitative variables are presented as mean ± SD or median (with interquartile range). For comparison between groups, the Mann-Whitney test, Student's *t* test or χ^2 test was employed where appropriate. The diagnostic performance of scores predicting ACS development, cut-off values and specificity and sensitivity of prognostic tools were established using receiver operating characteristic (ROC) curve analysis. Results with $P < 0.05$ were considered statistically significant.

RESULTS

A total of 44 patients with AP were included in the study. Demographic and clinical data of these patients are represented in Table 1.

All patients were divided into ACS and non-ACS groups. Median IAP in the ACS group at admission was 22.0 (18.5-25.0) mmHg and 9.25 (3.0-12.4) mmHg in the non-ACS group ($P < 0.01$) (Figure 1).

Differences of APACHE Ⅱ, Glasgow-Imrie and MODS median scores on admission and maximal scores (Max) during hospitalization period between ACS and non-ACS groups are presented in Table 2. The study revealed that patients in the ACS group had significantly higher multifactorial clinical scores on admission and higher maximal scores during hospitalization ($P < 0.01$). There were no significant differences between median admission and maximal scores of APACHE Ⅱ, Glasgow-Imrie and MODS within ACS or non-ACS groups ($P > 0.05$). The ACS group was characterized by

Table 1 Demographic and clinical variables of the whole series

Demographic and clinical variables	Values
Number of patents	44
Age (years ± SD)	49 ± 18
Gender male	70.4% (31/44)
SAP	70.4% (31/44)
Presence of necrosis	49.9% (18/44)
Extent (percentage) of necrosis	
< 30%	38.9% (7/18)
30%-50%	11.1% (2/18)
> 50%	50.0% (9/18)
IAH (IAP ⩾ 12 mmHg)	43.2% (19/44)
ACS (IAP ⩾ 20 mmHg + MOF)	13.6% (6/44)
Mortality	13.6% (6/44)
APACHE Ⅱ score at admission[1]	6.5 (4.0-10.0)
Max APACHE Ⅱ score during hospitalization[1]	8.0 (5.0-11.0)
Glasgow-Imrie score at admission[1]	3.0 (2.0-3.0)
Max Glasgow-Imrie score during hospitalization[1]	3.0 (2.0-4.0)
MODS score at admission[1]	2.0 (1.0-3.0)
Max MODS score during hospitalization[1]	2.0 (1.0-4.0)

[1]Values of clinical multifactorial scores is expressed as median (lower & upper quartiles).

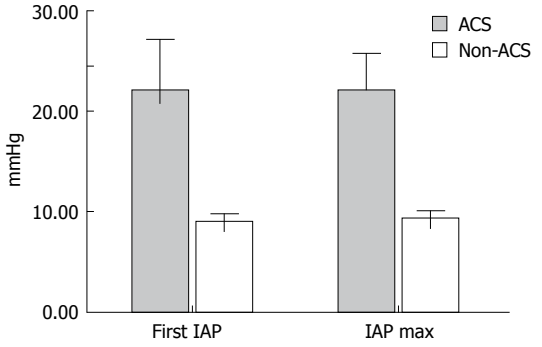


Figure 1 The 1st IAP and maximal IAP value in ACS and non-ACS groups. All patients were divided in ACS (grey colour) and non-ACS (white colour) groups. Median IAP at admission was 22.0 and 9.25 mmHg in ACS and non-ACS groups respectively ($P < 0.01$). There was no significant difference between value of the 1st measurement and maximum observed value of IAP within each group.

a markedly higher incidence of severe and necrotizing AP, and by the presence of high volume pancreatic necrosis in comparison to the non-ACS group. Mortality rate in the ACS group was also significantly higher, when compared to the non-ACS group (Table 3).

SAP was diagnosed in 70.4% (31/44) of all cases in this study group. We believe such a relatively high incidence of SAP is associated with the concentration of patients with severe disease in our tertiary care center referred from other regional hospitals, and a special focus on the patients with systemic inflammatory response syndrome and multiorgan failure (MOF). Nevertheless, the incidence of ACS in our study population was 19.35% (6/31) and did not exceed the prevalence of IAH and ACS shown in other clinical studies. Interestingly, the prevalence of IAH was significantly lower in the mild AP group, with only 7.69% (1/13) when compared to 58.06% (18/31) in the SAP group ($P > 0.01$). Specifically, all cases of ACS occurred in the SAP group with an incidence of 19.35% (6/31), while there were no cases of ACS in the mild AP group (0/13).

Table 2 Clinical scores (on admission and max value) in relation to presence of ACS

Clinical scores	ACS group median (lower & upper quartiles)	Non-ACS group median (lower & upper quartiles)	*P*
APACHE Ⅱ score on admission	12.0 (9.0-1.0]	6.0 (4.0-9.0)	< 0.01
Max APACHE score	14.0 (11.0-18.0)	7.0 (4.0-10.0)	< 0.01
Glasgow-Imrie score on admission	5.0 (4.0-5.0)	2.0 (2.0-3.0)	< 0.01
Max Glasgow-Imrie score	5.0 (4.0-5.0)	2.5 (1.0-3.0)	< 0.01
MODS score on admission	4.5 (3.0-8.0)	1.5 (1.0-3.0)	< 0.01
Max MODS score	4.5 (3.0-8.0)	2.0 (1.0-3.0)	< 0.01

Table 3 Clinical characteristics of patients with and without ACS

Clinical characteristics	ACS group (n = 6, %)	Non-ACS group (n = 38, %)	*P*
Severe AP	6 (100)	25 (65.8)	NS
Necrotizing AP	5 (83.3)	13 (34.2)	< 0.05
Necrosis > 30%	5 (83.3)	6 (15.8)	< 0.05
Deaths	4 (66.6)	2 (5.2)	< 0.01

Table 4 Areas under the ROC curves for prognostic factors of ACS

Variables	Area	Std. error	Asymptotic sig.	Confidence Interval	
				lower	upper
1st IAP on admission	0.932	0.065	0.001	0.805	1.059
Glasgow-Imrie score	0.921	0.054	0.001	0.816	1.026
APACHE Ⅱ score	0.866	0.062	0.004	0.745	0.987
MODS score	0.829	0.098	0.010	0.636	1.022

Table 5 Cut-off points, sensitivity and specificity for prognostic factors of ACS

Variable	Cut-off	Sensitivity (%)	Specificity (%)
APACHE Ⅱ score	> 7	100.0 (54.1-100.0)	60.5 (43.4-75.9)
Glasgow-Imrie score	> 3	83.3 (36.1-97.2)	86.8 (71.9-95.5)
MODS score	> 2	83.3 (36.1-97.2)	73.7 (56.9- 86.6)
1st IAP on admission	> 18	83.3 (36.1-97.2)	100.0 (90.7-100.0)

The diagnostic performance of multifactorial clinical scores and the first IAP measurement in predicting development of ACS during the course of AP were assessed using ROC curve analysis. ROC analysis revealed that all the analysed clinical scores are of good prognostic value in determining patients who are likely to further develop ACS (Figure 2). The areas under the ROC curves, cut-off values, specificity and sensitivity of prognostic tools are presented in Tables 4 and 5.

DISCUSSION

Our study confirmed the earlier published observations that AP is a risk factor for development of IAH and ACS[13,14,19,20]. Overall, somewhat higher rates of SAP in our institution could be explained by the concentration

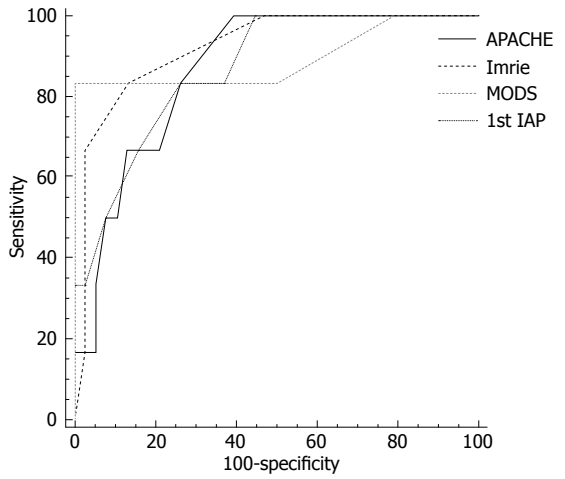


Figure 2 ROC curve analysis of prognostic factors for ACS development. ROC analysis revealed that clinical scores (APACHE Ⅱ, Glasgow-Imrie and MODS) and first IAP measurement on admission are good prognostic markers in determining patients who are likely to develop ACS. There was no significant difference between the areas under the ROC curves for these prognostic markers.

of the patients, because our hospital is a tertiary care center and many patients with suspected severe disease are referred to it from regional hospitals. However, the incidence of IAH and ACS in our study was similar to that observed in other studies, and as expected, it was associated with a higher incidence of MOF and higher mortality rates.

An early diagnosis of ACS and its adequate management is crucial[1,8,15,17]. The measurement and monitoring of IAP *via* urinary bladder catheter is a simple procedure, which requires virtually no technical skills and little resources. However, this procedure is invasive and is associated with significant discomfort for the patient[21,22]. It has also been shown that indwelling urinary catheters are associated with a higher incidence of infectious complications and prevalence of nosocomial pathogens[23-25]. Clearly, placement of a urinary catheter should not be routinely recommended for all patients, especially not for those who are unlikely to develop ACS. Therefore clinical assessment in selecting the patients that are likely to develop ACS is of particular importance. Our study demonstrated that development of IAH and ACS during the AP could be predicted by the use of clinical multifactorial scoring systems (APACHE Ⅱ, MODS, Glasgow-Imrie score), thus allowing a timely and appropriate selection of patients for this invasive procedure during the first hours and days of the disease. Clinical scores of patients who eventually suffered from IAH were higher during the first days in comparison to the group of patients with normal IAP. These findings are in accord with the observations of other groups[2,4,20,26]. The ROC analysis disclosed that APACHE Ⅱ, MODS, and Glasgow-Imrie scores have similar cut-off values to those used for the prediction of SAP. IAP > 18 mmHg on admission is also a valuable indicator that the patient has a higher risk for persistent IAH and development of ACS during the course of AP. All these prognostic markers had a good sensitivity, specificity and large area under the curve. Furthermore, the use of a clinical scoring system in combination with the first IAP measurement (eg. APACHE Ⅱ + first IAP on admission) allows us to identify nearly 100% of patients who are likely to develop SAP and suffer from ACS.

Previously published studies do not provide us with any useful recommendations or criteria for the selection of the AP patients for the IAP measurement and monitoring, although it would be unnecessary in the majority of cases when patients have a mild and self-limiting disease.

Based on the results of our study we recommend measuring the IAP only in cases when patients present with SAP (i.e. APACHE Ⅱ > 7; MODS > 2 or Glasgow-Imrie score > 3). We advocate a continuous monitoring of IAP in all cases when the patient suffers from SAP and has an IAP > 18 mmHg on first measurement. We would recommend utilizing the simpler Glasgow-Imrie or MODS scores in daily clinical practice and a more complex APACHE Ⅱ score in the clinical trial setting.

CONCLUSION

Placement of a urinary catheter for the monitoring of IAP would be unnecessary in the majority of AP cases, when patients have a mild and self-limiting disease.

We recommend measuring the IAP only in cases when patients present with SAP (i.e. APACHE Ⅱ > 7; MODS > 2 or Glasgow-Imrie score > 3). We advocate a continuous monitoring of IAP in all cases when the patient suffers from SAP and has an IAP > 18 mmHg on first measurement.

COMMENTS

Background

Acute pancreatitis (AP) remains a disease with an unpredictable clinical course, and significant associated morbidity and mortality. Recently, the elevated intra-abdominal pressure (IAP) after onset of AP has gained growing attention, because it is increasingly recognized as an important risk factor for mortality in the early phase of the disease. Intra-abdominal hypertension (IAH) has been recognized as a cause of organ dysfunction in critically ill patients, including those suffering from severe acute pancreatitis (SAP).

Research frontiers

It has been shown that early recognition and treatment of IAH and abdominal compartment syndrome (ACS) result in a significant improvement in patient survival and decreased morbidity, however, clinical abdominal assessment showed poor sensitivity and accuracy for identifying the elevated IAP. The essential approach to the diagnosis and management of ACS is a timely IAP measurement. It is still not clear whether early IAP measurement should be a routine for all AP patients and which patients would benefit most from the IAP monitoring.

Innovations and breakthroughs

An early diagnosis of ACS and its adequate management is crucial. The measurement and monitoring of IAP *via* urinary bladder catheter is a simple procedure, which requires virtually no technical skills and little resources. However, this procedure is invasive and is associated with significant discomfort for the patient. It has also been shown that indwelling urinary catheters are associated with a higher incidence of infectious complications and prevalence of nosocomial pathogens. Clearly, placement of a urinary catheter should not be routinely recommended for all patients, especially not for those who are unlikely to develop ACS. For the first time, our study demonstrated that development of IAH and ACS during the AP could be predicted by the use of clinical multifactorial scoring systems [Acute Physiology and Chronic Health

Evaluation Ⅱ (APACHE-Ⅱ), Multiorgan Dysfunction Score (MODS), Glasgow-Imrie score], thus allowing a timely and appropriate selection of patients for this invasive procedure during the first hours and days of the disease.

Applications

Based on the results of our study, we recommend measuring the IAP only in cases when patients present with SAP (i.e. APACHE Ⅱ > 7; MODS > 2 or Glasgow-Imrie score > 3). They advocate a continuous monitoring of IAP in all cases when the patient suffers from SAP and has an IAP > 18 mmHg on first measurement.

Terminology

ACS is a severe increase in the pressure within the abdomen (IAP) such that a patient's internal organs begin to fail and malfunction. This is a medical emergency. Untreated, ACS has a high mortality rate. There are a number of different methods that your doctor may use to treat the ACS. These may include giving medications to sedate or temporarily paralyze you or your loved one, placing tubes through the nose and into the stomach to remove fluid and air, placing tubes into the abdomen to remove fluid or blood, or opening the abdomen to release the increased pressure. Most patients with IAH and/or ACS will be cared for in an ICU where doctors and nurses constantly monitor for signs of illness and treat patients to keep their heart, lungs, kidneys, liver, and intestines functioning as normally as possible.

Peer review

An important aspect of AP has been addressed in this paper, as not much has been written about IAP and ACS in relation to AP. The study design is simple and clear.

REFERENCES

1 **Wilmer A**. ICU management of severe acute pancreatitis. *Eur J Intern Med* 2004; **15**: 274-280

2 **Buter A**, Imrie CW, Carter CR, Evans S, McKay CJ. Dynamic nature of early organ dysfunction determines outcome in acute pancreatitis. *Br J Surg* 2002; **89**: 298-302

3 **Dugernier T**, Reynaert M, Laterre PF. Early multi-system organ failure associated with acute pancreatitis: a plea for a conservative therapeutic strategy. *Acta Gastroenterol Belg* 2003; **66**: 177-183

4 **Khan AA**, Parekh D, Cho Y, Ruiz R, Selby RR, Jabbour N, Genyk YS, Mateo R. Improved prediction of outcome in patients with severe acute pancreatitis by the APACHE II score at 48 hours after hospital admission compared with the APACHE II score at admission. Acute Physiology and Chronic Health Evaluation. *Arch Surg* 2002; **137**: 1136-1140

5 **Burch JM**, Moore EE, Moore FA, Franciose R. The abdominal compartment syndrome. *Surg Clin North Am* 1996; **76**: 833-842

6 **Ivatury RR**, Diebel L, Porter JM, Simon RJ. Intra-abdominal hypertension and the abdominal compartment syndrome. *Surg Clin North Am* 1997; **77**: 783-800

7 **Meldrum DR**, Moore FA, Moore EE, Franciose RJ, Sauaia A, Burch JM. Prospective characterization and selective management of the abdominal compartment syndrome. *Am J Surg* 1997; **174**: 667-672; discussion 672-673

8 **Sugrue M**, Jones F, Deane SA, Bishop G, Bauman A, Hillman K. Intra-abdominal hypertension is an independent cause of postoperative renal impairment. *Arch Surg* 1999; **134**: 1082-1085

9 **Balogh Z**, McKinley BA, Cocanour CS, Kozar RA, Holcomb JB, Ware DN, Moore FA. Secondary abdominal compartment syndrome is an elusive early complication of traumatic shock resuscitation. *Am J Surg* 2002; **184**: 538-543; discussion 543-544

10 **Malbrain ML**, Chiumello D, Pelosi P, Wilmer A, Brienza N, Malcangi V, Bihari D, Innes R, Cohen J, Singer P, Japiassu A, Kurtop E, De Keulenaer BL, Daelemans R, Del Turco M, Cosimini P, Ranieri M, Jacquet L, Laterre PF, Gattinoni L. Prevalence of intra-abdominal hypertension in critically ill patients: a multicentre epidemiological study. *Intensive Care Med* 2004; **30**: 822-829

11 **Malbrain ML**, Chiumello D, Pelosi P, Bihari D, Innes R, Ranieri VM, Del Turco M, Wilmer A, Brienza N, Malcangi V, Cohen J, Japiassu A, De Keulenaer BL, Daelemans R, Jacquet L, Laterre PF, Frank G, de Souza P, Cesana B, Gattinoni L. Incidence and prognosis of intraabdominal hypertension in a mixed population of critically ill patients: a multiple-center epidemiological study. *Crit Care Med* 2005; **33**: 315-322

12 **Tao HQ**, Zhang JX, Zou SC. Clinical characteristics and management of patients with early acute severe pancreatitis: experience from a medical center in China. *World J Gastroenterol* 2004; **10**: 919-921

13 **Al-Bahrani AZ**, Abid GH, Holt A, McCloy RF, Benson J, Eddleston J, Ammori BJ. Clinical relevance of intra-abdominal hypertension in patients with severe acute pancreatitis. *Pancreas* 2008; **36**: 39-43

14 **De Waele JJ**, Hoste E, Blot SI, Decruyenaere J, Colardyn F. Intra-abdominal hypertension in patients with severe acute pancreatitis. *Crit Care* 2005; **9**: R452-R457

15 **Pupelis G**, Austrums E, Snippe K, Berzins M. Clinical significance of increased intraabdominal pressure in severe acute pancreatitis. *Acta Chir Belg* 2002; **102**: 71-74

16 **Malbrain ML**, Cheatham ML, Kirkpatrick A, Sugrue M, Parr M, De Waele J, Balogh Z, Leppaniemi A, Olvera C, Ivatury R, D'Amours S, Wendon J, Hillman K, Johansson K, Kolkman K, Wilmer A. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. *Intensive Care Med* 2006; **32**: 1722-1732

17 **Cheatham ML**, Malbrain ML, Kirkpatrick A, Sugrue M, Parr M, De Waele J, Balogh Z, Leppaniemi A, Olvera C, Ivatury R, D'Amours S, Wendon J, Hillman K, Wilmer A. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. II. Recommendations. *Intensive Care Med* 2007; **33**: 951-962

18 **Kirkpatrick AW**, Brenneman FD, McLean RF, Rapanos T, Boulanger BR. Is clinical examination an accurate indicator of raised intra-abdominal pressure in critically injured patients? *Can J Surg* 2000; **43**: 207-211

19 **Leppaniemi A**, Johansson K, De Waele JJ. Abdominal compartment syndrome and acute pancreatitis. *Acta Clin Belg Suppl* 2007; 131-135

20 **Rosas JM**, Soto SN, Aracil JS, Cladera PR, Borlan RH, Sanchez AV, Ros FB, Posa LG. Intra-abdominal pressure as a marker of severity in acute pancreatitis. *Surgery* 2007; **141**: 173-178

21 **Niel-Weise BS**, van den Broek PJ. Urinary catheter policies for short-term bladder drainage in adults. *Cochrane Database Syst Rev* 2005; CD004203

22 **Saint S**, Lipsky BA, Baker PD, McDonald LL, Ossenkop K. Urinary catheters: what type do men and their nurses prefer? *J Am Geriatr Soc* 1999; **47**: 1453-1457

23 **Hatt JK**, Rather PN. Role of bacterial biofilms in urinary tract infections. *Curr Top Microbiol Immunol* 2008; **322**: 163-192

24 **Nazarko L**. Effective evidence based catheter management. *Br J Community Nurs* 2008; **13**: 110, 112-110, 114

25 **Sheng WH**, Wang JT, Lin MS, Chang SC. Risk factors affecting in-hospital mortality in patients with nosocomial infections. *J Formos Med Assoc* 2007; **106**: 110-118

26 **Zhang WF**, Ni YL, Cai L, Li T, Fang XL, Zhang YT. Intra-abdominal pressure monitoring in predicting outcome of patients with severe acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 420-423

S- Editor Li LF **L- Editor** Logan S **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.722

World J Gastroenterol 2009 February 14; 15(6): 722-726
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Outcome of laparoscopic cholecystectomy is not influenced by chronological age in the elderly

Hyung Ook Kim, Jung Won Yun, Jun Ho Shin, Sang Il Hwang, Yong Kyun Cho, Byung Ho Son, Chang Hak Yoo, Yong Lai Park, Hungdai Kim

Hyung Ook Kim, Jun Ho Shin, Sang Il Hwang, Byung Ho Son, Chang Hak Yoo, Yong Lai Park, Hungdai Kim, Departments of Surgery, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul 110-746, South Korea
Jung Won Yun, Yong Kyun Cho, Departments of Internal Medicine, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul 110-746, South Korea
Author contributions: Kim HO and Yun JW contributed equally to the work; Shin JH conceived and designed the study; Cho YK and Hwang SI acquired the data; Kim HO, Yun JW, Hwang SI and Cho YK analyzed and interpreted the data; Kim HO and Yun JW drafted the manuscript; Shin JH, Son BH, Yoo CH, Park YL and Kim H revised the manuscript.
Correspondence to: Jun Ho Shin, MD, PhD, Department of Surgery, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 108, Pyung-Dong, Jongno-Ku, Seoul 110-746, South Korea. junho0521.shin@samsung.com
Telephone: +82-2-20012138 Fax: +82-2-20012131
Received: October 16, 2008 Revised: January 5, 2009
Accepted: January 12, 2009
Published online: February 14, 2009

Abstract

AIM: To evaluate the outcome of laparoscopic cholecystectomy (LC) in patients aged 80 years and older.

METHODS: A total of 353 patients aged 65 to 79 years (group 1) and 35 patients aged 80 years and older (group 2) underwent LC. Patients were further classified into two other groups: those with uncomplicated gallbladder disease (group A) or those with complicated gallbladder disease (group B).

RESULTS: There were no significant differences between the age groups (groups 1 and 2) with respect to clinical characteristics such as age, gender, comorbid disease, or disease presentation. Mean operative time, conversion rate, and the incidence of major postoperative complications were similar in groups 1 and 2. However, the percentage of high-risk patients was significantly higher in group 2 than in group 1 (20.0% *vs* 5.7%, $P < 0.01$). Group A comprised 322 patients with a mean age of 71.0 ± 5.3 years, and group B comprised 51 patients with a mean age of 69.9 ± 4.8 years. In group B, mean operative time (78.4 ± 49.3 min *vs* 58.3 ± 35.8 min, $P < 0.01$), mean postoperative hospital stay (7.9 ± 6.5 d *vs* 5.0 ± 3.7 d, $P < 0.01$), and the incidence of major postoperative complications (9.8% *vs* 3.1%, $P < 0.05$) were significantly greater than in group A. The conversion rate tended to be higher in group B, but this difference was not significant.

CONCLUSION: Perioperative outcomes in elderly patients who underwent LC seem to be influenced by the severity of gallbladder disease, and not by chronologic age. In octogenarians, LC should be performed at an earlier, uncomplicated stage of the disease whenever possible to improve perioperative outcomes.

Key words: Elderly; Laparoscopic cholecystectomy; Octogenarians; Gallbladder; Cholecystitis

Peer reviewer: Yasuji Arase, MD, Department of Gastroenterology, Toranomon Hospital, 2-2-2 Toranomonminato-ku, Tokyo 105-8470, Japan

Kim HO, Yun JW, Shin JH, Hwang SI, Cho YK, Son BH, Yoo CH, Park YL, Kim H. Outcome of laparoscopic cholecystectomy is not influenced by chronological age in the elderly. *World J Gastroenterol* 2009; 15(6): 722-726 Available from: URL: http://www.wjgnet.com/1007-9327/15/722.asp DOI: http://dx.doi.org/10.3748/wjg.15.722

INTRODUCTION

The Korean population is steadily aging. The percentage of the population 65 years of age and older was 5.8% in 1995 and was 9.1% in 2005. This age group is expected to grow from 14.3% in 2018 to 20.8% in 2026[1]. The prevalence of gallstone formation increases with age[2]. The reported incidence of cholelithiasis in the very elderly (80 years and older) is as high as 38%-53%[3,4]. This age group has a high incidence of complicated gallstone disease, such as acute cholecystitis, choledocholithiasis, and gallstone pancreatitis[5]. Laparoscopic cholecystectomy (LC) is currently the

procedure of choice for the management of gallbladder disease[6]. Many reports have demonstrated the relative safety and efficacy of LC, with low conversion rates and low postoperative morbidity compared to open cholecystectomy (OC)[7,8]. Currently, LC is being used with increasing frequency in elderly patients. Advanced age may be associated with increased postoperative complications and high conversion rates[9]. However, as life expectancy continues to increase, octogenarians are becoming a growing proportion of the population undergoing LC. Therefore, the purpose of this study was to evaluate the outcome of LC in patients aged 80 years and older compared to patients aged 65 to 79 years.

MATERIALS AND METHODS

Study group

A retrospective analysis of patients aged 65 years and older who underwent LC from November 1991 to January 2006 at Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea was performed. A total of 353 patients aged 65 to 79 years (group 1) and 35 patients aged 80 years and older (group 2) underwent LC. According to disease presentation, patients were further classified into two other groups: one with uncomplicated gallbladder disease (group A) and one with complicated gallbladder disease (group B). The diagnosis of gallbladder disease was based on a combination of clinical, laboratory, and radiologic findings. The most common imaging techniques used were ultrasonography and computed tomography. The diagnosis was confirmed by pathologic evaluation and surgical inspection. Whenever choledocholithiasis was suspected, preoperative endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography (MRCP) was performed. Endoscopic sphincterotomy with stone extraction was performed as needed. Since March 2004, percutaneous transhepatic gallbladder drainage (PTGBD) has been performed at our institution for patients admitted with suspected complicated acute cholecystitis (gallbladder empyema, gangrenous cholecystitis, perforated acute cholecystitis, emphysematous cholecystitis, and pericholecystic abscess).

Surgical technique

All LC procedures were carried out by one surgeon who had previous experience of more than 3000 laparoscopic cholecystectomies, assisted by a resident. A 10-mm Visiport® trocar (Tyco, Norwalk, USA) was inserted at the subumbilical area as the first port site. Two additional laparoscopic cannulae were inserted; one in the right upper quadrant (5 mm) and the other in the epigastric area (12 mm). A soft silastic drain was used in all patients.

Statistical analysis

The clinical characteristics, duration of LC surgery, conversion rate, complication rate, and postoperative hospital stay were analyzed using a statistical analysis program package (SPSS 15.0, SPSS Inc. Chicago, IL, USA). The results are expressed as mean ± SD. The statistical significance of observed differences was tested using the χ^2 test, Fisher's exact test, or Mann-Whitney test. A probability of 0.05 or less was $P < 0.05$ considered statistically significant.

Table 1 Preoperative clinical characteristics *n* (%)

	Group 1, 65-79 yr (*n* = 353)	Group 2, ≥ 80 yr (*n* = 35)	*P* value
Age	69.8 ± 3.7	82.9 ± 2.9	< 0.001
Male	152	13	0.499
Comorbid disease	166 (47.0)	18 (51.4)	0.619
Diabetes mellitus	67 (19.0)	11 (31.4)	0.080
Hypertension	111 (31.4)	6 (25.7)	0.484
Chronic liver disease	9 (2.5)	1 (2.9)	1.000
Ischemic heart disease	4 (1.1)	0 (0.0)	1.000
Cerebrovascular accident	2 (0.6)	0 (0.0)	1.000
Other	10 (2.8)	1 (2.9)	1.000
Diagnosis			
Uncomplicated gallbladder disease	294 (83.3)	28 (80.0)	0.622
Complicated gallbladder disease	47 (13.3)	4 (11.4)	1.000
Gallbladder cancer	12 (3.4)	3 (8.6)	0.144
Preoperative ERCP	33 (9.3)	4 (11.4)	0.761
CBD stone	29 (8.2)	4 (11.4)	0.522
Prior abdominal surgery	78 (22.1)	8 (22.9)	0.918
Preoperative PTGBD	12 (3.4)	1 (2.9)	1.000
ASA score			< 0.01
1 + 2	333 (94.3)	28 (80.0)	
3 + 4	20 (5.7)	7 (20.0)	

ERCP: Endoscopic retrograde cholangiopancreatography; CBD: Common bile duct; PTGBD: Percutaneous transhepatic gallbladder drainage; ASA: American society of anesthesiologists.

RESULTS

Overview of patients

There were no significant differences among the age groups (group 1 and 2) with respect to clinical characteristics such as age, gender, additional disease, and disease presentation. However, the percentage of high-risk patients was significantly higher in group 2 (20.0%) than in group 1 (5.7%). High risk was defined as an American Society of Anesthesiologists (ASA) score of 3 or 4 (Table 1).

Perioperative outcomes

Three hundred and twenty-two patients (group A) underwent LC for uncomplicated gallbladder disease (recurrent biliary colic, gallbladder polyp, and chronic cholecystitis), 51 patients (group B) underwent LC for complicated gallbladder disease (acute cholecystitis, gallbladder empyema, gangrenous cholecystitis, perforated acute cholecystitis, emphysematous cholecystitis, pericholecystic abscess, biliary pancreatitis, and cholangitis), and the remaining 15 patients underwent LC for gallbladder cancer. Not all gallbladder

Table 2 Perioperative outcome according to age group *n* (%)

	Group 1, 65-79 yr (*n* = 353)	Group 2, ≥ 80 yr (*n* = 35)	*P* value
Operative time (min)	60.8 ± 38.4	82.6 ± 47.6	0.087
Conversions	9 (2.5)	2 (5.7)	0.260
Complications	15 (4.2)	2 (5.7)	0.659
Bile leakage	6	1	
Intraabdominal fluid collection	3	1	
Wound infection	3	0	
Subumbilical wound hernia	1	0	
Bleeding	2	0	
Postoperative hospital stay (d)	5.5 ± 4.2	5.1 ± 4.0	0.873

cancers were diagnosed before surgery. One patient in group 1 had a pT2 tumor and underwent complete radical surgery of the gallbladder bed with lymph node dissection. Preoperative PTGBD was performed for 12 patients in group 1 and for one patient in group 2. The timing of LC in patients with acute cholecystitis was variable (early, delayed, or scheduled after PTGBD) according to clinical factors such as specialist or operating theater availability and the patient's medical condition.

Mean operative time and the conversion rate to OC were similar for the two age groups. The reasons for conversion were difficulty in gallbladder exposure or dissection at the Calot triangle because of dense adhesions. The incidence of major postoperative complications was similar in the two groups; 15 patients in group 1 (4.2%) and 2 patients in group 2 (5.7%). Six patients in group 1 and one patient in group 2 developed bile leakage after surgery. All patients were treated conservatively and the leakage subsided spontaneously within 7 d. Three patients in group 1 and one in group 2 had intraabdominal fluid collection; all patients were treated by ultrasound-guided percutaneous catheter drainage and parenteral antibiotics. Two patients in group 1 presented with bloody discharge from the subhepatic silastic drain on the first postoperative day, but the bleeding was controlled conservatively without blood transfusion. Postoperative wound infection occurred in three patients in group 1. One patient in group 1 developed a subumbilical wound hernia (10 mm cannula wound), which was surgically treated. The length of postoperative hospital stay was similar in the two groups (Table 2).

Group A (uncomplicated gallbladder disease) comprised 322 patients with a mean age of 71.0 ± 5.3 years, and group B (complicated gallbladder disease) comprised 51 patients with a mean age of 69.9 ± 4.8 years. In group B, the mean operative time was 78.4 ± 49.3 min, and this was significantly longer than that in group A (58.3 ± 35.8 min, $P < 0.01$). The incidence of major postoperative complications was significantly higher (9.8% *vs* 3.1%, $P < 0.05$) and mean postoperative hospital stay was significantly longer (7.9 ± 6.5 d *vs* 5.0 ± 3.7 d, $P < 0.01$) in group B compared to group A. The conversion rate tended to be higher in group B (5.9%) compared to group A (2.2%), but this difference was not significant ($P = 0.144$) (Table 3).

Table 3 Perioperative outcome according to disease presentation *n* (%)

	Group A (*n* = 322) Uncomplicated	Group B (*n* = 51) Complicated	*P* value
Age	71.0 ± 5.3	69.9 ± 4.8	0.125
Preoperative WBC count (/mm^3)	7048 ± 2166	11 928 ± 4269	< 0.001
Operative time (min)	58.3 ± 35.8	78.4 ± 49.3	< 0.01
Conversions	7 (2.2)	3 (5.9)	0.144
Complications	10 (3.1)	5 (9.8)	< 0.05
Postoperative hospital stay (d)	5.0 ± 3.7	7.9 ± 6.5	< 0.01

DISCUSSION

Gallbladder disease is the most common indication for abdominal surgery in the elderly[4,10,11]. In Korea, the age distribution of gallstones shows a peak incidence in the seventh decade and common bile duct stones show a peak incidence in the eighth decade[12]. Management of gallstones is important in the elderly because of a high rate of complicated biliary disease, increased postoperative morbidity, and prolonged hospital stay compared to younger patients[13,14]. However, recent reports have documented a mortality rate of 0% after LC in octogenarians[15,16]. In the present study, the mortality rate was 0% in octogenarians, with a 5.7% morbidity rate. This morbidity rate was comparable to that found in other studies (2.2-18.5%)[15-18]. Furthermore, there were no significant differences in operative time, conversion rate, complication rate, and postoperative hospital stay between the two age groups (group 1 *vs* group 2). The only difference observed was in the preoperative ASA score. Kwon *et al*[17] also found that there were no significant differences in the conversion rate, morbidity, mortality, and length of hospital stay between a group aged 65 to 79 years and a group aged ≥ 80 years. However, Pavlidis *et al*[16] reported that LC in the very elderly was associated with a higher conversion rate, increased morbidity, and longer hospital stay. Conversion to OC and postoperative complications may be associated with severe complicated gallbladder disease[19]. In the present study, operative time, postoperative complication rate, and postoperative hospital stay were also greater in patients who underwent LC for complicated gallbladder disease. In uncomplicated gallbladder disease, the overall conversion rate to OC was only 2.2% and the postoperative complication rate was also lower (3.1% *vs* 9.8%). These results may indicate that perioperative outcome is not influenced by chronologic age in the elderly, but is influenced by disease presentation.

When LC is performed in patients with severe cholecystitis, the rate of conversion to open surgery and postoperative complications is usually high. The

rate of conversion to open surgery in cases of severe cholecystitis is 8.7%-35%[20-23]. The complication rate associated with LC performed for acute cholecystitis ranges from 3% to 40%[19-22,24,25]. Therefore, since March 2004, we have adopted a protocol that includes PTGBD in patients admitted with suspected complicated acute cholecystitis (gallbladder empyema, gangrenous cholecystitis, perforated acute cholecystitis, emphysematous cholecystitis and pericholecystic abscess). Twelve patients in group 1 (3.4%) and one patient in group 2 (2.9%) underwent PTGBD before LC, and the conversion rate to OC was zero in both groups. Watanabe *et al*[26] reported that the combination of ultrasonography-guided PTGBD and LC was a safe and effective treatment for patients with acute suppurative cholecystitis. There were age no conversions to OC when LC was performed at a mean of 34.3 d after PTGBD. Moreover, a conversion rate of 3% was observed in patients who underwent surgery for acute cholecystitis 4 d after PTGBD[27]. In the present study, preoperative PTGBD may have had a positive effect on LC in complicated gallbladder disease.

Preoperative ERCP was performed in 33 patients in group 1 (9.3%) and 4 patients in group 2 (11.4%) who had clinical, laboratory, and radiological suspicion of choledocholithiasis, and 29 (87.9%) and 4 (100.0%), respectively, had common bile duct stones. All these patients underwent successful endoscopic sphincterotomy with stone extraction. The reported incidence of choledocholithiasis rises with age (26% and 43% in patients aged 65-79 years and 80-95 years, respectively)[10]. Therefore, preoperative ERCP in the elderly should be performed for patients with clinical, laboratory, and radiological suspicion of choledocholithiasis.

If elderly patients tend to have higher conversion and postoperative complication rates, and a longer postoperative hospital stay, this may be due to a higher incidence of complicated gallbladder disease[15]. In the present study, there was no difference in disease presentation between the two age groups; complicated gallbladder disease was 13.3% in group 1 and 11.4% in group 2. Therefore, despite higher ASA scores in group 2, perioperative outcomes might not be significantly different between the two groups.

In conclusion, perioperative outcomes in the elderly seem to be influenced by the severity of gallbladder disease, and not by chronologic age. In the very elderly, such as octogenarians, LC is also relatively safe, with acceptable morbidity compared to elderly patients younger than 80 years of age, if they have uncomplicated gallbladder disease. Therefore, in this age group, LC should be performed at an earlier, uncomplicated stage of the disease as often as possible to improve perioperative outcomes.

COMMENTS

Background

The Korean population is steadily aging. As life expectancy continues to increase, octogenarians are becoming a growing proportion of the population undergoing laparoscopic cholecystectomy (LC).

Research frontiers

Perioperative outcomes in the elderly seem to be influenced by the severity of gallbladder disease, and not by chronologic age. LC is relatively safe in the very elderly, such as octogenarians.

Related publications

Many reports have demonstrated the relative safety and efficacy of LC, with low conversion rates and low postoperative morbidity compared to open cholecystectomy. Currently, LC is being used with increasing frequency in elderly patients. Advanced age may be associated with increased postoperative complications and high conversion rates.

Innovations and breakthroughs

If elderly patients tend to have higher conversion and postoperative complication rates, and a longer postoperative hospital stay, this may be due to a higher incidence of complicated gallbladder disease.

Applications

In the very elderly, LC should be performed at an earlier, uncomplicated stage of the disease as often as possible to improve perioperative outcomes.

Peer review

The authors reported that outcome of LC is not influenced by chronologic age. This result is interesting.

REFERENCES

1 **Korean National Statistical Office**. Population Projections for Korea: 2005-2050. Korean National Statistical Office, Daejeon, Republic of Korea, 2006
2 **Bennion LJ**, Grundy SM. Risk factors for the development of cholelithiasis in man (second of two parts). *N Engl J Med* 1978; **299**: 1221-1227
3 **Evers BM**, Townsend CM Jr, Thompson JC. Organ physiology of aging. *Surg Clin North Am* 1994; **74**: 23-39
4 **Kahng KU**, Roslyn JJ. Surgical issues for the elderly patient with hepatobiliary disease. *Surg Clin North Am* 1994; **74**: 345-373
5 **Bingener J**, Richards ML, Schwesinger WH, Strodel WE, Sirinek KR. Laparoscopic cholecystectomy for elderly patients: gold standard for golden years? *Arch Surg* 2003; **138**: 531-535; discussion 535-536
6 **Barkun JS**, Barkun AN, Sampalis JS, Fried G, Taylor B, Wexler MJ, Goresky CA, Meakins JL. Randomised controlled trial of laparoscopic versus mini cholecystectomy. The McGill Gallstone Treatment Group. *Lancet* 1992; **340**: 1116-1119
7 **Buanes T**, Mjåland O. Complications in laparoscopic and open cholecystectomy: a prospective comparative trial. *Surg Laparosc Endosc* 1996; **6**: 266-272
8 **Williams LF Jr**, Chapman WC, Bonau RA, McGee EC Jr, Boyd RW, Jacobs JK. Comparison of laparoscopic cholecystectomy with open cholecystectomy in a single center. *Am J Surg* 1993; **165**: 459-465
9 **Fried GM**, Clas D, Meakins JL. Minimally invasive surgery in the elderly patient. *Surg Clin North Am* 1994; **74**: 375-387
10 **Brunt LM**, Quasebarth MA, Dunnegan DL, Soper NJ. Outcomes analysis of laparoscopic cholecystectomy in the extremely elderly. *Surg Endosc* 2001; **15**: 700-705
11 **Shamburek RD**, Farrar JT. Disorders of the digestive system in the elderly. *N Engl J Med* 1990; **322**: 438-443
12 **Kim MH**, Ohrr HC, Chung JB, Kim CD, Kang JK, Koh MS, Kim NJ, Kim DG, Kim SK, Kim YS, Kim YT, Kim JH, Roe IH, Kim YI, Park SH, Seol SY, Shim CS, Yang US, Yeo HS, Rew JS, Yoon YB, Lee SK, Chung MK, Choi SC. Epidemiologic Study on Korean Gallstone Disease a Natiinwide Cooperative Study. *Korean J Gastroenterol* 1998; **32**: 635-647
13 **Magnuson TH**, Ratner LE, Zenilman ME, Bender JS. Laparoscopic cholecystectomy: applicability in the geriatric population. *Am Surg* 1997; **63**: 91-96
14 **Maxwell JG**, Tyler BA, Rutledge R, Brinker CC, Maxwell BG, Covington DL. Cholecystectomy in patients aged 80

and older. *Am J Surg* 1998; **176**: 627-631
15 **Hazzan D**, Geron N, Golijanin D, Reissman P, Shiloni E. Laparoscopic cholecystectomy in octogenarians. *Surg Endosc* 2003; **17**: 773-776
16 **Pavlidis TE**, Marakis GN, Symeonidis N, Psarras K, Ballas K, Rafailidis S, Sakantamis AK. Considerations concerning laparoscopic cholecystectomy in the extremely elderly. *J Laparoendosc Adv Surg Tech A* 2008; **18**: 56-60
17 **Kwon AH**, Matsui Y. Laparoscopic cholecystectomy in patients aged 80 years and over. *World J Surg* 2006; **30**: 1204-1210
18 **Leandros E**, Alexakis N, Archontovasilis F, Albanopoulos K, Dardamanis D, Menenakos E, Tsigris C, Giannopoulos A. Outcome analysis of laparoscopic cholecystectomy in patients aged 80 years and older with complicated gallstone disease. *J Laparoendosc Adv Surg Tech A* 2007; **17**: 731-735
19 **Eldar S**, Sabo E, Nash E, Abrahamson J, Matter I. Laparoscopic cholecystectomy for the various types of gallbladder inflammation: a prospective trial. *Surg Laparosc Endosc* 1998; **8**: 200-207
20 **Habib FA**, Kolachalam RB, Khilnani R, Preventza O, Mittal VK. Role of laparoscopic cholecystectomy in the management of gangrenous cholecystitis. *Am J Surg* 2001; **181**: 71-75
21 **Hunt DR**, Chu FC. Gangrenous cholecystitis in the laparoscopic era. *Aust N Z J Surg* 2000; **70**: 428-430
22 **Kiviluoto T**, Sirén J, Luukkonen P, Kivilaakso E. Randomised trial of laparoscopic versus open cholecystectomy for acute and gangrenous cholecystitis. *Lancet* 1998; **351**: 321-325
23 **Merriam LT**, Kanaan SA, Dawes LG, Angelos P, Prystowsky JB, Rege RV, Joehl RJ. Gangrenous cholecystitis: analysis of risk factors and experience with laparoscopic cholecystectomy. *Surgery* 1999; **126**: 680-685; discussion 685-686
24 **Stevens KA**, Chi A, Lucas LC, Porter JM, Williams MD. Immediate laparoscopic cholecystectomy for acute cholecystitis: no need to wait. *Am J Surg* 2006; **192**: 756-761
25 **Tzovaras G**, Zacharoulis D, Liakou P, Theodoropoulos T, Paroutoglou G, Hatzitheofilou C. Timing of laparoscopic cholecystectomy for acute cholecystitis: a prospective non randomized study. *World J Gastroenterol* 2006; **12**: 5528-5531
26 **Watanabe Y**, Sato M, Abe Y, Iseki S, Sato N, Kimura S. Preceding PTGBD decreases complications of laparoscopic cholecystectomy for patients with acute suppurative cholecystitis. *J Laparoendosc Surg* 1996; **6**: 161-165
27 **Chikamori F**, Kuniyoshi N, Shibuya S, Takase Y. Early scheduled laparoscopic cholecystectomy following percutaneous transhepatic gallbladder drainage for patients with acute cholecystitis. *Surg Endosc* 2002; **16**: 1704-1707

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

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.727

World J Gastroenterol 2009 February 14; 15(6): 727-731
World Journal of Gastroenterology ISSN 1007-9327

Usefulness of anti-ulcer drugs for the prevention and treatment of peptic ulcers induced by low doses of aspirin

Sayaka Nakashima, Shinichi Ota, Shin Arai, Kiyoko Yoshino, Mie Inao, Keiko Ishikawa, Nobuaki Nakayama, Yukinori Imai, Sumiko Nagoshi, Satoshi Mochida

Sayaka Nakashima, Shinichi Ota, Shin Arai, Kiyoko Yoshino, Mie Inao, Keiko Ishikawa, Nobuaki Nakayama, Yukinori Imai, Sumiko Nagoshi, Satoshi Mochida, Department of Gastroenterology and Hepatology Faculty of Medicine, Saitama Medical University, Saitama, Japan

Author contributions: Ota S designed the research; Nakashima S and Yoshino K performed the research; Nakashima S, Yoshino K, Arai S, Ota S and Mochida S analyzed the data; Nakashima S wrote the manuscript; Inao M, Ishikawa K, Nakayama N, Imai Y and Nagoshi S gave suggestions for the descriptions; Mochida S completed the paper; All authors participated in the treatment of patients.

Correspondence to: Satoshi Mochida, MD, PhD, Department of Gastroenterology & Hepatology, Faculty of Medicine, Saitama Medical University, 38 Morohongo, Moroyama-cho, Iruma-gun, Saitama 350-0495, Japan. smochida@saitama-med.ac.jp

Telephone: +81-49-2761198 Fax: +81-49-2761198

Received: October 29, 2008 Revised: January 5, 2009
Accepted: January 12, 2009
Published online: February 14, 2009

Abstract

AIM: To investigate the usefulness of anti-ulcer drugs for the prevention and treatment of low-dose aspirin-induced peptic ulcer.

METHODS: Upper gastrointestinal endoscopy was performed in 68 patients receiving daily low-dose aspirin (81 or 100 mg/day). The endoscopic findings were classified according to the Lanza score, and the scores were compared between groups categorized according to the concomitant use of anti-ulcer drugs and the types of drugs used. In another study, 31 hemorrhagic peptic ulcer patients who had been receiving low-dose aspirin were enrolled. The patients were randomly classified into the proton pump inhibitor (PPI)-treated group and the H2 receptor antagonist (H2RA)-treated group. The administration of low-dose aspirin was continued concomitantly, and endoscopic examinations were performed 8 wk later.

RESULTS: The Lanza scores (mean ± SD) of the gastro-mucosal lesions were 1.0 ± 1.9 and 1.9 ± 2.3 in 8 and 16 patients receiving prevention therapy with a PPI and an H2RA, respectively. Both scores were significantly smaller than the scores in 34 patients who were not receiving prevention therapy (4.7 ± 1.0) and in 10 patients receiving cytoprotective anti-ulcer drugs (4.3 ± 1.6). In the prospective study, 18 and 13 patients received a PPI and an H2RA, respectively. Endoscopic examinations revealed that the tissue in the region of the gastro-mucosal lesions had reverted to normal in all patients in the PPI-treated group and in 12 patients (92%) in the H2RA-treated group; no significant differences were observed between the groups.

CONCLUSION: H2RA therapy was effective for both the prevention and treatment of low-dose aspirin-induced peptic ulcer, similar to the effects of PPIs, while cytoprotective anti-ulcer drugs were ineffective in preventing ulceration.

Key words: Hemorrhagic ulcer; H2 receptor Antagonist; Low-dose aspirin; Peptic ulcer; Proton pump inhibitor

Peer reviewer: Atsushi Nakajima, Professor, Division of Gastroenterology, Yokohama City University Graduate School of Medicine, 3-9 Fuku-ura, Kanazawa-ku, Yokohama 236-0004, Japan

Nakashima S, Ota S, Arai S, Yoshino K, Inao M, Ishikawa K, Nakayama N, Imai Y, Nagoshi S, Mochida S. Usefulness of anti-ulcer drugs for the prevention and treatment of peptic ulcers induced by low doses of aspirin. *World J Gastroenterol* 2009; 15(6): 727-731 Available from: URL: http://www.wjgnet.com/1007-9327/15/727.asp DOI: http://dx.doi.org/10.3748/wjg.15.727

INTRODUCTION

Two major causes of peptic ulcers are infection with *Helicobacter pylori* (*H pylori*) and the administration of non-steroidal anti-inflammatory drugs (NSAIDs). Recently, much attention has been paid to NSAID-induced peptic ulcers, since the trend toward *H pylori* eradication using proton pump inhibitors (PPIs) and antibiotics is likely to reduce the incidence of *H pylori*-induced peptic ulcers in the future. Moreover, the increasing proportion of elderly individuals among the Japanese population is likely to produce a simultaneous increase in the prescription

of NSAIDs for the treatment of pain arising from osteoporosis and/or osteoarthritis. This trend may highlight the significance of NSAID-induced peptic ulcers. Low-dose aspirin has also been shown to induce peptic ulcers, similar to the effects of regular-dose aspirin and other NSAIDs[1]; this finding suggests that NSAID-induced peptic ulcers may become more common among the elderly population, since many patients receive antithrombotic therapy using low-dose aspirin for the treatment of cardiovascular diseases. Previously, we reported that NSAIDs were associated with hemorrhagic peptic ulcers in 28% of the patients seen between 2001 and 2004; the rate of patients receiving low-dose aspirin in this population was 27%, while the rates of patients receiving regular-dose aspirin, loxoprofen, diclofenac and other NSAIDs were 6%, 16%, 10% and 21%, respectively[1]. These data suggest that among the various NSAIDs in use, low-dose aspirin is the most important drug provoking peptic ulcers in Japan.

Infection with *H pylori* is frequently found in patients with NSAID-induced peptic ulcers, including those with low-dose aspirin-induced ulcers. According to our previous survey[1], a total of 82% of patients with hemorrhagic peptic ulcers tested positive for *H pylori* infection; the positivity rate was higher among those not treated with NSAIDs (88.6%) than among those receiving NSAIDs (67.2%). Of note, 62.5% of the patients with hemorrhagic peptic ulcers induced by low-dose aspirin tested positive for *H pylori*[1]. However, whether *H pylori* eradication prevents the development of peptic ulcers induced by low-dose aspirin remains controversial[2]. Chan *et al*[3] reported that both *H pylori* eradication and PPI administration were effective in preventing peptic ulcers induced by low-dose aspirin. On the other hand, Lai *et al*[4] revealed that hemorrhage recurred in more than 10% of the patients with low-dose aspirin-induced peptic ulcers, even after *H pylori* eradication, whereas PPI administration was effective in reducing the risk of recurrence. A therapeutic strategy to prevent peptic ulcers induced by NSAIDs, especially low-dose aspirin, is needed.

In the present study, we evaluated the efficacies of various anti-ulcer drugs (PPIs, H2RA and cytoprotective drugs) for the prevention and treatment of peptic ulcers induced by low-dose aspirin, by comparing the endoscopic findings of the patients to establish a therapeutic strategy for low-dose aspirin-induced peptic ulcers in Japanese patients.

MATERIALS AND METHODS

Study-1

Patients receiving daily low-dose aspirin (81 or 100 mg/day) and undergoing an upper gastrointestinal endoscopy at Saitama Medical University Hospital between February 2001 and September 2006 were enrolled in the study. Patients receiving NSAIDs other than aspirin and/or receiving regular-dose aspirin (from 1.0 to 4.5 g/day) were excluded from the analysis. Patients with malignancies or those who had undergone a gastrectomy were also excluded. The endoscopic findings were classified according to the Lanza score, as show in Table 1[5]. The scores were compared between groups categorized according to the concomitant use of anti-ulcer drugs and the types of drugs used.

Table 1 Classification of endoscopic findings according to the Lanza score[5]

Score	Endoscopic findings
0	No lesion
1	Hemorrhagic erosion
2	One or two erosions
3	3-10 erosions
4	More than 10 erosions
5	Ulcer

Study-2

The subjects comprised hemorrhagic peptic ulcer patients who had been receiving low-dose aspirin daily and who had been admitted to Saitama Medical University Hospital between February 2001 and March 2008. The exclusion criteria were similar to those used in Study-1: patients receiving NSAIDs other than aspirin and/or regular-dose aspirin and those with malignancies or who had undergone a gastrectomy at enrolment. Informed consent was obtained from all patients, and the subjects were randomly classified into two groups. The patients classified as the PPI-treated group were given either lansoprazole (15 or 30 mg, daily), rabeprazole sodium (10, 20 or 40 mg, daily) or omeprazole (20 mg, daily). In contrast, the patients in the H2RA-treated group were given famotidine (40 mg, daily). In all patients, the administration of low-dose aspirin was continued concomitantly with the PPI or famotidine therapy. Infection with *H pylori* was determined using a serum antibody test (AP-960; Scimed Life Systems/Boston Scientific Corp, Natick, MA, USA) or a culture of gastric mucosal specimens obtained during an endoscopic examination. *H pylori* eradication with the administration of lansoprazole (60 mg, daily), amoxicillin hydrate (1500 mg, daily) and clarithromycin (800 mg, daily) was performed for 7 d in patients with a positive infection status within 2 wk of the occurrence of peptic ulcer hemorrhage. The therapeutic efficacy of *H pylori* eradication was assessed by the urea breath test 8 wk later. An upper gastrointestinal endoscopy was performed 8 wk after the initiation of PPI or famotidine therapy. The ulcer was diagnosed as healed once scar formation at the site of the lesion was complete.

Statistical Analysis

The Mann-Whitney *U* test and the Fisher's exact test were used to analyze the data. Statistical significance was defined as $P < 0.05$.

RESULTS

Study-1

Sixty-eight patients (45 men and 23 women) between

Table 2 Anti-ulcer drugs used in patients enrolled in study-1

Type of drug	Drug name	Doses (/day)	Number of patients
None			34
PPIs			8
	Rabeprazole sodium	10 mg	3
	Lansoprazole	30 mg	2
	Lansoprazole	15 mg	1
	Omeprazole	20 mg	2
H2RAs			16
	Famotidine	40 mg	1
	Famotidine	20 mg	8
	Famotidine	10 mg	1
	Lafutidine	20 mg	2
	Nizatidine	300 mg	2
	Ranitidine hydrochloride	75 mg	1
	Cimetidine	100 mg	1
Cytoprotective anti-ulcer drugs			10
	Rebamipide	300 mg	2
	Rebamipide	200 mg	1
	Rebamipide	100 mg	1
	Azulensulfonate sidium + L-Glutamine	2 g	2
	Teprenone	1.5 g	1
	Polaprezinc	150 mg	1
	Sofalcone	300 mg	1
	Alginate sodium	180 mL	1

the ages of 25 and 88 years were enrolled in the study. Thirty-four patients received no anti-ulcer drugs, while 8, 16 and 10 patients were given PPIs, H2RAs and cytoprotective anti-ulcer drugs, respectively. The types and doses of the anti-ulcer drugs are shown in Table 2. Duration of daily low-dose aspirin therapy ranged from 1 to 3650 d, however, therapy duration (days; mean ± SD) did not differ in patients receiving and not receiving anti-ulcer drugs (898 ± 1384 and 1165 ± 1389, respectively). In the case of patients receiving anti-ulcer drugs, the prevention therapies were initiated simultaneously with low-dose aspirin administration. Anti-platelet and anticoagulant drugs were used in 5 and 1 patients receiving and not receiving anti-ulcer drugs, respectively. No differences in age or sex were observed among the patients not treated with anti-ulcer drugs and those receiving PPI, H2RA or cytoprotective anti-ulcer drugs (Table 3). However, the endoscopic scores were significantly smaller in patients receiving PPIs and H2RAs, compared with those receiving cytoprotective anti-ulcer drugs and those not receiving anti-ulcer drugs. A grade 5 endoscopic score was observed in 31 of 34 patients (91%) not receiving anti-ulcer drugs and in 8 of 10 patients (80%) receiving cytoprotective anti-ulcer drugs, while the grades ranged between 0 and 3 in 11 of 16 patients (69%) receiving H2RAs and in 7 of 8 patients (88%) receiving PPIs. The scores in patients receiving PPIs and in those receiving H2RAs were statistically similar. In addition, no differences were observed between the scores in 5 patients receiving regular-dose H2RAs and in 11 patients receiving low-dose H2RAs. Moreover, the scores in patients receiving cytoprotective anti-ulcer drugs and in those not receiving anti-ulcer drugs were similar. One 72-year-old male patient had an endoscopic score of grade 5 despite the use of a PPI. *H pylori* infection was not detected in this patient, but a coronary artery bypass grafting procedure had been performed 39 d prior to the endoscopic examination, and the patient had been receiving daily doses of warfarin in addition to low-dose aspirin since that time.

Study-2

Forty patients were enrolled in the study: 20 patients in the PPI-treatment group and 20 patients in the H2RA-treatment group. An endoscopic examination was performed at 8 wk in 18 patients (13 men and 5 women) aged 69.2 ± 13.4 years (mean ± SD) in the PPI-treatment group and in 13 patients (10 men and 3 women) aged 69.7 ± 9.5 years in the H2RA-treatment group; the 9 remaining patients were transferred to other hospitals prior to endoscopic examination. A positive *H pylori* infection status was seen in 12 and 10 patients (67% and 77%) in the PPI-treatment and H2RA-treatment groups, respectively; *H pylori* infection rate was similar in both groups. *H pylori* eradication was achieved in all patients who had a positive *H pylori* infection status. Endoscopic examinations performed at 8 wk revealed that the peptic ulcers had completely healed in 18 patients (100%) in the PPI-treatment group and in 12 patients (92%) in the H2RA-treatment group; no difference in therapeutic efficacy was seen between the two groups. The peptic ulcer in one patient with a positive *H pylori* infection status in the H2RA-treatment group had not completely healed at 8 wk. This patient had repeatedly received loxoprofen for the treatment of a headache 1 wk prior to the endoscopic examination, suggesting that peptic ulcers might recur in the presence of loxoprofen administration, even if the low-dose aspirin-induced ulcer had healed during famotidine therapy. There were 3 and 6 patients with a negative *H pylori* infection status in the H2RA-treatment and PPI-treatment groups, respectively. Peptic ulcers were completely healed in all these patients.

DISCUSSION

In the US and Europe, drugs such as PPIs[6], prostaglandins[7] and regular-dose H2RAs[8] have been reported to be effective in the prevention of NSAID-induced peptic ulcers, and PPIs and prostaglandins have been reported to effectively attenuate such ulcers[6]. In addition, studies conducted in Hong Kong and the US have revealed that both *H pylori* eradication[3] and PPI treatment[4] are useful in preventing the recurrence of hemorrhagic peptic ulcers induced by low-dose aspirin. In Japan, however, the administration of PPIs, H2RAs and prostaglandins for the prevention of peptic ulcers induced by NSAIDs and low-dose aspirin is not covered by medical insurance, although the use of these drugs for the treatment of peptic ulcers is covered. Among these drugs, PPIs and prostaglandins are widely accepted as the most effective therapeutic agents for NSAID-induced peptic ulcers, however, prostaglandins are seldom used due to their adverse effects such as abdominal pain and diarrhea. Thus, in Japan, many

Table 3 Endoscopic findings classified according to the Lanza score in patients receiving low-dose aspirin with or without anti-ulcer drugs

Number of patients	Sex M:F	Age (yr) (mean ± SD)	Lanza scores (Number of patients)						Mean scores
			0	1	2	3	4	5	
No anti-ulcer drugs									
34	24:10	69.4 ± 11.2	1	0	1	0	1	31	4.74[1]
Cytoprotective drugs									
10	8:2	67.9 ± 8.6	1	0	0	1	0	8	4.30[1]
H2RAs									
16	7:9	67.8 ± 14.0	7	3	1	0	0	5	1.88[1]
At regular doses									
5	2:3	69.6 ± 10.8	2	1	1	0	0	1	1.60[1]
Less than regular doses									
11	5:6	66.9 ± 15.6	5	2	0	0	0	4	2.00[1]
PPIs									
8	6:2	62.0 ± 17.0	6	0	0	1	0	1	1.00[1]

[1]$P < 0.05$ *vs* both no anti-ulcer drugs and cytoprotective drugs, according to the Mann-Whitney *U* test.

physicians usually use PPIs for the treatment of peptic ulcers induced by NSAIDs and low-dose aspirin. For the prevention of peptic ulcers, cytoprotective anti-ulcer drugs other than prostaglandins are commonly used, although no evidence supporting this use has been reported in the medical literature. Therefore, a therapeutic strategy for the treatment and prevention of peptic ulcers induced by NSAIDs, especially low-dose aspirin, should be established for Japanese patients.

Thus, we performed a retrospective study (Study-1) to clarify the efficacy of various anti-ulcer drugs to prevent peptic ulcers induced by low-dose aspirin and a prospective study (Study-2) to compare the therapeutic efficacies of PPIs and H2RAs for treating such ulcers. The retrospective study revealed that H2RAs effectively prevented peptic ulcers induced by low-dose aspirin to a degree similar to that of PPIs, however, cytoprotective drugs were not effective in preventing peptic ulcers. To our surprise, H2RA administration at doses less than the regular dose was also effective in preventing low-dose aspirin-induced peptic ulcers. Furthermore, the prospective study demonstrated that the therapeutic efficacies of PPIs and H2RAs at regular doses were almost equivalent. The secretion of gastric acids from the gastric mucosa has been shown to be lower in Japanese patients than in European and American patients[9,10], since marked atrophy of the gastric mucosa is often found in many Japanese patients due to the prevalence of *H pylori* infection. Thus, low-dose H2RAs, such as famotidine 20 mg daily, seem to be effective in the prevention of peptic ulcers induced by low-dose aspirin, and regular-dose H2RAs, such as famotidine 40 mg, may be sufficient to treat such peptic ulcers in Japanese patients. However, it should be noted that grade-5 endoscopic findings were observed in a 72-year-old patient in Study-1 receiving both warfarin and low-dose aspirin despite concomitant prevention therapy with a PPI. In addition, peptic ulcer healing did not occur in one patient receiving loxoprofen as well as low-dose aspirin in the H2RA-treated group in Study-2. The usefulness of H2RAs for the treatment and prevention of low-dose aspirin-induced peptic ulcers should be further investigated, focusing on elderly patients and those receiving warfarin as well as NSAIDs[11]. Moreover, the therapeutic and prevention efficacy of H2RA should be studied in relation to *H pylori* infection status, therapeutic effect of *H pylori* infection, history of upper gastrointestinal diseases and the types of peptic ulcer such as acute and chronic disorders in future research.

In conclusion, H2RA therapy was effective for both the prevention and treatment of low-dose aspirin-induced peptic ulcers, similar to the effects of PPIs, while cytoprotective anti-ulcer drugs were ineffective in preventing peptic ulcers. Considering the cost and adverse effects of PPIs and prostaglandins, H2RA may be the most beneficial anti-ulcer drug for the prevention and treatment of peptic ulcers induced by low-dose aspirin in Japan.

ACKNOWLEDGMENTS

We thank Dr. Michio Shiibashi (Medical Information Center, Saitama Medical University) and Dr. Hiromi Bamba (Medical Research Center, Saitama Medical University) for their technical help.

COMMENTS

Background

The incidence of low-dose aspirin-induced peptic ulcer seems to be increasing in Japan in conjunction with the increasing proportion of elderly individuals, in whom metabolic syndrome frequently develops. However, a therapeutic and prevention strategy for such peptic ulcers has not yet been established.

Research frontiers

The effect of *Helicobacter pylori* (*H pylori*) eradication in the prevention of peptic ulcers induced by low-dose aspirin remains controversial. Chan *et al*[3] reported that both *H pylori* eradication and proton pump inhibitor (PPI) administration were effective in preventing peptic ulcers induced by low-dose aspirin. On the other hand, Lai *et al*[4] revealed that hemorrhage recurred in more than 10% of patients with low-dose aspirin-induced peptic ulcers, even after *H pylori* eradication, whereas PPI administration was effective in reducing the risk of recurrence.

Innovations and breakthroughs

H2 receptor antagonist (H2RA) was effective in both the prevention and treatment of low-dose aspirin-induced peptic ulcers, similar to the effects of

PPIs, while cytoprotective anti-ulcer drugs were ineffective in preventing ulcers.

Applications

Considering the cost and adverse effects of PPIs and prostaglandins, H2RAs may be the most beneficial anti-ulcer drugs for the prevention and treatment of peptic ulcers induced by low-dose aspirin in Japan.

Terminology

Low-dose aspirin: aspirin at regular doses (from 1.0 to 4.5 g/day) is administered to patients with fever, headache and arthralgia. In contrast, daily low-dose aspirin (81 or 100 mg/day) is used as antithrombotic therapy for patients with cardiovascular diseases.

Peer review

The incidence of low-dose aspirin-induced peptic ulcers is increasing in Japan, but the evidence is still lacking. In this paper, the authors evaluated the efficacies of various anti-ulcer drugs for the prevention and treatment of low-dose aspirin-induced peptic ulcers. The author concluded that the efficacies of H2RAs and PPIs for the prevention and treatment of L-Asp-induced peptic ulcers.

REFERENCES

1 **Nakashima S**, Arai S, Mizuno Y, Yoshino K, Ando S, Nakamura Y, Sugawara K, Koike M, Saito E, Naito M, Nakao M, Ito H, Hamaoka K, Rai F, Asakura Y, Akamatu M, Fujimori K, Inao M, Imai Y, Ota S, Fujiwara K, Shiibashi M. A clinical study of Japanese patients with ulcer induced by low-dose aspirin and other non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther* 2005; **21** Suppl 2: 60-66

2 **Sung JJ**. Should we eradicate Helicobacter pylori in non-steroidal anti-inflammatory drug users? *Aliment Pharmacol Ther* 2004; **20** Suppl 2: 65-70

3 **Chan FK**, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, Wu JC, Lau JY, Hui Y, Lai MS, Chan HL, Sung JJ. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. *N Engl J Med* 2001; **344**: 967-973

4 **Lai KC**, Lam SK, Chu KM, Wong BC, Hui WM, Hu WH, Lau GK, Wong WM, Yuen MF, Chan AO, Lai CL, Wong J. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. *N Engl J Med* 2002; **346**: 2033-2038

5 **Lanza F**, Peace K, Gustitus L, Rack MF, Dickson B. A blinded endoscopic comparative study of misoprostol versus sucralfate and placebo in the prevention of aspirin-induced gastric and duodenal ulceration. *Am J Gastroenterol* 1988; **83**: 143-146

6 **Hawkey CJ**, Karrasch JA, Szczepanski L, Walker DG, Barkun A, Swannell AJ, Yeomans ND. Omeprazole compared with misoprostol for ulcers associated with nonsteroidal antiinflammatory drugs. Omeprazole versus Misoprostol for NSAID-induced Ulcer Management (OMNIUM) Study Group. *N Engl J Med* 1998; **338**: 727-734

7 **Graham DY**, Agrawal NM, Roth SH. Prevention of NSAID-induced gastric ulcer with misoprostol: multicentre, double-blind, placebo-controlled trial. *Lancet* 1988; **2**: 1277-1280

8 **Taha AS**, Hudson N, Hawkey CJ, Swannell AJ, Trye PN, Cottrell J, Mann SG, Simon TJ, Sturrock RD, Russell RI. Famotidine for the prevention of gastric and duodenal ulcers caused by nonsteroidal antiinflammatory drugs. *N Engl J Med* 1996; **334**: 1435-1439

9 **Tahir H**, Sumii K, Haruma K, Tari A, Uemura N, Shimizu H, Sumioka M, Inaba Y, Kumamoto T, Matsumoto Y. A statistical evaluation on the age and sex distribution of basal serum gastrin and gastric acid secretion in subjects with or without peptic ulcer disease. *Hiroshima J Med Sci* 1984; **33**: 125-130

10 **Feldman M**, Richardson CT, Lam SK, Samloff IM. Comparison of gastric acid secretion rates and serum pepsinogen I and II concentrations in Occidental and Oriental duodenal ulcer patients. *Gastroenterology* 1988; **95**: 630-635

11 **Wolfe MM**, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1999; **340**: 1888-1899

S- Editor Li LF **L- Editor** Webster JR **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.732

World J Gastroenterol 2009 February 14; 15(6): 732-736
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Establishment of an animal model of ischemic type intrahepatic biliary lesion in rabbits

Qin-Song Sheng, Da-Zhi Chen, Ren Lang, Qiang He, Yong-Jiu Yang, Zhao-Wei Qu, De-Fang Zhao, Xiao-Sheng Zhang

Qin-Song Sheng, Da-Zhi Chen, Ren Lang, Qiang He, Yong-Jiu Yang, Zhao-Wei Qu, De-Fang Zhao, Xiao-Sheng Zhang, Department of Hepatobiliary and Pancreatosplenic Surgery, Beijing Chaoyang Hospital affiliated to the Capital Medical University, Beijing 100020, China

Author contributions: Chen DZ, Sheng QS, Yang YJ and Qu ZW performed the operation; He Q, Lang R, Zhao DF and Zhang XS assisted in the reference search and data analysis; Sheng QS wrote the paper.

Correspondence to: Da-Zhi Chen, Department of Hepatobiliary and Pancreatosplenic Surgery, Beijing Chaoyang Hospital affiliated to the Capital Medical University, Beijing 100020, China. chendazhi@medmail.com.cn

Telephone: +86-10-85231503 Fax: +86-10-85231503

Received: September 16, 2008 Revised: December 7, 2008
Accepted: December 14, 2008
Published online: February 14, 2009

Abstract

AIM: To explore a method to establish an animal model of ischemic type intrahepatic biliary lesion in rabbits.

METHODS: Forty Japanese white rabbits of clean grade were divided randomly into four groups (10 rabbits per group) including sham operation (SO) group, and artery-bile obstruction (ABO)-1 h group, ABO-2 h group and ABO-3 h group. All the rabbits in this study underwent the same initial surgical procedure in which the liver was prepared as for graft removal during liver transplantation. Subsequently in the SO group, no additional vascular intervention was performed, while in groups ABO-1 h, ABO-2 h and ABO-3 h, the animals underwent combined clamping of the hepatic artery and common bile duct with microvascular clips for 1, 2 and 3 h, respectively. After the scheduled occlusion time, the clip was removed to recover blood supply. The animals were killed 4 wk after operation. The survival rate, liver function, cholangiography and histopathological manifestation of the rabbits in each group were observed.

RESULTS: The survival rate was 100% in groups SO, ABO-1 h and ABO-2 h, while it was 60% in group ABO-3 h. At each observation time, the change degree of the indexes of liver function was proportional to the clamping time (ABO-3 h > ABO-2 h > ABO-1 h > SO, $P < 0.05$). Cholangiographical and histopathologic manifestations both showed that intrahepatic biliary lesion aggravated proportionally with the increase of the clamping time.

CONCLUSION: An animal model of ischemic type intrahepatic biliary lesion in rabbits is successfully established, which may provide a reliable technique for basic and clinical research into the etiology, development and prophylaxis of ischemic type intrahepatic biliary lesion after liver transplantation.

Key words: Biliary complication; Ischemic type biliary lesion; Animal model; Liver transplantation; Intrahepatic biliary stricture; Ischemic reperfusion injury

Peer reviewers: Paul J Ciclitira, Professor, The Rayne Institute (GKT), St Thomas' hospital, London NW32QG, United Kingdom; Hongjin Huang, PhD, Celera Diagnostics, 1401 Harbor Bay Parkway, Alameda, California 94502, United States

Sheng QS, Chen DZ, Lang R, He Q, Yang YJ, Qu ZW, Zhao DF, Zhang XS. Establishment of an animal model of ischemic type intrahepatic biliary lesion in rabbits. *World J Gastroenterol* 2009; 15(6): 732-736 Available from: URL: http://www.wjgnet.com/1007-9327/15/732.asp DOI: http://dx.doi.org/10.3748/wjg.15.732

INTRODUCTION

Biliary complications, which occur at a rate of approximately 6%-35%[1], have long been recognized as a major cause of morbidity and graft failure in patients after orthotopic liver transplantation (OLT). The most troublesome is the so-called ischemic type biliary lesion (ITBL), with an incidence varying between 5% and 15%[2]. ITBL is a special biliary complication with non-anastomotic biliary tree destruction, which is one of the most important reasons for liver re-transplantation. For intrahepatic ITBL, especially in the presence of extensive intrahepatic ITBL, endoscopic and radiological techniques and surgical approaches are usually unsuccessful and re-transplantation is mostly unavoidable[2]. Therefore, it is urgent to establish an animal model of ischemic type intrahepatic biliary lesion to study the etiology, development and prophylaxis

of ITBL. In the present study, by combined clamping of the common bile duct and hepatic artery for 2 h, producing the biliary ischemia reperfusion injury, and raising the animals for 4 wk, an animal model of ischemic type intrahepatic biliary lesion in rabbits was successfully established.

MATERIALS AND METHODS

Animals and groups

Animal care and experimental procedures were carried out strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and the related ethical regulations of our university. Forty Japanese white rabbits of clean grade, weighing 2.0-2.5 kg, were selected (provided by Institute of Laboratory Animal Science), irrespective of male or female gender. All of the rabbits were raised under the same condition including a temperature of 18-23℃, the relative humidity of 50%-60%, 12 h diurnal rhythm and freedom to eat and drink. The rabbits were divided randomly into four groups (10 rabbits per group) including sham operation (SO) group, and artery-bile obstruction (ABO)-1 h group, ABO-2 h group and ABO-3 h group.

Establishment of an animal model

The rabbits were prohibited to eat for 12 h and drink for 6 h before operation. They were anesthetized by injecting 1% pentobarbital (1-2 mL/kg) intravenously. The skin was prepared and disinfected routinely. Then, a median incision of the epigastrium about 6 cm in length was formed. All the rabbits in this study underwent the same initial surgical procedure in which the liver was prepared as for graft removal during liver transplantation. Upon the completion of this procedure, the liver was isolated from all vascular supply except for the main hepatic artery, the extra-hepatic peribiliary plexus and the portal vein. In the SO group, no additional vascular intervention was performed. In groups ABO-1 h, ABO-2 h and ABO-3 h, the animals underwent combined clamping of the hepatic artery and common bile duct with microvascular clips for 1, 2 and 3 h, respectively. After the scheduled occlusion time, the clip was removed to recover blood supply. All the animals from these groups were killed 4 wk after operation.

Observation indexes

Survival rate: The animals in each group were raised for 4 wk after operation, and the survival rates of each group were observed.

Examination of liver function: biochemical liver tests were monitored at different time points up to 4 wk including preoperation, and the 1 d, the 1, 2, 3 and 4 wk post-operation. Serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), total bilirubin (TBIL) and direct bilirubin (DBIL) were measured, using standard analytical methods.

Cholangiography: Four weeks after operation, all the rabbits in the four groups underwent cholangiography. Under routine anesthesia, the abdominal cavity was opened according to the former incision. Three to five milliliters of 30% meglucamine diatrizoate solution was injected slowly through the distal end of the common bile duct, and a X-ray photograph was taken to observe the intrahepatic biliary lesion.

Histopathologic examination: After cholangiography, the animals were killed to collect liver tissue samples at the hepatic hilum for histopathological examination. Serial 4-μm-thick sections of formalin-fixed, paraffin-embedded liver tissues were stained with hematoxylin-eosin.

Statistical analysis

Quantitative data were shown as mean ± SD. SPSS statistical software 13.0 was used to conduct analysis of variance of multiple means. For all analyses, a P value less than 0.05 was considered statistically significant.

RESULTS

Survival rates

The animals in groups SO, ABO-1 h and ABO-2 h lived throughout the period of the experiment with a survival rate of 100%. However, four rabbits in group ABO-3 h died on the days 5, 7, 12 and 14 after operation, respectively, with a survival rate of 60%. The causes of death included hepatic ischemic necrosis, bile leakage and abdominal infection, which was supported by autopsy and histopathological examination.

Examination of liver function

All the biochemical indexes (AST, ALT, ALP, GGT, TBIL and DBIL) in groups ABO-1 h, ABO-2 h and ABO-3 h increased with different degrees after operation. No biochemical abnormality was observed in group SO during the entire follow-up. The indexes reached a peak on the day 1 after operation, and then decreased gradually. At the end of the observation period (4 wk after operation), all biochemical abnormalities were spontaneously resolved except for the ALP and GGT in group ABO-2 h and ABO-3 h, which were still higher than that in group SO. At each observation time, the change in the indexes was proportional to clamping time (ABO-3 h > ABO-2 h > ABO-1 h > SO, $P < 0.05$) (Table 1).

Cholangiography

Four weeks after operation, all the rabbits in the four groups underwent cholangiography. The images of the intra-hepatic bile duct indicated that it was normal and no intrahepatic biliary lesion was visualized in groups of SO and ABO-1 h. However, a biliary lesion was observed obviously in groups ABO-2 h and ABO-3 h. It showed that intrahepatic biliary lesion aggravated proportionally with the increase in the clamping time (Figure 1).

Table 1 The change of biochemical indexes of rabbits in all groups (mean ± SD)

Groups	Preoperation	1 d post-operation	1 wk post-operation	2 wk post-operation	3 wk post-operation	4 wk post-operation
AST (U/L)						
SO	38.3 ± 2.4	38.1 ± 2.6	38.0 ± 2.4	38.7 ± 2.3	38.5 ± 2.1	39.1 ± 3.3
ABO-1 h	38.2 ± 2.3	260.3 ± 19.5[1]	85.0 ± 11.1[1]	39.0 ± 1.6	38.2 ± 2.5	39.0 ± 3.3
ABO-2 h	40.1 ± 1.7	359.9 ± 11.9[2]	154.8 ± 14.4[2]	86.9 ± 5.7[2]	39.1 ± 1.7	38.8 ± 1.9
ABO-3 h	39.8 ± 4.6	461.9 ± 11.4[3]	199.2 ± 16.9[3]	112.0 ± 16.7[3]	82.7 ± 9.7[3]	40.1 ± 3.6
ALT (U/L)						
SO	42.2 ± 2.2	42.0 ± 2.3	43.2 ± 2.0	40.0 ± 3.7	40.7 ± 3.4	42.0 ± 2.3
ABO-1 h	42.0 ± 2.4	200.5 ± 13.0[1]	169.8 ± 14.3[1]	65.9 ± 11.5[1]	42.3 ± 2.8	43.2 ± 2.0
ABO-2 h	43.9 ± 2.3	400.1 ± 12.3[2]	200.2 ± 12.0[2]	92.2 ± 8.6[2]	42.7 ± 3.1	42.2 ± 3.7
ABO-3 h	43.8 ± 4.6	505.9 ± 14.0[3]	292.0 ± 15.8[3]	116.6 ± 9.0[3]	87.7 ± 8.8[3]	44.6 ± 2.5
ALP (U/L)						
SO	117.0 ± 14.7	115.9 ± 15.9	114.3 ± 14.8	110.3 ± 9.6	113.3 ± 12.7	111.4 ± 11.7
ABO-1 h	117.6 ± 14.5	203.9 ± 18.8[1]	139.8 ± 10.3[1]	119.6 ± 9.0[1]	112.5 ± 9.3	109.5 ± 7.5
ABO-2 h	118.8 ± 14.2	304.8 ± 16.8[2]	173.1 ± 11.2[2]	136.2 ± 6.6[2]	138.8 ± 6.3[2]	139.6 ± 7.4[2]
ABO-3 h	113.9 ± 11.7	440.0 ± 29.4[3]	226.0 ± 13.7[3]	183.7 ± 9.8[3]	149.4 ± 10.2[3]	149.7 ± 11.5[3]
GGT (U/L)						
SO	16.7 ± 2.1	17.5 ± 2.5	18.0 ± 2.1	18.5 ± 2.6	18.8 ± 3.9	17.3 ± 2.3
ABO-1 h	18.7 ± 2.2	62.8 ± 10.2[1]	20.3 ± 3.4	17.3 ± 2.8	19.7 ± 3.5	17.5 ± 2.5
ABO-2 h	17.7 ± 2.8	111.5 ± 9.2[2]	106.5 ± 11.3[2]	81.9 ± 7.6[2]	84.7 ± 9.4[2]	59.6 ± 13.6[2]
ABO-3 h	17.9 ± 3.0	214.6 ± 18.6[3]	182.5 ± 8.6[3]	153.7 ± 14.4[3]	155.9 ± 19.3[3]	98.0 ± 6.8[3]
TBIL (μmol/L)						
SO	10.0 ± 2.0	9.5 ± 1.7	10.4 ± 2.0	10.1 ± 1.9	10.6 ± 2.2	10.8 ± 2.3
ABO-1 h	10.1 ± 1.8	21.0 ± 3.1[1]	10.6 ± 2.4	9.8 ± 1.6	9.5 ± 2.0	10.5 ± 2.3
ABO-2 h	9.9 ± 2.2	30.7 ± 2.3[2]	19.8 ± 1.9[2]	10.5 ± 2.2	9.3 ± 1.5	9.9 ± 2.0
ABO-3 h	9 .7 ± 1.9	40.7 ± 4.3[3]	30.6 ± 2.4[3]	18.1 ± 2.2[3]	10.3 ± 1.8	11.2 ± 2.3
DBIL (μmol/L)						
SO	3.9 ± 1.0	3.8 ± 1.5	3.9 ± 1.3	4.9 ± 1.6	4.5 ± 1.1	5.5 ± 1.2
ABO-1 h	4.7 ± 1.3	13.5 ± 2.5[1]	4.2 ± 1.3	4.8 ± 0.8	4.1 ± 1.2	4.6 ± 0.8
ABO-2 h	4.3 ± 1.3	20.4 ± 2.8[2]	13.4 ± 2.2[2]	4.9 ± 1.1	3.9 ± 1.1	4.6 ± 1.3
ABO-3 h	4.5 ± 1.1	33.7 ± 3.0[3]	19.6 ± 1.7[3]	11.2 ± 2.0[3]	5.0 ± 1.4	4.8 ± 1.5

[1]ABO-1 h *vs* SO, $P < 0.05$; [2]ABO-2 h *vs* ABO-1 h, $P < 0.05$; [3]ABO-3 h *vs* ABO-2 h, $P < 0.05$.

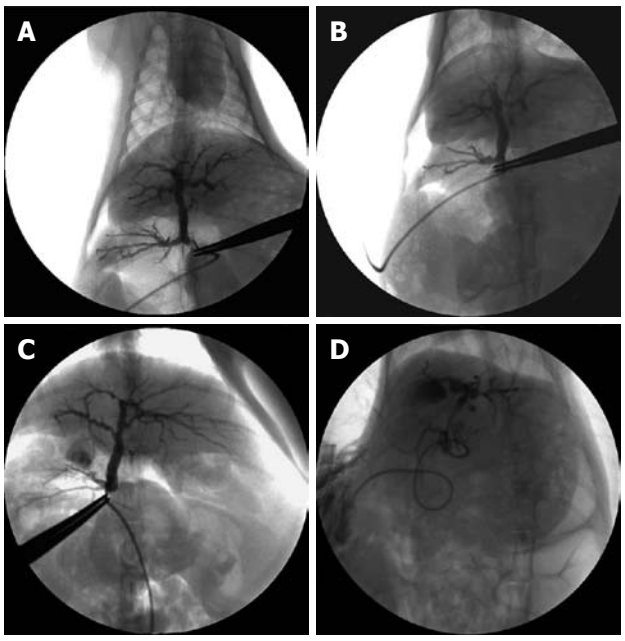


Figure 1 Cholangiography of the four groups 4 wk after operation. A (SO): The image of the intra-hepatic bile duct was normal. No biliary stricture or dilation was observed; B (ABO-1 h): The image of the intrahepatic bile duct resembled that of SO, no intrahepatic biliary lesion was visualized either; C (ABO-2 h): All the branches of the intrahepatic bile duct appeared loose, with the "string beads manifestation" in part of them; D (ABO-3 h): The image of the intrahepatic bile duct was obviously abnormal, with a loose appearance and severe damage.

Histopathological examination

After cholangiography, the sample of liver tissues at the hepatic hilum was taken for histopathological examination. The results indicated that the morphology of the intrahepatic bile duct epithelial cells was normal and there was no cell necrosis in groups SO and ABO-1 h. However, epithelial cells were obviously damaged and sloughed into the bile duct lumen in groups ABO-2 h and ABO-3 h. It also showed that the intrahepatic biliary lesion was aggravated proportionally with the clamping time (Figure 2).

DISCUSSION

ITBL is defined as non-anastomotic destruction of the graft's biliary tree after OLT and characterized by the formation of sludge or stone, bile duct destruction, and even non-function of the allograft[3,4]. A classification of ITBL has been proposed based on the localization of the abnormalities, distinguishing type I (extrahepatic lesions), type Ⅱ (intrahepatic lesions) and type Ⅲ (intra- and extrahepatic alterations)[1]. For type Ⅱ and type Ⅲ, especially for multiple and diffuse ITBL, there is poor prognosis and graft survival, with the result of liver function failure and inevitable re-transplantation in most patients. Therefore, it is of clinical significance to establish an animal model of ischemic type intrahepatic

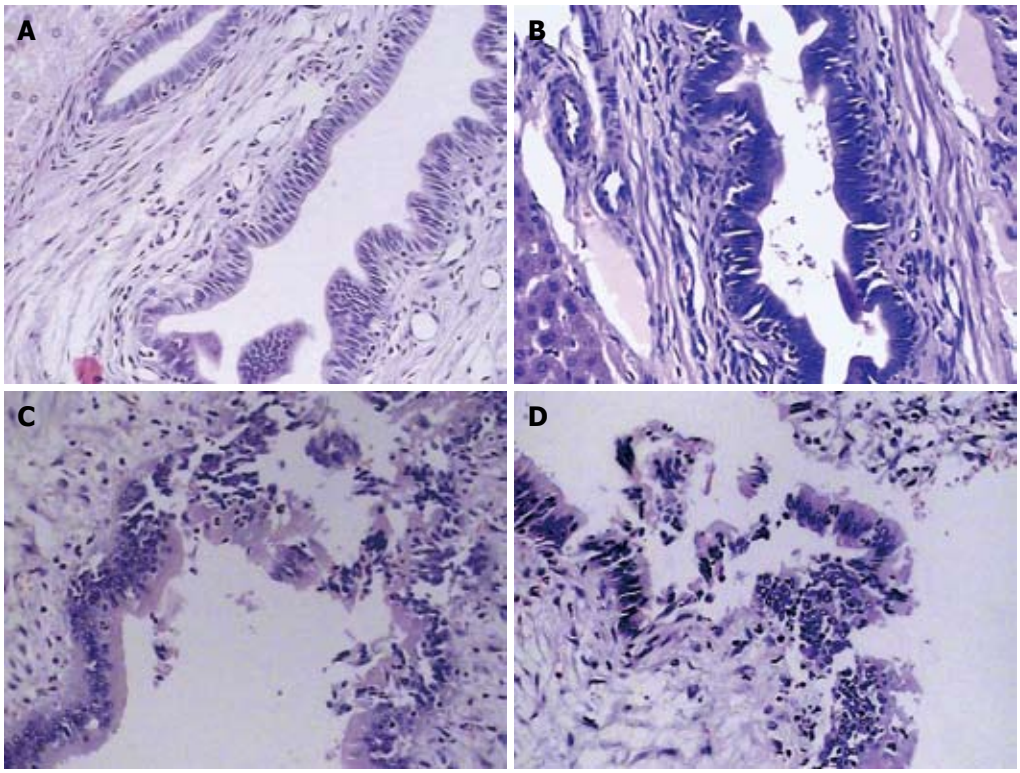


Figure 2 Histopathological manifestations of the four groups 4 wk after operation. A (SO): The intrahepatic bile duct epithelial cells were eumorphic and no cell necrosis was observed; B (ABO-1 h): The intrahepatic bile duct epithelial cells were almost normal. Small amounts of bile duct epithelial cells sloughed into the bile duct lumen; C (ABO-2 h): The intrahepatic bile duct epithelial cells were damaged obviously, and some of them were necrotic and sloughed into the bile duct lumen; D (ABO-3 h): The intrahepatic bile duct epithelial cells were damaged severely; many of them were necrotic and sloughed into the bile duct lumen. The normal epithelial structures disappeared (HE, × 200).

biliary lesion for studying the etiology, development and prophylaxis of ITBL.

Over the past years, several risk factors of this complication have been identified, strongly suggesting a multi-factorial origin, although the exact pathophysiological mechanism of ITBL is still unknown. The generally accepted risk factors include prolonged cold ischemic time warm ischemic time, reperfusion injury, disturbed blood flow in the peribiliary vascular plexus, ABO incompatibility, cytomegalovirus infection, chemokine polymorphism CCR5 delta 32, and bile salt-induced injury[2,5]. Obviously, ischemic injury is of vital importance for the occurrence of ITBL.

Several studies[6-8] have suggested that the peribiliary plexus (PBP) and hepatic artery branches are both the blood supply of the intrahepatic bile duct. Furthermore, it is demonstrated by scanning electron microscopy that there is no arterio-portal anastomosis in the liver of rabbits[9]. That is to say, the intrahepatic bile duct of rabbits do not receive their blood supply from portal vein. Therefore, in this study, by combined clamping of common bile duct and hepatic artery, as well as isolating the liver from all peripheral vascular connections, the blood supply of the intrahepatic bile duct was occluded nearly completely. After removing the clip, the intrahepatic bile duct underwent warm ischemia-reperfusion, which better simulated the clinical procedure of intrahepatic biliary warm ischemia and reperfusion injury in liver transplantation.

In this study, with the increase of the clamping time (1 h, 2 h, 3 h), it was found that intrahepatic biliary lesions were aggravated proportionally, as observed by biochemical indexes, cholangiography and histopathologicial examination. As for the clamping time, in group ABO-1 h, biliary lesions were mild and no imaging and histopathological changes were found, while in group ABO-3 h, the success rate of the animal model was only 60%, in spite of the obvious intrahepatic biliary lesion. However, significant biliary lesions and a high success rate of the model (100%) were both observed in group ABO-2 h. Therefore, combined clamping of the common bile duct and hepatic artery for 2 h was considered the optimal clamping time to establish the model of ischemic-type intrahepatic biliary lesion in rabbits.

Generally, the time from biliary ischemic necrosis and fibrosis to stricture is about 30 d after clinical liver transplantation. Zhao *et al*[10] established an animal model of biliary ischemic stenosis with clamping in mice and observed the significant extrahepatic biliary ischemic stenosis on day 21 after operation. Therefore, the time interval of 4 wk was chosen in this study for the initial observation time. It was not confirmed that the animals in group ABO-2 h underwent more significant intrahepatic stricture when prolonging the observation time after operation.

Overall, the advantages of this animal model included the following. (1) By combined clamping of the common bile duct and hepatic artery, as well as isolating the liver from all peripheral vascular connections, the intrahepatic bile duct is in complete warm ischemia, which can better reflect the clinical procedure of intrahepatic biliary warm ischemia and reperfusion injury in liver transplantation. (2) Surgery with occlusion of the common bile duct and hepatic artery and without that of portal vein, is easy to perform, and has less trauma and higher survival rate. (3) The common bile duct and hepatic artery of all the animals are clamped by the same microvascular clip, which can control

the strength and time precisely. (4) The animal model excluded the influence of other surgical operations (biliary anastomosis, hepatic artery anastomosis, T tube detaining), rejection, biliary cold conservation and drug toxicity. (5) The intrahepatic biliary anatomy, structure and microcirculation of the rabbits are similar to those of humans. Moreover, this animal is cheap and easy to obtain sufficient samples[11].

In conclusion, in the present study, by combined clamping of the common bile duct and hepatic artery for 2 h, producing the biliary ischemia-reperfusion injury, and raising the rabbits for 4 wk, the animal model of ischemic-type intrahepatic biliary lesion in rabbits was successfully established, which may provide a reliable technique for basic and clinical research into the etiology, development and prophylaxis of ischemic type intrahepatic biliary lesion after liver transplantation.

COMMENTS

Background

Biliary complications are a major cause of morbidity and graft failure in patients after orthotopic liver transplantation (OLT). The most troublesome is the so-called ischemic type biliary lesion (ITBL), which is one of the most important reasons for liver re-transplantation. Therefore, it is of clinical significance to establish an animal model of ischemic type intrahepatic biliary lesion for studying the etiology, development and prophylaxis of ITBL.

Research frontier

ITBL, with an incidence varying between 5% and 15% after OLT, is defined as non-anastomotic destruction of the graft's biliary tree after OLT. Although the exact pathophysiological mechanism of ITBL is still unknown, several risk factors of this often cumbersome complication have been identified, strongly suggesting a multi-factorial origin. Therefore, the etiology, development and prophylaxis of ITBL have been research hotspots.

Innovations and breakthrough

This animal model of ITBL is easy to establish, and has less trauma and a higher survival rate. Moreover, it can better reflect the clinical procedure of intrahepatic biliary warm ischemia and reperfusion injury in liver transplantation. In addition, the model excluded the influence of other operations, rejection and biliary cold conservation.

Applications

This animal model of ITBL may provide a reliable technique for basic and clinical research into the etiology, development and prophylaxis of ischemic type intrahepatic biliary lesion after liver transplantation.

Peer review

The manuscript is of interest as a measure of assessing ischemia of the liver and bile ducts, and the methods appear acceptable. It is an interesting study.

REFERENCES

1 **Verdonk RC**, Buis CI, Porte RJ, Haagsma EB. Biliary complications after liver transplantation: a review. *Scand J Gastroenterol Suppl* 2006; **(243)**: 89-101

2 **Buis CI**, Hoekstra H, Verdonk RC, Porte RJ. Causes and consequences of ischemic-type biliary lesions after liver transplantation. *J Hepatobiliary Pancreat Surg* 2006; **13**: 517-524

3 **Boraschi P**, Donati F, Gigoni R, Urbani L, Femia M, Cossu MC, Filipponi F, Falaschi F. Ischemic-type biliary lesions in liver transplant recipients: evaluation with magnetic resonance cholangiography. *Transplant Proc* 2004; **36**: 2744-2747

4 **Hoffman A**, Kiesslich R, Moench C, Bittinger F, Otto G, Galle PR, Neurath MF. Methylene blue-aided cholangioscopy unravels the endoscopic features of ischemic-type biliary lesions after liver transplantation. *Gastrointest Endosc* 2007; **66**: 1052-1058

5 **Moench C**, Uhrig A, Lohse AW, Otto G. CC chemokine receptor 5delta32 polymorphism-a risk factor for ischemic-type biliary lesions following orthotopic liver transplantation. *Liver Transpl* 2004; **10**: 434-439

6 **Beaussier M**, Wendum D, Fouassier L, Rey C, Barbu V, Lasnier E, Lienhart A, Scoazec JY, Rosmorduc O, Housset C. Adaptative bile duct proliferative response in experimental bile duct ischemia. *J Hepatol* 2005; **42**: 257-265

7 **Gaudio E**, Franchitto A, Pannarale L, Carpino G, Alpini G, Francis H, Glaser S, Alvaro D, Onori P. Cholangiocytes and blood supply. *World J Gastroenterol* 2006; **12**: 3546-3552

8 **Nishida S**, Nakamura N, Kadono J, Komokata T, Sakata R, Madariaga JR, Tzakis AG. Intrahepatic biliary strictures after liver transplantation. *J Hepatobiliary Pancreat Surg* 2006; **13**: 511-516

9 **Motta PM**. The three-dimensional microanatomy of the liver. *Arch Histol Jpn* 1984; **47**: 1-30

10 **Zhao DF**, Chen DZ, Lv JS, Lang R, Jin ZK, Qing H. Establishment of an animal model of biliary ischemic stenosis with clamping in mice. *Transplant Proc* 2008; **40**: 1303-1305

11 **Kanoria S**, Glantzounis G, Jalan R, Davies NA, Seifalian AM, Williams R, Davidson BR. A model to study total hepatic ischemia-reperfusion injury. *Transplant Proc* 2004; **36**: 2586-2589

S- Editor Li LF **L- Editor** Ma JY **E- Editor** Zheng XM

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.737

World J Gastroenterol 2009 February 14; 15(6): 737-741
World Journal of Gastroenterology ISSN 1007-9327

Synergetic anticancer effect of combined gemcitabine and photodynamic therapy on pancreatic cancer *in vivo*

Qi Xie, Lin Jia, Yan-Hong Liu, Cheng-Gang Wei

Qi Xie, Cheng-Gang Wei, Department of Radiology, Nan Sha Center Hospital, Guangzhou Municipal First People's Hospital, Guangzhou Medical College, Guangzhou 510180, Guangdong Province, China
Lin Jia, Yan-Hong Liu, Department of Digestive Diseases, Guangzhou Municipal First People's Hospital, Guangzhou Medical College, Guangzhou 510180, Guangdong Province, China
Author contributions: Xie Q and Jia L designed the research; Liu YH performed the research; Jia L, Liu YH and Wei CG analyzed the data; Xie Q, Jia L and Wei CG wrote the paper.
Correspondence to: Dr. Lin Jia, Department of Digestive Diseases, Guangzhou Municipal First People's Hospital, Guangzhou Medical College, No. 1 Panfu Road, Guangzhou 510180, Guangdong Province, China. jialin@medmail.com.cn
Telephone: +86-20-81628678 Fax: +86-20-81628809
Received: July 31, 2008 Revised: December 31, 2008
Accepted: January 7, 2009
Published online: February 14, 2009

Abstract

AIM: To investigate the anti-tumor effects of combined cytotoxic drug (gemcitabine) and photodynamic therapy (PDT) on human pancreatic cancer xenograft in nude mice.

METHODS: Human pancreatic cancer cell line SW1990 was used in the investigation of the *in vivo* effect of combined gemcitabine and PDT on human pancreatic cancer xenograft in mice. Sixty mice were randomly allocated into a control group (without treatment), photosensitizer treatment group (2 mg/kg photosan, without illumination), chemotherapy group (50 mg/kg gemcitabine i.p.), PDT group (2 mg/kg photosan + laser irradiation) and combined treatment group (photosan + chemotherapy), with 12 mice in each group. Tumor size was measured twice every week. Anti-tumor activity in different groups was evaluated by tumor growth inhibition (TGI).

RESULTS: No significant anti-tumor effect was observed either in photosensitizer treatment group or in chemotherapy group. PDT led to necrosis in cancer lesions and significantly reduced tumor volume compared with photosensitizer on day 6 and at the following time points after initialization of therapy (0.24 ± 0.15-0.49 ± 0.08 *vs* 0.43 ± 0.18-1.25 ± 0.09, $P < 0.05$). PDT significantly reduced tumor volume in combined treatment group compared with photosensitizer treatment group (0.12 ± 0.07-0.28 ± 0.12 *vs* 0.39 ± 0.15-1.20 ± 0.11, $P < 0.05$), small dose chemotherapy group (0.12 ± 0.07-0.28 ± 0.12 *vs* 0.32 ± 0.14-1.16 ± 0.08, $P < 0.05$) and control group (0.12 ± 0.07-0.28 ± 0.12 *vs* 0.43 ± 0.18-1.25 ± 0.09, $P < 0.05$). TGI was higher in the combined treatment group (82.42%) than in the PDT group (58.18%).

CONCLUSION: PDT has a significant anti-tumor effect, which is maintained for a short time and can be significantly enhanced by small doses of gemcitabine.

Key words: Pancreatic carcinoma; Nude mice; Animal model; Photodynamic therapy; Gemcitabine

Peer reviewer: Dr. Terumi Kamisawa, Department of Internal Medicine, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo, Japan

Xie Q, Jia L, Liu YH, Wei CG. Synergetic anticancer effect of combined gemcitabine and photodynamic therapy on pancreatic cancer *in vivo*. *World J Gastroenterol* 2009; 15(6): 737-741 Available from: URL: http://www.wjgnet.com/1007-9327/15/737.asp DOI: http://dx.doi.org/10.3748/wjg.15.737

INTRODUCTION

Pancreatic cancer remains a lethal disease. Most pancreatic cancer patients are at advanced stage when apparent symptoms occur. Pancreatic cancer patients undergoing resection at the time of initial diagnosis account for less than 20%[1], and the 5-year survival rates after complete and partial pancreatic resection are 15%-25%[2-3] and 8%-14%[4-5], respectively. Treatment modalities for inoperable patients are largely limited to radiotherapy, chemotherapy, or their combination. Pancreas is a retroperitoneal organ, with stomach, intestine, and spinal cord around it. Since the sensitivity of pancreatic cancer to radiotherapy is poor, and the tolerant dosage of pancreas tissue is low, radiotherapy does not lead to a convincing beneficial effect on survival. Chemotherapy is the main therapeutic method for advanced pancreatic cancer. 5-fluorouacil is probably

the most useful single agent for symptomatic relief[6]. Gemcitabine may also have a value for palliation[7-9]. Overall, the prognosis of pancreatic cancer patients is poor with a 1-year survival rate of about 10%[10]. Therefore, new treatment modalities are urgently needed.

Photodynamic therapy (PDT) produces localized tissue necrosis with light (most conveniently from a laser), after administration of a photosensitizing agent in the presence of oxygen[10-12], based on the use of photosensitizing compounds that localize quite selectively in neoplastic/hyperplastic tissues and become cytotoxic when exposed to light[12-14]. In view of the antitumor effect of single treatment, PDT is local. PDT in combination with surgery[15], radiotherapy[16] or chemotherapy[17], has become a subject of research. New photosensitizers with improved spectroscopic, photochemical and tissue-localizing properties and improved laser instrumentation have stimulated attempts to establish clinical protocols for incorporation of PDT into multi-treatment modalities[18-24].

Most studies on PDT to date have been on lesions of the skin or in the wall of hollow organs, but recent interest is more in its potential for treating lesions of solid organs such as the pancreas[10,11,20]. We have undertaken experiments on treating pancreatic cancer of mice with different doses of gemcitabine[25]. The results indicate that the growth of transplanted tumors can be inhibited by gemcitabine at 100 mg/kg, which shows severe side effects such as diarrhea, dehydration and loss of weight[25]. When gemcitabine at 100 mg/kg is used, the growth of transplanted tumors could not be controlled[25]. On the other hand, combined angiogenetic inhibitors can decrease side effects of gemcitabine, meanwhile the growth and metastasis of transplanted tumors are effectively inhibited[25].

In this study, gemcitabine was used as a cytotoxic drug. The cytotoxic and antitumor effects of combined gemcitabine and PDT were evaluated. Human pancreatic cancer cell line SW1990 was used in experiments to assess the effect of gemcitabine or PDT, or their combination, on pancreatic cancer in accordance with institutional guidelines.

MATERIALS AND METHODS

Tumor line, animals and drugs

SW1990, a high transferred human pancreatic cancer cell line (ATCC, Kyriazis MD, USA), was maintained in Laboratory of Sun Yat-Sen Memorial Hospital and serially passed in nude mice. Five- to six-week-old male BALB/c nude mice were obtained from Animal Center Sun Yat-Sen University. Photosan, a second generation of photosensitizer, was provided by Diolitec Pharmaceutical Company (Germany). Gemcitabine was provided by Lilly Pharmaceutical Company (USA).

Animal model and therapeutic group

Two male nude mice (6 wk of age) were inoculated subcutaneously with 0.5×10^7 SW1990 cells. Tumors in subcutaneous tissue were excised and tumor tissue was implanted subcutaneously in 60 nude mice.

A tumor model was established and observed for 10-14 d after implantation of tumor tissue. Tumor-bearing nude mice were divided into control group (group A), photosensitizer group (group B), chemotherapy group (group C), PDT group (group D), and combined group (group E), with 12 mice in each group.

Forty-eight hours after the mice had photosan, tumor masses in mice of the PDT group and combined group were exposed to light (λ = 630 nm, 120 J/cm^2) from a PDT630 semiconductor laser (Diolitec Pharmaceutical Company) for 20 min. Gemcitabine (50 mg/kg) was injected into the peritoneal cavity of mice in the combined group 1 h prior to light exposure and on days 3, 6 and 9 after light exposure. The same dose of gemcitabine was given to mice in the chemotherapy group at the same time point as in the combined group.

Data collection

All experimental mice were weighed and tumor diameters were measured with vernier calipers before treatment and twice a week after treatment. On day 21 after treatment, all experimental mice were sacrificed with their tumors removed and weighed to obtain tumor weight (TW). Tumor volume (TV) was determined according to the formula: $TV = 3/4 \times \pi \times (b/2)^2 \times a/2$, where a is the length and b is the height of the tumor. Antitumor activity was evaluated by tumor growth inhibition (TGI), calculated according to the formula: $TGI = (1 - TW_T/TW_C) \times 100\%$ in treated (T) and control (C) mice.

Statistical analysis

Data analysis was performed using SPSS11.0 statistical package (SPSS, Chicago, USA). Tumor response to treatment was compared using two-way ANOVA and Student-Neuman-Keuls test. $P < 0.05$ was considered statistically significant.

RESULTS

Tumor volume

Tumor volume increased with time after treatment in the photosensitizer group, small dose chemotherapy group and control group (Table 1 and Figure 1). Tumor volume had no significant difference at the same time point in three groups (ANOVA).

PDT led to necrosis in cancer lesions. Partial tumor necrotic tissue was exfoliated and a necrotic edge of volcano-like uplift was formed 1 wk after treatment. Tumor volume was significantly reduced in PDT treatment group compared with the photosensitizer treatment group and control group at different time points after initialization of therapy (ANOVA, $P < 0.05$). No significant difference in tumor volume was found on days 3, 6, 9 and 12, but tumor volume increased significantly on days 15, 18 and 21 ($P < 0.05$) in the PDT group after treatment.

Table1 Tumor volume in different groups after treatment with PDT and/or gemcitabine (cm^3) (mean ± SE)

Groups	Pre-therapy	3 d	6 d	9 d	12 d	15 d	18 d	21 d	*P*
A	0.14 ± 0.09	0.26 ± 0.13	0.43 ± 0.18	0.56 ± 0.23	0.66 ± 0.23	0.80 ± 0.10	1.01 ± 0.12	1.25 ± 0.09	< 0.01
B	0.12 ± 0.06	0.26 ± 0.11	0.39 ± 0.15	0.51 ± 0.18	0.62 ± 0.17	0.75 ± 0.09	0.93 ± 0.08	1.20 ± 0.11	< 0.01
C	0.13 ± 0.07	0.23 ± 0.10	0.32 ± 0.14	0.44 ± 0.14	0.57 ± 0.12	0.72 ± 0.10	0.91 ± 0.12	1.16 ± 0.08	< 0.01
D	0.14 ± 0.08	0.22 ± 0.12	0.24 ± 0.15	0.24 ± 0.16	0.28 ± 0.12	0.35 ± 0.10	0.42 ± 0.12	0.49 ± 0.08	< 0.01
E	0.12 ± 0.07	0.13 ± 0.09	0.14 ± 0.10	0.15 ± 0.09	0.17 ± 0.08	0.18 ± 0.10	0.22 ± 0.10	0.28 ± 0.12	< 0.01
P	0.951	0.038	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	

Variance-test, $P > 0.05$.

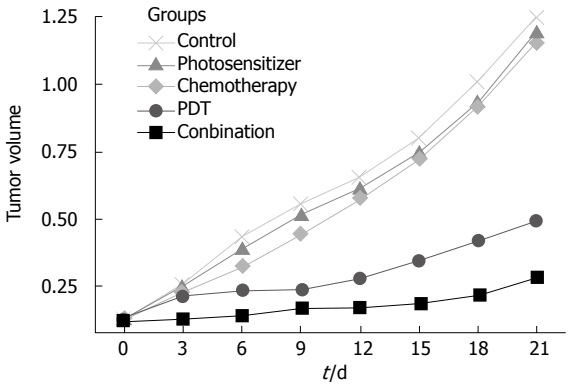


Figure 1 Tumor volume in different groups after treatment with PDT and/or gemcitabine (cm^3) (mean ± SE).

Tumor volume was significantly decreased in the combined PDT and gemcitabine treatment group compared with photosensitizer treatment group, small dose chemotherapy group and control group ($P < 0.05$). Tumor volume was significantly smaller in the combined treatment group than in the PDT group at different time points after treatment.

Tumor weight and TGI

Tumor weight and TGI of mice in five groups are listed in Table 2. Tumor weights of mice in the combination group (0.29 ± 0.20 g) and PDT group (0.69 ± 0.23 g) were significantly lower than those in the photosensitizer treatment group (1.62 ± 0.12 g), chemotherapy group (1.37 ± 0.19 g) and control group (1.65 ± 0.21 g) ($P < 0.01$). Tumor weights of mice in the combined group were obviously lower than those in the PDT group ($P < 0.01$). The mean tumor weight of mice in the two groups was significantly different ($P < 0.01$). Tumor weights were not significantly different in the photosensitizer treatment group, small dose chemotherapy group and control group. TGI (82.42%) was higher in the combined treatment group than in the PDT treatment group (58.18%).

Toxicity

The mice in four experimental groups had a loss of weight during the experiments. The weight in the four experimental groups was not significantly different from that in the control group.

Four treatment modalities did not induce signs of toxicity such as diarrhea and vomiting. No treatment-related deaths occurred. Mice in the PDT group and combined treatment group had no skin photosensitivity to light.

Table 2 Tumor weight (g) and TGI (%) response to PDT and/or gemcitabine

Group	Animals (*n*)	TW (mean ± SE)	TGI
A	12	1.65 ± 0.21	-
B	12	1.62 ± 0.12	1.80
C	12	1.37 ± 0.19	17.00
D	12	0.69 ± 0.23	58.18
E	12	0.29 ± 0.20	82.42
P	-	< 0.01	-

Variance-test, $P = 0.1$.

DISCUSSION

PDT may be defined as a treatment based on an oxygen-dependent reaction between a photosensitizing dye and light, that is, the light combination of two absolutely non-toxic elements, drug and light, in the presence of oxygen, can result in selective destruction of tissue[11,26]. The technique consists of administration of a tumor-localizing photosensitizing agent, which most often requires metabolic synthesis followed by activation of the agent by light with a specified wavelength. Photosensitizing agents used in PDT are macromolecular materials, which contribute to preferential location in neoplastic tissues and delay clearance of neoplastic tissues[27]. Therefore, PDT aims at a sequence of photochemical and photobiological processes that cause irreversible damage to tumor tissues and little damage to connective tissues, and maintain the mechanical integrity of organs[12,24]. It was reported that PDT has a selective effect on malignant pancreas but no significant effect on normal pancreas, and could well match other treatment modalities, except for radical surgery[28]. In a pilot clinical trail, Bown *et al*[10] used PDT in the palliative treatment of 16 patients with cancers in the pancreatic head that could not be treated with surgery because of the advanced nature of the disease or the general condition of the patients, and they concluded that PDT may be of value for treating localized cancers in patients who are poor candidates for definitive surgery or in whom the location of tumor makes pancreatic resection inappropriate. Abulafi *et al*[29] and Tseng *et al*[30] reported that patients with pancreatic and ampullary carcinoma

who are not suitable for surgery should be treated with PDT, since PDT is both feasible and safe for small tumors.

PDT, on the other hand, has some disadvantages and limitations. Little information is currently available concerning the uptake of photosensitizer by pancreas or pancreatic cancer. Local spread of photodynamic agents to vital organs is common, and may cause perforation of the duodenum and jejunum, leading to death after treatment with PDT[11,31]. In addition, large tumor mass limits PDT to penetrate into the effective depth of tissue and needs multiple interstitial optical fibers to increase its volume[32]. Therefore, the ability of chemotherapeutic agents to enhance the effects of PDT in cell culture and transplantable mouse tumors has been studied by several groups[20-22]. Kirveliene *et al*[20] used murine hepatoma MH-22A to investigate *in vitro* and *in vivo* cytotoxic and anti-tumor effects of doxorubicin (Dox), a conventional anticancer drug, and PDT, showing that Dox potentiates therapeutic efficacy of PDT and *vice versa*, and that the degree of potentiation is influenced by Dox. Peterson *et al*[21] and Snyder *et al*[22] reported that combined treatment with PDT and Dox is more effective than treatment with either PDT or Dox alone. *In vitro* studies have revealed a significant effect of fluoropyrimidines[18] and mitomycin C[19] on the viability of mTHPC-photosensitized cells. Some studies focused on the effects of PDT on pancreas cancer[10,11]. As we know, no study about the effect of therapy with photodynamic-cytotoxic agents on pancreatic cancer has been reported.

Gemcitabine is an active nucleoside analogue against a wide variety of cancers, including non-small cell lung cancer and pancreatic cancer[7-9]. Gemcitabine, acting as an antimetabolite, can inhibit ribonucleotide reductase and DNA synthesis, and induce apoptosis[33]. Gemcitabine today remains a first-line drug for patients with advanced pancreatic cancer[7-9]. However, it has a narrow therapeutic index due to rapid enzyme deamination by deoxytidine deaminase into its corresponding inactive uracil derivative, and can also induce drug resistance[34,35]. Therefore, a high dose of gemcitabine is needed to achieve the desired therapeutic response with different adverse effects[36]. To overcome such drawbacks, based on the relation between quantity and effect of gemcitabine[25], we used photosan and gemcitabine as representatives of photosensitizing and cytotoxic agents to investigate the cytotoxic and antitumor effects of gemcitabine and PDT on pancreatic cancer *in vivo*. The results indicate that small dose gemcitabine or photosensitizer can not inhibit the growth of pancreatic cancer. PDT had a significant anti-tumor effect, but lasted a short time. Photodynamic-cytotoxic therapy (small dose gemcitabine) could significantly inhibit the growth of pancreatic cancer, and showed a relative long-duration anti-tumor effect compared with PDT. The inhibition rate of photodynamic-cytotoxic agents and PDT for tumors was 82.42% and 58.18%, respectively. The four treatment modalities did not induce any signs of toxicity such as diarrhea and vomiting. No treatment-related death occurred. Animals in the PDT group and combined treatment group had no skin photosensitivity to light.

In conclusion, low dose gemcitabine increases the anticancer effect of PDT with no obvious adverse effects. Combined PDT and gemcitabine can be used in treatment of pancreatic cancer in patients who are poor candidates for surgery.

ACKNOWLEDGMENTS

The authors thank Wen-Ge Huang and Fen-Fen Guo for their excellent technical assistance.

COMMENTS

Background

Most patients with pancreatic cancer are at advanced stage when apparent symptoms occur. Treatment of pancreatic cancer remains a great challenge, and new treatment modalities are urgently needed. In this study, gemcitabine was used as a cytotoxic drug, and cytotoxic and antitumor effects of combined gemcitabine and photodynamic therapy (PDT) were evaluated.

Research frontiers

Gemcitabine may have a value in treatment of pancreatic cancer. Recently, more studies on PDT have been focused on solid pancreatic caner. The prognosis of pancreatic cancer is poor. This is the first report on the synergetic anticancer effect of combined gemcitabine and PDT on pancreatic cancer *in vivo*.

Innovations and breakthroughs

The results of this study indicate that PDT-cytotoxic therapy (small dose gemcitabine) could significantly inhibit the growth of pancreatic cancer, and showed a relative long duration of anti-tumor effect compared with PDT. This study first demonstrated that low dose gemcitabine could increase the anticancer effect of PDT with no obvious adverse effects.

Applications

Combined PDT and gemcitabine therapy can be used in treatment of patients who are poor candidates for surgery.

Terminology

PDT is a way to produce local tissue necrosis with light (most conveniently from a laser) after administration of a photosensitizing agent in the presence of oxygen. PDT is based on the use of photosensitizing compounds that localize quite selectively in neoplastic/hyperplastic tissues and become cytotoxic when exposed to light.

Peer review

The study revealed that PDT can significantly inhibit the growth of pancreatic cancer, and its effect could be significantly enhanced by a small dose of gemcitabine. The findings are of great interest and provide a foundation for its application in clinical practice. The data are reliable and valuable.

REFERENCES

1 **Kern S**, Hruban R, Hollingsworth MA, Brand R, Adrian TE, Jaffee E, Tempero MA. A white paper: the product of a pancreas cancer think tank. *Cancer Res* 2001; **61**: 4923-4932

2 **Sohn TA**, Yeo CJ, Cameron JL, Koniaris L, Kaushal S, Abrams RA, Sauter PK, Coleman J, Hruban RH, Lillemoe KD. Resected adenocarcinoma of the pancreas-616 patients: results, outcomes, and prognostic indicators. *J Gastrointest Surg* 2000; **4**: 567-579

3 **Richter A**, Niedergethmann M, Sturm JW, Lorenz D, Post S, Trede M. Long-term results of partial pancreaticoduodenectomy for ductal adenocarcinoma of the pancreatic head: 25-year experience. *World J Surg* 2003; **27**: 324-329

4 **Dalton RR**, Sarr MG, van Heerden JA, Colby TV. Carcinoma of the body and tail of the pancreas: is curative resection justified? *Surgery* 1992; **111**: 489-494

5 **Brennan MF**, Moccia RD, Klimstra D. Management of adenocarcinoma of the body and tail of the pancreas. *Ann Surg* 1996; **223**: 506-511; discussion 511-512
6 **DiMagno EP**, Reber HA, Tempero MA. AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. American Gastroenterological Association. *Gastroenterology* 1999; **117**: 1464-1484
7 **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
8 **Cardenes HR**, Chiorean EG, Dewitt J, Schmidt M, Loehrer P. Locally advanced pancreatic cancer: current therapeutic approach. *Oncologist* 2006; **11**: 612-623
9 **Haefner M**, Bluethner T, Niederhagen M, Moebius C, Wittekind C, Mossner J, Caca K, Wiedmann M. Experimental treatment of pancreatic cancer with two novel histone deacetylase inhibitors. *World J Gastroenterol* 2008; **14**: 3681-3692
10 **Bown SG**, Rogowska AZ, Whitelaw DE, Lees WR, Lovat LB, Ripley P, Jones L, Wyld P, Gillams A, Hatfield AW. Photodynamic therapy for cancer of the pancreas. *Gut* 2002; **50**: 549-557
11 **Fan BG**, Andren-Sandberg A. Photodynamic therapy for pancreatic cancer. *Pancreas* 2007; **34**: 385-389
12 **Dougherty TJ**, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, Moan J, Peng Q. Photodynamic therapy. *J Natl Cancer Inst* 1998; **90**: 889-905
13 **Ayaru L**, Wittmann J, Macrobert AJ, Novelli M, Bown SG, Pereira SP. Photodynamic therapy using verteporfin photosensitization in the pancreas and surrounding tissues in the Syrian golden hamster. *Pancreatology* 2007; **7**: 20-27
14 **Dolmans DE**, Fukumura D, Jain RK. Photodynamic therapy for cancer. *Nat Rev Cancer* 2003; **3**: 380-387
15 **Herrera-Ornelas L**, Petrelli NJ, Mittelman A, Dougherty TJ, Boyle DG. Photodynamic therapy in patients with colorectal cancer. *Cancer* 1986; **57**: 677-684
16 **Kostron H**, Fritsch E, Grunert V. Photodynamic therapy of malignant brain tumours: a phase I/II trial. *Br J Neurosurg* 1988; **2**: 241-248
17 **Gonzalez S**, Arnfield MR, Meeker BE, Tulip J, Lakey WH, Chapman JD, McPhee MS. Treatment of Dunning R3327-AT rat prostate tumors with photodynamic therapy in combination with misonidazole. *Cancer Res* 1986; **46**: 2858-2862
18 **Zimmermann A**, Walt H, Haller U, Baas P, Klein SD. Effects of chlorin-mediated photodynamic therapy combined with fluoropyrimidines in vitro and in a patient. *Cancer Chemother Pharmacol* 2003; **51**: 147-154
19 **van Geel IP**, Oppelaar H, Oussoren YG, Schuitmaker JJ, Stewart FA. Mechanisms for optimising photodynamic therapy: second-generation photosensitisers in combination with mitomycin C. *Br J Cancer* 1995; **72**: 344-350
20 **Kirveliene V**, Grazeliene G, Dabkeviciene D, Micke I, Kirvelis D, Juodka B, Didziapetriene J. Schedule-dependent interaction between Doxorubicin and mTHPC-mediated photodynamic therapy in murine hepatoma in vitro and in vivo. *Cancer Chemother Pharmacol* 2006; **57**: 65-72
21 **Peterson CM**, Shiah JG, Sun Y, Kopeckova P, Minko T, Straight RC, Kopecek J. HPMA copolymer delivery of chemotherapy and photodynamic therapy in ovarian cancer. *Adv Exp Med Biol* 2003; **519**: 101-123
22 **Snyder JW**, Greco WR, Bellnier DA, Vaughan L, Henderson BW. Photodynamic therapy: a means to enhanced drug delivery to tumors. *Cancer Res* 2003; **63**: 8126-8131
23 **Streckyte G**, Didziapetriene J, Grazeliene G, Prasmickiene G, Sukeliene D, Kazlauskaite N, Characiejus D, Griciute L, Rotomskis R. Effects of photodynamic therapy in combination with Adriamycin. *Cancer Lett* 1999; **146**: 73-86
24 **Hopper C**. Photodynamic therapy: a clinical reality in the treatment of cancer. *Lancet Oncol* 2000; **1**: 212-219
25 **Jia L**, Zhang MH, Yuan SZ, Huang WG. Antiangiogenic therapy for human pancreatic carcinoma xenografts in nude mice. *World J Gastroenterol* 2005; **11**: 447-450
26 **Sheng C**, Pogue BW, Wang E, Hutchins JE, Hoopes PJ. Assessment of photosensitizer dosimetry and tissue damage assay for photodynamic therapy in advanced-stage tumors. *Photochem Photobiol* 2004; **79**: 520-525
27 **Laftavi MR**, Lens P, Margonari J, Cathingol D, Dubernard JM, Martin X. Photodynamic therapy can selectively eradicate pancreas exocrine secretion. *Transplant Proc* 1998; **30**: 596-598
28 **Bown SG**, Lovat LB. The biology of photodynamic therapy in the gastrointestinal tract. *Gastrointest Endosc Clin N Am* 2000; **10**: 533-550
29 **Abulafi AM**, Allardice JT, Williams NS, van Someren N, Swain CP, Ainley C. Photodynamic therapy for malignant tumours of the ampulla of Vater. *Gut* 1995; **36**: 853-856
30 **Tseng WW**, Saxton RE, Deganutti A, Liu CD. Infrared laser activation of indocyanine green inhibits growth in human pancreatic cancer. *Pancreas* 2003; **27**: e42-e45
31 **Schroder T**, Chen IW, Sperling M, Bell RH Jr, Brackett K, Joffe SN. Hematoporphyrin derivative uptake and photodynamic therapy in pancreatic carcinoma. *J Surg Oncol* 1988; **38**: 4-9
32 **Wilson BC**. Potential applications of photodynamic therapy in regenerative medicine. *J Craniofac Surg* 2003; **14**: 278-283
33 **Cappella P**, Tomasoni D, Faretta M, Lupi M, Montalenti F, Viale F, Banzato F, D'Incalci M, Ubezio P. Cell cycle effects of gemcitabine. *Int J Cancer* 2001; **93**: 401-408
34 **Reid JM**, Qu W, Safgren SL, Ames MM, Krailo MD, Seibel NL, Kuttesch J, Holcenberg J. Phase I trial and pharmacokinetics of gemcitabine in children with advanced solid tumors. *J Clin Oncol* 2004; **22**: 2445-2451
35 **Moog R**, Burger AM, Brandl M, Schuler J, Schubert R, Unger C, Fiebig HH, Massing U. Change in pharmacokinetic and pharmacodynamic behavior of gemcitabine in human tumor xenografts upon entrapment in vesicular phospholipid gels. *Cancer Chemother Pharmacol* 2002; **49**: 356-366
36 **Reddy LH**, Khoury H, Paci A, Deroussent A, Ferreira H, Dubernet C, Decleves X, Besnard M, Chacun H, Lepetre-Mouelhi S, Desmaele D, Rousseau B, Laugier C, Cintrat JC, Vassal G, Couvreur P. Squalenoylation favorably modifies the in vivo pharmacokinetics and biodistribution of gemcitabine in mice. *Drug Metab Dispos* 2008; **36**: 1570-1577

S- Editor Tian L **L- Editor** Wang XL **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.742

World J Gastroenterol 2009 February 14; 15(6): 742-747
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Diagnosis of chest pain with foregut symptoms in Chinese patients

Bo Deng, Ru-Wen Wang, Yao-Guang Jiang, Qun-You Tan, Xiang-Li Liao, Jing-Hai Zhou, Yun-Ping Zhao, Tai-Qian Gong, Zheng Ma

Bo Deng, Ru-Wen Wang, Yao-Guang Jiang, Qun-You Tan, Xiang-Li Liao, Jing-Hai Zhou, Yun-Ping Zhao, Tai-Qian Gong, Zheng Ma, Thoracic Surgery Department, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China
Author contributions: Deng B, Wang RW and Jiang YG diagnosed patients, analyzed the data and wrote the manuscript; Tan QY, Liao XL, Zhou JH, Zhao YP, Gong TQ and Ma Z collected the data.
Correspondence to: Ru-Wen Wang, MD, Thoracic Surgery Department, Institute of Surgery Research, Daping Hospital, The Third Military Medical University, Chongqing 400042, China. superdb@163.com
Telephone: +86-23-68757983 Fax: +86-23-68890331
Received: September 18, 2008 Revised: November 16, 2008
Accepted: November 23, 2008
Published online: February 14, 2009

Key words: Chest pain; Esophageal manometric; Twenty-four-hour intra-esophageal pH monitoring; Holter electrocardiography

Peer reviewer: Diego Garcia-Compean, MD, Professor, Faculty of Medicine, University Hospital, Department of Gastroenterology, Autonomous University of Nuevo Leon, Ave Madero y Gonzalitos, 64700 Monterrey, NL, México

Deng B, Wang RW, Jiang YG, Tan QY, Liao XL, Zhou JH, Zhao YP, Gong TQ, Ma Z. Diagnosis of chest pain with foregut symptoms in Chinese patients. *World J Gastroenterol* 2009; 15(6): 742-747 Available from: URL: http://www.wjgnet.com/1007-9327/15/742.asp DOI: http://dx.doi.org/10.3748/wjg.15.742

Abstract

AIM: To evaluate the diagnosis of chest pain with foregut symptoms in Chinese patients.

METHODS: Esophageal manometric studies, 24-h introesophageal pH monitoring and 24-h electrocardiograms (Holter electrocardiography) were performed in 61 patients with chest pain.

RESULTS: Thirty-nine patients were diagnosed with non-specific esophageal motility disorders (29 patients with abnormal gastroesophageal reflux and eight patients with myocardial ischemia). Five patients had diffuse spasm of the esophagus plus abnormal gastroesophageal reflux (two patients had concomitant myocardial ischemia), and one patient was diagnosed with nutcracker esophagus.

CONCLUSION: The esophageal manometric studies, 24-h intra-esophageal pH monitoring and Holter electrocardiography are significant for the differential diagnosis of chest pain, particularly in patients with foregut symptoms. In cases of esophageal motility disorders, pathological gastroesophageal reflux may be a major cause of chest pain with non-specific esophageal motility disorders. Spasm of the esophageal smooth muscle might affect the heart-coronary smooth muscle, leading to myocardial ischemia.

INTRODUCTION

Recent reports[1-2] have indicated that recurrent chest pain is often a result of esophageal motility disorders or gastroesophageal reflux diseases (GERD), which is known as esophageal chest pain. Esophageal chest pain is very similar to symptoms seen during myocardial ischemia. It is also observed in patients with coronary artery disease with similar incidence (i.e. linked angina)[3]. As a result, differential diagnosis is often difficult and many patients with esophageal chest pain are misdiagnosed as having coronary artery disease[4]. Hence, the percentage of patients that are correctly treated and cured is relatively low. It is well known that esophageal manometry and 24-h intra-esophageal pH monitoring provide accurate diagnoses of esophageal motility disorders. As an incentive, Holter electrocardiography is more economical for these patients from developing countries compared to coronary artery opacification.

Previous reports have focused on the diagnosis of unclear chest pain via both coronary artery 24-h intra-esophageal pH monitoring and coronary artery opacification, which is very expensive to the patient, particularly in developing countries. In addition, very few studies have shown the efficacy of combined esophageal manometry, 24-h intra-esophageal pH monitoring, and electrocardiograms from Holter

monitoring and the results have been inconclusive. Paterson *et al*[5] reported that there was no correlation between the incidence of chest pain and changes in esophageal manometry, 24-h intra-esophageal pH monitoring, and Holter electrocardiography. Wright *et al*[6] reported that physiologic gastroesophageal reflux does not induce electrocardiographic changes from Holter monitoring. However, Dobrzycki *et al*[7] and Patai *et al*[8] reported distinguishable alterations in 24-h intra-esophageal pH monitoring and Holter electrocardiography during chest pain. As a result, it was thought that simultaneous 24-h esophageal pH manometry and electrocardiograms from Holter monitoring could contribute to the diagnosis of atypical chest pain. However, up to now, many questions remain unaddressed. Can esophageal motility disorders affect myocardial ischemia? Can GERD affect myocardial ischemia? All of these diseases can cause chest pain, so it is unclear how to differentiate between cause and effect in these patients. Is there any clinical significance to the combination of these three types of monitoring in the differential diagnosis of unclear chest pain? Here, we present our experience in the combined application of monitoring over the past 6 years.

MATERIALS AND METHODS

Patients

From September 2001 to May 2007, 61 Chinese patients with chest pain from the thoracic surgery department were enrolled into this study. The patients were both male (27) and female (34), ranging in age from 18 to 69 years (average age: 45.5 years). All patients had complaints of varying degrees of chest pain and most had symptoms of retrosternal pain or backache, ranging in duration from 60 d to 5 years (average duration of pain: 14.5 mo). Twenty patients had intermittent dysphagia and 40 had other symptoms such as regurgitation. There were no abnormalities in their hemogram, chest X-rays, routine electrocardiograms or esophago-gastroscopy.

Methods

Esophageal motility was studied by standard water perfusion and stationary manometry (Medtronic DPT-6000, Smith Medical, Sweden) with computer-assisted analysis (Polygram 2.0, Smith Medical, Sweden) of the tracings according to a previously published protocol[9-10]. Briefly, a station pull-through technique was applied, and measurements were made at the pressure levels of the lower esophageal sphincter (LESP), the relaxation rate of the lower esophageal sphincter (LESRR), the esophageal body, the upper esophageal sphincter and the pharynx.

Twenty-four-hour intra-esophageal pH was monitored using the classical DeMeester criteria[11]. A single channel, nasoesophageal, antimony pH-probe (Synectics Medical, Sweden) was positioned 5 cm above the lower esophageal sphincter and was connected to a portable data acquisition system (Digitrapper Mark II Gold, Synectics Medical, Sweden). Following 24-h intra-esophageal pH monitoring, chest leads at CM1, CM5 and CMF of a digital ambulatory 24-h Holter monitor (Life Card CF, Reynolds Medical, England) were positioned. Holter studies and 24-h pH monitoring were performed simultaneously. Following 24-h monitoring, data from the two monitors were analyzed by software (supplied by Life Card CF, Reynolds Medical, England and Digitrapper Mark Ⅱ Gold, Synectics Medical, Sweden).

RESULTS

Results interpretation

Esophageal contraction waves following swallowing were classified as (1) peristaltic, (2) simultaneous, (3) interrupted, or (4) dropped. Primary esophageal motility disorders were classified according to Chinese standards[12]: (1) Diffuse esophageal spasm; (2) Nutcracker esophagus; or (3) Non-specific esophageal motility disorders. Abnormal gastro-esophageal reflux was considered when the score was > 14.72. If the decreasing amplitude of the ST segment was above 0.1 mV, chest pain arousing from myocardial ischemia was considered, according to an electrocardiogram obtained from the Holter monitor.

Results of combined examination

In the present case report, 45 of 61 patients had different types of esophageal motility disorders (Table 1). The episodes of pain are shown in Table 1. Results of esophageal manometric studies and 24-h intra-esophageal pH monitoring are presented in Table 2.

Eight patients were diagnosed with myocardial ischemia and non-specific esophageal motility disorders. Two patients were diagnosed with myocardial ischemia and diffuse spasm of the esophagus. Of the above 10 patients, eight had myocardial ischemia, which occurred with simultaneous abnormal gastroesophageal reflux (GERD) (Figure 1).

DISCUSSION

Recurrent chest pain is typically general and difficult to identify. Shrestha *et al*[4] reported that 30% of non-cardiac chest pain was caused by esophageal diseases such as GERD. Since the innervation and location of the esophageal nervous system in the body overlaps with the cardiac nervous system, symptoms are often similar. As a result, patients with esophageal chest pain are often misdiagnosed as having coronary artery disease[13]. Therefore, it is important for physicians to pay particular attention to the differential diagnosis of inconclusive esophageal chest pain.

As for the differential diagnosis of chest pain, there are very few studies investigating combined 24-h intra-esophageal pH and electrocardiograms from Holter monitoring. Several studies[5-6] have found that there is minimal correlation between the incidence of

Table 1 Results of esophageal manometric studies, 24-h intra-esophageal pH monitoring and electrocardiograms from Holter monitoring in 77 patients

Diagnosis	Number cases and pain episodes		Cases combined with abnormal gastroesophageal reflux			Cases combined with myocardial ischemia		
	Cases	Pain episodes	Cases	Pain episodes	Pain episodes with changes in 24-h pH monitoring	Cases	Pain episodes	Pain episodes with changes in Holter monitoring
Non-specific esophageal motility disorders	39	312	29	235	156	8	61	10
Diffuse spasm of esophagus	5	65	5	65	53	2	25	13
Nutcracker esophagus	1	18	0	18	0	0	0	0
Normal case	16	83	0	0	0	0	0	0
Consolidation of table	61	478	34	366	0	10	86	23

Table 2 Results of esophageal manometric studies and 24-h intra-esophageal pH monitoring

	Cases	LESP	LESRR	Overall length of LES	Abdominal length of LES	Swallow waves	pH monitoring DeMeester scores
Non-specific esophageal motility disorders	39	Reduced in 29 patients (9.32 ± 1.53 mmHg)	Reduced in 28 patients (52.18 ± 20.51%)	Reduced in 21 patients (2.3 ± 0.1 cm)	Reduced in 18 patients (1.2 ± 0.1 cm)	39 patients with simultaneous non-transmitted waves	60.2 ± 12.4
Diffuse spasm of esophagus	5	Normal in 5 patients (18.35 ± 2.92 mmHg)	Reduced in 5 patients (30.50% ± 6.65%)	Normal in 5 patients (3.3 ± 0.3 cm)	Normal in 5 patients (2.0 ± 0.1 cm)	20% or more simultaneous contractions in response to wet swallows appearing in all five patients	80.4 ± 35.5
Nutcracker esophagus	1	12.2 mmHg	64.0%	4.0 cm	2.5 cm	High amplitude contracting wave appearing above 229.7 mmHg	

chest pain and changes in esophageal dysfunction and myocardial ischemia monitoring, while others have reported the opposite[7,8]. Therefore, in the present study, we reassessed the significance of the combined monitoring. In our study, 45 of 61 patients had esophageal disorders (the rate was 73.7%, which was rather high since the patients were recruited from the thoracic surgery outpatient clinic and some of them had pre-existing upper gastrointestinal symptoms such as dysphagia or regurgitation), and 10 (16.4%) patients had myocardial ischemia. We think that the main reason for the weak correlation between incidences of chest pain and changes in the combined monitoring reported by Paterson *et al*[5] and Wright *et al*[6] may be as follows: (1) the number of samples that were selected randomly was relatively small; and (2) there may have been an unknown heterogeneity of patient populations.

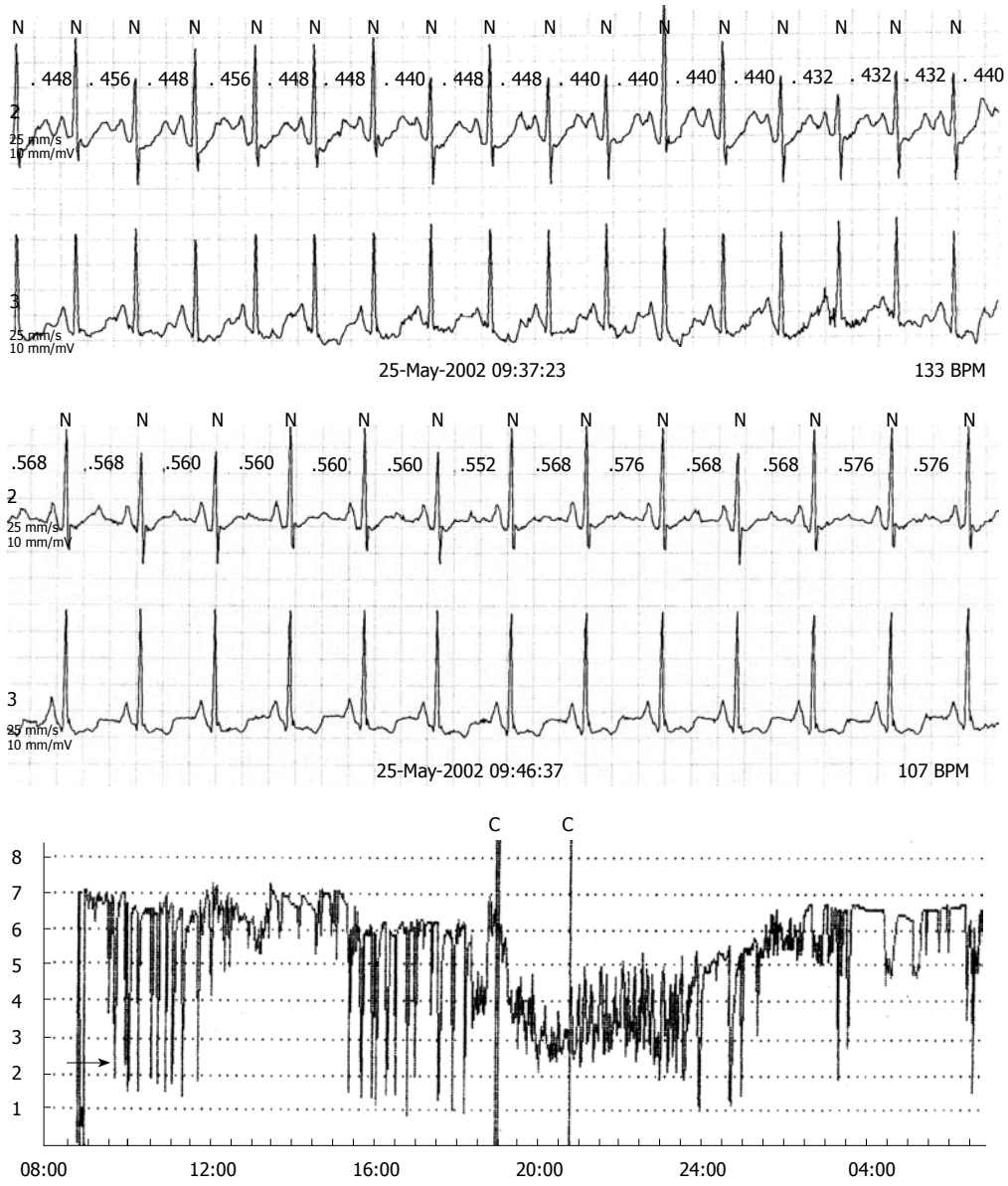
Esophageal motility disorders are the most common cause of esophageal chest pain[14]. Non-transmitted contraction waves appeared in all of the 39 patients with non-specific esophageal motility disorders, while 29 patients had abnormal gastroesophageal reflux. Among the total 235 pain episodes, 156 had changes in 24-h pH monitoring, indicating that abnormal gastroesophageal reflux may be the main cause of chest pain in these patients, confirmed by the observation by Patai *et al*[8] showing that proton pump inhibition with omeprazole alleviated gastroesophageal reflux as well as spontaneous chest pain. Interestingly, exercise potentiated the effect of intra-esophageal pH monitoring on electrocardiogram abnormalities (Badzynski *et al*[15]).

In the five patients with diffuse spasm of the esophagus, 20% or more had simultaneous contractions in response to wet swallows. However, some degree of peristaltic function was retained and the differential diagnosis could be made between diffuse spasm of the esophagus and achalasia. All of the five cases were defined as secondary diffuse spasm of the esophagus due to abnormal gastroesophageal reflux[13]. Two patients were diagnosed as having simultaneous diffuse spasm of the esophagus, myocardial ischemia, and GERD. We hereby hypothesize that diffuse spasm of the esophageal smooth muscle might affect spasm of the heart-coronary smooth muscle, leading to myocardial ischemia. All of these could be caused by a disturbance of the nervous system controlling the esophagus and cardiovascular system. It is interesting that this presumption is supported by Manfrini *et al*[16], in which esophageal spasm was considered to be related to myocardial ischemia ($P < 0.05$). Bidirectional analysis of causal effects showed that the influence between esophageal and coronary spasms was mutual and reciprocal in seven patients with variant angina. In two patients in our study, 25 pain episodes occurred with eight episodes causing changes in 24-h pH and Holter monitoring. This fact indicates that myocardial ischemia may occur with GERD, especially in patients with diffuse spasm of the esophagus.

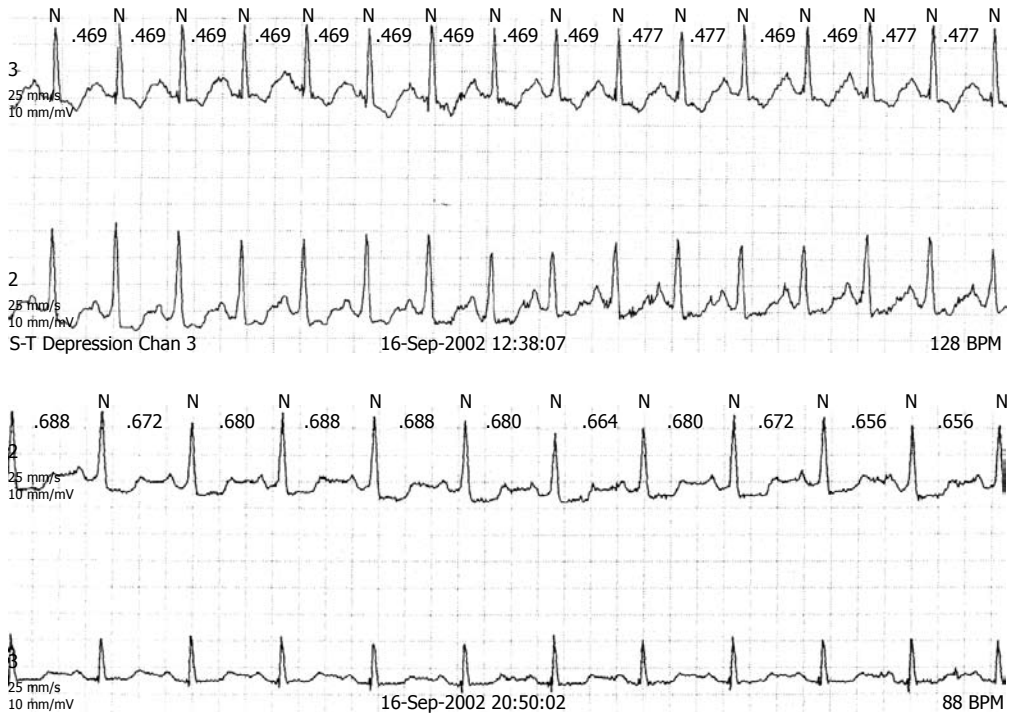
Amplitude of the contractive wave in the patients with nutcracker esophagus was 229.7 mmHg, consistent with the diagnostic criteria[17].

Hence, combined monitoring of esophageal manometry, 24-h intra-esophageal pH and electrocardiograms from Holter monitoring are very

A


N N N N N N N N N N N N N N N N N N N
. 448 . 456 . 448 . 456 . 448 . 448 . 440 . 448 . 448 . 440 . 440 . 440 . 440 . 432 . 432 . 432 . 440
2
25 mm/s
10 mm/mV
3
25 mm/s
10 mm/mV
25-May-2002 09:37:23
133 BPM
N N N N N N N N N N N N N N N N N N N
.568 .568 .560 .560 .560 .560 .552 .568 .576 .568 .568 .576 .576
2
25 mm/s
10 mm/mV
3
25 mm/s
10 mm/mV
25-May-2002 09:46:37
107 BPM
C C
8
7
6
5
4
3
2
1
08:00
12:00
16:00
20:00
24:00
04:00

B


N N N N N N N N N N N N N N N N N N N
.469 .469 .469 .469 .469 .469 .469 .469 .469 .469 .477 .477 .469 .469 .477 .477
3
25 mm/s
10 mm/mV
2
25 mm/s
10 mm/mV
S-T Depression Chan 3
16-Sep-2002 12:38:07
128 BPM
N N N N N N N N N N N N N N N N N N N
.688 .672 .680 .688 .688 .680 .664 .680 .672 .656 .656
2
25 mm/s
10 mm/mV
3
25 mm/s
10 mm/mV
16-Sep-2002 20:50:02
88 BPM

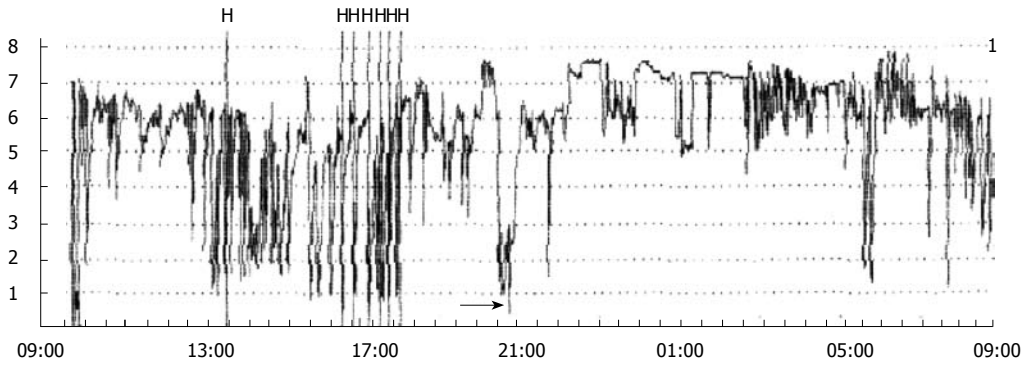


Figure 1 Curves of 24-h intra-esophageal pH monitoring and Holter electrocardiography in a patient with abnormal gastroesophageal reflux show the decreasing amplitude of the ST segment. A: Ranged from 0.05 mV to 0.15 mV in 9 min. The results also indicate that abnormal gastroesophageal reflux occurred during this period; B: 0.1 mV to 0.3 mV in from 12:38:07 to 20:50:02. The results also indicate that abnormal gastro-esophageal reflux occurred during this period.

significant for the diagnosis of recurrent chest pain, particularly for patients with foregut symptoms. We therefore, suggest that the diagnostic procedures of cloudy chest pain should be undertaken as follows. Firstly, the patients with cloudy chest pain should undergo routine examination including hemogram, chest X-rays, routine electrocardiogram and esophagogastroscopy, in order to exclude severe diseases such as tumor or myocardial infarction. Secondly, the combined examinations should be recommended to the patients without the positive results of the routine examinations and with foregut symptoms as the "final step" in the diagnostic procedures. The detection rate is satisfactorily high, as shown in our cases. In conclusion, as an added incentive, the combined monitoring is very cost-effective for the patients, with a total cost of approximately 50 US dollars.

COMMENTS

Background

Studies on unclear chest pain are timely and important with the growing age of the world's population. However, very few studies have been performed about esophageal manometric studies, 24-h intra-esophageal pH monitoring and a Holter electrocardiography for the differential diagnosis of chest pain caused by esophageal dysfunctional and/or myocardial ischemia. Interestingly, the results of published papers on the combined monitoring have been inconclusive.

Research frontiers

The aim of the present study was to evaluate the significance of combined monitoring in the diagnosis of chest pain with foregut symptoms in Chinese patients.

Innovations and breakthroughs

The study indicated that spasm of the esophageal smooth muscle might affect the heart-coronary smooth muscle, leading to myocardial ischemia. The combination of esophageal manometric studies, 24-h intra-esophageal pH monitoring and Holter electrocardiography are significant for the differential diagnosis of chest pain, particularly with foregut symptoms. As an added incentive, combined monitoring is very cost-effective for the patients, especially those from developing countries.

Peer review

The manuscript describes an interesting study that aimed to demonstrate the etiology of chest pain. In fact, it is well known that non-cardiac chest pain is associated with GERD in about 50% of cases and that spastic esophageal motility disorders are related to chest pain. This study revisits the conflicting situation concerning the precise primary cause of chest pain, especially in patients in whom myocardial ischemia, GERD and esophageal spastic motility disorders are simultaneously found. As the authors pointed out, it has been suggested that spastic motility disorders may cause myocardial ischemia.

REFERENCES

1 **Mudipalli RS**, Remes-Troche JM, Andersen L, Rao SS. Functional chest pain: esophageal or overlapping functional disorder. *J Clin Gastroenterol* 2007; **41**: 264-269
2 **Rencoret G**, Csendes A, Henríquez A. [Esophageal manometry in patients with non cardiac chest pain] *Rev Med Chil* 2006; **134**: 291-298
3 **Fang J**, Bjorkman D. A critical approach to noncardiac chest pain: pathophysiology, diagnosis, and treatment. *Am J Gastroenterol* 2001; **96**: 958-968
4 **Shrestha S**, Pasricha PJ. Update on noncardiac chest pain. *Dig Dis* 2000; **18**: 138-146
5 **Paterson WG**, Abdollah H, Beck IT, Da Costa LR. Ambulatory esophageal manometry, pH-metry, and Holter ECG monitoring in patients with atypical chest pain. *Dig Dis Sci* 1993; **38**: 795-802
6 **Wright RA**, McClave SA, Petruska J. Does physiologic gastroesophageal reflux affect heart rate or rhythm? *Scand J Gastroenterol* 1993; **28**: 1021-1024
7 **Dobrzycki S**, Baniukiewicz A, Korecki J, Bachórzewska-Gajewska H, Prokopczuk P, Musial WJ, Kamiński KA, Dabrowski A. Does gastro-esophageal reflux provoke the myocardial ischemia in patients with CAD? *Int J Cardiol* 2005; **104**: 67-72
8 **Patai A**, Sipos E, Döbrönte Z. [Sinoatrial block caused by gastroesophageal reflux. The role of simultaneous 24 hr. esophageal pH-metry and Holter-ECG in the differential diagnosis of angina pectoris] *Orv Hetil* 1996; **137**: 687-690
9 **Deng B**, Wang RW, Jiang YG, Liao XL. The application of esophageal manometry and ambulatory esophageal pH monitoring test in the esophageal chest pain: 44 cases report. *Zhonghua Xiongxin Xueguan Waike Zazhi* 2003; **19**: 341-342
10 **Deng B**, Wang RW, Jiang YG, Tan QY, Zhao YP, Zhou JH, Liao XL, Ma Z. Functional and menometric study of side-to-side stapled anastomosis and traditional hand-sewn anastomosis in cervical esophagogastrostomy. *Eur J Cardiothorac Surg* 2009; **35**: 8-12
11 **DeMeester TR**, Wang CI, Wernly JA, Pellegrini CA, Little AG, Klementschitsch P, Bermudez G, Johnson LF, Skinner DB. Technique, indications, and clinical use of 24 hour esophageal pH monitoring. *J Thorac Cardiovasc Surg* 1980; **79**: 656-670
12 **Deng B**, Wang RW, Jiang YG, Ma Z, Liao XL. The significance of esophagealmotility testing and 24-hour esophageal pH monitoring in the diagnosis of chest pain. *Zhongguo Xiongxin Xueguan Waike Linchuang Zazhi* 2004; **11**: 262-264
13 **Szarka LA**, DeVault KR, Murray JA. Diagnosing

gastroesophageal reflux disease. *Mayo Clin Proc* 2001; **76**: 97-101

14 **Kim SH**, Lee JS, Im HH, Hwang KR, Jung IS, Hong SJ, Ryu CB, Kim JO, Jo JY, Lee MS, Shim CS, Kim BS. [The relationship between ineffective esophageal motility and gastro-esophageal reflux disease] *Korean J Gastroenterol* 2005; **46**: 255-261

15 **Budzynski J**, Kłopocka M, Pulkowski G, Suppan K, Fabisiak J, Majer M, Swiatkowski M. The effect of double dose of omeprazole on the course of angina pectoris and treadmill stress test in patients with coronary artery disease-a randomised, double-blind, placebo controlled, crossover trial. *Int J Cardiol* 2008; **127**: 233-239

16 **Manfrini O**, Bazzocchi G, Luati A, Borghi A, Monari P, Bugiardini R. Coronary spasm reflects inputs from adjacent esophageal system. *Am J Physiol Heart Circ Physiol* 2006; **290**: H2085-H2091

17 **Kamberoglou DK**, Xirouchakis ES, Margetis NG, Delaporta EE, Zambeli EP, Doulgeroglou VG, Tzias VD. Correlation between esophageal contraction amplitude and lower esophageal sphincter pressure in patients with nutcracker esophagus. *Dis Esophagus* 2007; **20**: 151-154

S- Editor Li LF **L- Editor** Ma JY **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.748

World J Gastroenterol 2009 February 14; 15(6): 748-752
World Journal of Gastroenterology ISSN 1007-9327

BRIEF ARTICLES

Combined therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation for small hepatocellular carcinoma

Wei-Zhu Yang, Na Jiang, Ning Huang, Jing-Yao Huang, Qu-Bin Zheng, Quan Shen

Wei-Zhu Yang, Na Jiang, Ning Huang, Jing-Yao Huang, Qu-Bin Zheng, Quan Shen, Interventional Radiology Department, Union Hospital, Fujian Medical University, Fuzhou 350001, Fujian Province, China
Author contributions: Yang WZ designed the research and wrote the paper; Jiang N, Huang N, Huang JY, Zheng QB performed the research; and Shen Q analyzed data.
Correspondence to: Na Jiang, Interventional Radiology Department, Union Hospital, Fujian Medical University, Fuzhou 350001, Fujian Province, China. hjy999@126.com
Telephone: +86-591-83357896 Fax: +86-591-83357896
Received: August 25, 2008 Revised: December 5, 2008
Accepted: December 12, 2008
Published online: February 14, 2009

Abstract

AIM: To assess the efficacy of combined transcatheter arterial chemoembolization (TACE) and percutaneous microwave coagulation therapy (PMCT) for small hepatocellular carcinoma (HCC).

METHODS: Thirty-five patients with a total of 41 HCC nodules (≤ 3 cm in diameter) were treated with TACE followed by computed tomograghy (CT)-guided percutaneous microwave coagulation therapy (PMCT) within 1-3 wk.

RESULTS: By biopsies and enhanced CT scans, complete necrosis of the tumor and 3-5 mm of the surrounding non-cancerous area were observed in 34 foci. In seven foci, incomplete necrosis of the surrounding parenchyma was observed. Serum alpha-fetoprotein (AFP) levels returned to normal 10 d after treatment in 25 patients who originally had high serum AFP levels. The follow-up period was 6-31 mo, and all patients remained alive. One patient had a recurrence in the subsegments of the liver, and another patient had a recurrence near the original lesion.

CONCLUSION: Combined therapy with TACE and PMCT is a safe and effective treatment without severe complications for small HCC.

Key words: Liver neoplasms; Therapy; Hepatocellular carcinoma; Transcatheter arterial chemoembolization; Microwave coagulation therapy; Percutaneous local treatment

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

Yang WZ, Jiang N, Huang N, Huang JY, Zheng QB, Shen Q. Combined therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation for small hepatocellular carcinoma. *World J Gastroenterol* 2009; 15(6): 748-752 Available from: URL: http://www.wjgnet.com/1007-9327/15/748.asp DOI: http://dx.doi.org/10.3748/wjg.15.748

INTRODUCTION

Surgical resection[1], transcatheter arterial chemoembolization (TACE), and percutaneous ethanol injection (PEI)[2-4] are effective therapies for small hepatocellular carcinoma (HCC). However, for patients with poor liver functions, hepatectomy is not the first treatment choice. TACE is not effective for HCC with a poor blood supply[5], and PEI only yields incomplete necrosis of the tumor due to the uneven distribution of ethanol[6]. Although percutaneous microwave coagulation therapy (PMCT) can lead to complete necrosis of the hepatocarcinoma cells, the induced area of necrosis is small[7,8]. TACE has the advantage of reducing the local blood supply of the tumor foci, resulting in tissue necrosis and inflammatory edema. Therefore, it can decrease the cooling effect of blood flow on the heating action of microwaves, and enhance the coagulation action of microwaves[9]. In the present study, we investigate the therapeutic efficacy of the combied therapy of TACE and PMCT for the treatment of small HCC.

MATERIALS AND METHODS

Patient data

A total of 35 patients received the combined therapy of TACE and PMCT in our hospital between April 2005 and May 2007. These patients comprised 27 males

and eight females, with an age range of 27-78 years, and a mean age of 56.69 ± 14.02 years. All patients had a history of hepatitis B and liver cirrhosis, with liver functions categorized as class A by Child-Pugh classification. All of the cases met the diagnostic criteria of primary HCC. Twenty-eight cases were confirmed by pathological examination of puncture-biopsied specimens under the guidance of computed tomograghy (CT) before PMCT. Twenty-five cases had elevated levels of serum alpha-fetoprotein (AFP). The size of the tumor focus was determined by measurement of the largest and shortest diameters of the largest section. All tumor foci were less than 3 cm in diameter.

Therapeutic methods

All patients were initially treated with TACE. Hepatic artery angiography was performed using the Seldinger technique. Femoral arterial catheterization was conducted through the common hepatic artery or proper hepatic artery, and the location, number, size, and blood supply of the tumors were evaluated. Subsequently, a microcatheter was super-selectively inserted into the hepatic lobe or hepatic segmental artery branch, and mixed suspensions of iodized oil (3-6 mL) and epirubicin (20-30 mg) were infused into the artery through the catheter. Finally, gelatin sponge particles were infused to embolize the artery until the arterial blood flow supplying the tumor was completely blocked.

PMCT was initiated 1-3 wk after TACE. The microwave therapeutic device, FORSEA MTC-3 for inter-tissue tumor treatment, was produced by Nanjing Qinghai Microwave Electric Institute (Nanjing, China). The diameter of its electrode needle was 14 G, and the instrument was cooled by a cold water cycling system. To relieve pain in the patients, dolantin (100 mg) and valium (10 mg) were injected intramuscularly 5 min before the PMCT.

The puncture site and pathway were determined under the guidance of CT. After anesthetizing the puncture site with 2% lidocaine, a 12 G guide-needle was placed at its opposite side, across the tumor focus. The electrode needle of the microwave device was connected to the output machine of the microwave instrument via a flexible coaxial cable, and the cooling water tube was connected. The constant-flow pump was switched on to test the functioning of the cold water cycling system. The inner needle of the guide-needle was withdrawn, and the electrode needle of the microwave machine was inserted through the outer needle of the guide to place the electrode in the tumor area. The microwave power was set at 60-70 W. The coagulation time for each focus was 10-15 min, and the coagulation area covering the tumor focus and its surrounding area measured 5 mm or more. If a single needle coagulation treatment did not produce satisfactory results, a second PMCT therapy was conducted a week later.

After TACE and PMCT treatment, liver protection, anti-inflammatory and sedation therapies were prescribed. A follow-up study by repeat CT (plain and enhanced) and serum AFP level measurement was conducted once every 1-2 mo.

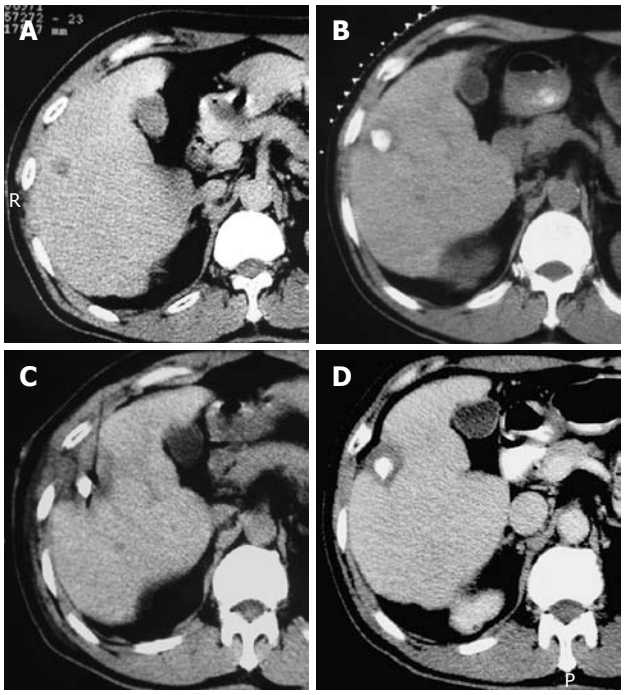


Figure 1 Pathological examinations of biopsied specimens and enhanced CT after PMCT showed complete tumor necrosis in 34 foci, together with complete ring-shaped necrosis of the surrounding non-cancerous hepatic parenchymal tissue, measuring 3-5 mm in width. A: CT scan of a primary HCC in the right anterior lobe of the liver, with a diameter of 1.6 cm × 1.4 cm; B: After TACE, the accumulation of iodized oil in the tumor area was satisfactory; C: PMCT was initiated 17 d later; D: One month after PMCT, an enhanced CT scan showed a complete non-enhanced area of the tumor, and a non-cancerous ring-shaped area surrounding the tumor (measuring 4-5 mm), indicating complete necrosis of the tumor lesion.

RESULTS

One to three weeks after all 41 foci were treated with TACE, these foci received PMCT treatment once. Among them, six foci were treated with a second PMCT within 1 wk.

Pathological examinations of biopsied specimens and enhanced CT after PMCT showed complete tumor necrosis in 34 foci, together with complete ring-shaped necrosis of the surrounding non-cancerous hepatic parenchymal tissue, measuring 3-5 mm in width (Figure 1). Seven other foci showed complete necrosis of the tumor, with incomplete necrosis of the surrounding non-cancerous hepatic parenchyma.

Among the 35 patients, serum AFP levels were elevated in 25; the AFP levels in these 25 patients returned to normal within 10 d after treatment.

Post-embolization symptoms included fever, pain, nausea, and vomiting after TACE, all of which were relieved by anti-inflammatory, liver protection, and sedation treatment. In the process of PMCT, most patients complained of local burning pain, which was generally tolerable, but in some patients, the microwave coagulation therapy had to be carried out intermittently.

After PMCT, some patients reported having fever and local pain, but these were not severe. Complications such as hemorrhage, subcapsular hematoma of the liver, or biliary duct damage were not found, nor was local dissemination of cancer cells along the puncture needle pathway.

All patients were followed up for 6-31 mo. No recurrence was found in 33 patients. One patient had a gradual increase of serum AFP levels 10 mo after the treatment, but repeat CT and magnetic resonance imaging (MRI) examinations revealed no recurrence. However, hepatic arteriography detected new tumor foci in the segments of the liver. Another patient showed an elevation of serum AFP levels 8 mo after treatment, and a CT scan showed recurrence in the lateral side of the original focus. The recurring foci were all treated with TACE and PMCT again. All patients remained alive during the follow-up period.

DISCUSSION

The mechanism of PMCT for HCC is based on the heating effect of microwaves and the sensitivity of the tumor to the heating action. The high temperature produced by the electrodes inserted into the tumor tissue results in the rapid coagulation and necrosis of the tumor tissue, thus achieving the goal of destroying the tumor. The area of PMCT is related to the diameter and number of the microwave electrode, the output power of the machine, coagulation time, and local blood circulation[7,10-13]. Increases in the diameter of the electrodes, the output power, and the coagulation time may enlarge the treatment areas of PMCT. However, increasing the diameter of the electrodes may also lead to increased rates of post-puncture hemorrhage, and an extension of the coagulation time may result in more pain for the patients. In practice, the diameter of the electrode and the output power of the microwave therapeutic machine are constant. Therefore, reducing the local blood supply is the only approach to achieving the goal of increasing the PMCT area.

It is well known that primary HCC possesses an abundant blood supply. TACE through the hepatic artery, especially the injection of iodine oil and gelatin sponge particles, blocks the artery supply to the tumor. The iodine oil can fill up the portal vein surrounding the tumor through the communication branches connecting the arterial and portal veins[14,15]. Thus, it can decrease the blood flow volume of the portal vein, and alleviate the adverse cooling effect induced by abundant blood flow[16,17]. In addition, TACE can result in ischemia of the tumor tissue and inflammatory edema, which accelerates tumor necrosis and enhances the coagulation effect of microwaves[18]. This forms the theoretical basis for the combined therapy of transcatheter hepatic artery chemoembolization with percutaneous microwave ablation for small HCC. Ishida *et al*[19] used hepatic artery embolization combined with a temporal block of the hepatic vein to reduce the segmental blood supply to the tumor. The therapeutic range of PMCT was increased; however, the procedures were too complicated to be practiced often.

Primary HCC is a malignancy that usually develops from liver cirrhosis, and is likely to have multiple foci. Although modern advanced diagnostic imaging techniques (spiral CT and MRI) can detect tumor nodules smaller than 1 cm, the detection rate is quite low. TACE therapy prior to PMCT enhances the efficacy of the latter. In addition, TACE can also help detect nodules that are undetectable by conventional imaging techniques, because digital subtraction angiography and the so-called iodine oil-CT are still the best methods for the detection of small HCC. Another advantage of TACE therapy prior to PMCT is that TACE can quickly and effectively control the foci that are inaccessible to PMCT.

Seki *et al*[20] treated 18 cases of small HCC of less than 3 cm using PMCT, and achieved complete necrosis in 17 of them. After a follow-up of 12-31 mo, all the patients were still alive. Lu *et al*[21] treated 67 cases of small HCC of less than 3 cm using the microwave therapy device produced by Nanjing Qinghai Company (Nanjing, China), and achieved a complete necrosis rate of 94% (63 cases). We used a combined therapy of transcatheter hepatic artery chemoembolization with PMCT for 35 cases of small HCC, and achieved complete necrosis in all cases. After a follow-up of 6-31 mo, only one patient had a recurrence at the original tumor site. Liang *et al*[22] analyzed the correlative factors for the prognosis of 288 HCC patients who received PMCT. They concluded that HCC patients with a single focus of a diameter $\leqslant$ 4 cm, and a liver function of Child-Pugh class A can have long-term survival after PMCT. Therefore, PMCT is ideal for small HCC. It can completely substitute for surgical resection, especially for elderly patients or those with a poor general condition or poor liver function[23].

Pathological examinations indicate that HCC is very likely to invade the capsule or extracapsular tissues[24,25]. Therefore, to achieve the goal of complete necrosis of tumor tissue and to increase its overall therapeutic efficacy, PMCT must not only destroy the tumor cells in the center of the tumor, but also destroy the adjacent non-cancerous hepatic parenchyma tissue. The microwave therapy device used in our study could produce coagulative tissue necrosis with a diameter of 4-5 cm under the conditions of an output power of 60-70 W, and a coagulation time of 15 min. By TACE, the area of tissue necrosis using a single needle electrode was even larger, completely covering a liver cancer nodule of $\leqslant$ 3 cm, with a tumor-free margin of 5 mm. Among our patients, there was one case of recurrence at the original tumor site, with incomplete liver necrosis of the surrounding non-cancerous liver parenchyma. This was probably due to the invasion of the tumor into the capsule or subcapsular tissues, or an insufficient coagulation time, which resulted in the incomplete killing of the tumor cells.

Compared with ultrasound-guided treatment, the

present study was carried out under guidance of CT and has multiple advantages[26,27]. It allows for determination of the precise locations of the foci, especially after TACE because iodine oil accumulation in the tumor foci makes the identification of their location and range easier. Additionally, when a plain CT scan is unable to clearly show the location of a tumor due to its small size, CT-guided puncturing of the focus can be performed based on the result of the MRI scan, thus reducing the "blindness" of the therapy. Finally, CT-guided liver puncturing has almost no "blind area", and is not affected by the adjacent organ full of gas.

Only mild adverse reactions were reported by our patients treated by PMCT. Local pain, during and after the treatment, was not very severe. No internal bleeding, subcapsular hematoma, biliary duct damage, or spreading of the tumor along the puncturing pathway were found. This suggests that PMCT is a safe therapy. Our results show that the combined TACE and PMCT for small HCC ($\leqslant$ 3 cm in diameter) is minimally invasive, simple and efficacious. Although further studies are needed, this combined therapy appears to be the first choice of treatment for small HCC that is unsuitable for surgical resection.

COMMENTS

Background

Surgical resection, transcatheter arterial chemoembolization (TACE), and percutaneous ethanol injection (PEI) are effective therapies for small hepatocellular carcinoma (HCC). However, for patients with poor liver functions, hepatectomy is not the first treatment choice. TACE is not effective for HCC with a poor blood supply, and PEI only yields incomplete necrosis of the tumor due to the uneven distribution of ethanol.

Research frontiers

TACE has the advantage of reducing the local blood supply of the tumor foci, resulting in tissue necrosis and inflammatory edema. Therefore, it can decrease the cooling effect of blood flow on the heating action of microwaves, and enhance the coagulating action of microwaves.

Innovations and breakthroughs

In the present study, the authors investigated the efficacy of the combined TACE and PMCT for small HCC.

Applications

The study shows that combined TACE and PMCT for treatment of small HCC ($\leqslant$ 3 cm in diameter) is minimally invasive, simple and efficacious. Although further studies are needed, this combined therapy appears to be the first choice of treatment for small HCC that is unsuitable for surgical resection.

Peer review

This is an interesting study which shows the advantages of combined TACE and PMCT for small HCC. It may provide useful information for us.

REFERENCES

1 **Wakai T**, Shirai Y, Suda T, Yokoyama N, Sakata J, Cruz PV, Kawai H, Matsuda Y, Watanabe M, Aoyagi Y, Hatakeyama K. Long-term outcomes of hepatectomy vs percutaneous ablation for treatment of hepatocellular carcinoma <=4 cm. *World J Gastroenterol* 2006; **12**: 546-552

2 **Shiina S**, Tagawa K, Unuma T, Fujino H, Uta Y, Niwa Y, Hata Y, Komatsu Y, Shiratori Y, Terano A. Percutaneous ethanol injection therapy of hepatocellular carcinoma: analysis of 77 patients. *AJR Am J Roentgenol* 1990; **155**: 1221-1226

3 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929

4 **Shiina S**, Tagawa K, Unuma T, Takanashi R, Yoshiura K, Komatsu Y, Hata Y, Niwa Y, Shiratori Y, Terano A. Percutaneous ethanol injection therapy for hepatocellular carcinoma. A histopathologic study. *Cancer* 1991; **68**: 1524-1530

5 **Kuroda C**, Sakurai M, Monden M, Marukawa T, Hosoki T, Tokunaga K, Wakasa K, Okamura J, Kozuka T. Limitation of transcatheter arterial chemoembolization using iodized oil for small hepatocellular carcinoma. A study in resected cases. *Cancer* 1991; **67**: 81-86

6 **Ishii H**, Okada S, Nose H, Okusaka T, Yoshimori M, Takayama T, Kosuge T, Yamasaki S, Sakamoto M, Hirohashi S. Local recurrence of hepatocellular carcinoma after percutaneous ethanol injection. *Cancer* 1996; **77**: 1792-1796

7 **Seki T**, Wakabayashi M, Nakagawa T, Imamura M, Tamai T, Nishimura A, Yamashiki N, Okamura A, Inoue K. Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. *Cancer* 1999; **85**: 1694-1702

8 **Peng QR**, Zhang LS, Xie SM, Chen GQ, Duan YX. A study on therapeutical effect of CT-guided percutaneous microwave coagulation therapy (PMCT) for primary liver cancer. *Xiandai Xiaohua Ji Jieru Zhenliao* 2005; **10**: 200-202

9 **Xiao ZY**, Chen XP, Huang ZY. Treatment of liver cancer with microwave coagulation after interruption of cancerous blood supply: A clinical analysis of 120 cases. *Zhonghua Gandan Waike Zazh*i 2005; **11**: 806-808

10 **Dong BW**, Liang P, Yu XL, Zeng XQ, Wang PJ, Su L, Wang XD, Xin H, Li S. Sonographically guided microwave coagulation treatment of liver cancer: an experimental and clinical study. *AJR Am J Roentgenol* 1998; **171**: 449-454

11 **Yu NC**, Raman SS, Kim YJ, Lassman C, Chang X, Lu DS. Microwave liver ablation: influence of hepatic vein size on heat-sink effect in a porcine model. *J Vasc Interv Radiol* 2008; **19**: 1087-1092

12 **Hines-Peralta AU**, Pirani N, Clegg P, Cronin N, Ryan TP, Liu Z, Goldberg SN. Microwave ablation: results with a 2.45-GHz applicator in ex vivo bovine and in vivo porcine liver. *Radiology* 2006; **239**: 94-102

13 **Simon CJ**, Dupuy DE, Iannitti DA, Lu DS, Yu NC, Aswad BI, Busuttil RW, Lassman C. Intraoperative triple antenna hepatic microwave ablation. *AJR Am J Roentgenol* 2006; **187**: W333-W340

14 **Kan Z**, Sato M, Ivancev K, Uchida B, Hedgpeth P, Lunderquist A, Rosch J, Yamada R. Distribution and effect of iodized poppyseed oil in the liver after hepatic artery embolization: experimental study in several animal species. *Radiology* 1993; **186**: 861-866

15 **Chung JW**. Transcatheter arterial chemoembolization of hepatocellular carcinoma. *Hepatogastroenterology* 1998; **45** Suppl 3: 1236-1241

16 **Patterson EJ**, Scudamore CH, Owen DA, Nagy AG, Buczkowski AK. Radiofrequency ablation of porcine liver in vivo: effects of blood flow and treatment time on lesion size. *Ann Surg* 1998; **227**: 559-565

17 **Goldberg SN**, Hahn PF, Tanabe KK, Mueller PR, Schima W, Athanasoulis CA, Compton CC, Solbiati L, Gazelle GS. Percutaneous radiofrequency tissue ablation: does perfusion-mediated tissue cooling limit coagulation necrosis? *J Vasc Interv Radiol* 1998; **9**: 101-111

18 **He W**, Liang XN, Zhang XR, Jiang XH, Zheng YC, Zhang Y. Evaluation of ultrasound-guided microwave coagulation in combination with transarterial chemoembolization for large hepatic cancers. *Zhongguo Weichuang Waike Zazhi* 2005; **5**: 31-33

19 **Ishida T**, Murakami T, Shibata T, Inoue Y, Takamura M, Niinobu T, Sato T, Nakamura H. Percutaneous microwave tumor coagulation for hepatocellular carcinomas with

interruption of segmental hepatic blood flow. *J Vasc Interv Radiol* 2002; **13**: 185-191

20 **Seki T**, Tamai T, Nakagawa T, Imamura M, Nishimura A, Yamashiki N, Ikeda K, Inoue K. Combination therapy with transcatheter arterial chemoembolization and percutaneous microwave coagulation therapy for hepatocellular carcinoma. *Cancer* 2000; **89**: 1245-1251

21 **Lu MD**, Xu HX, Kuang M, Xie XY, Liu GJ, Xu ZF, Zheng YL, Liang JY. Research of the improved microwave ablation therapy for treatment of hepatocellular carcioma. *Zhongguo Shiyong Waike Zazhi* 2004; **24**: 678-680

22 **Liang P**, Dong B, Yu X, Yu D, Wang Y, Feng L, Xiao Q. Prognostic factors for survival in patients with hepatocellular carcinoma after percutaneous microwave ablation. *Radiology* 2005; **235**: 299-307

23 **Aramaki M**, Kawano K, Ohno T, Sasaki A, Tahara K, Kai S, Iwashita Y, Kitano S. Microwave coagulation therapy for unresectable hepatocellular carcinoma. *Hepatogastroenterology* 2004; **51**: 1784-1787

24 **Nakajima Y**, Nagabuchi E, Sato N, Kamiyama T, Matsuoka S, Une Y, Uchino J. [A clinical study of liver resections in patients with small hepatocellular carcinoma less than three centimeter in diameter] *Nippon Geka Gakkai Zasshi* 1992; **93**: 1087-1090

25 **Kanai T**, Hirohashi S, Upton MP, Noguchi M, Kishi K, Makuuchi M, Yamasaki S, Hasegawa H, Takayasu K, Moriyama N. Pathology of small hepatocellular carcinoma. A proposal for a new gross classification. *Cancer* 1987; **60**: 810-819

26 **Guan YS**, Sun L, Zhou XP, Li X, Zheng XH. Hepatocellular carcinoma treated with interventional procedures: CT and MRI follow-up. *World J Gastroenterol* 2004; **10**: 3543-3548

27 **Huang N**, Yang WZ, Jiang N, Zheng QB, Huang JY. Treatment of portal vein tumor emboli of hepatocellular carcinoma with CT-guided percutaneous ethanol injection. *Jieru Fangshexue Zazhi* 2006; **15**: 670-672

S- Editor Li JL **L- Editor** Ma JY **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.753

World J Gastroenterol 2009 February 14; 15(6): 753-755
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naive patients

Rebecca Pastor, François Habersetzer, Samira Fafi-Kremer, Michel Doffoël, Thomas F Baumert, Jean-Pierre Gut, Françoise Stoll-Keller, Evelyne Schvoerer

Rebecca Pastor, Samira Fafi-Kremer, Jean-Pierre Gut, Françoise Stoll-Keller, Evelyne Schvoerer, Laboratoire de Virologie, CHRU de Strasbourg, 3 Rue Koeberlé, 67000 Strasbourg, France
Samira Fafi-Kremer, Thomas F Baumert, Jean-Pierre Gut, Françoise Stoll-Keller, Evelyne Schvoerer, Inserm U 748, Strasbourg, 3 Rue Koeberlé, 67000 Strasbourg, France
François Habersetzer, Michel Doffoël, Thomas F Baumert, Service d'Hépato-gastroentérologie, Nouvel Hôpital Civil, 1 Place de l'Hôpital, 67091 Strasbourg, France
Samira Fafi-Kremer, Thomas F Baumert, Jean-Pierre Gut, Françoise Stoll-Keller, Evelyne Schvoerer, Université Louis Pasteur, Faculté de Médecine, 4 Rue Kirschleger, 67084 Strasbourg, France
Author contributions: Pastor R performed the experiments, analyzed the data and wrote the manuscript; Habersetzer F, Doffoël M and Baumert TF contributed to the manuscript writing as experts in Hepato-gastroenterology and in viral hepatitis field; Fafi-Kremer S, Gut JP and Stoll-Keller F contributed to the virological analyses; Schvoerer E conducted the study and wrote the manuscript.
Supported by Siemens Medical Solutions Diagnostics, France, provided the reagents
Correspondence to: Dr. Evelyne Schvoerer, Institut de Virologie, 3 Rue Koeberlé, 67000 Strasbourg, France. evelyne.schvoerer@chru-strasbourg.fr
Telephone: +33-390243696 Fax: +33-390243750
Received: September 26, 2008 Revised: December 17, 2008
Accepted: December 24, 2008
Published online: February 14, 2009

Abstract

Anti-hepatitis B virus (HBV) therapy leads to the emergence of mutant viral strains during the treatment of chronic hepatitis B with nucleos(t)ides analogues. The existence of HBV variants with primary antiviral resistance may be important for treatment choice. We studied two patients with chronic HBV infection by sequencing the HBV polymerase gene. They had adefovir- and tenofovir-related mutations in the viral polymerase, although they had never been treated. These mutations were rtV214A/rtN238T in one patient and rtA194T in the other. Thus, mutations in untreated patients deserve cautious surveillance. These data indicate that mutations that can theoretically confer adefovir or tenofovir resistance may emerge in treatment-naive patients.

Key words: Hepatitis B virus; Viral polymerase mutations; Treatment-naive patients

Peer reviewers: George Papatheodoridis, MD, Assistant Professor in Medicine & Gastroenterology, 2nd Department of Internal Medicine, Athens University Medical School, Hippokration General Hospital of Athens, 114 Vas. Sophias Ave., 11527 Athens, Greece; Rosemary Joyce Burnett, MPH, Department of Epidemiology National School of Public Health, University of Limpopo, Medunsa Campus PO Box 173, MEDUNSA, Pretoria 0204, South Africa

Pastor R, Habersetzer F, Fafi-Kremer S, Doffoël M, Baumert TF, Gut JP, Stoll-Keller F, Schvoerer E. Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naive patients. *World J Gastroenterol* 2009; 15(6): 753-755 Available from: URL: http://www.wjgnet.com/1007-9327/15/753.asp DOI: http://dx.doi.org/10.3748/wjg.15.753

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem, with approximately 350 million individuals chronically infected worldwide. Chronic HBV carriers are exposed to the risk of complications, including chronic hepatitis, cirrhosis and hepatocellular carcinoma. Up to one million people die every year from complications of HBV infection[1].

The discovery and clinical use of antiviral agents, targeting in particular the viral reverse transcriptase, have revolutionized therapy for patients chronically infected with HBV[2]. While each nucleotide or nucleoside produces efficient viral suppression, they induce only modest rates of HBV surface antigen (HBsAg) seroconversion and require long-term administration to control disease in patients. The need for long-term therapy necessitates drug safety and the ability to delay or manage the emergence of resistant HBV strains[3-5]. The clinical benefit of these therapies has been compromised by the emergence of resistant viral strains that carry specific mutations in the polymerase gene[6,7]. Elsewhere, mutations can be observed in treatment-naive patients, but very little is known about the frequency and impact

of these HBV variants[8-11].

In this context, different treatment options for the optimal management of chronic hepatitis B deserve to be mentioned. According to the European Association for the Study of the Liver, interferon alpha or nucleos(t)ides analogues can be used[12]. The indications for treatment, for patients with HBe antigen (HBeAg)-positive or -negative chronic HBV infection, are based on three criteria: serum HBV DNA > 2000 IU/mL, serum alanine aminotransferase (ALT) above the upper limit of normal, and liver biopsy showing at least grade A2 or stage F2 by METAVIR scoring. As a summary of recommendations, interferon can be proposed in patients with high baseline ALT (> 3 times upper limit of normal) and HBV DNA < 2×10^6 IU/mL at baseline. Interferon gives higher rates of HBeAg and HBsAg seroconversion but has frequent side effects. The second option is treatment with nucleos(t)ides analogues with potent antiviral efficacy, good tolerance but lower rates of HBe and HBs seroconversion, possibly indefinite duration of treatment, and risk of HBV resistance.

In the context of the introduction of HBV sequencing in the Virology Laboratory of Strasbourg University Hospital, with the aim to explore genotypic viral resistance, 14 untreated patients with chronic HBV infection were investigated for the HBV polymerase gene. Two of 14 patients showed, in the absence of treatment pressure, mutations theoretically linked to adefovir and tenofovir pressure.

CASE REPORT

In December 2005, a 50-year-old Vietnamese man was diagnosed with chronic HBV infection, without co-infection by hepatitis delta virus, hepatitis C virus (HCV) or human immunodeficiency virus (HIV). This diagnosis was established by exploring asthenia associated with arthralgia. As mentioned during the medical investigation when the patient was admitted to the University Hospital of Strasbourg, the infection was acquired by vertical transmission, which is common in Asia[13,5]. At the time of diagnosis, HBsAg and HBeAg were positive and anti-HBc antibodies were detected. The patient's ALT and aspartate aminotransferase (AST) values were seven times the upper limit of normal. The viral load was 1.08×10^8 IU/mL (COBAS TaqMan HBV test; Roche Diagnostics). In March 2006, a liver biopsy confirmed the diagnosis of chronic hepatitis B with moderate activity and extensive fibrosis (Metavir score A2F3). Sequencing of the HBV polymerase (TRUGENE® HBV genotyping kit; Siemens Medical Solutions Diagnostics, France) revealed two mutations potentially linked to adefovir resistance: rtV214A and rtN238T (confirmed by a second sequencing).

The second patient, a 35-year-old Moroccan man, was diagnosed with HBV infection in May 2003, without co-infection by hepatitis delta virus, HCV or HIV. He presented no past history of hospitalization, transfusion or drug addiction. HBsAg was positive, HBeAg was negative, and anti-HBc antibodies were detected in the absence of anti-HBs antibodies. The viral load was 43 200 copies/mL (Amplicor HBV test; Roche Diagnostics). ALT and AST values were normal. A liver biopsy performed in July 2003 confirmed the diagnosis of chronic hepatitis B (Metavir score A1F0). Therefore, a simple follow-up was proposed to the patient. In December 2005, sequencing of the viral genome revealed an rtA194T mutation of the polymerase (confirmed by a second sequencing), possibly linked to tenofovir resistance.

DISCUSSION

In these two cases, viral variants with mutations that confer potential adefovir or tenofovir resistance were discovered in patients who had never been treated.

The rate of selection of adefovir resistance is around 30% after 5 years of treatment. Adefovir resistance is associated with a primary mutation in the D domain at rtN236T. In addition, a number of other mutations have been detected that cluster into three distinct regions of the polymerase: the D and A domains (rtP237H, rtN238T/D, rtV84M and rtS85A); the B domain at rtA181T/V; and the C-D interdomain (rtV214A, rtQ215S). These mutations may be regarded as secondary resistance mutations, as they are associated only with very low-level resistance *in vitro*. These secondary mutations have also been detected in the absence of rtN236T (both alone and in combination) in patients who have either not responded or have had a virological breakthrough during adefovir treatment[6]. The rtN238T mutation may be involved in disruption of triphosphate binding in viral polymerase. However, other authors have suggested that background polymorphisms including rtV214A and rtN238T could exist without any impact on antiviral treatment failure[8].

Tenofovir resistance conferred by rtA194T in association with the changes that cause lamivudine resistance, i.e. rtL180M and rtM204V, has been observed in individuals who are co-infected with HBV and HIV-1[14,15]. However, the analysis reported by other authors has not provided a clear association between rtA194T and viral load rebound[4]. Thus, the potential impact of this mutation on tenofovir susceptibility deserves further study.

Two hypotheses come to mind. The first one is that the mutations appeared in the course of the chronic infection. The probability of selecting antiviral resistance is usually proportional to the intensity of selection pressure and the diversity of HBV quasispecies[6]. In our cases, treatment was not the means of selection pressure that allowed the mutated clones to take over. In our study, the pressure may correspond to the patients' immune system, or the mutant clones that bear the rtV214A, rtN238T or rtA194T mutations may confer a replication advantage on the wild-type virus. This hypothesis suggests that there is a natural polymorphism in the population with chronic hepatitis B, which might predispose to resistance to certain antiviral agents. The second hypothesis is that the patients may have been

infected with strains from other patients who had been treated with the corresponding nucleotide analogues.

Although the fact that two treatment-naive patients were infected by HBV strains with mutations in viral polymerase is interesting, two limitations have to be considered. First, since only 14 patients were studied, the prevalence of these changes in treatment-naive patients cannot be safely established. Large-scale investigations in HBV-infected patients, before any anti-HBV treatment**,** should be conducted in order to determine this prevalence. Second, the changes observed in positions 194, 214 and 238 of HBV polymerase do not represent well-established HBV resistance mutations[4,8]. Based on *in vitro* results, with controversial data reported for the rtA194T change by Delaney *et al*[4] and for rtV214A and rtN238T by Borroto-Esoda *et al*[8], the clinical significance of these mutants remains questionable.

In conclusion, our results concerning HBV mutations in treatment-naive patients that potentially confer resistance suggest the need for studies on large cohorts. By analyzing HBV sequences before antiviral therapy with analogues in treatment-naive patients, the clinical impact of pre-treatment mutations on the efficacy of antiviral therapy may be better characterized[16].

REFERENCES

1 **Pallier C**, Castéra L, Soulier A, Hézode C, Nordmann P, Dhumeaux D, Pawlotsky JM. Dynamics of hepatitis B virus resistance to lamivudine. *J Virol* 2006; **80**: 643-653

2 **Durantel D**, Brunelle MN, Gros E, Carrouée-Durantel S, Pichoud C, Villet S, Trepo C, Zoulim F. Resistance of human hepatitis B virus to reverse transcriptase inhibitors: from genotypic to phenotypic testing. *J Clin Virol* 2005; **34** Suppl 1: S34-S43

3 **Buti M**, Elefsiniotis I, Jardi R, Vargas V, Rodriguez-Frias F, Schapper M, Bonovas S, Esteban R. Viral genotype and baseline load predict the response to adefovir treatment in lamivudine-resistant chronic hepatitis B patients. *J Hepatol* 2007; **47**: 366-372

4 **Delaney WE**, Ray AS, Yang H, Qi X, Xiong S, Zhu Y, Miller MD. Intracellular metabolism and in vitro activity of tenofovir against hepatitis B virus. *Antimicrob Agents Chemother* 2006; **50**: 2471-2477

5 **Wai CT**, Fontana RJ. Clinical significance of hepatitis B virus genotypes, variants, and mutants. *Clin Liver Dis* 2004; **8**: 321-352, vi

6 **Bartholomeusz A**, Locarnini S. Hepatitis B virus mutations associated with antiviral therapy. *J Med Virol* 2006; **78** Suppl 1: S52-S55

7 **Tenney DJ**, Levine SM, Rose RE, Walsh AW, Weinheimer SP, Discotto L, Plym M, Pokornowski K, Yu CF, Angus P, Ayres A, Bartholomeusz A, Sievert W, Thompson G, Warner N, Locarnini S, Colonno RJ. Clinical emergence of entecavir-resistant hepatitis B virus requires additional substitutions in virus already resistant to Lamivudine. *Antimicrob Agents Chemother* 2004; **48**: 3498-3507

8 **Borroto-Esoda K**, Miller MD, Arterburn S. Pooled analysis of amino acid changes in the HBV polymerase in patients from four major adefovir dipivoxil clinical trials. *J Hepatol* 2007; **47**: 492-498

9 **Chen CH**, Wang JH, Lee CM, Hung CH, Hu TH, Wang JC, Lu SN, Changchien CS. Virological response and incidence of adefovir resistance in lamivudine-resistant patients treated with adefovir dipivoxil. *Antivir Ther* 2006; **11**: 771-778

10 **Schildgen O**, Sirma H, Funk A, Olotu C, Wend UC, Hartmann H, Helm M, Rockstroh JK, Willems WR, Will H, Gerlich WH. Variant of hepatitis B virus with primary resistance to adefovir. *N Engl J Med* 2006; **354**: 1807-1812

11 **Yamazhan T**, Sertöz R, Pullukçu H, Taşbakan M, Ulusoy S, Erensoy S. [A case of chronic hepatitis B with primary adefovir resistance] *Mikrobiyol Bul* 2007; **41**: 297-301

12 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242

13 **Lavanchy D**. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; **34** Suppl 1: S1-S3

14 **Shaw T**, Bartholomeusz A, Locarnini S. HBV drug resistance: mechanisms, detection and interpretation. *J Hepatol* 2006; **44**: 593-606

15 **Sheldon J**, Camino N, Rodés B, Bartholomeusz A, Kuiper M, Tacke F, Núñez M, Mauss S, Lutz T, Klausen G, Locarnini S, Soriano V. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. *Antivir Ther* 2005; **10**: 727-734

16 **Wu GY**, Chen HS. Novel approaches towards conquering hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 830-836

S- Editor Li LF **L- Editor** Kerr C **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.756

World J Gastroenterol 2009 February 14; 15(6): 756-757
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Complete peritonectomy and intraperitoneal chemotherapy for recurrent rectal cancer with peritoneal metastasis

Jung Wook Huh, Young Jin Kim, Hyeong Rok Kim

Jung Wook Huh, Young Jin Kim, Hyeong Rok Kim, Department of Surgery, Chonnam National University Hwasun Hospital and Medical School, Gwangju 501-757, South Korea
Author contributions: All authors contributed to the intellectual content and approved the final version; Huh JW wrote the paper; Kim YJ performed the operation.
Correspondence to: Young Jin Kim, MD, PhD, Department of Surgery, Chonnam National University Hwasun Hospital and Medical School, Gwangju 501-757, South Korea. kimyjin@chonnam.ac.kr
Telephone: +82-61-3797646 Fax: +82-61-3797661
Received: November 17, 2008 Revised: January 6, 2009
Accepted: January 13, 2009
Published online: February 14, 2009

Abstract

A 68-year-old man underwent laparoscopic low anterior resection for rectal carcinoma in December 2006. Nearly 19 mo after the operation, he developed recurrent rectal cancer with peritoneal metastasis. In September 2008, he subsequently underwent a laparotomy with a peritonectomy, omentectomy, splenectomy, and a Hartmann procedure. Hyperthermic intraperitoneal oxaliplatin 750 mg was administered. The patient was discharged with no postoperative complications and has been well with postoperative FOLFOX chemotherapy.

Key words: Peritonectomy; Peritoneal metastasis; Recurrent rectal cancer

Peer reviewers: Dr. Bernardino Rampone, Department of General Surgery and Surgical Oncology, University of Siena, viale Bracci, Siena 53100, Italy; Gerardo Rosati, MD, Medical Oncology Unit, "S. Carlo" Hospital, Via Potito Petrone, 1, Potenza 85100, Italy

Huh JW, Kim YJ, Kim HR. Complete peritonectomy and intraperitoneal chemotherapy for recurrent rectal cancer with peritoneal metastasis. *World J Gastroenterol* 2009; 15(6): 756-757 Available from: URL: http://www.wjgnet.com/1007-9327/15/756.asp DOI: http://dx.doi.org/10.3748/wjg.15.756

INTRODUCTION

Peritoneal carcinomatosis has a poor prognosis, and survival is generally less than 6 mo once diagnosed[1,2]. Recent advances in the treatment of this disease have resulted in improved, and even long-term, survival for select patients[3-5]. Optimal cytoreduction and adjuvant intraperitoneal chemotherapy have been credited with much of the recent success in the treatment of this cohort of patients. We would like to share our own experience with a complete peritonectomy and intraperitoneal chemotherapy for recurrent rectal cancer with peritoneal metastasis, and to review the relevant literature.

CASE REPORT

A 68-year-old man underwent laparoscopic low anterior resection (LAR) for upper rectal cancer. Histological examination revealed a poorly differentiated mucinous adenocarcinoma with a 4-cm distal margin and the TNM staging was T2N0M0. On routine follow-up, an anastomotic induration was felt on digital rectal examination 19 mo later, which was confirmed to be adenocarcinoma upon endoscopic biopsy. Abdominopelvic computed tomography and positron emission tomography showed multiple soft tissue infiltrations throughout the peritoneum. At laparotomy, a bulky mass around the previous rectal anastomosis and multiple peritoneal metastatic nodules were identified. A complete peritonectomy (Figure 1), complete omentectomy, splenectomy, and a Hartmann procedure were performed successfully with no visible gross disease at the completion of the procedure. Two catheters were inserted in the abdomen and secured: one on the left side aimed toward the pelvis and the other on the right directed up over the liver. The abdomen was closed, and the peritoneal cavity was filled with heated saline at 42℃. Continuous hyperthermic intraoperative intraperitoneal perfusion with 750 mg of heated oxaliplatin was then performed over 90 min. The operating time was 515 min with a moderate blood loss of 500 mL. Histopathology revealed a recurrent mucinous adenocarcinoma in the rectum with a clear resection margin and multiple metastatic nodules in the diaphragmatic, pelvic, and pericolic peritoneum and omentum. His postoperative course was uneventful, and he was discharged on the 25th postsurgical day. He had difficulty voiding, but this was managed conservatively. For 2 mo after the second

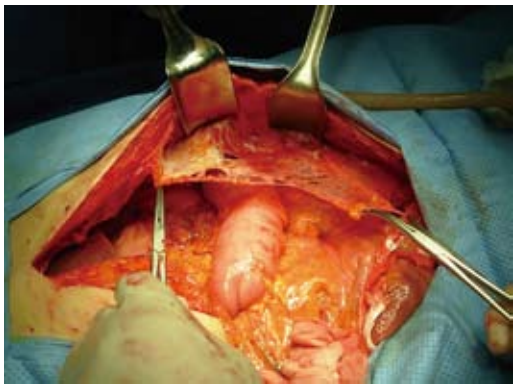


Figure 1 The peritonectomy.

operation, the patient has been well with postoperative FOLFOX chemotherapy.

DISCUSSION

Traditionally, there has been consensus in the oncology community that those patients with peritoneal carcinomatosis of colorectal origin were incurable. Neither systemic chemotherapy nor intraperitoneal chemotherapy alone had any significant impact on survival.

Recently, there has been increased interest in re-examining the management of peritoneal metastatic disease, and in utilizing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy[6-9]. Aggressive cytoreductive surgery attempts to eradicate all residual tumor cells, or to significantly reduce the tumor burden, and the infusion of hyperthermic intraoperative intraperitoneal chemotherapy allows a favorable drug distribution to all surfaces at risk, without delay. A randomized trial by a Dutch group demonstrated superior survival with the combined approach over traditional 5-fluorouracil-based systemic chemotherapy, for peritoneal carcinomatosis of colorectal cancer[6]. Moreover, with proper patient selection, minimal morbidity can be achieved, with good overall survival and prolonged disease-free periods. The 11.1% rate of major perioperative complications and no perioperative mortality achieved in this cohort of patients compares favorably with the 27% quoted in the literature[10]. Elias *et al*[11] recently suggested that preoperative intraperitoneal chemohyperthermia with oxaliplatin is better tolerated than early postoperative intraperitoneal chemotherapy with mitomycin C and 5-FU, and is twice as efficient in curing peritoneal carcinomatosis.

This report describes our initial experience with peritonectomy and hyperthermic intraperitoneal chemotherapy in a patient with recurrent rectal cancer. This combined approach can be a feasible treatment option for the traditionally inoperable recurrent rectal cancer patient with peritoneal metastasis.

REFERENCES

1 **Loggie BW**, Fleming RA, McQuellon RP, Russell GB, Geisinger KR. Cytoreductive surgery with intraperitoneal hyperthermic chemotherapy for disseminated peritoneal cancer of gastrointestinal origin. *Am Surg* 2000; **66**: 561-568

2 **McQuellon RP**, Loggie BW, Fleming RA, Russell GB, Lehman AB, Rambo TD. Quality of life after intraperitoneal hyperthermic chemotherapy (IPHC) for peritoneal carcinomatosis. *Eur J Surg Oncol* 2001; **27**: 65-73

3 **Feldman AL**, Libutti SK, Pingpank JF, Bartlett DL, Beresnev TH, Mavroukakis SM, Steinberg SM, Liewehr DJ, Kleiner DE, Alexander HR. Analysis of factors associated with outcome in patients with malignant peritoneal mesothelioma undergoing surgical debulking and intraperitoneal chemotherapy. *J Clin Oncol* 2003; **21**: 4560-4567

4 **Mohamed F**, Chang D, Sugarbaker PH. Third look surgery and beyond for appendiceal malignancy with peritoneal dissemination. *J Surg Oncol* 2003; **83**: 5-12; discussion 12-13

5 **Yan TD**, Morris DL. Cytoreductive surgery and perioperative intraperitoneal chemotherapy for isolated colorectal peritoneal carcinomatosis: experimental therapy or standard of care? *Ann Surg* 2008; **248**: 829-835

6 **Verwaal VJ**, van Ruth S, de Bree E, van Sloothen GW, van Tinteren H, Boot H, Zoetmulder FA. Randomized trial of cytoreduction and hyperthermic intraperitoneal chemotherapy versus systemic chemotherapy and palliative surgery in patients with peritoneal carcinomatosis of colorectal cancer. *J Clin Oncol* 2003; **21**: 3737-3743

7 **Glehen O**, Cotte E, Schreiber V, Sayag-Beaujard AC, Vignal J, Gilly FN. Intraperitoneal chemohyperthermia and attempted cytoreductive surgery in patients with peritoneal carcinomatosis of colorectal origin. *Br J Surg* 2004; **91**: 747-754

8 **Teo M**, Foo KF, Koo WH, Wong LT, Soo KC. Lessons learned from initial experience with peritonectomy and intra-peritoneal chemotherapy infusion. *World J Surg* 2006; **30**: 2132-2135

9 **Qu ZB**, Liu LX. Management of pseudomyxoma peritonei. *World J Gastroenterol* 2006; **12**: 6124-6127

10 **Ahmad SA**, Kim J, Sussman JJ, Soldano DA, Pennington LJ, James LE, Lowy AM. Reduced morbidity following cytoreductive surgery and intraperitoneal hyperthermic chemoperfusion. *Ann Surg Oncol* 2004; **11**: 387-392

11 **Elias D**, Benizri E, Di Pietrantonio D, Menegon P, Malka D, Raynard B. Comparison of two kinds of intraperitoneal chemotherapy following complete cytoreductive surgery of colorectal peritoneal carcinomatosis. *Ann Surg Oncol* 2007; **14**: 509-514

S- Editor Li LF **L- Editor** Stewart GJ **E- Editor** Lin YP

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.758

World J Gastroenterol 2009 February 14; 15(6): 758-760
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Gastric pneumatosis intestinalis associated with malignancy: An unusual case report

Ahmet Bilici, Berrin Karadag, Alper Doventas, Mesut Seker

Ahmet Bilici, Mesut Seker, Department of Medical Oncology, Dr.Lutfi Kirdar Kartal Education and Research Hospital, Kartal, 34210 Istanbul, Turkey
Berrin Karadag, Department of Internal Medicine, Sisli Etfal Education and Research Hospital, 34210 Istanbul, Turkey
Alper Doventas, Department of Internal Medicine, Istanbul Education and Research Hospital, 34210 Istanbul, Turkey
Author contributions: Bilici A, Doventas A and Karadag B designed the work; Bilici A wrote the paper; Bilici A and Karadag B followed up the patient; Bilici A and Seker M reviewed the article.
Correspondence to: Ahmet Bilici, MD, Department of Medical Oncology, Menderes mh. 23.sok.Caglar Apt. No. 16/1, Esenler, 34210 Istanbul, Turkey. drknower@hotmail.com
Telephone: +90-505-7988132 Fax: +90-216-4232134
Received: June 22, 2008 Revised: December 30, 2008
Accepted: January 7, 2009
Published online: February 14, 2009

Abstract

Pneumatosis intestinalis (PI) is an uncommon disease defined as gas-filled cysts that are found in the wall of the gastrointestinal (GI) tract. The exact causes of PI are still unclear, but it may associated with coexisting diseases, such as some GI disorders, connective tissue disease, some medication and drugs, and rarely malignancy. The most common localization is the small intestine. Gastric PI secondary to malignancy has been rarely documented. We report on a 94-year-old man with gastric PI associated with inoperable adenocarcinoma localized in the duodenum. Following the gastrojejunostomy and choledochojejunostomy bypass, his general condition improved and PI disappeared, but he died due to poor performance status and malignancy 6 mo later. We suggest that in patients presenting with PI, malignancy should be considered in the differential diagnosis.

Key words: Pneumatosis intestinalis; Malignancy; Adenocarcinoma

Peer reviewers: Toru Ishikawa, MD, Department of Gastroenterology, Saiseikai Niigata Second Hospital, Teraji 280-7, Niigata, Niigata 950-1104, Japan; Dr. Shinji Tanaka, Director, Department of Endoscopy, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

Bilici A, Karadag B, Doventas A, Seker M. Gastric pneumatosis intestinalis associated with malignancy: An unusual case report. *World J Gastroenterol* 2009; 15(6): 758-760 Available from: URL: http://www.wjgnet.com/1007-9327/15/758.asp DOI: http://dx.doi.org/10.3748/wjg.15.758

INTRODUCTION

Pneumatosis intestinalis (PI) is a rare disorder characterized by multilocular gas-filled cysts localized in the submucosa and subserosa of the alimentary tract. It can occur in any part of the gastrointestinal tract (GI), from the esophagus to the rectum, but the small intestine is the most common localization[1-5]. PI may be primarily and secondarily associated with a coexisting disease. Eighty-five percent of patients have secondary PI[1,6,7]. Gastric PI, defined as air within the wall of the stomach, is an uncommon localization[8-12]. On the other hand, gastric PI, secondary to malignancy, has been very rarely reported in the literature[13]. We described here a case of gastric PI associated with adenocarcinoma localized in duodenum.

CASE REPORT

A 94-year-old man was admitted to our hospital with anorexia, nausea, vomiting, fatigue, constipation, weight loss, abdominal bloating and discomfort which started 1 mo previously, in May 2003. He had a prior history of peptic ulcer 15 years ago. He reported a history of upper GI bleeding in 1990. He had no the other systemic diseases, such as chronic obstructive lung diseases, connective tissue diseases, and no history of abdominal surgery. His family history was not contributory. On physical examination, he was dehydrated and afebrile. His blood pressure was 100/80 mm/Hg, pulse was 84/min, and heart sounds and jugular venous pressure were normal. No murmur was detected. Breath sounds were normal and there were no organomegaly or lympadenomegaly. Epigastric and periumblical sensitivity were noted. There was an abdominal distension and hypertympanism found on epigastric sites.

Initial laboratory results were as follows: blood urea nitrogen 23 mg/dL, creatinine 1.1 mg/dL, sodium 140 mEq/L, potassium 3.8 mEq/L, calcium 9.3 mg/dL, alanine aminotransferase 33 U/L, aspartate

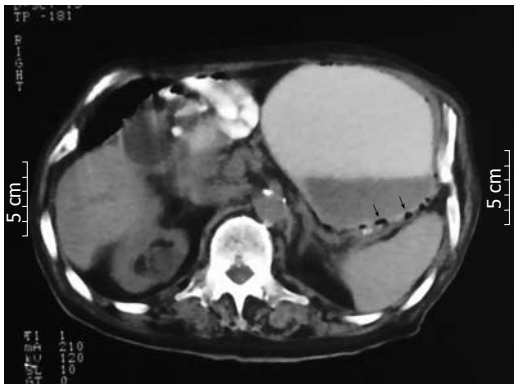


Figure 1 Abdomino-pelvic CT scan showing big stomach and multilocular gas within the wall of the stomach (arrows).

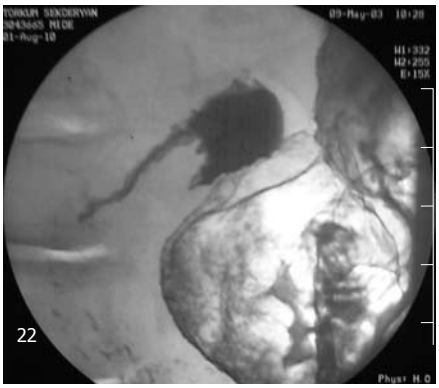


Figure 2 Barium-enema study showing luminal obstruction of 80% in the bulber apex.

aminotransferase 27 U/L, γ- glutamyltransferase 428 U/L (N:7-49) alkaline phosphatase 317 U/L (N:38-155), lactate dehydrogenase 412 IU/L, glucose 105 mg/dL, erythrocyte sedimentation rate 40 mm/h, C-reactive protein 136.4 mg/L, total bilirubin 0.78 mg/dL, direct bilirubin 0.13 mg/dL, white blood cells (WBCs) 12400/mm^3, platelets 217000/mm^3, hematocrit 36.3%, mean corpuscular volume 84.9 fL, Fe 47 μ/dL, total iron binding capacity 214 μ/dL, and ferritin 55.4 ng/mL. Serum protein electrophoresis was normal. The patient's tumor marker profile was as follows: carbohydrate antigen 19-9 903.1 U/mL (0-37), carcinoembryonic antigen 12.3 ng/mL (0-3.4). Urine analysis revealed a urine specific gravity of 1010, protein negative, pH 5, and two WBCs per high-power field on urine sediment. Hepatitis B and C serology, and anti-HIV assays were negative. Fecal occult blood test was positive for the one stool sample. Other laboratory values were within normal limits.

The patient was treated with nasogastric intubation, parenteral fluids, ranitidine i.v. and broad-spectrum antibiotics. Because of elevated tumor markers, a diagnostic work-up for possible malignancy was initiated. The plain abdominal radiography (PAR) was normal. Abdominal ultrasonography (US) revealed sludge within the lumen of the gall bladder with findings of hydrops. The intrahepatic bile ducts were dilated (right, 5.3 mm; left, 4.5 mm). Abdomen and pelvic CT scans showed an enlarged stomach and multilocular gas-filled cysts localized in the wall of the stomach (Figure 1). The diagnosis of PI was made. Subsequent magnetic resonance cholangiopancreatography imaging demonstrated sludge with findings of hydrops and dilatation of intrahepatic bile ducts and choledochus. There was a calculius of 2 cm diameter at the neck of gall bladder.

A barium-enema study revealed luminal obstruction of 80% (Figure 2). As a result of these findings, gastroduodenoscopy and biopsy were performed. In the antrum, the ulcer had a 4-5 cm diameter, likely a malignant ulcer, and it was established that the lesion obstructed the lumen in the postbulbar duodenum. Malignancy was not detected in biopsy specimens. After the patient's general condition markedly improved, exploratory laporatomy was performed in the department of general surgery. An inoperable tumor originating from the duodenum was detected. In addition, the tumor was infiltrated to extra-hepatic bile ducts and vascular structures. Gastrojejunostomy and choledochojejunostomy were carried out. The histopathological examination of biopsy specimens revealed adenocarcinoma infiltration originating from the GI tract. As a result of his age and performance status, we decided to treat with supportive management. Following the surgical procedure, the gas-filled cysts disappeared and his symptoms improved. After 6 mo of follow-up, he died from the the influence of malignancy and poor general condition.

DISCUSSION

PI is an uncommon entity in which gas-filled lesions are accumulated within the GI wall. In 42% of the cases with PI, the small intestine is the most common and the large intestine (36%) the second most common localization; in 22% of patients both small and large intestine are affected[1,2,6]. On the other hand, gastric PI has been rarely documented[8-12]. It can be classified as either idiopathic or of primary unknown etiology (15%) or the secondary type (85%)[1,6,7]. Fifty-five percent of secondary causes are peptic ulcer related to pyloric obstruction, and 3.3% of cases are GI malignancy[2]. In our patient, the gastric secondary type of PI associated with GI malignancy was detected. Gastric PI related to malignancy has been documented extremely rarely[13].

Although the pathogenesis of PI is not well understood, many theories have been proposed. First, the mechanical theory suggests that gas under pressure diffuses into the bowel wall from the intestinal lumen or the pulmonary airway. This theory is probably related to PI caused by trauma, surgery, endoscopy and bowel obstruction. The bacterial theory proposes that bacteria enter the bowel wall and produce gas within the intestinal wall[3,14]. The pulmonary theory proposes that alveolar rupture results in the diffusion of air through the mediastinum into the retroperitoneal space, through the diaphragm, along major vessels in the mesentery, and into the bowel wall. It may explain the occurrence of PI in patients with chronic obstructive pulmonary disease. Finally, there is the mucosal damage theory. It suggests that gas under pressure is forced into the bowel wall

by mucosal disruption. PI associated with peptic ulcer disease, pyloric stenosis and malignancy may probably be explained by this theory[2,3].

PI is usually asymptomatic, but patients can have gastrointestinal symptoms variying in severity, such as abdominal discomfort, distension and pain, rectal bleeding, constipation, meteorism and weight loss. The symptoms are dependent on the localization of PI and on the presence or non-presence of underlying disease[2,3,6]. Our patient also presented with gastrointestinal symptoms and elevated tumor markers. After PI was detected, we initialy started a diagnostic work-up to exclude malignancy as a cause of PI. The work-up revealed an inoperable adenocarcinoma originating from the duodenum. After gastrojejunostomy and choledochojejunostomy, the gas-filled cysts disappeared and the patient improved.

In two-thirds of patients with PI, PAR shows characteristic changes in the bowel. The PAR with our patient was normal, but an abdomino-pelvic CT scan revealed PI. In the literature, additional diagnostic procedures, such as US, CT, MRI, endoscopy and barium contrast studies, have been documented[1,2,5]. There is no special treatment for PI. If underlying diseases are present, it is necessary to treat them[6]. Conservative therapy can be causal or symptomatic. Causal treatments consist of normobaric or hyperbaric oxygen therapy, antibiotic treatment, especially metronidazole, endoscopic puncture and cysts sclerotherapy[6,15-17]. The aim of these therapies is to suppress the etiological mechanisms. Surgical treatments should be avoided unless there are serious diseases, such as malignancy, metabolic acidosis, portal venous gas and severe inflammation[18].

This report constitutes a rare case of gastric PI caused by duodenal adenocarcinoma. In patients with PI, especially localized in the stomach, who have serious GI symptoms and elevated tumor markers, malignancy-induced PI should be considered in the differential diagnosis.

REFERENCES

1 **Jamart J**. Pneumatosis cystoides intestinalis. A statistical study of 919 cases. *Acta Hepatogastroenterol* (Stuttg) 1979; **26**: 419-422

2 **Heng Y**, Schuffler MD, Haggitt RC, Rohrmann CA. Pneumatosis intestinalis: a review. *Am J Gastroenterol* 1995; **90**: 1747-1758

3 **Gagliardi G**, Thompson IW, Hershman MJ, Forbes A, Hawley PR, Talbot IC. Pneumatosis coli: a proposed pathogenesis based on study of 25 cases and review of the literature. *Int J Colorectal Dis* 1996; **11**: 111-118

4 **Boerner RM**, Fried DB, Warshauer DM, Isaacs K. Pneumatosis intestinalis. Two case reports and a retrospective review of the literature from 1985 to 1995. *Dig Dis Sci* 1996; **41**: 2272-2285

5 **Rennenberg RJ**, Koek GH, Van Hootegem P, Stockbrugger RW. Pneumatosis cystoides intestinalis, four cases of a rare disease. *Neth J Med* 2002; **60**: 22-25

6 **Voboril R**. Pneumatosis cystoides intestinalis--a review. *Acta Medica* (Hradec Kralove) 2001; **44**: 89-92

7 **Kreiss C**, Forohar F, Smithline AE, Brandt LJ. Pneumatosis intestinalis complicating C. difficile pseudomembranous colitis. *Am J Gastroenterol* 1999; **94**: 2560-2561

8 **Cordum NR**, Dixon A, Campbell DR. Gastroduodenal pneumatosis: endoscopic and histological findings. *Am J Gastroenterol* 1997; **92**: 692-695

9 **Travadi JN**, Patole SK, Simmer K. Gastric pneumatosis in neonates: revisited. *J Paediatr Child Health* 2003; **39**: 560-562

10 **Kawano S**, Tanaka H, Daimon Y, Niizuma T, Terada K, Kataoka N, Iwamura Y, Aoyama K. Gastric pneumatosis associated with duodenal stenosis and malrotation. *Pediatr Radiol* 2001; **31**: 656-658

11 **Franquet T**, Gonzalez A. Gastric and duodenal pneumatosis in a child with annular pancreas. *Pediatr Radiol* 1987; **17**: 262

12 **Leonidas JC**. Gastric pneumatosis in infancy. *Arch Dis Child* 1976; **51**: 395-398

13 **Holt RW**, Dekker J. Gastric pneumatosis intestinalis associated with cholangiocarcinoma. *South Med J* 1986; **79**: 79-80

14 **Yale CE**, Balish E. The natural course of Clostridium perfringens--induced pneumatosis cystoides intestinalis. *J Med* 1992; **23**: 279-288

15 **Tak PP**, Van Duinen CM, Bun P, Eulderink F, Kreuning J, Gooszen HG, Lamers CB. Pneumatosis cystoides intestinalis in intestinal pseudoobstruction. Resolution after therapy with metronidazole. *Dig Dis Sci* 1992; **37**: 949-954

16 **Paw HG**, Reed PN. Pneumatosis cystoides intestinalis confined to the small intestine treated with hyperbaric oxygen. *Undersea Hyperb Med* 1996; **23**: 115-117

17 **Johansson K**, Lindstrom E. Treatment of obstructive pneumatosis coli with endoscopic sclerotherapy: report of a case. *Dis Colon Rectum* 1991; **34**: 94-96

18 **Knechtle SJ**, Davidoff AM, Rice RP. Pneumatosis intestinalis. Surgical management and clinical outcome. *Ann Surg* 1990; **212**: 160-165

S- Editor Cheng JX **L- Editor** Rippe RA **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
doi:10.3748/wjg.15.761

World J Gastroenterol 2009 February 14; 15(6): 761-763
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Coexistence of tuberculous peritonitis and primary papillary serous carcinoma of the peritoneum: A case report and review of the literature

Xiang-Qian Hou, Hai-Hong Cui, Xing Jin

Xiang-Qian Hou, Xing Jin, Department of General Surgery, Shandong Provincial Hospital, Shandong University, Jinan 250021, Shandong Province, China
Hai-Hong Cui, Department of Digestive Disease, the 456 hospital of PLA, Jinan 250031, Shandong Province, China
Author contributions: Hou XQ, Cui HH and Jin X contributed equally to this work; Hou XQ, Cui HH performed research; Hou XQ, Cui HH, Jin X wrote the paper.
Correspondence to: Xing Jin, Department of General Surgery, Shandong Provincial Hospital, Shandong University, Jinan 250021, Shandong Province, China. xingjinsd@yahoo.cn
Telephone: +86-531-88382783 Fax: +86-531-87060696
Received: June 29, 2008 Revised: August 3, 2008
Accepted: August 10, 2008
Published online: February 14, 2009

Abstract

A major diagnostic challenge to the evaluation of an incomplete intestinal obstruction is to distinguish between infectious and malignant etiologies. We present a case of an elderly woman complaining of abdominal pain accompanied with nausea and vomiting, and failure to pass gas or stools. Anti-tuberculosis drugs were used to relieve her abdominal pain, and a needle biopsy of the peritoneal cavity showed evidence of primary papillary serous carcinoma of the peritoneum (PSCP). This is a rare description of tuberculosis in the setting of PSCP. This report illustrates the potential complex nature of malignancies, and emphasizes the need to consider coexistence of malignancy and infection in patients, especially in those with risk factors for malignancy who fail with antibiotic therapy.

Key words: Primary papillary serous carcinoma; Peritoneum; Tuberculous peritonitis

Peer reviewer: 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

Hou XQ, Cui HH, Jin X. Coexistence of tuberculous peritonitis and primary papillary serous carcinoma of the peritoneum: A case report and review of the literature. *World J Gastroenterol* 2009; 15(6): 761-763 Available from: URL: http://www.wjgnet.com/1007-9327/15/761.asp DOI: http://dx.doi.org/10.3748/wjg.15.761

INTRODUCTION

A major diagnostic challenge to the evaluation of peritonitis is to determine whether it represents an infectious or malignant process. In the majority of cases, it is difficult to distinguish them from each other based on their clinical and radiographic data alone, and more invasive testing is usually required to reach a definitive diagnosis. In this report, we describe our experience with a case of peritonitis resulting from the coexistence of two distinct pathological processes.

CASE REPORT

A 59-year-old Chinese woman who was previously healthy presented to our clinic with a 2-mo history of intermittent abdominal pain accompanying general fatigue, subjective low-grade fever, weight loss and night sweats, which were not improved after treatment with over-the-counter antibiotics and traditional Chinese herbal drugs. Ten days before she visited our clinic, she felt her symptoms getting worse with nausea and vomiting, and weight loss of 10 kg within 2 mo. Thereafter, she failed to pass gas or stools which prompted her to seek medical care. She was admitted to 263 Hospital of PLA. A biochemical test showed 6500/mL white blood cells (WBCs), 78% N, 90 g/L hemoglobin, normal tumor markers (including carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, CA125, alpha fetoprotein (AFP), erythrocyte sedimentation rate (ESR) 148 mm/h), and 135.8 mg/L CRP. Abdominal plain film displayed ascites and incomplete intestinal obstruction. Chest X-ray showed a high density of WBCs in the right middle outer lobe and lymph node calcification in the right trachea bifurcation. Abdominal paracentesis was arranged for further evaluation. Routine test of ascites showed that yellow fluid was translucent, 95% mononuclear cells, 5% polynuclear cells, 6400/μL erythrocytes and 210/μL WBCs, and 1.010 gravity. Protein concentration was 44.7 g/L, glucose was 1.82 mmoL/L, lactate

dehydrogenase was 334 U/L, and adenosine deaminase was 15 U/L. Acid-fast bacillus could not be found in the fluid. Culture of bacteria was negative. Cytology of the fluid was also negative. CEA and AFP of ascites were normal. Ten days after treatment with antibiotics, her abdominal pain was aggravated, and she was transferred to our hospital.

The patient was a cotton spinner prior to retirement and underwent appendectomy 30 years ago. She denied any history of hepatitis or alcohol, tobacco or recreational drug use and had no knowledge of sick contacts. Physical examination showed symptoms of tenacious abdominal wall, deep and rebound tenderness, decreased intestinal sound, but no actual palpable abdominal mass. Liver and spleen were not palpable, shifting dullness was negative.

After admission, tuberculin was positive. Ultrasonography and pelvic cavity computed tomography (CT) showed ascites and mesentery thickening and peritonitis could not be excluded. Blood test results were 100/mL WBCs, 83.8% N, 85 g/L hemoglobin, and 545 000/mL platelets. Routine urine and feces tests were normal. Feces occult blood tests was negative (140 mm/h ESR, 218 mg/L CRP, and 29.6 g/L albumin). Two-thirds of anti-tuberculosis test parameters were positive. Tumor markers (CEA, CA199, CA125, and AFP) were normal. CA, CYFRA, FRER, and TSGF were 153 100 U/mL, 21-1 16.44 ng/mL, > 2000 ng/mL, and 121.45 U/mL, respectively. LTA was positive. Autoimmune antibodies were negative. Abdominal ultrasound showed small encapsulated effusion in abdominal and pelvic cavity. Parietal peritoneum was thick. Ultrasound of the vagina showed metratrophia with no occupying lesions in the pelvic cavity. Chest X-ray showed old tuberculous foci on both lungs and mediastinum. Due to the suspicious appearance of the lesion, a positron emission tomography (PET) scan was performed along with induced peritoneal cavity CT. Diagnostic percutaneous needle biopsy was discussed with the patient and her family, but she denied any invasive tests. PET scan showed an increased metabolic activity in peritoneum, mesentery, and areas of omentum with thick and diffusive lesser tubercles. Local intestinal adhesions or distensions showed incomplete intestinal obstruction.

She began to cough and had a low fever, but biochemical tests could not identify any infectious bacteria in her sputum and feces. Since the patient remained symptomatic, we decided to treat her disease with oral isoniazid, rifabutin, ethambutol, and pyrazinamide. During the first 6 wk, she noticed significant clinical improvements, such as reduction in abdominal pain and ascites, and resolution of tenacious abdominal wall. Body temperature became normal. However, 8 wk after antibiotic therapy, as well as enteral and parenteral nutrition, her condition began to deteriorate. In addition to CT and ultrasonography, the patient underwent percutaneous CT-guided biopsy of the thick peritoneum, which revealed primary papillary serous carcinoma of the peritoneum (PSCP). Although subsequent staging suggested that it was amenable to chemotherapy, the patient was too weak to tolerate the treatment. Finally, therapy failed to improve her condition and she developed liver and kidney failure.

DISCUSSION

The main differential diagnostic considerations of diffuse peritoneal involvement associated with peritonitis include infectious processes (mainly tuberculosis) and malignant neoplastic conditions. Peritoneal involvement is a rare form of abdominal tuberculosis. Peritoneal tuberculosis occurs predominantly in patients aged 20-40 years and is always secondary to other tuberculous lesions and appears to be more common in females than in males. Tuberculosis in females commonly reaches the peritoneum through tubal infection and attacks the tubes during the sexually active period of life[1]. Diagnosis of any extrapulmonary forms of tuberculosis is quite difficult. For example, difficult diagnosis of peritoneal tuberculosis is due to its non-specific clinical manifestations, such as weight loss, abdominal pain, fever, ascites, and vomiting[2-3]. Positive culture of 7.8% *Mycobacterium. tuberculosis* has been reported[4]. Although our patient denied previous medical history of pulmonary or extra-pulmonary tuberculosis, tuberculous infection was diagnosed based on her positive tuberculin test and chest X-ray scan.

PSCP is a rare malignancy that predominantly affects postmenopausal women[5]. Reports suggest that approximately 10% of women diagnosed with ovarian, endometrial or sigmoid carcinoma actually have PSCP[6-8]. Multicentric peritoneal involvement is typical, and omental involvement is particularly common. Extensive calcification of omental caking present in many cases is a useful CT finding for excluding mesothelioma. The absence of ovarian mass is critical for excluding metastatic papillary serous ovarian carcinoma, which otherwise has a similar appearance at CT and is histologically identical to its primary peritoneal counterpart[9]. Some reports indicate a poor prognosis for women with peritoneal carcinomatosis[10-13]. Patients suffering from PSCP usually complain of abdominal distention, pain or pressure, associated with ascites and gastrointestinal symptoms (loss of appetite, nausea, vomiting, and change in bowel habits)[14]. On physical examination, the most common finding is ascites. The clinical presentation is usually indistinguishable from advanced ovarian cancer. Reports suggest that approximately 10% of women diagnosed with ovarian carcinoma actually have PSCP[15].

In this case, a moderate amount of ascites located between intestinal canals was observed by ultrasonography, and a thickened intestinal wall and pronounced enhancement of peritoneum were seen at CT scanning. Most nodules coalesced to form large omental plaques (omental cakes) (Figure 1). The largest plaque was located in the left lower quadrant of the

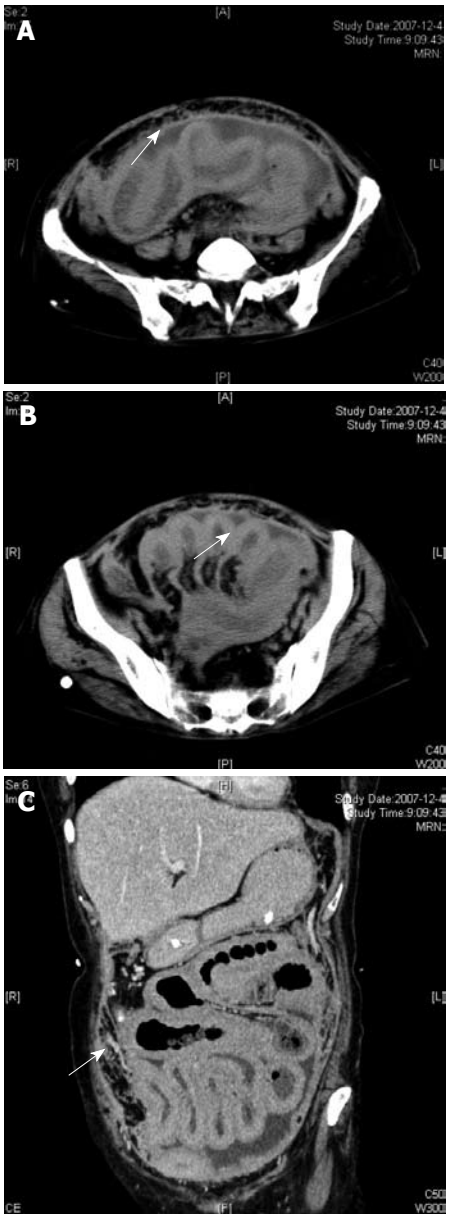


Figure 1 CT showing pronounced contrast enhancement of peritoneum and thickened intestinal wall. A: Most nodules coalesced to form large omental plaques (omental cakes); B: A moderate amount of ascites located between intestinal canals; C: No actual large abdominal masses.

abdomen, and extended to the pelvis, but did not involve the ovary. There was no calcification within the masses. The size of ovaries was normal. After treatment with anti-tuberculous drugs, the ascites decreased. Two months later, ascites stopped decreasing, suggesting that tissue biopsy is necessary to help its diagnosis. A delayed diagnosis or inadequate treatment, may promote progression to the malignant disease and the risk of life-threatening complications. To the best of our knowledge, this is the first report on the coexistence of PSCP and tuberculous peritonitis in humans.

REFERENCES

1 **Bernhard JS**, Bhatia G, Knauer CM. Gastrointestinal tuberculosis: an eighteen-patient experience and review. *J Clin Gastroenterol* 2000; **30**: 397-402
2 **Riquelme A**, Calvo M, Salech F, Valderrama S, Pattillo A, Arellano M, Arrese M, Soza A, Viviani P, Letelier LM. Value of adenosine deaminase (ADA) in ascitic fluid for the diagnosis of tuberculous peritonitis: a meta-analysis. *J Clin Gastroenterol* 2006; **40**: 705-710
3 **Khan R**, Abid S, Jafri W, Abbas Z, Hameed K, Ahmad Z. Diagnostic dilemma of abdominal tuberculosis in non-HIV patients: an ongoing challenge for physicians. *World J Gastroenterol* 2006; **12**: 6371-6375
4 **Demir K**, Okten A, Kaymakoglu S, Dincer D, Besisik F, Cevikbas U, Ozdil S, Bostas G, Mungan Z, Cakaloglu Y. Tuberculous peritonitis--reports of 26 cases, detailing diagnostic and therapeutic problems. *Eur J Gastroenterol Hepatol* 2001; **13**: 581-585
5 **Stafford-Johnson DB**, Bree RL, Francis IR, Korobkin M. CT appearance of primary papillary serous carcinoma of the peritoneum. *AJR Am J Roentgenol* 1998; **171**: 687-689
6 **Chand M**, Moore PJ, Clarke AD, Nash GF, Hickisk T. A diagnostic dilemma following risk-reducing surgery for BRCA1 mutation - a case report of primary papillary serous carcinoma presenting as sigmoid cancer. *World J Surg Oncol* 2007; **5**: 102
7 **Alberti N**, Serrano-Egea A, García-García E, Ballestín C, Pérez-Barrios A, López-Ríos F, de Agustín P. Primary papillary serous carcinoma of the peritoneum: report of a case with diagnosis by fine needle aspiration and immunocytochemistry. *Acta Cytol* 2007; **51**: 203-206
8 **Altaras MM**, Bernheim J, Zehavi T, Drucker L, Uziel O, Fishman A. Papillary serous carcinoma of the peritoneum coexisting with or after endometrial carcinoma. *Gynecol Oncol* 2002; **84**: 245-251
9 **Pickhardt PJ**, Bhalla S. Primary neoplasms of peritoneal and sub-peritoneal origin: CT findings. *Radiographics* 2005; **25**: 983-995
10 **Taus P**, Petru E, Gucer F, Pickel H, Lahousen M. Primary serous papillary carcinoma of the peritoneum: a report of 18 patients. *Eur J Gynaecol Oncol* 1997; **18**: 171-172
11 **Look M**, Chang D, Sugarbaker PH. Long-term results of cytoreductive surgery for advanced and recurrent epithelial ovarian cancers and papillary serous carcinoma of the peritoneum. *Int J Gynecol Cancer* 2004; **14**: 35-41
12 **Bell KA**, Smith Sehdev AE, Kurman RJ. Refined diagnostic criteria for implants associated with ovarian atypical proliferative serous tumors (borderline) and micropapillary serous carcinomas. *Am J Surg Pathol* 2001; **25**: 419-432
13 **Iavazzo C**, Vorgias G, Katsoulis M, Kalinoglou N, Dertimas V, Akrivos T. Primary peritoneal serous papillary carcinoma: clinical and laboratory characteristics. *Arch Gynecol Obstet* 2008; **278**: 53-56
14 **Zhou J**, Iwasa Y, Konishi I, Kan N, Kannagi R, Kobashi Y, Kim YC, Yamabe H. Papillary serous carcinoma of the peritoneum in women. A clinicopathologic and immunohistochemical study. *Cancer* 1995; **76**: 429-436
15 **Rothacker D**, Mobius G. Varieties of serous surface papillary carcinoma of the peritoneum in northern Germany: a thirty-year autopsy study. *Int J Gynecol Pathol* 1995; **14**: 310-318

S- Editor Li JL **L- Editor** Wang XL **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2009 February 14; 15(6): 764
World Journal of Gastroenterology ISSN 1007-9327

ACKNOWLEDGMENTS

Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Mauro Bernardi, Professor
Internal Medicine, Cardioangiology, Hepatology, University of Bologna, Semeiotica Medica - Policlinico S. Orsola-Malpighi - Via Massarenti, 9, Bologna 40138, Italy

Julio H Carri, Professor
Internal Medicine - Gastroenterology, Universidad Nacional de Córdoba, Av.Estrada 160-P 5-Department D, Córdoba 5000, Argentina

Mark J Czaja, MD
Liver Research Center, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461, United States

Sharon DeMorrow, Assistant Professor
Division of Research and Education, Scott and White Hospital and The Texas A&M University System, Health Science Center College of Medicine, Temple, Texas 76504, United States

Isabel Fabregat, PhD, Associate Professor
Laboratori d'Oncologia Molecular, Institut d'Investigación Biomèdica de Bellvitge, Gran Via, Km 2,7, L'Hospitalet, 08907 Barcelona, Spain

Hartmut Jaeschke, Professor
Liver Research Institute, University of Arizona, College of Medicine, 1501 N Campbell Ave, Room 6309, Tucson, Arizona 85724, United States

Peter L Lakatos, MD, PhD, Assistant Professor
1st Department of Medicine, Semmelweis University, Koranyi S 2A, Budapest H1083, Hungary

Juan-Ramón Larrubia, PhD
Gastroenterology Unit and Liver Research Unit., Guadalajara University Hospital, Donante de Sangre s/n, 19002 Guadalajara, 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

Sri P Misra, Professor
Gastroenterology, Moti Lal Nehru Medical College, Allahabad 211001, India

Yuji Naito, Professor
Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan

Ian C Roberts-Thomson, Professor
Department of Gastroenterology and Hepatology, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South 5011, Australia

Philip Rosenthal, MD, Professor of Pediatrics & Surgery
UCSF, 500 Parnassus Avenue, Box 0136, MU 4-East, San Francisco, CA 94143-0136, United States

Chifumi Sato, Professor
Department of Analytical Health Science, Tokyo Medical and Dental University, Graduate School of Health Sciences, 1-5-45 Yushima, Bunkyoku, Tokyo 113-8519, Japan

World J Gastroenterol 2009 February 14; 15(6): 765
World Journal of Gastroenterology ISSN 1007-9327

Meetings

Events Calendar 2009

January 12-15, 2009
Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
http://www.apasl2009hongkong.org/en/home.aspx

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
http://www.easl.ch/

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
http://www.ddw.org

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
http://www.chinamed.com.cn/wcio2009/

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
http://www.asge.org/index.aspx?id=5040

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists(IASGO)
http://iasgo2009.org/en/index.shtml

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
http://www.apdwcongress.org/2009/index.shtml

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
The Liver Meeting

November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
London, UK
Gastro 2009 UEGW/World Congress of Gastroenterology
www.gastro2009.org

Global Collaboration for Gastroenterology

For the first time in the history of gastroenterology, an international conference will take place which joins together the forces of four pre-eminent organisations: Gastro 2009, UEGW/WCOG London. The United European Gastroenterology Federation (UEGF) and the World Gastroenterology Organisation (WGO), together with the World Organisation of Digestive Endoscopy (OMED) and the British Society of Gastroenterology (BSG), are jointly organising a landmark meeting in London from November 21-25, 2009. This collaboration will ensure the perfect balance of basic science and clinical practice, will cover all disciplines in gastroenterology (endoscopy, digestive oncology, nutrition, digestive surgery, hepatology, gastroenterology) and ensure a truly global context; all presented in the exciting setting of the city of London. Attendance is expected to reach record heights as participants are provided with a compact "all-in-one" programme merging the best of several GI meetings. Faculty and participants from all corners of the earth will merge to provide a truly global environment conducive to the exchange of ideas and the forming of friendships and collaborations.

Online Submissions: wjg.wjgnet.com
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2009 February 14; 15(6): 766-768
World Journal of Gastroenterology ISSN 1007-9327

Instructions to authors

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol* ISSN 1007-9327 CN 14-1219/R) is a weekly open-access (OA) peer-reviewed journal supported by an editorial board consisting of 1212 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

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

The major task of *WJG* is to rapidly report the most recent results in basic and clinical research on gastroenterology, hepatology, endoscopy and gastrointestinal surgery fields, specifically including autoimmune, cholestatic and biliary disease, esophageal, gastric and duodenal disorders, cirrhosis and its complications, celiac disease, dyspepsia, gastroesophageal reflux disease, esophageal and stomach cancers, carcinoma of the colon and rectum, gastrointestinal bleeding, gastrointestinal infection, intestinal inflammation, intestinal microflora and immunity, irritable bowel syndrome; liver biology/pathobiology, liver failure, growth and cancer; liver failure/cirrhosis/portal hypertension, liver fibrosis; *Helicobacter pylori*, hepatitis B and C virus, hepatology elsewhere; pancreatic disorders, pancreas and biliary tract disease, pancreatic cancer; transplantation, genetics, epidemiology, microbiology and inflammatory disorders, molecular and cell biology, nutrition; geriatric gastroenterology, pediatric gastroenterology, steatohepatitis and metabolic liver disease; diagnosis and screening, endoscopy, imaging and advanced technology.

The columns in the issues of *WJG* will be adjusted in 2009, which will include: (1) Editorial: Introduce and comment on the substantial advance and its importance in the fast-developing areas; (2) Leader Frontier: Comment on excitement and existing problems of core fields, and offer suggestions for the future research; (3) Topic Highlight: Experts in gastroenterology and hepatology to focus on certain individual hot topics and try to provide answers to the clinical questions on the topics; (4) Observation: Which updates the development of old and new questions, highlights unsolved questions, and provides strategies on how to solve the questions; (5) Guidelines for Basic Research: Which provides Guidelines for basic research; (6) Guidelines for Clinical Practice: Which provides guidelines for clinical diagnosis and treatment; (7) Review Articles: Summarize the representative progress in core scientific disciplines, comment on the research status, and make suggestions for the future work; (8) Original Articles: Originally report the innovative and valuable findings in gastroenterology and hepatology; (9) Brief Articles: Briefly report the novel and innovative findings in gastroenterology and hepatology; (10) Case Report: Report a rare or typical case; (11) Letters to the Editor: Discuss and make reply to the contributions published in *WJG*, or introduce and comment on a controversial issue of general interest; (12) Book Reviews: Introduce and comment on quality monographs of gastroenterology and hepatology; and (13) Guidelines: Guidelines or common understanding for gastroenterology and hepatology from international academic committee.

Indexed and abstracted in

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

Published by

The WJG Press and Baishideng

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of The WJG Press and Baishideng, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials. gov sponsored by the United States National Library of Medicine, and we encourage all potential contributors to register with it. However, in the event that other registers become available, you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the corresponding author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://wjg.wjgnet.com/wjg. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/wjg/help/instructions.jsp) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to submission@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of

supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-59080039, Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words). Available from:http://www.wjgnet.com/wjg/help/8.doc

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/wjg/help/instructions.jsp.

Illustrations

Figures should be numbered as 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc*. It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension,

insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/wjg/help/14.doc.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.
Biology: *H pylori*, *E coli*, *etc*.

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Gastroenterology

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

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/wjg/help/10.doc.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/wjg/help/9.doc.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.