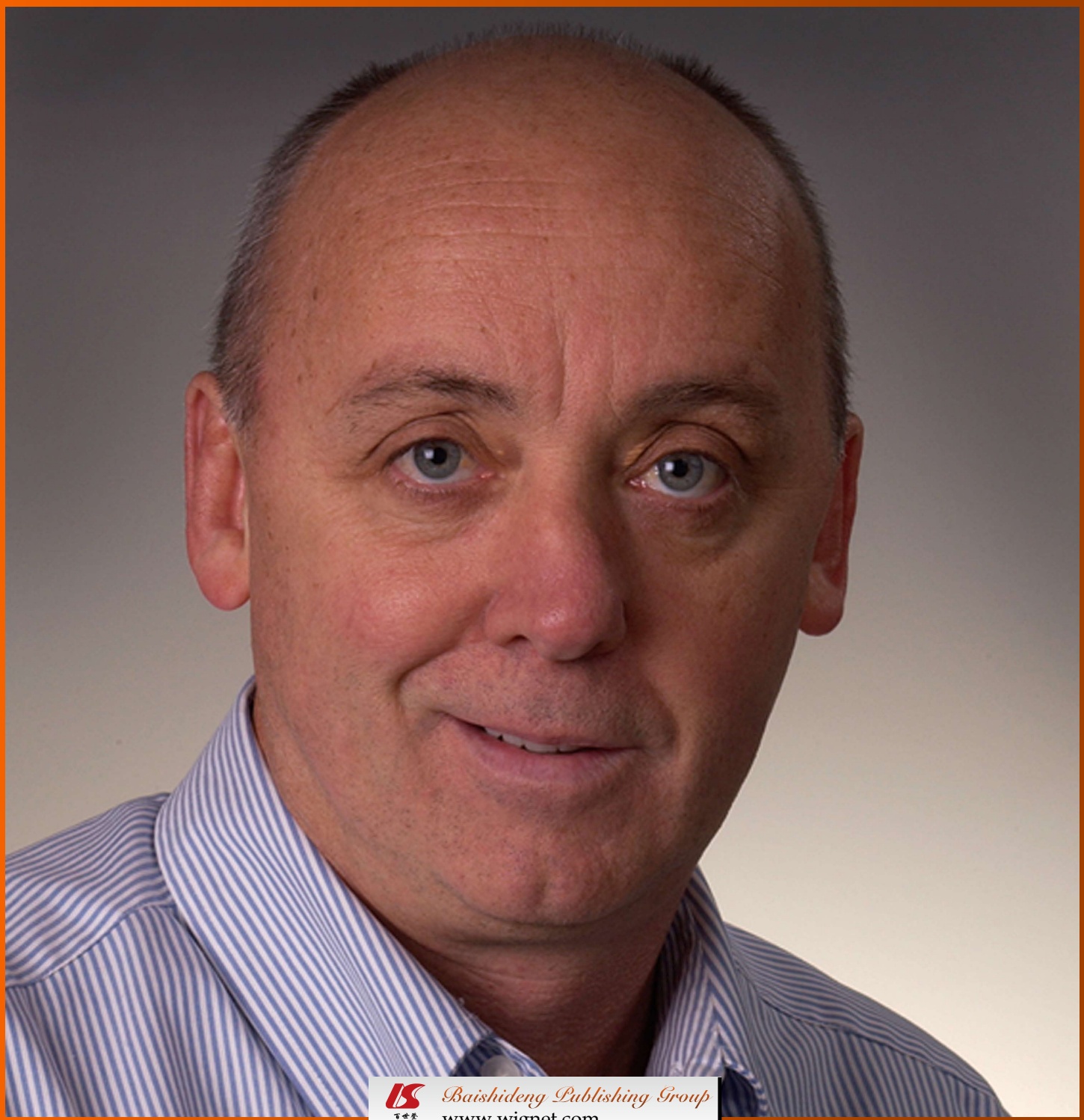
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 March 28; 19(12): 1855-2010



Baishideng Publishing Group
www.wjgnet.com

WJG World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2010-2013

The *World Journal of Gastroenterology* Editorial Board consists of 1352 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 64 countries, including Albania (1), Argentina (8), Australia (33), Austria (15), Belgium (14), Brazil (13), Brunei Darussalam (1), Bulgaria (2), Canada (21), Chile (3), China (82), Colombia (1), Croatia (2), Cuba (1), Czech (6), Denmark (9), Ecuador (1), Egypt (4), Estonia (2), Finland (8), France (29), Germany (87), Greece (22), Hungary (11), India (32), Indonesia (2), Iran (10), Ireland (6), Israel (13), Italy (124), Japan (140), Jordan (2), Kuwait (1), Lebanon (4), Lithuania (2), Malaysia (1), Mexico (11), Morocco (1), Moldova (1), Netherlands (32), New Zealand (2), Norway (13), Pakistan (2), Poland (11), Portugal (6), Romania (4), Russia (1), Saudi Arabia (3), Serbia (3), Singapore (11), Slovenia (1), South Africa (3), South Korea (46), Spain (43), Sri Lanka (1), Sweden (17), Switzerland (12), Thailand (1), Trinidad and Tobago (1), Turkey (30), United Arab Emirates (2), United Kingdom (95), United States (285), and Uruguay (1).

Phillip S Oates, *Perth*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Philip G Dinning, *Koagarah*
Christopher Christophi, *Melbourne*
Cuong D Tran, *North Adelaide*
Shan Rajendra, *Tasmania*
Rajvinder Singh, *Adelaide*
William Kemp, *Melbourne*
Phil Sutton, *Melbourne*
Richard Anderson, *Victoria*
Vance Matthews, *Melbourne*
Alexander G Heriot, *Melbourne*
Debbie Trinder, *Fremantle*
Ian C Lawrance, *Perth*
Adrian G Cummins, *Adelaide*
John K Olynyk, *Fremantle*
Alex Boussioutas, *Melbourne*
Emilia Prakoso, *Sydney*
Robert JL Fraser, *Daw Park*

Austria

Wolfgang Mikulits, *Vienna*
Alfred Gangl, *Vienna*
Dietmar Öfner, *Salzburg*
Georg Roth, *Vienna*
Herwig R Cerwenka, *Graz*
Ashraf Dahaba, *Graz*
Markus Raderer, *Vienna*
Alexander M Hirschl, *Wien*
Thomas Wild, *Kapellerfeld*
Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Michael Trauner, *Vienna*
Stefan Riss, *Vienna*

Belgium

Rudi Beyaert, *Gent*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Benedicte Y De Winter, *Antwerp*
Etienne M Sokal, *Brussels*
Marc Peeters, *De Pintelaan*
Eddie Wisse, *Keerbergen*
Jean-Yves L Reginster, *Liège*
Mark De Ridder, *Brussel*
Freddy Penninckx, *Leuven*
Kristin Verbeke, *Leuven*
Lukas Van Oudenhove, *Leuven*
Leo van Grunsven, *Brussels*
Philip Meuleman, *Ghent*

Brazil

Heitor Rosa, *Goiania*
Roberto J Carvalho-Filho, *Sao Paulo*
Damiao Carlos Moraes Santos, *Rio de Janeiro*
Marcelo Lima Ribeiro, *Braganca Paulista*
Eduardo Garcia Vilela, *Belo Horizonte*
Jaime Natan Eisig, *São Paulo*
Andre Castro Lyra, *Salvador*
José Liberato Ferreira Caboclo, *Brazil*
Yukie Sato-Kuwabara, *São Paulo*
Raquel Rocha, *Salvador*
Paolo R Salvalaggio, *Sao Paulo*
Ana Cristina Simões e Silva, *Belo Horizonte*
Joao Batista Teixeira Rocha, *Santa Maria*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Zahariy Krastev, *Sofia*
Mihaela Petrova, *Sofia*

Canada

Eldon Shaffer, *Calgary*
Nathalie Perreault, *Sherbrooke*
Philip H Gordon, *Montreal*
Ram Prakash Galwa, *Ottawa*
Baljinder Singh Salh, *Vancouver*
Claudia Zwingmann, *Montreal*
Alain Bitton, *Montreal*
Pingchang Yang, *Hamilton*
Michael F Byrne, *Vancouver*
Andrew L Mason, *Alberta*
John K Marshall, *Hamilton Ontario*
Kostas Pantopoulos, *Montreal*
Waliul Khan, *Ontario*
Eric M Yoshida, *Vancouver*
Geoffrey C Nguyen, *Toronto*
Devendra K Amre, *Montreal*
Tedros Bezabeh, *Winnipeg*
Wangxue Chen, *Ottawa*
Qiang Liu, *Saskatoon*

Chile

De Aretxabala Xabier, *Santiago*
Marcelo A Beltran, *La Serena*
Silvana Zanlungo, *Santiago*

China

Chi-Hin Cho, *Hong Kong*
Chun-Qing Zhang, *Jinan*
Ren Xiang Tan, *Nanjing*
Fei Li, *Beijing*
Hui-Jie Bian, *Xi'an*
Xiao-Peng Zhang, *Beijing*
Xing-Hua Lu, *Beijing*
Fu-Sheng Wang, *Beijing*
An-Gang Yang, *Xi'an*
Xiao-Ping Chen, *Wuhan*
Zong-Jie Cui, *Beijing*
Ming-Liang He, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
Qin Su, *Beijing*
Jian-Zhong Zhang, *Beijing*
Paul Kwong-Hang Tam, *Hong Kong*
Wen-Rong Xu, *Zhenjiang*
Chun-Yi Hao, *Beijing*
San-Jun Cai, *Shanghai*
Simon Law, *Hong Kong*
Yuk Him Tam, *Hong Kong*
De-Liang Fu, *Shanghai*
Eric WC Tse, *Hong Kong*
Justin CY Wu, *Hong Kong*
Nathalie Wong, *Hong Kong*
Jing Yuan Fang, *Shanghai*
Yi-Min Mao, *Shanghai*
Wei-Cheng You, *Beijing*
Xiang-Dong Wang, *Shanghai*
Xuan Zhang, *Beijing*
Zhao-Shen Li, *Shanghai*
Guang-Wen Cao, *Shanghai*
En-min Li, *Shantou*
Yu-Yuan Li, *Guangzhou*
Fook Hong Ng, *Hong Kong*
Hsiang-Fu Kung, *Hong Kong*
Wai Lun Law, *Hong Kong*
Eric CH Lai, *Hong Kong*
Jun Yu, *Hong Kong*
Ze-Guang Han, *Shanghai*
Bian zhao-xiang, *Hong Kong*
Wei-Dong Tong, *Chongqing*

Colombia

Germán Campuzano-Maya, *Medellín*

Croatia

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

Cuba

Damian C Rodriguez, *Havana*

Czech

Milan Jirsa, *Praha*
Pavel Trunečka, *Prague*
Jan Bures, *Hradec Kralove*
Marcela Kopacova, *Hradec Kralove*
Ondrej Slaby, *Brno*
Radan Bruha, *Prague*

Denmark

Asbjørn M Drewes, *Aalborg*
Leif Percival Andersen, *Copenhagen*
Jan Mollenhauer, *Odense C*
Morten Frisch, *Copenhagen S*
Jorgen Rask-Madsen, *Skodsborg*
Morten Hylander Møller, *Holte*
Søren Rafaelsen, *Vejle*
Vibeke Andersen, *Aabenraa*
Ole Haagen Nielsen, *Herlev*

Ecuador

Fernando E Sempértegui, *Quito*

Egypt

Zeinab Nabil Ahmed Said, *Cairo*
Hussein M Atta, *El-Minia*
Asmaa Gaber Abdou, *Shebein Elkom*

Maha Maher Shehata, *Mansoura*

Estonia

Riina Salupere, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Saila Kauhanen, *Turku*
Pauli Antero Puolakkainen, *Turku*
Minna Nyström, *Helsinki*
Juhani Sand, *Tampere*
Jukka-Pekka Mecklin, *Jyvaskyla*
Lea Veijola, *Helsinki*
Kaija-Leena Kolho, *Helsinki*
Thomas Kietzmann, *Oulu*

France

Boris Guiu, *Dijon*
Baumert F Thomas, *Strasbourg*
Alain L Servin, *Châtenay-Malabry*
Patrick Marcellin, *Paris*
Jean-Jacques Tuech, *Rouen*
Francoise L Fabiani, *Angers*
Jean-Luc Faucheron, *Grenoble*
Philippe Lehours, *Bordeaux*
Stephane Supiot, *Nantes*
Lionel Bueno, *Toulouse*
Flavio Maina, *Marseille*
Paul Hofman, *Nice*
Abdel-Majid Khatib, *Paris*
Annie Schmid-Alliana, *Nice cedex 3*
Frank Zerbib, *Bordeaux Cedex*
Rene Gerolami Santandera, *Marseille*
Sabine Colnot, *Paris*
Catherine Daniel, *Lille Cedex*
Thabut Dominique, *Paris*
Laurent Huwart, *Paris*
Alain Braillon, *Amiens*
Bruno Bonaz, *Grenoble*
Evelyne Schvoerer, *Strasbourg*
M Coeffier, *Rouen*
Mathias Chamaillard, *Lille*
Hang Nguyen, *Clermont-Ferrand*
Veronique Vitton, *Marseille*
Alexis Desmoulière, *Limoges*
Juan Iovanna, *Marseille*

Germany

Hans L Tillmann, *Leipzig*
Stefan Kubicka, *Hannover*
Elke Cario, *Essen*
Hans Scherubl, *Berlin*
Harald F Teutsch, *Ulm*
Peter Konturek, *Erlangen*
Thilo Hackert, *Heidelberg*
Jurgen M Stein, *Frankfurt*
Andrej Khandoga, *Munich*
Karsten Schulmann, *Bochum*
Jutta Elisabeth Lüttges, *Riegelsberg*
Wolfgang Hagmann, *Heidelberg*
Hubert Blum, *Freiburg*
Thomas Bock, *Berlin*
Christa Buechler, *Regensburg*
Christoph F Dietrich, *Bad Mergentheim*
Ulrich R Fölsch, *Kiel*
Nikolaus Gassler, *Aachen*
Markus Gerhard, *Munich*
Dieter Glebe, *Giessen*
Klaus R Herrlinger, *Stuttgart*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Ludwigshafen*
Joachim Labenz, *Siegen*
Peter Malfertheiner, *Magdeburg*
Sabine Mihm, *Göttingen*
Markus Reiser, *Bochum*
Steffen Rickes, *Magdeburg*
Andreas G Schreyer, *Regensburg*
Henning Schulze-Bergkamen, *Heidelberg*
Ulrike S Stein, *Berlin*
Wolfgang R Stremmel, *Heidelberg*
Fritz von Weizsäcker, *Berlin*
Stefan Wirth, *Wuppertal*
Dean Bogoevski, *Hamburg*
Bruno Christ, *Halle/Saale*
Peter N Meier, *Hannover*
Stephan Johannes Ott, *Kiel*
Arndt Vogel, *Hannover*
Dirk Haller, *Freising*
Jens Standop, *Bonn*
Jonas Mudter, *Erlangen*
Jürgen Büning, *Lübeck*
Matthias Ocker, *Erlangen*
Joerg Trojan, *Frankfurt*
Christian Trautwein, *Aachen*
Jorg Kleeff, *Munich*
Christian Rust, *Munich*
Claus Hellerbrand, *Regensburg*
Elke Roeb, *Giessen*
Erwin Biecker, *Siegburg*
Ingmar Königsrainer, *Tübingen*
Jürgen Borlak, *Hannover*
Axel M Gressner, *Aachen*
Oliver Mann, *Hamburg*
Marty Zdichavsky, *Tübingen*
Christoph Reichel, *Bad Brückenau*
Nils Habbe, *Marburg*
Thomas Wex, *Magdeburg*
Frank Ulrich Weiss, *Greifswald*
Manfred V Singer, *Mannheim*
Martin K Schilling, *Homburg*
Philip D Hard, *Giessen*
Michael Linnebacher, *Rostock*
Ralph Graeser, *Freiburg*
Rene Schmidt, *Freiburg*
Robert Obermaier, *Freiburg*
Sebastian Mueller, *Heidelberg*
Andrea Hille, *Goettingen*
Klaus Mönkemüller, *Bottrop*
Elfriede Bollschweiler, *Köln*
Siegfried Wagner, *Deggendorf*
Dieter Schilling, *Mannheim*
Joerg F Schlaak, *Essen*
Michael Keese, *Frankfurt*
Robert Grützmann, *Dresden*
Ali Canbay, *Essen*
Dirk Domagk, *Muenster*
Jens Hoeppner, *Freiburg*
Frank Tacke, *Aachen*
Patrick Michl, *Marburg*
Alfred A Königsrainer, *Tübingen*
Kilian Weigand, *Heidelberg*
Mohamed Hassan, *Duesseldorf*
Gustav Paumgartner, *Munich*
Philipe N Khalil, *Munich*
Martin Storr, *Munich*

Greece

Andreas Larentzakis, *Athens*
Tsianos Epameinondas, *Ioannina*
Elias A Kouroumalis, *Heraklion*
Helen Christopoulou-Aletra, *Thessaloniki*
George Papatheodoridis, *Athens*
Ioannis Kanellos, *Thessaloniki*
Michael Koutsilieris, *Athens*
T Choli-Papadopoulou, *Thessaloniki*
Emanuel K Manesis, *Athens*
Evangelos Tsiambas, *Ag Paraskevi Attiki*
Konstantinos Mimidis, *Alexandroupolis*
Spilios Manolakopoulos, *Athens*
Spiros Sgouros, *Athens*
Ioannis E Koutroubakis, *Heraklion*
Stefanos Karagiannis, *Athens*
Spiros Ladas, *Athens*
Elena Vezali, *Athens*
Dina G Tiniakos, *Athens*
Ekaterini Chatzaki, *Alexandroupolis*
Dimitrios Roukos, *Ioannina*
George Sgourakis, *Athens*
Maroulio Talieri, *Athens*

Hungary

Peter L Lakatos, *Budapest*
Yvette Mándi, *Szeged*
Ferenc Sipos, *Budapest*
György M Buzás, *Budapest*
László Czakó, *Szeged*
Peter Hegyi, *Szeged*
Zoltan Rakonczay, *Szeged*
Gyula Farkas, *Szeged*
Zsuzsa Szondy, *Debrecen*
Gabor Veres, *Budapest*
Zsuzsa Schaff, *Budapest*

India

Philip Abraham, *Mumbai*
Sri P Misra, *Allahabad*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Jai Dev Wig, *Chandigarh*
Uday C Ghoshal, *Lucknow*
Pramod Kumar Garg, *New Delhi*
Barjesh Chander Sharma, *New Delhi*
Gopal Nath, *Varanasi*
Bhupendra Kumar Jain, *Delhi*
Devinder Kumar Dhawan, *Chandigarh*
Ashok Kumar, *Lucknow*
Benjamin Perakath, *Tamil Nadu*
Debidas Ghosh, *Midnpore*
Pankaj Garg, *Panchkula*
Samiran Nundy, *New Delhi*
Virendra Singh, *Chandigarh*
Bikash Medhi, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Vandana Panda, *Mumbai*
Vineet Ahuja, *New Delhi*
SV Rana, *Chandigarh*

Deepak N Amarapurkar, *Mumbai*
Abhijit Chowdhury, *Kolkata*
Jasbir Singh, *Kurukshetra*
B Mittal, *Lucknow*
Sundeep Singh Saluja, *New Delhi*
Pradyumna Kumar Mishra, *Mumbai*
Runu Chakravarty, *Kolkata*
Nagarajan Perumal, *New Delhi*

Indonesia

David handojo Muljono, *Jakarta*
Andi Utama, *Tangerang*

Iran

Seyed-Moayed Alavian, *Tehran*
Reza Malekzadeh, *Tehran*
Peyman Adibi, *Isfahan*
Alireza Mani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Mohammad Abdollahi, *Tehran*
Majid Assadi, *Bushehr*
Arezoo Aghakhani, *Tehran*
Marjan Mohammadi, *Tehran*
Fariborz Mansour-Ghanaei, *Rasht*

Ireland

Ross McManus, *Dublin*
Billy Bourke, *Dublin*
Catherine Greene, *Dublin*
Ted Dinan, *Cork*
Marion Rowland, *Dublin*

Israel

Abraham R Eliakim, *Haifa*
Simon Bar-Meir, *Tel Hashomer*
Ami D Sperber, *Beer-Sheva*
Boris Kirshtein, *Beer Sheva*
Mark Pines, *Bet Dagan*
Menachem Moshkowitz, *Tel-Aviv*
Ron Shaoul, *Haifa*
Shmuel Odes, *Beer Sheva*
Sigal Fishman, *Tel Aviv*
Alexander Becker, *Afula*
Assy Nimer, *Safed*
Eli Magen, *Ashdod*
Amir Shlomai, *Tel-Aviv*

Italy

Mauro Bortolotti, *Bologna*
Gianlorenzo Dionigi, *Varese*
Fiorucci Stefano, *Perugia*
Roberto Berni Canani, *Naples*
Ballarin Roberto, *Modena*
Bruno Annibale, *Roma*
Vincenzo Stanghellini, *Bologna*
Giovanni B Gaeta, *Napoli*
Claudio Bassi, *Verona*
Mauro Bernardi, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Dario Conte, *Milano*
Francesco Costa, *Pisa*
Giovanni D De Palma, *Naples*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Edoardo G Giannini, *Genoa*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Pietro Invernizzi, *Milan*
Ezio Laconi, *Cagliari*
Giuseppe Montalto, *Palermo*
Giovanni Musso, *Torino*
Gerardo Nardone, *Napoli*
Valerio Nobili, *Rome*
Raffaele Pezzilli, *Bologna*
Alberto Piperno, *Monza*
Anna C Piscaglia, *Roma*
Piero Portincasa, *Bari*
Giovanni Tarantino, *Naples*
Cesare Tosetti, *Porretta Terme*
Alessandra Ferlini, *Ferrara*
Alessandro Ferrero, *Torino*
Donato F Altomare, *Bari*
Giovanni Milito, *Rome*
Giuseppe Sica, *Rome*
Guglielmo Borgia, *Naples*
Giovanni Latella, *L'Aquila*
Salvatore Auricchio, *Naples*
Alberto Biondi, *Rome*
Alberto Tommasini, *Trieste*
Antonio Basoli, *Roma*
Giuliana Decorti, *Trieste*
Marco Silano, *Roma*
Michele Reni, *Milan*
Pierpaolo Sileri, *Rome*
Achille Iolascon, *Naples*
Alessandro Granito, *Bologna*
Angelo A Izzo, *Naples*
Giuseppe Currò, *Messina*
Pier Mannuccio Mannucci, *Milano*
Marco Vivarelli, *Bologna*
Massimo Levrero, *Rome*
Massimo Rugge, *Padova*
Paolo Angeli, *Padova*
Silvio Danese, *Milano*
Antonello Trecca, *Rome*
Antonio Gasbarrini, *Rome*
Cesare Ruffolo, *Treviso*
Massimo Falconi, *Verona*
Fausto Catena, *Bologna*
Francesco Manguso, *Napoli*
Giancarlo Mansueto, *Verona*
Luca Morelli, *Trento*
Marco Scarpa, *Padova*
Mario M D'Elios, *Florence*
Francesco Luzza, *Catanzaro*
Franco Roviello, *Siena*
Guido Torzilli, *Rozzano Milano*
Luca Frulloni, *Verona*
Lucia Malaguarnera, *Catania*
Lucia Ricci Vitiani, *Rome*
Mara Massimi, *L'Aquila*
Mario Pescatori, *Rome*
Mario Rizzetto, *Torino*
Mirko D'Onofrio, *Verona*
Nadia Peparini, *Rome*
Paola De Nardi, *Milan*
Paolo Aurello, *Rome*
Piero Amodio, *Padova*
Riccardo Nascimbeni, *Brescia*
Vincenzo Villanacci, *Brescia*
Vittorio Ricci, *Pavia*
Silvia Fargion, *Milan*
Luigi Bonavina, *Milano*
Oliviero Riggio, *Rome*
Fabio Pace, *Milano*
Gabrio Bassotti, *Perugia*
Giulio Marchesini, *Bologna*
Roberto de Franchis, *Milano*
Giovanni Monteleone, *Rome*
C armelo Scarpignato, *Parma*
Luca VC Valenti, *Milan*
Urgesi Riccardo, *Rome*
Marcello Persico, *Naples*
Antonio Moschetta, *Bari*
Luigi Muratori, *Bologna*
Angelo Zullo, *Roma*
Vito Annese, *Florence*
Simone Lanini, *Rome*
Alessandro Grasso, *Savona*
Giovanni Targher, *Verona*
Domenico Girelli, *Verona*
Alessandro Cucchetti, *Bologna*
Fabio Marra, *Florence*
Michele Milella, *Rome*
Francesco Franceschi, *Rome*
Giuseppina De Petro, *Brescia*
Salvatore Leonardi, *Catania*
Cristiano Simone, *Santa Maria Imbaro*
Bernardino Rampone, *Salerno*
Francesco Crea, *Pisa*
Walter Fries, *Messina*
Antonio Craxì, *Palermo*
Gerardo Rosati, *Potenza*
Mario Guslandi, *Milano*
Gianluigi Giannelli, *Bari*
Paola Loria, *Modena*
Paolo Sorrentino, *Avellino*
Armando Santoro, *Rozzano*
Gabriele Grassi, *Trieste*
Antonio Orlacchio, *Rome*

Japan

Tsuneo Kitamura, *Chiba*
Katsutoshi Yoshizato, *Higashihiroshima*
Masahiro Arai, *Tokyo*
Shinji Tanaka, *Hiroshima*
Keiji Hirata, *Kitakyushu*
Yoshio Shirai, *Niigata*
Susumu Ohmada, *Maebashi*
Kenichi Ikejima, *Tokyo*
Masatoshi Kudo, *Osaka*
Yoshiaki Murakami, *Hiroshima*
Masahiro Tajika, *Nagoya*
Kentaro Yoshika, *Toyoake*
Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Hitoshi Asakura, *Tokyo*
Mitsuhiro Fujishiro, *Tokyo*
Toru Hiyama, *Higashihiroshima*
Yutaka Inagaki, *Kanagawa*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Terumi Kamisawa, *Tokyo*

Norihiro Kokudo, *Tokyo*
Shin Maeda, *Tokyo*
Yasushi Matsuzaki, *Ibaraki*
Kenji Miki, *Tokyo*
Hiroto Miwa, *Hyogo*
Yoshiharu Motoo, *Kanazawa*
Kunihiko Murase, *Tusima*
Atsushi Nakajima, *Yokohama*
Yuji Naito, *Kyoto*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Hirohide Ohnishi, *Akita*
Kazuichi Okazaki, *Osaka*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Yasushi Sano, *Kobe*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Toyama*
Shinji Shimoda, *Fukuoka*
Masayuki Sho, *Nara*
Hidekazu Suzuki, *Tokyo*
Shinji Togo, *Yokohama*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Akihito Nagahara, *Tokyo*
Hiroaki Takeuchi, *Kochi*
Keiji Ogura, *Tokyo*
Kotaro Miyake, *Tokushima*
Mitsunori Yamakawa, *Yamagata*
Naoaki Sakata, *Sendai*
Naoya Kato, *Tokyo*
Satoshi Mamori, *Hyogo*
Shogo Kikuchi, *Aichi*
Shoichiro Sumi, *Kyoto*
Susumu Ikehara, *Osaka*
Taketo Yamaguchi, *Chiba*
Tokihiko Sawada, *Tochigi*
Tomoharu Yoshizumi, *Fukuoka*
Toshiyuki Ishiwata, *Tokyo*
Yasuhiro Fujino, *Akashi*
Yasuhiro Koga, *Isehara city*
Yoshihisa Takahashi, *Tokyo*
Yoshitaka Takuma, *Okayama*
Yutaka Yata, *Maebashi-city*
Itaru Endo, *Yokohama*
Kazuo Chijiiwa, *Miyazaki*
Kouhei Fukushima, *Sendai*
Masahiro Iizuka, *Akita*
Mitsuyoshi Urashima, *Tokyo*
Munechika Enjoji, *Fukuoka*
Takashi Kojima, *Sapporo*
Takumi Kawaguchi, *Kurume*
Yoshiyuki Ueno, *Sendai*
Yuichiro Eguchi, *Saga*
Akihiro Tamori, *Osaka*
Atsushi Masamune, *Sendai*
Atsushi Tanaka, *Tokyo*
Hitoshi Tsuda, *Tokyo*
Takashi Kobayashi, *Tokyo*
Akimasa Nakao, *Nagogya*
Hiroyuki Uehara, *Osaka*
Masahito Uemura, *Kashihara*
Satoshi Tanno, *Sapporo*
Toshinari Takamura, *Kanazawa*
Yohei Kida, *Kainan*
Masanori Hatakeyama, *Tokyo*
Satoru Kakizaki, *Gunma*
Shuhei Nishiguchi, *Hyogo*
Yuichi Yoshida, *Osaka*
Manabu Morimoto, *Japan*
Mototsugu Kato, *Sapporo*
Naoki Ishii, *Tokyo*
Noriko Nakajima, *Tokyo*
Nobuhiro Ohkohchi, *Tsukuba*
Takanori Kanai, *Tokyo*
Kenichi Goda, *Tokyo*
Mitsugi Shimoda, *Mibu*
Zenichi Morise, *Nagoya*
Hitoshi Yoshiji, *Kashihara*
Takahiro Nakazawa, *Nagoya*
Utaroh Motosugi, *Yamanashi*
Nobuyuki Matsuhashi, *Tokyo*
Yasuhiro Kodera, *Nagoya*
Takayoshi Ito, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Haruhiko Sugimura, *Hamamatsu*
Hiroki Yamaue, *Wakayama*
Masao Ichinose, *Wakayama*
Takaaki Arigami, *Kagoshima*
Nobuhiro Zaima, *Nara*
Naoki Tanaka, *Matsumoto*
Satoru Motoyama, *Akita*
Tomoyuki Shibata, *Toyoake*
Tatsuya Ide, *Kurume*
Tsutomu Fujii, *Nagoya*
Osamu Kanauchi, *Toky*
Atsushi Irisawa, *Aizuwakamatsu*
Hikaru Nagahara, *Tokyo*
Keiji Hanada, *Onomichi*
Keiichi Mitsuyama, *Fukuoka*
Shin Maeda, *Yokohama*
Takuya Watanabe, *Niigata*
Toshihiro Mitaka, *Sapporo*
Yoshiki Murakami, *Kyoto*
Tadashi Shimoyama, *Hirosaki*

Jordan

Ismail Matalka, *Irbid*
Khaled Jadallah, *Irbid*

Kuwait

Islam Khan, *Safat*

Lebanon

Bassam N Abboud, *Beirut*
Rami Moucari, *Beirut*
Ala I Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania

Giedrius Barauskas, *Kaunas*
Limas Kupcinskas, *Kaunas*

Malaysia

Andrew Seng Boon Chua, *Ipoh*

Mexico

Saúl Villa-Trevio, *Mexico*
Omar Vergara-Fernandez, *Mexico*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Jalisco*
Miguel Angel Mercado, *Distrito Federal*
Richard A Awad, *Mexico*
Aldo Torre Delgadillo, *Mexico*
Paulino Martínez Hernández Magro, *Celaya*
Carlos A Aguilar-Salinas, *Mexico*
Jesus K Yamamoto-Furusho, *Mexico*

Morocco

Samir Ahboucha, *Khouribga*

Moldova

Igor Mishin, *Kishinev*

Netherlands

Ulrich Beuers, *Amsterdam*
Albert Frederik Pull ter Gunne, *Tilburg*
Jantine van Baal, *Heidelberglaan*
Wendy Wilhelmina Johanna de Leng, *Utrecht*
Gerrit A Meijer, *Amsterdam*
Lee Bouwman, *Leiden*
J Bart A Crusius, *Amsterdam*
Frank Hoentjen, *Haarlem*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Karel van Erpecum, *Utrecht*
BW Marcel Spanier, *Arnhem*
Misha Luyer, *Sittard*
Pieter JF de Jonge, *Rotterdam*
Robert Christiaan Verdonk, *Groningen*
John Plukker, *Groningen*
Maarten Tushuizen, *Amsterdam*
Wouter de Herder, *Rotterdam*
Erwin G Zoetendal, *Wageningen*
Robert J de Knegt, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Annemarie de Vries, *Rotterdam*
Astrid van der Velde, *Ede*
Lodewijk AA Brosens, *Utrecht*
James CH Hardwick, *Leiden*
Loes van Keimpema, *Nijmegen*
WJ de Jonge, *Amsterdam*
Zuzana Zelinkova, *Rotterdam*
LN van Steenbergen, *Eindhoven*
Frank G Schaap, *Amsterdam*
Jeroen Maljaars, *Leiden*

New Zealand

Andrew S Day, *Christchurch*
Max S Petrov, *Auckland*

Norway

Espen Melum, *Oslo*

Trine Olsen, *Tromsø*
Eyvind J Paulssen, *Tromsø*
Rasmus Goll, *Tromsø*
Asle W Medhus, *Oslo*
Jon Arne Søreide, *Stavanger*
Kjetil Soreide, *Stavanger*
Reidar Fossmark, *Trondheim*
Trond Peder Flaten, *Trondheim*
Olav Dalgard, *Oslo*
Ole Høie, *Arendal*
Magdy El-Salhy, *Bergen*
Jørgen Valeur, *Oslo*

Pakistan

Shahab Abid, *Karachi*
Syed MW Jafri, *Karachi*

Poland

Beata Jolanta Jablońska, *Katowice*
Halina Cichoż-Lach, *Lublin*
Tomasz Brzozowski, *Cracow*
Hanna Gregorek, *Warsaw*
Marek Hartleb, *Katowice*
Stanislaw J Konturek, *Krakow*
Andrzej Dabrowski, *Bialystok*
Jan Kulig, *Kraków*
Julian Swierczynski, *Gdansk*
Marek Bebenek, *Wroclaw*
Dariusz M Lebensztejn, *Bialystok*

Portugal

Ricardo Marcos, *Porto*
Guida Portela-Gomes, *Estoril*
Ana Isabel Lopes, *Lisboa Codex*
Raquel Almeida, *Porto*
Rui Tato Marinho, *Lisbon*
Ceu Figueiredo, *Porto*

Romania

Dan L Dumitrascu, *Cluj*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Anca Trifan, *Iasi*

Russia

Vasiliy I Reshetnyak, *Moscow*

Saudi Arabia

Ibrahim A Al Mofleh, *Riyadh*
Abdul-Wahed Meshikhes, *Qatif*
Faisal Sanai, *Riyadh*

Serbia

Tamara M Alempijevic, *Belgrade*
Dusan M Jovanovic, *Sremska Kamenica*
Zoran Krivokapic, *Belgrade*

Singapore

Brian Kim Poh Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Fock Kwong Ming, *Singapore*
Francis Seow-Choen, *Singapore*
Kok Sun Ho, *Singapore*
Kong Weng Eu, *Singapore*
Madhav Bhatia, *Singapore*
London Lucien Ooi, *Singapore*
Wei Ning Chen, *Singapore*
Richie Soong, *Singapore*
Kok Ann Gwee, *Singapore*

Slovenia

Matjaz Homan, *Ljubljana*

South Africa

Rosemary Joyce Burnett, *Pretoria*
Michael Kew, *Cape Town*
Roland Ndip, *Alice*

South Korea

Byung Chul Yoo, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Marie Yeo, *Suwon*
Jeong Min Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Joong-Won Park, *Goyang*
Hoon Jai Chun, *Seoul*
Myung-Gyu Choi, *Seoul*
Sang Kil Lee, *Seoul*
Sang Yeoup Lee, *Gyeongsangnam-do*
Won Ho Kim, *Seoul*
Dae-Yeul Yu, *Daejeon*
Donghee Kim, *Seoul*
Sang Geon Kim, *Seoul*
Sun Pyo Hong, *Geonggi-do*
Sung-Gil Chi, *Seoul*
Yeun-Jun Chung, *Seoul*
Ki-Baik Hahm, *Incheon*
Ji Kon Ryu, *Seoul*
Kyu Taek Lee, *Seoul*
Yong Chan Lee, *Seoul*
Seong Gyu Hwang, *Seongnam*
Seung Woon Paik, *Seoul*
Sung Kim, *Seoul*
Hong Joo Kim, *Seoul*
Hyoung-Chul Oh, *Seoul*
Nayoung Kim, *Seongnam-si*
Sang Hoon Ahn, *Seoul*
Seon Hahn Kim, *Seoul*
Si Young Song, *Seoul*
Young-Hwa Chung, *Seoul*
Hyo-Cheol Kim, *Seoul*
Kwang Jae Lee, *Swon*
Sang Min Park, *Seoul*
Young Chul Kim, *Seoul*
Do Hyun Park, *Seoul*
Dae Won Jun, *Seoul*
Dong Wan Seo, *Seoul*
Soon-Sun Hong, *Incheon*
Hoguen Kim, *Seoul*
Ho-Young Song, *Seoul*
Joo-Ho Lee, *Seoul*
Jung Eun Lee, *Seoul*
Jong H Moon, *Bucheon*

Spain

Eva Vaquero, *Barcelona*
Andres Cardenas, *Barcelona*
Laureano Fernández-Cruz, *Barcelona*
Antoni Farré, *Spain*
Maria-Angeles Aller, *Madrid*
Raul J Andrade, *Málaga*
Fernando Azpiroz, *Barcelona*
Josep M Bordas, *Barcelona*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Isabel Fabregat, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
María IT López, *Jaén*
Jesús M Prieto, *Pamplona*
Mireia Miquel, *Sabadell*
Ramon Bataller, *Barcelona*
Fernando J Corrales, *Pamplona*
Julio Mayol, *Madrid*
Matias A Avila, *Pamplona*
Juan Macías, *Seville*
Juan Carlos Laguna Egea, *Barcelona*
Juli Busquets, *Barcelona*
Belén Beltrán, *Valencia*
José Manuel Martin-Villa, *Madrid*
Lisardo Boscá, *Madrid*
Luis Grande, *Barcelona*
Pedro Lorenzo Majano Rodriguez, *Madrid*
Adolfo Benages, *Valencia*
Domínguez-Muñoz JE, *Santiago de Compostela*
Gloria González Aseguinolaza, *Navarra*
Javier Martin, *Granada*
Luis Bujanda, *San Sebastián*
Matilde Bustos, *Pamplona*
Luis Aparisi, *Valencia*
José Julián calvo Andrés, *Salamanca*
Benito Velayos, *Valladolid*
Javier Gonzalez-Gallego, *León*
Ruben Ciria, *Córdoba*
Francisco Rodriguez-Frias, *Barcelona*
Manuel Romero-Gómez, *Sevilla*
Albert Parés, *Barcelona*
Joan Roselló-Catafau, *Barcelona*

Sri Lanka

Arjuna De Silva, *Kelaniya*

Sweden

Stefan G Pierzynowski, *Lund*
Hanns-Ulrich Marschall, *Stockholm*
Lars A Pahlman, *Uppsala*
Helena Nordenstedt, *Stockholm*
Bobby Tingstedt, *Lund*
Evangelos Kalaitzakis, *Gothenburg*
Lars Erik Agréus, *Huddinge*
Annika Lindblom, *Stockholm*

Roland Andersson, *Lund*
Zongli Zheng, *Stockholm*
Mauro D'Amato, *Huddinge*
Greger Lindberg, *Stockholm*
Pär Erik Myrelid, *Linköping*
Sara Lindén, *Göteborg*
Sara Regnér, *Malmö*
Åke Nilsson, *Lund*

Switzerland

Jean L Frossard, *Geneva*
Andreas Geier, *Zürich*
Bruno Stieger, *Zürich*
Pascal Gervaz, *Geneva*
Paul M Schneider, *Zurich*
Felix Stickel, *Berne*
Fabrizio Montecucco, *Geneva*
Inti Zlobec, *Basel*
Michelangelo Foti, *Geneva*
Pascal Bucher, *Geneva*
Andrea De Gottardi, *Berne*
Christian Toso, *Geneva*

Thailand

Weekitt Kittisupamongkol, *Bangkok*

Trinidad and Tobago

Shivananda Nayak, *Mount Hope*

Turkey

Tarkan Karakan, *Ankara*
Yusuf Bayraktar, *Ankara*
Ahmet Tekin, *Mersin*
Aydin Karabacakoglu, *Konya*
Osman C Ozdogan, *Istanbul*
Özlem Yilmaz, *Izmir*
Bülent Salman, *Ankara*
Can GONEN, *Kutahya*
Cuneyt Kayaalp, *Malatya*
Ekmel Tezel, *Ankara*
Eren Ersoy, *Ankara*
Hayrullah Derici, *Balıkesir*
Mehmet Refik Mas, *Etlik-Ankara*
Sinan Akay, *Tekirdag*
A Mithat Bozdayi, *Ankara*
Metin Basaranoglu, *Istanbul*
Mesut Tez, *Ankara*
Orhan Sezgin, *Mersin*
Mukaddes Esrefoglu, *Malatya*
Ilker Tasci, *Ankara*
Kemal Kismet, *Ankara*
Selin Kapan, *Istanbul*
Seyfettin Köklü, *Ankara*
Murat Sayan, *Kocaeli*
Sabahattin Kaymakoglu, *Istanbul*
Yucel Ustundag, *Zonguldak*
Can Gonen, *Istanbul*
Yusuf Yilmaz, *Istanbul*
Müge Tecder-Ünal, *Ankara*
İlhami Yüksel, *Ankara*

United Arab Emirates

Fikri M Abu-Zidan, *Al-Ain*
Sherif M Karam, *Al-Ain*

United Kingdom

Anastasios Koulaouzidis, *Edinburgh*
Sylvia LF Pender, *Southampton*
Hong-Xiang Liu, *Cambridge*
William Dickey, *Londonderry*
Simon D Taylor-Robinson, *London*
James Neuberger, *Birmingham*
Frank I Tovey, *London*
Kevin Robertson, *Glasgow*
Chew Thean Soon, *Manchester*
Geoffrey Burnstock, *London*
Vamsi R Velchuru, *United Kingdom*
Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Lesley A Anderson, *Belfast*
Anthony TR Axon, *Leeds*
Jim D Bell, *London*
Alastair D Burt, *Newcastle*
Tatjana Crnogorac-Jurcevic, *London*
Daniel R Gaya, *Edinburgh*
William Greenhalf, *Liverpool*
Indra N Guha, *Southampton*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
Janusz AZ Jankowski, *Oxford*
Peter Karayiannis, *London*
Patricia F Lalor, *Birmingham*
Giorgina Mieli-Vergani, *London*
D Mark Pritchard, *Liverpool*
Marco Senzolo, *Padova*
Roger Williams, *London*
M H Ahmed, *Southampton*
Christos Paraskeva, *Bristol*
Emad M El-Omar, *Aberdeen*
A M El-Tawil, *Birmingham*
Anne McCune, *Bristol*
Charles B Ferguson, *Belfast*
Chin Wee Ang, *Liverpool*
Clement W Imrie, *Glasgow*
Dileep N Lobo, *Nottingham*
Graham MacKay, *Glasgow*
Guy Fairbairn Nash, *Poole*
Ian Lindsey, *Oxford*
Jason CB Goh, *Birmingham*
Jeremy FL Cobbold, *London*
Julian RF Walters, *London*
Jamie Murphy, *London*
John Beynon, *Swansea*
John B Schofield, *Kent*
Anil George, *London*
Aravind Suppiah, *East Yorkshire*
Basil Ammori, *Salford*
Catherine Walter, *Cheltenham*
Chris Briggs, *Sheffield*
Jeff Butterworth, *Shrewsbury*
Nawfal Hussein, *Nottingham*
Patrick O'Dwyer, *Glasgow*
Rob Glynne-Jones, *Northwood*
Sharad Karandikar, *Birmingham*
Venkatesh Shanmugam, *Derby*
Yeng S Ang, *Wigan*
Alberto Quaglia, *London*
Andrew Fowell, *Southampton*
Gianpiero Gravante, *Leicester*
Piers Gatenby, *London*
Kondragunta Rajendra Prasad, *Leeds*
Sunil Dolwani, *Cardiff*
Andrew McCulloch Veitch, *Wolverhampton*
Brian Green, *Belfast*
Noriko Suzuki, *Middlesex*
Richard Parker, *North Staffordshire*
Shahid A Khan, *London*
Akhilesh B Reddy, *Cambridge*
Jean E Crabtree, *Leeds*
John S Leeds, *Sheffield*
Paul Sharp, *London*
Sumita Verma, *Brighton*
Thamara Perera, *Birmingham*
Donald Campbell McMillan, *Glasgow*
Kathleen B Bamford, *London*
Helen Coleman, *Belfast*
Eyad Elkord, *Manchester*
Mohammad Ilyas, *Nottingham*
Simon R Carding, *Norwich*
Ian Chau, *Sutton*
Claudio Nicoletti, *Norwich*
Hendrik-Tobias Arkenau, *London*
Muhammad Imran Aslam, *Leicester*
Giuseppe Orlando, *Oxford*
John S Leeds, *Aberdeen*
S Madhusudan, *Nottingham*
Amin Ibrahim Amin, *Dunfermline*
David C Hay,*Edinburgh*
Alan Burns, *London*

United States

Tauseef Ali, *Oklahoma City*
George Y Wu, *Farmington*
Josef E Fischer, *Boston*
Thomas Clancy, *Boston*
John Morton, *Stanford*
Luca Stocchi, *Cleveland*
Kevin Michael Reavis, *Orange*
Shiu-Ming Kuo, *Buffalo*
Gary R Lichtenstein, *Philadelphia*
Natalie J Torok, *Sacramento*
Scott A Waldman, *Philadelphia*
Georgios Papachristou, *Pittsburgh*
Carla W Brady, *Durham*
Robert CG Martin, *Louisville*
Eugene P Ceppa, *Durham*
Shashi Bala, *Worcester*
Imran Hassan, *Springfield*
Klaus Thaler, *Columbia*
Andreas M Kaiser, *Los Angeles*
Shawn D Safford, *Norfolk*
Massimo Raimondo, *Jacksonville*
Kazuaki Takabe, *Richmond VA*
Stephen M Kavic, *Baltimore*
T Clark Gamblin, *Pittsburgh*
BS Anand, *Houston*
Ananthanarayanan M, *New York*
Anthony J Bauer, *Pittsburgh*
Edmund J Bini, *New York*
Xian-Ming Chen, *Omaha*
Ramsey Chi-man Cheung, *Palo Alto*
Parimal Chowdhury, *Arkansas*
Mark J Czaja, *New York*

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 12 March 28, 2013

EDITORIAL

1855 New strategies for colorectal cancer screening
Di Lena M, Travaglio E, Altomare DF

FRONTIER

1861 Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy
Wallace JL

REVIEW

1877 Role of T cell death in maintaining immune tolerance during persistent viral hepatitis
Larrubia JR, Lokhande MU, García-Garzón S, Miquel J, Subirá D, Sanz-de-Villalobos E

1890 Clinical application of liver stiffness measurement using transient elastography in chronic liver disease from longitudinal perspectives
Kim BK, Fung J, Yuen MF, Kim SU

ORIGINAL ARTICLE

1901 Promising effect of Magliasa, a traditional Iranian formula, on experimental colitis on the basis of biochemical and cellular findings
Rahimi R, Baghaei A, Baeeri M, Amin G, Shams-Ardekani MR, Khanavi M, Abdollahi M

1912 Expression and clinical significance of CD73 and hypoxia-inducible factor-1α in gastric carcinoma
Lu XX, Chen YT, Feng B, Mao XB, Yu B, Chu XY

BRIEF ARTICLE

1919 Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*
Vieira-Lopes DA, Pinheiro NL, Sales A, Ventura A, Araújo FG, Gomes ID, Nascimento AA

1930 Decreased prevalence of celiac disease among Brazilian elderly
Almeida LM, Castro LC, Uenishi RH, de Almeida FC, Fritsch PM, Gandolfi L, Pratesi R, de Medeiros Nóbrega YK

1936 Conventional endoscopic retrograde cholangiopancreaticography *vs* the Olympus V-scope system
Raithel M, Nägel A, Maiss J, Wildner D, Hagel AF, Braun S, Diebel H, Hahn EG

1943 Factors associated with early virological response to peginterferon-α-2a/ribavirin in chronic hepatitis C
García-Samaniego J, Romero M, Granados R, Alemán R, Jorge Juan M, Suárez D, Pérez R, Castellano G, González-Portela C

1953 Endoscopic submucosal dissection for the treatment of neoplastic lesions in the gastrointestinal tract
Białek A, Wiechowska-Kozłowska A, Pertkiewicz J, Karpińska K, Marlicz W, Milkiewicz P, Starzyńska T

1962 Curcumin attenuated paracetamol overdose induced hepatitis
Somanawat K, Thong-Ngam D, Klaikeaw N

1968 Loss of BRCA1 expression leads to worse survival in patients with gastric carcinoma
Zhang ZZ, Liu YJ, Yin XL, Zhan P, Gu Y, Ni XZ

1975 Prognostic assessment of different metastatic lymph node staging methods for gastric cancer after D2 resection
Xu J, Bian YH, Jin X, Cao H

1984 Effect evaluation of interleukin-1 receptor antagonist nanoparticles for mesenchymal stem cell transplantation
Shi XL, Zhu W, Tan JJ, Xiao JQ, Zhang L, Xu Q, Ma ZL, Ding YT

CASE REPORT

1992 Usefulness of positron emission tomography in primary intestinal follicular lymphoma
Tari A, Asaoku H, Kunihiro M, Tanaka S, Yoshino T

1997 Transmesocolic hernia with strangulation in a patient without surgical history: Case report
Jung P, Kim MD, Ryu TH, Choi SH, Kim HS, Lee KH, Park JH

2000 Rare case of *Helicobacter pylori*-related gastric ulcer: Malignancy or pseudomorphism?
Li TT, Qiu F, Wang ZQ, Sun L, Wan J

2005 Coexistence of gastrointestinal stromal tumor, esophageal and gastric cardia carcinomas
Zhou Y, Wu XD, Shi Q, Jia J

LETTERS TO THE EDITOR 2009

Tumor rupture during surgery for gastrointestinal stromal tumors: Pay attention!

Peparini N, Chirletti P

APPENDIX I-VI Instructions to authors

ABOUT COVER John L Wallace, PhD, MBA, FRSC, Farncombe Family Digestive Health Research Institute, McMaster University, 1280 Main Street West, HSC-3N4, Hamilton, ON L8S 4K1, Canada

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF I-IX Editorial Board

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
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

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
March 28, 2013

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

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

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1855

World J Gastroenterol 2013 March 28; 19(12): 1855-1860
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

New strategies for colorectal cancer screening

Maria Di Lena, Elisabetta Travaglio, Donato F Altomare

Maria Di Lena, Elisabetta Travaglio, Donato F Altomare, Department of Emergency and Organ Transplantation, University Aldo Moro of Bari, 11-70124 Bari, Italy
Author contributions: Di Lena M, Travaglio E and Altomare DF contributed equally to this paper.
Correspondence to: Donato F Altomare, MD, Professor, Department of Emergency and Organ Transplantation, University Aldo Moro of Bari, Piazza G Cesare, 11-70124 Bari, Italy. donatofrancesco.altomare@uniba.it
Telephone: +39-8-5592107 Fax: +39-8-5478735
Received: August 21, 2012 Revised: September 30, 2012
Accepted: November 6, 2012
Published online: March 28, 2013

Abstract

Colorectal cancer (CRC) is still one of the leading causes of cancer-related death in Western countries, despite major improvements in its treatment. The dramatically high social and economic impact of CRC on human health makes the identification of a reliable screening tool of paramount importance. Current screening methods, such as the fecal occult blood test and colonoscopy do not adequately meet the ideal requisites of a screening test because, even if they are effective, they are limited first by too low specificity and sensitivity, or second by high invasiveness, costs and risk. Nowadays extended efforts are made by researchers to look for more reliable and effective screening tests based on a systems biology approach, using biological samples easily available, such as urine, breath, serum and feces. The effectiveness and reliability of several new attempts to screen these patients by non-invasive analysis of their biological samples using genomic (genetic and epigenetic alteration), transcriptomic (miRNA), proteomic (cancer-related antigens, new antibodies against tumor-associated antigens, mutated proteins) and metabolomic (volatile organic metabolites) methods are discussed in this review. Among the most interesting new screening tools, fecal fluorescent long-DNA, fecal miRNA and metabolomic evaluation in breath and/or serum seem to be most promising.

Key words: Colorectal cancer; Screening; Fecal occult blood test; Systems biology; Metabolomics; Proteomic; microRNA; Volatile organic compounds

Di Lena M, Travaglio E, Altomare DF. New strategies for colorectal cancer screening. *World J Gastroenterol* 2013; 19(12): 1855-1860 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1855.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1855

TRADITIONAL APPROACH TO COLORECTAL CANCER SCREENING

Colorectal cancer (CRC) is the second most commonly diagnosed cancer and the second leading cause of cancer death in Europe, with an incidence of 43 600 new cases between 2007 and 2008[1]. The dramatically high social and economic impact of CRC on human health makes the identification of a reliable screening tool of paramount importance. CRC, as a cancer actually fulfills the World Health Organization conditions required for mass screening, since it is a very common disease, with major morbidity and mortality rates and is almost always preceded by a slow progressive premalignant lesion (the adenomatous polyp) which can readily be removed leading to true cancer prevention[2]. Screening strategies for CRC involve the separation of the population into two main categories: average risk and high risk populations. Each of these categories is targeted using a different screening program. In the first group, adults over 50 years without a personal or family history of CRC, polyps or inflammatory bowel diseases (IBD) are screened. The high risk population includes subjects with a family history of CRC, a personal history of CRC or polyps or are index cases affected by IBD. There is, however, a third category, more specifically characterized by an heredofamilial risk and represented by hereditary cancer syndromes such

as familial adenomatous polyposis and hereditary non-polyposis CRC[3,4]. Such cases should be screened directly with total colonoscopy (TC). The average risk population reflects the vast majority of the population and needs to be screened by less-invasive, low-cost techniques with acceptable patient compliance[5]. For that reason, in the last decade, there has been a great interest and research effort in developing the optimal CRC screening tool.

Clinically validated screening strategies currently available in practice include fecal occult blood testing (FOBT), TC, flexible sigmoidoscopy (FS) and radiographic imaging, such as double contrast barium enema and virtual TC. FOBT is the most commonly used method for CRC screening. In this respect, it is non-invasive, inexpensive and matches patient compliance better than other screening tools. In 2008, Hewitson *et al*[6] published a systematic review comparing the results of four randomized controlled trials, using FOBT as a screening tool, and in approximately 320 000 patients screened, there was an overall reduction of the relative risk of dying of CRC of 16%. Despite this, FOBT has demonstrated an unacceptably low specificity rate. To improve its reliability in this regard, fecal immunohistochemistry testing (FIT), which specifically detects non-degraded human globin using anti-human hemoglobin antibodies, has replaced the older guaiac-based FOBT (which identified the heme group by pseudoperoxidase). Despite this major improvement, the search for occult blood in the feces still has severe limitations as a screening tool, mainly because of its low specificity, hence leading to a high number of unnecessary colonoscopies[7,8]. FS has been proposed as a balance between the invasiveness of a given test (such as low invasive tests like FOBT and FIT), their accuracy and their potential complications (*e.g.*, TC), considering that about two-thirds of the screened CRCs detected are located in the rectum and sigmoid colon. It may be possible to increase the performance characteristics of FS by combining it with FOBT/FIT, however, the risk of leaving undetected CRC in other colonic sites is currently unacceptable[9,10].

TC still remains the gold standard for the diagnosis of both colorectal polyps and malignancies. The National Polyp Study demonstrated that the incidence of CRC was reduced from 76% to 90%[11] after polypectomy. Although very effective for diagnosis and treatment, TC has the limitations of low patient compliance, high cost, a high level of invasiveness and a moderate incidence of serious complications in specific subgroups (an incidence of 0.1%-0.3% of life-threatening complications including bleeding and perforation). TC colonography (or virtual TC) involves the use of helical TC to generate high-resolution 3D images of the abdomen and pelvis, replacing the older barium enema in providing full structural evaluation of the entire colon. A study conducted by Fenlon *et al*[12] in a high risk population, reported a sensitivity of 71% for TC colonography, although this was strongly influenced by polyp size where only 55% of polyps between 1 and 5 mm in maximal diameter were correctly identified. The sensitivity for virtual diagnosis was significantly higher when polyps ranged between 6 and 9 mm or were larger than 10 mm in size (82% and 91%, respectively; $P = 0.001$)[12]. This investigation, however, had the drawbacks of considerable exposure to ionizing radiation, discomfort of the bowel preparation and the necessity to complete the procedure by TC in cases of polyp or cancer detection, as well as being expensive (with inherent derivative costs) and currently not suitable for screening purposes.

From these considerations it is clear that current screening methods do not properly meet the ideal requisites of a screening test, so that extended effort has been dedicated by researchers at looking for more reliable and effective screening tests based on the systems biology approach using biological samples easily available such as urine, breath, serum and feces. Since the human genome was completely identified in 2003, the entire set of genes and proteins expressed have been extensively studied using genomic, transcriptomic or proteomic approaches.

GENOMIC APPROACH TO CRC SCREENING

Several authors have attempted to identify cancer-related mutated DNA/RNA, mutated proteins or normal proteins abnormally synthesized [*e.g.*, carcinoembryonic antigen (CEA), cytokeratins] in different biological samples as potential biomarkers for CRC. Colorectal carcinogenesis is characterized by genetic alteration (gene mutation or gene amplification) and epigenetic alteration (gene *hypermethylation* or *chromatin* modification), which both transform normal epithelial cells into cancer cells. CRC cells are continuously shed in the feces, due to a high proliferative rate, so that mutated DNA can be readily detected in the feces of these patients. This issue is complex, where mutation in the *APC*, *K-ras* and *p53* genes were initially investigated in stool samples of CRC patients, in accordance with the Volgenstein model of *CRC* genesis[13]. Other markers have also been studied by Imperiale *et al*[14] who conducted a large population-based study comparing the fecal DNA test with FOBT, using a DNA marker panel formed by 21 mutations and demonstrated a sensitivity of 52% for invasive cancers compared with 13% for FOBT in the same population. Fecal DNA testing has been commercially available in the United States since 2003, but so far has rarely been adopted for screening despite preliminary studies showing that the use of a large pool of genetic markers results in a sensitivity of 71%-91% and a specificity of more than 93%[15]. A recent interesting approach involves the use of fluorescent long DNA (FL-DNA) measurement, designed to identify cancer DNA fragments greater than 150-200 db pairs. Changes are noted since cancer cells do not undergo apoptosis, which in normal epithelial cells typically initiate DNA cleavage and degradation producing small measurable fragments. This FL-DNA technique has shown a performance sensitivity up to 80% in detecting CRC[16]. Such mutated DNA can also

be demonstrated in the urine of CRC patients. Human urine has been shown to contain two types of DNA: large type, greater than 1 kb, presumably derived from cells shed into the urine from the urinary tract and small type, between 150 bp and 250 bp, derived from the circulation, which can cross the renal barrier. Sample urine collection is non-invasive and isolation of DNA from urine is easier than from others specimens, due its low extraneous protein content. The comparison of mutated *K-ras* sequences, in particular the mutation in codon 12, between tumor, blood and urine from CRC patients and healthy controls showed an 83% correspondence of mutated DNA in urine and tumor tissue in the same patients[17]. Epigenetic changes which characterize CRC cells have only been studied in urine samples; most notably, the *hypermethylated vimentin* (*m-VIM*) gene. The detection of *m-VIM* in urine samples is significantly associated with CRC when compared with healthy controls[18].

TRANSCRIPTOMIC APPROACH TO CRC SCREENING

The most recent transcriptomic approach to identify potential biomarkers for CRC involves the study of microRNAs (miRNA), short non-coding 18-22 nucleotide RNA molecules involved in regulation of gene expression through post-transcriptional processing. Their expression is deregulated in cancer cells where altered miRNA expression leads to altered expression of their target gene including a range of potential oncogenes and oncosuppressors during carcinogenesis. Chen *et al*[19] showed that levels of miRNA in the serum are stable, reproducible and consistent in humans, concluding that they can be potential biomarkers for different diseases. Recent studies have indicated that circulating microRNAs incorporated into microvesicles and exosomes may be involved in genetic informational exchange between cells and may regulate extracellular matrix degradation, immunologic response and angiogenic factors which favor cancer cell growth and metastasis[20]. MiR-145, miR-143, miR-135a and b, miR-17-92, miR-21 have been most studied in CRC where Ng *et al*[21] were able to identify a significant increase of miR92 in the plasma of CRC patients compared with controls. Similar results have been reported by Huang *et al*[22] demonstrated a significant increase in miR29a and miR92a in patients with adenomas and CRC compared with controls, supporting the hypothesis that the miR17-92 cluster could have a role in cell proliferation, tumor angiogenesis and apoptotic suppression. Altered miRNA[23] expression has been examined in the stools of CRC patients and could represent an optimal screening tool for this cancer where colonic cancer cells exfoliate in greater quantity and their nucleic acid can be extracted and distinguished from those of bacteria. In this regard, Link *et al*[24] compared fecal specimens of patients with CRC, patients with adenomas and normal controls, showing a specific miRNA pattern in the three groups where miR21, miR106 were over expressed in CRC patients compared with controls, but where levels were higher in patients with adenomas and tended to decrease in cancer cases. Other researchers, however, were unable to confirm the higher expression of miR21, whilst the clusters miR17-92 and miR135 have been found to be significantly higher in the feces of CRC patients when compared with controls[25]. Another fecal mRNA frequently investigated as a potential CRC marker in stool is the prostaglandin-synthase 2, which showed a sensitivity between 50% and 90% and a specificity of 93% or higher in the diagnosis of CRC, although the reliability of this study was limited by the small number of CRC patients evaluated[26,27].

PROTEOMIC APPROACH TO CRC SCREENING

A further method for early detection and screening of CRC is to look at the modified "proteome" as a direct effect of mutated gene expression or as the occurrence of new antibodies against tumor-associated antigens (TAAs) identified in CRC. Hundt *et al*[28] have published a systematic review of 19 studies, in which 52 protein markers were analyzed, using common standard procedures such as enzyme-linked immunoassay, radioimmunoassay or more recent approaches like chromatographic and mass spectrometric assays based on surface-enhanced laser desorption/ionization time-of-flight (TOF) and matrix-assisted laser desorption/ionization TOF technologies. These compounds can be divided into antigens, antibodies, cytokines and other CRC-relevant proteins. CEA is the most investigated marker. High CEA levels are derived from embryonic tissues and CRC, but they also increase in other malignancies, including gastric and pancreatic cancer, as well as in IBD and in smokers. Its role for screening is limited because CEA evaluation has been shown to have a sensitivity of only 43%-69% in detecting early CRC, whilst its reliability increases in metastatic cancer where assessment lies outside the screening purpose. Carbohydrate antigens such as CA 19-9, CA195, CA 50 or CA 72-4 have been investigated in many studies, but with comparatively disappointing results. The best performance amongst these antigens is that of CA 19-9, with a sensitivity ranging between 18% and 65% and a specificity of over 90%. Other antigens considered for screening purposes include the sialylated Lewis antigen X, CO 29.11[29], urokinase-type plasminogen activator[30] and small intestinal mucin antigen[31], but none of these serological antigens have so far demonstrated an acceptable reliability in clinical testing. Recently Matsubara *et al*[32] studying the proteome of CRC patients compared with healthy controls, using label-free quantitative mass spectrometry and protein microarray, identified the adipophilin or adipose differentiation-related protein, a protein involved in the cancer pathway and normally expressed in cancer cells but not by the normal mucosa. This protein has been investigated as a potential plasma biomarker for early CRC stages, showing high receiver

operating characteristics[32].

Other studies have focused on the use of autoantibodies antibodies against TAAs as serological markers for cancer diagnosis, because they are absent in healthy subjects and other non-cancer conditions. Many autoantibodies against known or unknown TAAs, have been found in the sera of patients with a range of malignancies[33-35]. Various technologies such as serologic analysis of recombinant cDNA expression libraries, first described in 1995 by Sahin *et al*[36], and protein arrays or phage display techniques have been used in their measurement. The occurrence of several serum autoantibodies against TAAs, such as epithelial cell adhesion molecule or cytokeratin, p53, p62, CEA, HER-2/neu, Ras, topoisomerase Ⅱ-alpha, histone deacetylases 3 and 5, ubiquitin C-terminal hydrolase L3, tyrosinase, tropomyosin and cyclin B1 have all been evaluated in CRC patients[37], but were detected only in a limited proportion of patients (< 40%). Mutated or abnormal proteins have been detected also in the feces as potential biomarkers for screening, including tumor pyruvate kinase type M2, which has good sensitivity for CRC (85%), but not for adenomas (28%)[38], S100 calcium binding protein A12 and metallopeptidase inhibitor 1. The latter showed a sensitivity for cancer of around 85% and a specificity of 95%[39] compared with healthy controls.

METABOLOMIC APPROACH TO CRC SCREENING

More recently, the study of specific metabolomic biomarkers for cancers has developed as a new frontier in cancer screening. Metabolomics are the endpoint of the "omics" cascade and incorporate the comprehensive study of low-molecular-weight metabolites, using high-throughput technologies, such as gas chromatography-mass spectrometry, or other analytical platforms. Ikeda *et al*[40] investigated the differences in serum metabolite profiles of esophageal, gastric and CRC patients and healthy volunteers, using the metabolomic approach to determine specific metabolomic biomarker candidates. They showed a different distribution of L-alanine, glucuronic lactone and L-glutamine in CRC patients, with a sensitivity of 54.5%-81.8% and a specificity of 6.7%-91.6%[40]. Specific metabolomes can be identified in several types of biologic samples, including feces, urine, serum, sputum and breath. In this regard, breath analysis could be considered the favored option for medical diagnostic purposes mostly because of its non-invasive nature, its low cost and its ready patient compliance[41]. Volatile organic compounds (VOCs) in exhaled breath were first isolated by Pauling *et al*[42] in 1971, and alteration in VOC production in cancer patients has been postulated to relate to (per)oxygenation of cell membrane-based polyunsaturated fatty acids resulting from genetic and/or protein mutations within tumor cells and the increased relative prevalence of reactive oxygen species within cancer cells[43,44]. Urine and serum are ideal tools for metabolomic analyses. Some studies using high-throughput techniques and artificial neural network statistics have identified some volatile organic metabolites as potential biomarkers for CRC in urine[45], and very recently, a Japanese group has developed a CRC-prediction model based on serum metabolomic analysis and which demonstrated a high sensitivity (82.8%) as a novel potential screening test for CRC[46]. A similar metabolomic approach was carried out by our group[47], looking at the VOCs contained in breath. In this study, 15 of the 58 VOCs identified formed a specific pattern in CRC patients and, using a probabilistic neural network, the ability to identify CRC patients showed a sensitivity of 86%, a specificity of 83% and an accuracy of 85% (area under the receiver operating characteristics curve: 0.85) for the diagnosis of CRC.

In conclusion, despite their usefulness and effectiveness, traditional methods for CRC screening are still far from fulfilling the optimal requisites for a screening test. The FOBT/FIT both have too low a sensitivity or specificity whilst the high sensitivity of CT is counterbalanced by its invasiveness and high cost. TC colonography is still improving its technical performance but is expensive and, in cases of positivity, a traditional TC is still required to remove polyps or for biopsies. New hopes are rapidly growing in this field with the application of the systems biology approach using biological samples which are readily available. Among these, the search for fecal FL-DNA, fecal miRNA and metabolomic evaluation in the breath and/or serum seems to be the most promising.

REFERENCES

1 **Ferlay J**, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. *Eur J Cancer* 2010; **46**: 765-781 [PMID: 20116997]

2 **Pignone M**, Rich M, Teutsch SM, Berg AO, Lohr KN. Screening for colorectal cancer in adults at average risk: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002; **137**: 132-141 [PMID: 12118972]

3 **Pant KD**, McCracken JD. Noninvasive colorectal cancer screening. *Dig Dis Sci* 2002; **47**: 1236-1240 [PMID: 12064797]

4 **Nelson RS**, Thorson AG. Colorectal cancer screening. *Curr Oncol Rep* 2009; **11**: 482-489 [PMID: 19840526]

5 **Burt RW**, Barthel JS, Dunn KB, David DS, Drelichman E, Ford JM, Giardiello FM, Gruber SB, Halverson AL, Hamilton SR, Ismail MK, Jasperson K, Lazenby AJ, Lynch PM, Martin EW, Mayer RJ, Ness RM, Provenzale D, Rao MS, Shike M, Steinbach G, Terdiman JP, Weinberg D. NCCN clinical practice guidelines in oncology. Colorectal cancer screening. *J Natl Compr Canc Netw* 2010; **8**: 8-61 [PMID: 20064289]

6 **Hewitson P**, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. *Am J Gastroenterol* 2008; **103**: 1541-1549 [PMID: 18479499]

7 **Allison JE**, Tekawa IS, Ransom LJ, Adrain AL. A comparison of fecal occult-blood tests for colorectal-cancer screening. *N Engl J Med* 1996; **334**: 155-159 [PMID: 8531970]

8 **van Rossum LG**, van Rijn AF, Laheij RJ, van Oijen MG, Fockens P, van Krieken HH, Verbeek AL, Jansen JB, Dekker E. Random comparison of guaiac and immunochemical fecal occult blood tests for colorectal cancer in a screening population. *Gastroenterology* 2008; **135**: 82-90 [PMID: 18482589]

9 **Lieberman DA**, Weiss DG. One-time screening for colorec-

tal cancer with combined fecal occult-blood testing and examination of the distal colon. *N Engl J Med* 2001; **345**: 555-560 [PMID: 11529208]
10 **Selby JV**, Friedman GD, Quesenberry CP, Weiss NS. A case-control study of screening sigmoidoscopy and mortality from colorectal cancer. *N Engl J Med* 1992; **326**: 653-657 [PMID: 1736103]
11 **Winawer SJ**, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, Waye JD, Schapiro M, Bond JH, Panish JF. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med* 1993; **329**: 1977-1981 [PMID: 8247072]
12 **Fenlon HM**, Nunes DP, Schroy PC, Barish MA, Clarke PD, Ferrucci JT. A comparison of virtual and conventional colonoscopy for the detection of colorectal polyps. *N Engl J Med* 1999; **341**: 1496-1503 [PMID: 10559450]
13 **Bosch LJ**, Carvalho B, Fijneman RJ, Jimenez CR, Pinedo HM, van Engeland M, Meijer GA. Molecular tests for colorectal cancer screening. *Clin Colorectal Cancer* 2011; **10**: 8-23 [PMID: 21609931]
14 **Imperiale TF**, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; **351**: 2704-2714 [PMID: 15616205]
15 **Ahlquist DA**, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, Thibodeau SN, Shuber AP. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. *Gastroenterology* 2000; **119**: 1219-1227 [PMID: 11054379]
16 **Calistri D**, Rengucci C, Casadei Gardini A, Frassineti GL, Scarpi E, Zoli W, Falcini F, Silvestrini R, Amadori D. Fecal DNA for noninvasive diagnosis of colorectal cancer in immunochemical fecal occult blood test-positive individuals. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2647-2654 [PMID: 20929882]
17 **Su YH**, Wang M, Brenner DE, Ng A, Melkonyan H, Umansky S, Syngal S, Block TM. Human urine contains small, 150 to 250 nucleotide-sized, soluble DNA derived from the circulation and may be useful in the detection of colorectal cancer. *J Mol Diagn* 2004; **6**: 101-107 [PMID: 15096565]
18 **Song BP**, Jain S, Lin SY, Chen Q, Block TM, Song W, Brenner DE, Su YH. Detection of hypermethylated vimentin in urine of patients with colorectal cancer. *J Mol Diagn* 2012; **14**: 112-119 [PMID: 22251609]
19 **Chen X**, Ba Y, Ma L, Cai X, Yin Y, Wang K, Guo J, Zhang Y, Chen J, Guo X, Li Q, Li X, Wang W, Zhang Y, Wang J, Jiang X, Xiang Y, Xu C, Zheng P, Zhang J, Li R, Zhang H, Shang X, Gong T, Ning G, Wang J, Zen K, Zhang J, Zhang CY. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res* 2008; **18**: 997-1006 [PMID: 18766170]
20 **Kosaka N**, Iguchi H, Ochiya T. Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci* 2010; **101**: 2087-2092 [PMID: 20624164]
21 **Ng EK**, Chong WW, Jin H, Lam EK, Shin VY, Yu J, Poon TC, Ng SS, Sung JJ. Differential expression of microRNAs in plasma of patients with colorectal cancer: a potential marker for colorectal cancer screening. *Gut* 2009; **58**: 1375-1381 [PMID: 19201770]
22 **Huang Z**, Huang D, Ni S, Peng Z, Sheng W, Du X. Plasma microRNAs are promising novel biomarkers for early detection of colorectal cancer. *Int J Cancer* 2010; **127**: 118-126 [PMID: 19876917]
23 **Altomare DF**, Di Lena M, Giuratrabocchetta S. MicroRNA: future perspectives in colorectal cancer. *Colorectal Dis* 2012; **14**: 133-134 [PMID: 22233118 DOI: 10.1111/j.1463-1318.2011.02874.x]
24 **Link A**, Balaguer F, Shen Y, Nagasaka T, Lozano JJ, Boland CR, Goel A. Fecal MicroRNAs as novel biomarkers for colon cancer screening. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1766-1774 [PMID: 20551304]
25 **Koga Y**, Yasunaga M, Takahashi A, Kuroda J, Moriya Y, Akasu T, Fujita S, Yamamoto S, Baba H, Matsumura Y. MicroRNA expression profiling of exfoliated colonocytes isolated from feces for colorectal cancer screening. *Cancer Prev Res* (Phila) 2010; **3**: 1435-1442 [PMID: 20959518]
26 **Kanaoka S**, Yoshida K, Miura N, Sugimura H, Kajimura M. Potential usefulness of detecting cyclooxygenase 2 messenger RNA in feces for colorectal cancer screening. *Gastroenterology* 2004; **127**: 422-427 [PMID: 15300574]
27 **Leung WK**, To KF, Man EP, Chan MW, Hui AJ, Ng SS, Lau JY, Sung JJ. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. *Am J Gastroenterol* 2007; **102**: 1070-1076 [PMID: 17378912]
28 **Hundt S**, Haug U, Brenner H. Blood markers for early detection of colorectal cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1935-1953 [PMID: 17932341]
29 **Kawahara M**, Chia D, Terasaki PI, Roumanas A, Sugich L, Hermes M, Iguro T. Detection of sialylated LewisX antigen in cancer sera using a sandwich radioimmunoassay. *Int J Cancer* 1985; **36**: 421-425 [PMID: 2995258]
30 **Huber K**, Kirchheimer JC, Sedlmayer A, Bell C, Ermler D, Binder BR. Clinical value of determination of urokinase-type plasminogen activator antigen in plasma for detection of colorectal cancer: comparison with circulating tumor-associated antigens CA 19-9 and carcinoembryonic antigen. *Cancer Res* 1993; **53**: 1788-1793 [PMID: 8467497]
31 **Eskelinen M**, Pasanen P, Janatuinen E, Pettersson N, Linnane A, Alhava E. Small intestinal mucin antigen (SIMA); a novel tumour marker in colorectal cancer? *Anticancer Res* 1995; **15**: 2351-2356 [PMID: 8572651]
32 **Matsubara J**, Honda K, Ono M, Sekine S, Tanaka Y, Kobayashi M, Jung G, Sakuma T, Nakamori S, Sata N, Nagai H, Ioka T, Okusaka T, Kosuge T, Tsuchida A, Shimahara M, Yasunami Y, Chiba T, Yamada T. Identification of adipophilin as a potential plasma biomarker for colorectal cancer using label-free quantitative mass spectrometry and protein microarray. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 2195-2203 [PMID: 21828233]
33 **Zhang JY**, Tan EM. Autoantibodies to tumor-associated antigens as diagnostic biomarkers in hepatocellular carcinoma and other solid tumors. *Expert Rev Mol Diagn* 2010; **10**: 321-328 [PMID: 20370589]
34 **Farlow EC**, Patel K, Basu S, Lee BS, Kim AW, Coon JS, Faber LP, Bonomi P, Liptay MJ, Borgia JA. Development of a multiplexed tumor-associated autoantibody-based blood test for the detection of non-small cell lung cancer. *Clin Cancer Res* 2010; **16**: 3452-3462 [PMID: 20570928]
35 **Desmetz C**, Bascoul-Mollevi C, Rochaix P, Lamy PJ, Kramar A, Rouanet P, Maudelonde T, Mangé A, Solassol J. Identification of a new panel of serum autoantibodies associated with the presence of in situ carcinoma of the breast in younger women. *Clin Cancer Res* 2009; **15**: 4733-4741 [PMID: 19584157]
36 **Sahin U**, Türeci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc Natl Acad Sci USA* 1995; **92**: 11810-11813 [PMID: 8524854]
37 **Lu H**, Goodell V, Disis ML. Targeting serum antibody for cancer diagnosis: a focus on colorectal cancer. *Expert Opin Ther Targets* 2007; **11**: 235-244 [PMID: 17227237]
38 **Mulder SA**, van Leerdam ME, van Vuuren AJ, Francke J, van Toorenenbergen AW, Kuipers EJ, Ouwendijk RJ. Tumor pyruvate kinase isoenzyme type M2 and immunochemical fecal occult blood test: performance in screening for colorectal cancer. *Eur J Gastroenterol Hepatol* 2007; **19**: 878-882 [PMID: 17873612]

39 **Karl J**, Wild N, Tacke M, Andres H, Garczarek U, Rollinger W, Zolg W. Improved diagnosis of colorectal cancer using a combination of fecal occult blood and novel fecal protein markers. *Clin Gastroenterol Hepatol* 2008; **6**: 1122-1128 [PMID: 18928937]

40 **Ikeda A**, Nishiumi S, Shinohara M, Yoshie T, Hatano N, Okuno T, Bamba T, Fukusaki E, Takenawa T, Azuma T, Yoshida M. Serum metabolomics as a novel diagnostic approach for gastrointestinal cancer. *Biomed Chromatogr* 2012; **26**: 548-558 [PMID: 21773981 DOI: 10.1002/bmc.1671]

41 **Patel K**. Noninvasive tools to assess liver disease. *Curr Opin Gastroenterol* 2010; **26**: 227-233 [PMID: 20179592]

42 **Pauling L**, Robinson AB, Teranishi R, Cary P. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proc Natl Acad Sci USA* 1971; **68**: 2374-2376 [PMID: 5289873]

43 **Kneepkens CM**, Lepage G, Roy CC. The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic Biol Med* 1994; **17**: 127-160 [PMID: 7959173]

44 **Toyokuni S**. Molecular mechanisms of oxidative stress-induced carcinogenesis: from epidemiology to oxygenomics. *IUBMB Life* 2008; **60**: 441-447 [PMID: 18465793]

45 **Silva CL**, Passos M, Câmara JS. Investigation of urinary volatile organic metabolites as potential cancer biomarkers by solid-phase microextraction in combination with gas chromatography-mass spectrometry. *Br J Cancer* 2011; **105**: 1894-1904 [PMID: 22085842]

46 **Nishiumi S**, Kobayashi T, Ikeda A, Yoshie T, Kibi M, Izumi Y, Okuno T, Hayashi N, Kawano S, Takenawa T, Azuma T, Yoshida M. A novel serum metabolomics-based diagnostic approach for colorectal cancer. *PLoS One* 2012; **7**: e40459 [PMID: 22792336]

47 **Altomare DF**, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, Dragonieri S, Memeo V, de Gennaro G. Exhaled volatile organic compounds identify patients with colorectal cancer. *Br J Surg* 2013; **100**: 144-150 [PMID: 23212621]

P- Reviewer Piso P **S- Editor** Wen LL **L- Editor** Cant MR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1861

World J Gastroenterol 2013 March 28; 19(12): 1861-1876
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

FRONTIER

Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy

John L Wallace

John L Wallace, Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON L8S 4K1, Canada
John L Wallace, Inflammation Research Network, University of Calgary, Calgary, AB T2N 4N1, Canada
Author contributions: Wallace JL solely contributed to this paper.
Supported by A grant from the Canadian Institutes of Health Research
Correspondence to: John L Wallace, PhD, MBA, FRSC, Farncombe Family Digestive Health Research Institute, McMaster University, 1280 Main Street West, HSC-3N4, Hamilton, ON L8S 4K1, Canada. altapharm@hotmail.com
Telephone: +1-905-5156132 Fax: +1-905-5289862
Received: January 23, 2013 Revised: March 6, 2013
Accepted: March 7, 2013
Published online: March 28, 2013

Abstract

This article reviews the latest developments in understanding the pathogenesis, detection and treatment of small intestinal damage and bleeding caused by nonsteroidal anti-inflammatory drugs (NSAIDs). With improvements in the detection of NSAID-induced damage in the small intestine, it is now clear that this injury and the associated bleeding occurs more frequently than that occurring in the stomach and duodenum, and can also be regarded as more dangerous. However, there are no proven-effective therapies for NSAID-enteropathy, and detection remains a challenge, particularly because of the poor correlation between tissue injury and symptoms. Moreover, recent studies suggest that commonly used drugs for protecting the upper gastrointestinal tract (*i.e.*, proton pump inhibitors) can significantly worsen NSAID-induced damage in the small intestine. The pathogenesis of NSAID-enteropathy is complex, but studies in animal models are shedding light on the key factors that contribute to ulceration and bleeding, and are providing clues to the development of effective therapies and prevention strategies. Novel NSAIDs that do not cause small in-

Biography

John L Wallace is a Professor in the Department of Medicine at McMaster University. He received his BSc and MSc from Queen's University (Canada) and his PhD from the University of Toronto. He completed his post-doctoral studies in the Department of Mediator Pharmacology at Wellcome Research Laboratories in London, England. That work was carried out in a group led by Sir John Vane, Sir Salvador Moncada and Dr. Brendan Whittle. In 2007, Dr. Wallace completed his MBA degree from the University of Birmingham (United Kingdom).

In 1996, he co-founded NicOX SA, based in Nice, France. He served as the Chair of the company's Scientific Advisory Board from 1996-2003, overseeing the development of nitric oxide-releasing drugs. NicOx went public on the Paris Stock Exchange in 1999, and has a ophthalmic drug in phase 3 clinical trials. In 2004, Dr. Wallace founded Antibe Therapeutics Inc., a company developing hydrogen sulfide-releasing drugs.

Dr. Wallace's research is focused on mediators of inflammation and their contribution to mucosal injury and dysfunction. He is also interested in the mechanisms of injury induced by the gastrointestinal tract by anti-inflammatory drugs, and the factors that regulate healing of ulcers. Dr. Wallace is attempting to develop gastrointestinal-sparing anti-inflammatory drugs.

He is a Fellow of the Royal Society of Canada, a member of the Brazilian Academy of Science and a Fellow of the British Pharmacological Society. He has won numerous international awards for his research, including the 2009 Premier's Summit Award ($5 million) and the 2012 William Harvey Medal for Outstanding Contributions to Science. From 2000-2002, Dr. Wallace was President of the Canadian Association of Gastroenterology.

Dr. Wallace has published approximately 400 peer-reviewed papers 100 book chapters, and is among the top 0.5 percent of biomedical scientists in the world in terms of citations (Hirsch factor of 88, > 25 000 citations).

testinal damage in animal models offer hope for a solution to this serious adverse effect of one of the most widely used classes of drugs.

Key words: Anti-inflammatory; Ulcer; Prostaglandin; Non-steroidal; Bleeding; Intestinal; Bile; Enterohepatic; Bacteria; Hydrogen sulfide; Aspirin; Hemorrhage

Wallace JL. Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy. *World J Gastroenterol* 2013; 19(12): 1861-1876 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1861.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1861

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the widely used prescription and over-the-counter medications. They are used to treat the symptoms of a variety of inflammatory conditions, most notably osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and gout. In such conditions, NSAIDs are used chronically, and the affected patients frequently have co-morbidities such as hypertension, diabetes and obesity, as well as often also taking glucocorticoids or anti-coagulants.

By inhibiting the activity of cyclo-oxygenase (COX), NSAIDs prevent the formation of prostaglandin (PG) H_2, which is the precursor for the production of all other PG and thromboxane subtypes. Most NSAIDs inhibit COX activity in a competitive fashion, whereas aspirin is an irreversible inhibitor of the enzyme. Indeed, the ability of aspirin to irreversibly inhibit thromboxane synthesis by platelets, and the lack of capacity of platelets to synthesize more COX, underlie the utility of chronic, low-dose aspirin as an anti-thrombotic drug, reducing the incidence of several adverse cardiovascular events (*e.g.*, stroke, myocardial infarction).

Inhibition of COX is central to the major anti-inflammatory actions of NSAIDs. By inhibiting the production of PGs (particularly PGE_2 and PGI_2), NSAIDs reduce two key elements of inflammation: vasodilation and pain. By reducing blood flow to a damaged and inflamed site, NSAIDs also contribute to a reduction of edema.

Unfortunately, PGs do not only contribute to the cardinal signs of inflammation. They also play important roles in many physiological processes. In the gastrointestinal (GI) tract, PGs are very important mediators of mucosal defence and repair[1]. Inhibition of their synthesis renders GI tissues much more susceptible to damage induced by luminal irritants (including gastric acid and bile), and less able to restore mucosal structure and function after injury[1]. Suppression of PG synthesis is the key effect of NSAIDs that leads to gastro-duodenal ulceration and bleeding. However, several other effects of NSAIDs appear to be central to the ability of these drugs to cause damage in the small intestine.

OVERVIEW OF THE CLINICAL PROBLEM

For several decades, the ability of NSAIDs to induce significant damage to the small intestine was largely unappreciated, being over-shadowed by the attention paid to damage induced by these agents in the stomach and proximal duodenum. The prevalence and clinical significance of NSAID-enteropathy continues to be greatly underrecognized. NSAID-induced enteropathy and bleeding occur more frequently that NSAID-induced gastropathy[2,3]. Significant small intestinal damage and bleeding can be observed in about 70% of chronic NSAID users[4,5], and in the majority of patients the injury is sub-clinical[6].

Unlike the case for NSAID-gastropathy, there are no proven-effective preventative therapies for NSAID-enteropathy, and the pathogenesis is poorly understood[7]. Iron-deficient anemia is a common first presentation of NSAID-enteropathy, and serious complications can include massive bleeding, perforation and strictures, sometimes leading to death[2,6,8].

Aspirin is the most commonly used NSAID, and it is a very frequent cause of small intestinal bleeding. In the United States and Europe, in over 50% of cases, aspirin has been identified as the precipitator of GI bleeding leading to hospital admissions[3,9,10]. Aspirin-induced small intestinal damage appears to occur more frequently when the aspirin is enteric-coated[8,11]. There is a lack of recognition of the frequency and potential severity of aspirin-induced lower GI injury, particularly when the aspirin is given at low doses for cardiovascular protection. In a recent clinical trial that involved over 1200 patients taking aspirin and another anti-platelet therapy for cardiovascular protection, lower GI bleeding was found to occur 3-times more frequently than upper GI bleeding[12]. Zhu *et al*[13] reported that only about 3.5% of patients prescribed low-dose aspirin also received a prescription for a proton pump inhibitor (PPI), histamine H_2 receptor antagonist (H_2RA) or muco-protective drug, suggesting that the prescribing physicians did not recognize the potential for GI adverse effects of low-dose aspirin. The pathogenesis of aspirin-induced small intestinal damage differs in several respects to that of the ulceration caused by other NSAIDs (discussed in more detail below).

Selective COX-2 inhibitors were introduced to the marketplace at the beginning of this century with a promise of GI safety[14,15]. While some selective COX-2 inhibitors produce less gastroduodenal damage in some circumstances, the promise of these drugs has been largely unfulfilled[16,17]. Selective COX-2 inhibitors cause small intestinal damage and bleeding (the latter effect is somewhat surprising given the minimal inhibitory effects these drugs of these drugs on platelet function). McCarthy[3] noted that in the VIGOR study, the majority of the GI bleeds originated from lesions in the small intestine (distal to the ligament of Treitz): 58% of the GI bleeds in patients taking rofecoxib and 52% of the GI bleeds in patients taking naproxen[13].

There are several reasons for the lack of recognition of the prevalence and seriousness of NSAID-enteropa-

thy. First, it is more difficult to detect small bowel damage than that induced by NSAIDs in the stomach and proximal duodenum: "The single most important reason for underestimating the clinical importance of NSAID enteropathy is the difficulty in making a diagnosis"[2]. Second, there is a poor correlation between NSAID-induced small intestinal damage and clinical symptoms.The vast majority of NSAID-enteropathy is sub-clinical[6], and when there are symptoms, they are largely non-specific (including iron deficiency anemia, occult blood, diarrhea, hypoalbuminemia, and malabsorption of vitamin B_{12} and/or bile acids). Thirdly, some researchers have argued that the focus of large pharmaceutical companies on the development of "gastroprotective" drugs, such as H_2RA, PPI, and putative gastric-sparing drugs (selective COX-2 inhibitors, NSAID pro-drugs) has led to a preoccupation of physicians and researchers with the stomach and proximal duodenum, at the expense of consideration of the detrimental effects of NSAIDs in the small (and large) intestine. The fact that there are no proven-effective treatments for NSAID-enteropathy likely also contributes to the lack of recognition of this serious condition[7].

DETECTION OF NSAID-ENTEROPATHY

Until recently, detection of NSAID-enteropathy has been very difficult, with most of the evidence for its occurrence coming from post-mortem studies or through indirect measures of intestinal bleeding[4,18,19]. Several indirect methods for detecting and measuring the severity of NSAID-enteropathy were developed, prior to improved endoscopic techniques for viewing the small intestine becoming widely available. These included measuring small intestinal permeability with sugars[20,21] or small molecular weight radioactive probes[22], measuring bleeding (presumed of intestinal origin) with radiolabelled red blood cells[23], and measuring leukocyte markers in the small intestine (radiographically)[24] or in feces[25]. All of these methods provide useful information, but none have become recognized as a "gold standard" for detecting and quantifying enteropathy, because of lack of specificity and/or sensitivity. However, with video capsule endoscopy (VCE) and double-balloon enteroscopy, it is now possible to directly visualize of NSAID-induced damage and bleeding throughout the small intestine. Using these methods, it has become clear that NSAID-enteropathy occurs frequently, even in low-risk subjects (healthy, young volunteers) with low-risk treatment protocols (short-term ingestion of NSAIDs, sometimes together with a "gastro-protective" agent). For example, using VCE, Graham *et al*[5] found a high prevalence of ulcers in long-term NSAID users. More than 70% of these patients (taking NSAID for more than 3 mo) had intestinal inflammation accompanied by bleeding and protein loss. Symptoms persisted after stopping the therapy (by as long as 16 mo in some patients). Maiden *et al*[25] reported gross damage in 68% of healthy volunteers taking diclofenac plus omeprazole for 2 wk. Even low-dose aspirin was found to cause significant small intestinal damage with short-term administration; thus, Endo *et al*[26] reported that 80% of patients taking low-dose aspirin for 2 wk had intestinal damage.

POLYPHARMACY CONUNDRUM: SHIFTING GI INJURY MORE DISTALLY

Animal studies of NSAID injury to the GI tract usually involve the use of healthy animals. Of course, the people most commonly taking NSAIDs on a chronic basis are those with chronic illnesses, and more often than not, they are affected by more than one disease. It is also the case that disorders such as rheumatoid arthritis, obesity and diabetes can increase the susceptibility of the patient to the GI (and other) adverse effects of NSAIDs[27-29]. Moreover, these patients are often taking a number of different drugs, which can also affect susceptibility to NSAID-induce GI injury and bleeding. Polypharmacy is now commonplace, even in patients that do not have disorders other than the one for which NSAID therapy is indicated. Consider a disorder like osteoarthritis, which is more common in the elderly. Cardiovascular diseases are common in this group of patients, often leading to co-prescription of low-dose aspirin and sometimes of other anticoagulants. Low-dose aspirin is also frequently co-prescribed with selective COX-2 inhibitors and conventional NSAIDs because of concerns about the elevated risk of serious cardiovascular events in patients taking those drugs[30]. Of course, co-administration of low-dose aspirin together with a selective COX-2 inhibitor essentially eliminates any advantage, in terms of upper GI safety, of the selective COX-2 inhibitor as compared to a conventional NSAID[15,31-33]. To reduce the expected upper GI toxicity of the combination of an NSAID and low-dose aspirin, PPIs are typically prescribed as well. Indeed, there are now fixed-dose, enteric-coated, combination tablets available that contain an NSAID and a PPI[34]. While there is strong evidence for PPIs reducing the severity of damage and bleeding in the stomach and duodenum, where the role of acid in the production of damage has been clearly demonstrated[1,35], there is no evidence to suggest that a PPI (or other anti-secretory drug) would reduce the severity of NSAID-induced enteropathy. Indeed, antisecretory drugs have been described as "useless either in preventing or treating mucosal lesions" induced by NSAIDs in the intestine[36]. It is worth repeating that the majority of damage and bleeding caused by NSAIDs occurs in the small intestine, distal to the ligament of Treitz[3,13].

Using a rat model, we attempted to replicate common clinical scenarios of polypharmacy to determine the effects on the small intestine[37]. Groups of rats were treated with combinations of anti-inflammatory doses of NSAIDs (naproxen, celecoxib or a novel hydrogen sulfide-releasing NSAID, ATB-346)[38], a PPI (omeprazole or lanzoprazole) and an anti-thrombotic dose of aspirin. In rats that received only the NSAID, the levels of small

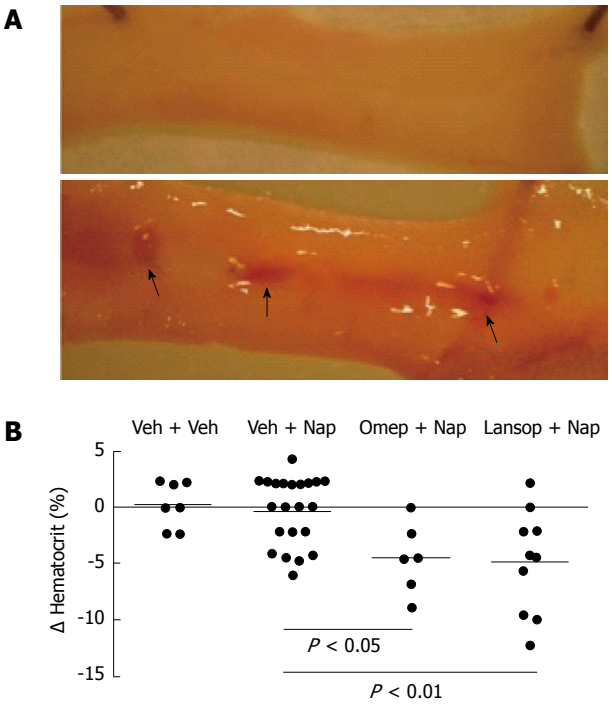


Figure 1 Proton pump inhibitors exacerbate naproxen-induced ulceration and bleeding. In panel A, the top image is of the jejunum of a rat treated with naproxen for 4.5 d (10 mg/kg twice-daily). There are no ulcers present. The bottom image is of a jejunum of a rat receiving the same naproxen treatment, but also treated with omeprazole at a dose that suppressed gastric acid secretion[37]. The arrows indicate the numerous hemorrhagic ulcers that form with this combination of treatments; Panel B shows the change in hematocrit of rats treated with naproxen (Nap) plus vehicle (Veh), omeprazole (Omep) or lansoprazole (Lansop)[37]. The two proton pump inhibitors significantly enhanced the decrease in hematocrit when co-administered with naproxen (no decrease in hematocrit was observed in rats treated with a proton pump inhibitors alone).

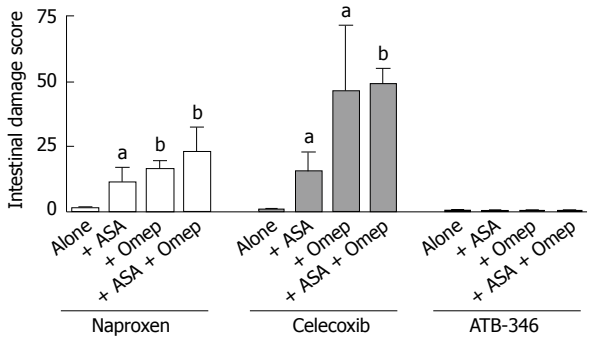


Figure 2 Proton pump inhibitors and low-dose aspirin significantly exacerbate nonsteroidal anti-inflammatory drug-induced small intestinal ulceration. Rats were treated orally, twice-daily for 4.5 d with equi-effective anti-inflammatory doses of naproxen (10 mg/kg), celecoxib (10 mg/kg) or ATB-346 (14.5 mg/kg). ATB-346 is a hydrogen sulfide-releasing derivative of naproxen[38]. Starting 5 d before the nonsteroidal anti-inflammatory drugs (NSAIDs), the rats began receiving twice-daily treatments with omeprazole (Omep) (10 mg/kg) or vehicle. Starting 3 d before the NSAIDs, the rats began receiving daily doses of low-dose aspirin (10 mg/kg) or vehicle. The results are shown as the mean ± SE of at least 6 rats per group. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the corresponding group treated with the NSAID alone. No intestinal damage was observed in rats treated with aspirin (ASA) alone. The exacerbation of small intestinal ulceration with omeprazole was also observed with another proton pump inhibitor, lanzoprazole[37]. This figure was constructed using data from Blackler *et al*[175].

intestinal damage and bleeding were very low (Figures 1 and 2). However, when co-administered with a PPI or with low-dose aspirin, the levels of small intestinal damage and bleeding in rats treated with naproxen or celecoxib increased significantly (Figures 1 and 2). This effect has been confirmed in a recent study by Satoh *et al*[39]. The combination of an NSAID with both a PPI and low-dose aspirin resulted in extensive damage and bleeding (the latter was evident post-mortem and also by marked decreases in hematocrit). ATB-346 did not produce small intestinal damage alone or in combination with a PPI and/or low-dose aspirin (Figure 2).

We then performed experiments to try to determine the mechanisms underlying the exacerbation of small intestinal damage by the PPIs. As discussed in more detail below, there is evidence that the bacteria residing in the small intestine play a significant role in the pathogenesis of NSAID-enteropathy. Given the evidence that marked suppression of gastric acid secretion by PPIs can alter the numbers of bacteria in the small intestine[40-42], we focused our investigation on potential changes in intestinal microbiota. Treatment of rats with omeprazole resulted in a dramatic shift in the types of bacteria in the small intestine (dysbiosis). In particular, there was a marked reduction of the Actinobacteria, particularly of *Bifidobacteria spp.* (> 80% reduction in the jejunum). This diminution of *Bifidobacteria* was an important factor in the PPI-induced increase in NSAID-induced intestinal damage: replenishment of intestinal *Bifidobacteria* in PPI-treated rats reduced levels of naproxen-induced intestinal damage those seen in rats not receiving a PPI. Further evidence that it was the dysbiosis induced by the PPI that resulted in elevated susceptibility to NSAID-enteropathy came from studies of germ-free mice[37]. Groups of germ-free mice were colonized with intestinal contents from rats that had been treated with a PPI or vehicle. Beginning one week later, the mice were treated with naproxen for 4 d, and the severity of intestinal damage was then blindly evaluated. Mice that had been colonized with bacteria from PPI-treated rats developed significantly worse intestinal damage than those colonized with bacteria from vehicle-treated rats.

While no clinical studies have been published that directly tested the hypothesis that treatment with PPIs could cause dysbiosis and thereby exacerbate NSAID-induced intestinal damage, there are several reports with data that are consistent with our hypothesis, as summarized by Daniell[43]. In addition to numerous studies documenting that PPIs altering the gut microbiota, resulting in diarrhea[40-42,44], there is evidence from two studies for the presence of intestinal inflammation (detected by elevated fecal calprotectin levels) in patients taking PPIs[45,46], and evidence for microscopic colitis in patients taking NSAIDs or PPIs[47-49], and particularly in patients taking both types of drugs concurrently[49]. In addition, two studies reported greater small intestinal damage in healthy volunteers taking an NSAID plus a PPI as compared to a group taking only a selective COX-2 inhibitor[50,51], and it

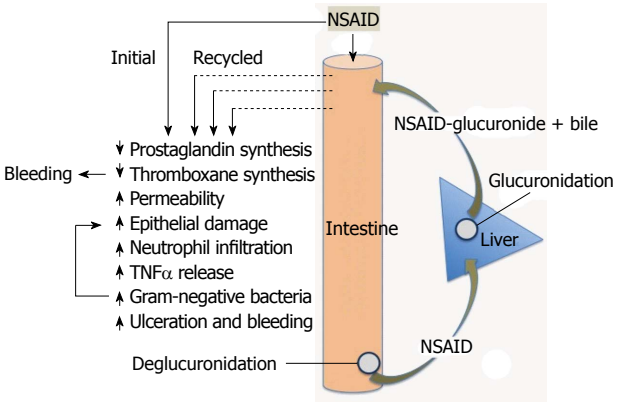


Figure 3 Pathogenesis of nonsteroidal anti-inflammatory drugs-Induced enteropathy. Nonsteroidal anti-inflammatory drugs (NSAIDs) produce effects during their initial exposure to the small intestine, and when secreted back into the proximal small intestine, along with bile, following their absorption in the distal intestine, and glucuronidation in the liver. Suppression of thromboxane synthesis likely plays an important role in promoting bleeding (especially with aspirin, an irreversible inhibitor of platelet thromboxane synthesis). Repeated exposure of the intestinal epithelium to the combination of NSAIDs and bile will promote damage, and the damage is likely exacerbated by the shift in intestinal bacteria stimulated by the NSAID (elevated gram-negative bacteria). These effects appear to be mediated by endotoxin, acting at least in part through toll-like receptor-4. The interplay among bile, bacteria and recirculation of the NSAID is complex. For example, bacterial enzymes convert primary bile acids to secondary bile acids (which may be more damaging) and bacterial enzymes are necessary for deglucuronidation, which permits reabsorption and enterohepatic recirculation of NSAIDs. TNFα: Tumor necrosis factor-alpha.

is now clear that the ability of selective COX-2 inhibitors to damage the small intestine is comparable to that of non-selective NSAIDs[17].

PATHOGENESIS

The key to development of treatments and prevention strategies for NSAID-enteropathy lies in better understanding of the pathogenesis of this injury. Fortunately, the animal models of NSAID enteropathy are very good, reproducible and simple, and can serve as useful tools for gaining a better understanding of the pathogenesis of this disorder and for testing potential therapeutic/preventative agents. Administration of NSAIDs to rats, for example, results in ulceration predominantly in the distal jejunum and ileum[52], the same regions where ulcers are concentrated humans[53,54]. While there will undoubtedly be some differences between rodent models and humans, the existing data suggest that the animal models will be predictive in terms of treatment and prevention strategies. Figure 3 shows some of the key mechanisms suggested to be involved in NSAID-enteropathy, which are discussed in more detail below.

Inhibition of cyclooxygenase activity

Flower *et al*[55] first suggested the existence of more than one isoform of COX in 1972. It was almost 20 years later that the two isoforms, now known as COX-1 and COX-2, were sequenced[56,57]. In the decade that followed, a tremendous amount of research was focused on understanding the physiology and pharmacology of these enzymes, largely fueled by the interest of several large pharmaceutical companies in the notion that selective inhibitors of COX-2 would provide all of the anti-inflammatory activities of NSAIDs without the major adverse effects. However, as the science of COX-2 caught up with the marketing of COX-2, it became evident that the delineation of functions of the two COX isoforms was not so clear-cut as had been proposed and heavily promoted. COX-1 contributes significantly to inflammation while COX-2 contributes significantly to many physiological functions, including mucosal defence[58]. This was shown clearly both by studies of mice lacking the gene for one of the COX isoforms and pharmacological studies[59-63]. A striking finding from our laboratory was that injection of carrageenan into the hind-paw of COX-2-deficient mice resulted in inflammation that did not resolve, as it would in a normal mouse[60], suggesting an important role for COX-2-derived prostanoids in resolution of inflammation and healing. Gilroy *et al*[61] provided compelling evidence from animal models of pleurisy showing the same, and identifying specific COX-2-derived prostanoids that contributed significantly to down-regulating inflammation. Serhan *et al*[64] described a family of previously unrecognized lipid mediators (lipoxins, resolvins, protectins), some of which were derived from COX-2, that act at several levels of the inflammatory cascade to "turn off" inflammation and allow for a coordinated restoration of tissue homeostasis[65].

The same was true in the GI tract, as COX-2-derived prostanoids were found to contribute significantly to maintenance of the integrity of the tissue, to repair of mucosal injury and to resolution of inflammation[58]. Thus, COX-2 is the isoform that produces PGs at the margins of gastric ulcers, which contribute significantly to the healing of those ulcers[66,67]. In the colon, prostaglandins derived from COX-2 play a very important role in down-regulating inflammation and promoting repair of mucosal injury[52,68,69]. Suppression of COX-2 activity has been shown to exacerbate experimental colitis[52,69]. Indeed, COX-2 is up-regulated throughout the GI tract when the tissue is injured or when there is insufficient PG production *via* COX-1[52,63,70]. For example, COX-2 is rapidly induced in the stomach in response to suppression of COX-1 by aspirin[70], and it helps to enhance mucosal defence in such circumstances. One of the mechanisms through which this is achieved is *via* the production, *via* COX-2, of a potent gastroprotective and anti-inflammatory substance, 15-epi-lipoxin A_4[71,72]. Induction of damage in the stomach, in the absence of any other toxic challenge, requires suppression of both COX-1 and COX-2[62], and this also appears to be the case in the small intestine[63].

Clinical studies generally show that selective COX-2 inhibitors produce less gastroduodenal injury and bleeding than conventional NSAIDs, but the small intestinal damage may not differ substantially between the two sub-classes of NSAIDs. For example, Maiden *et al*[73] performed a VCE study comparing the enteropathy

produced in patients on long-term NSAID or selective COX-2 inhibitor therapy, and the key finding was that NSAIDs and selective COX-2 inhibitors produced comparable levels of small bowel damage (small intestinal injury was observed in 50% of the patients treated with a selective COX-2 inhibitor *vs* 62% of patents treated with a conventional NSAID; not significantly different).

While suppression of COX activity undoubtedly contributes to the pathogenesis of NSAID-enteropathy, it is clear that other factors probably play a more significant role. Suppression of COX activity likely contributes to this disorder mainly through the impairment of repair processes, such as angiogenesis[74], and through inhibition of platelet aggregation, leading to bleeding. The latter effect, however, is most apparent with aspirin, which irreversibly inhibits platelet COX-1, and with NSAIDs that have a long half-life.

Mitochondrial injury

One of the earliest changes that can be detected after NSAID administration, in addition to inhibition of COX activity, is mitochondrial injury[75]. Morphological evidence of mitochondrial damage can be detected within 1 h of administration of an NSAID to rats, and *in vitro* studies of liver showed that the NSAID could rapidly cause uncoupling of oxidative phosphorylation[75]. This provides a mechanistic explanation for the ability of NSAIDs to damage intestinal epithelial cells and to increase epithelial permeability, as have been demonstrated by several groups[22,52,76]. On the other hand, this mechanism does not explain the localization of ulcers in the jejunum and ileum in animal models and in humans. In their endoscopic study of diclofenac-induced small intestinal injury, Fujimori *et al*[54] observed denuded regions throughout the small intestine (perhaps indicative of a topical erosive effect), but ulcers were concentrated in the distal jejunum and ileum.

Role of bile and enterohepatic circulation

Several observations suggest important roles for bile and for enterohepatic circulation of NSAIDs in the pathogenesis of NSAID-enteropathy (Figure 3). Ligation of the bile duct in rats prevents NSAID-induced intestinal damage[75-78]. There have also been reports that NSAIDs that do not re-circulate enterohepatically do not cause small intestinal damage[52,75], although aspirin is a notable exception, at least when administered intraduodenally or in an enteric-coated formulation[11,78]. Also, in rats lacking the hepatocanalicular conjugate export pump, which is required for excretion of conjugated NSAIDs into bile, but not for the flow of bile itself, intestinal damage induced by an NSAID (diclofenac) was prevented[79]. On the other hand, induction of higher expression of the export pump aggravated NSAID-induced intestinal damage[79]. A number of studies have demonstrated that a combination of an NSAID and bile is damaging to intestinal epithelial cells[80,81] and non-GI cells[82]. It is noteworthy that in all of these studies, it was secondary bile acids that were found, in combination with NSAIDs, to be effective in damaging cells. Moreover, it has been shown that administration of an NSAID to rats results in increased concentrations of secondary bile acids in bile[83]. Thus, when an NSAID recirculates enterohepatically, the intestinal epithelium is repeatedly exposed to a damaging combination of the NSAID and bile. If this were the primary mechanism of injury in NSAID-enteropathy, however, one would expect to see ulcers produced where the highest concentrations of NSAID and bile would be found (*i.e.*, near the Sphincter of Oddi), whereas the most severe tissue injury is concentrated in the more distal parts of the small intestine[54]. It has been suggested that the sites of ulceration correspond to the sites of NSAID re-absorption, and related to the deconjugation of the NSAIDs at those sites by bacterial β-glucuronidases[79,84-86].

Role of bacteria

There is an abundance of evidence that intestinal bacteria contribute to the pathogenesis of NSAID-enteropathy, but it remains unclear if there is a primary role, initiating the tissue damage, or just a secondary role, exacerbating tissue injury and impeding repair. One of the key observations leading some to propose a primary role of bacteria in NSAID-enteropathy is that germ-free rats and mice develop little or no intestinal damage when given an NSAID, but when colonized by gram-negative bacteria, these animals become susceptible to NSAID-enteropathy[87,88]. Several studies have documented dramatic shifts in the types of bacteria in the small intestine following NSAID administration, with increases in gram-negative bacteria generally being observed, and a concomitant reduction in gram-positive bacteria[89-93]. In some studies, there appeared to be an enrichment of specific bacteria, such as *Enterococcus faecalis*, *Clostridium*, *Bacteroides* and *Escherichia coli* (*E. coli*)[89-91]. A number of studies reported protective effects of antibiotics against NSAID-enteropathy, particularly when the antibiotics were effective in reducing number of gram-negative bacteria[88,89,93,94]. Similarly, some probiotics have been reported to reduce the severity of NSAID-enteropathy, especially when they prevent increases in the number of gram-negative bacteria in the intestine[93,95,96]. Despite a considerable number of studies examining the potential contribution of bacteria to NSAID-enteropathy, there remains a lack of clear evidence for a primary role of bacteria in initiation of tissue injury. Bacteria rapidly colonize sites of ulceration and can interfere with ulcer healing[97,98]. In one of the earliest papers on the pathogenesis of NSAID-enteropathy, Kent *et al*[89] remarked "since the antibiotics do not prevent completely the ulceration, we think that these agents reduce the severity of the lesion by allowing healing to start sooner". A similar conclusion was drawn by Yamada *et al*[99].

The apparent importance of gram-negative bacteria in the pathogenesis of NSAID-enteropathy is consistent with reports of a role for lipopolysaccharide (LPS) in driving tissue inflammation and impairment of ulcer healing. Hagiwara *et al*[91] showed that heat-killed *E. coli*

and their purified LPS caused "deterioration" of NSAID-induced ileal ulcers, but could not cause ulcers themselves in the absence of the NSAID). Koga *et al*[94] reported that systemic administration of LPS reversed the beneficial effects of an antibiotic in reducing the severity of NSAID-enteropathy in rats, and further demonstrated that T cell function was not required for NSAIDs to induce intestinal ulceration. Watanabe *et al*[93] demonstrated that mice lacking the endotoxin receptor, toll-like receptor-4, developed much less (about 80%) intestinal damage when given an NSAID than the normal counterparts. These data are once again consistent with the notion that bacteria play a secondary role in NSAID-enteropathy, exacerbating tissue injury and interfering with ulcer healing. These effects may be in part attributable to activation of neutrophils in the mucosal microcirculation, which has been shown to contribute significantly to ulceration[100-104], and local generation of tumor necrosis factor-alpha may be one of the main triggers leading to neutrophil recruitment and/or activation[93,105-107].

As mentioned above, one of the key observations supporting an important role of bacteria in the pathogenesis of NSAID-enteropathy was that germ-free animals do not develop significant small intestinal damage following NSAID administration[75-78]. However, one must bear in mind that ligation of the bile duct blocks the secretion of bile and the enterohepatic circulation of NSAIDs, both of which have been implicated in intestinal injury by these drugs (Figure 3). The conversion of primary bile acids to secondary bile acids is dependent on intestinal bacterial enzymes. Thus, germ-free animals lack secondary bile acids. As mentioned above, most studies that have shown that bile acids (alone or in combination with an NSAID) can cause damage to intestinal epithelial cells have used secondary, rather than primary bile acids[80,81]. Moreover, the re-absorption of NSAIDs in the distal small intestine is largely dependent on bacterial β-glucuronidase activity, which de-conjugates NSAID-glucuronides, allowing the NSAID to be transported across the epithelium[84]. Enterohepatic circulation of NSAIDs is negligible in animals that lack intestinal bacteria, resulting in decreased exposure of the intestine to the NSAID, and therefore decreased tissue injury. Recently, LoGuidice *et al*[85] demonstrated that an inhibitor of bacterial β-glucuronidase could significantly reduce the severity of diclofenac-induced small intestinal injury in mice. β-glucuronidase has been shown to be expressed in *Clostridium*, *Peptostreptococcus*, *Staphylococcus* and *E. coli*[85,108].

TREATING AND PREVENTING NSAID-ENTEROPATHY

In sharp contrast to NSAID-induced gastroduodenal damage, where several options are available to provide protection to a patient, no treatments or prevention strategies for NSAID-enteropathy have been convincingly shown to be effective.As outlined above, PPIs provide upper GI protection against NSAIDs but worsen NSAID-enteropathy in animals, and there is emerging evidence that the same is the case in humans. There are novel NSAIDs in development that do not cause enteropathy in animals (discussed below).

Misoprostol, metronidazole and sulfasalazine have all been suggested to be beneficial in treatment or prevention of NSAID-enteropathy in humans, but the studies suggesting this had significant limitations (open-label, not controlled, and/or small sample sizes)[22,24,109-111]. Misoprostol, H_2RA and sucralfate were found to be ineffective in reducing NSAID-induced intestinal permeability in humans[112,113], though in one, open-label study misoprostol reduced the elevated intestinal permeability induced by indomethacin[114]. Based on the animal data showing beneficial effects of metronidazole in reducing NSAID-enteropathy[99], Bjarnason *et al*[24] performed an open-label human study of chronic NSAID users. The patients took metronidazole for 2-12 wk while continuing their NSAID treatment. The endpoints were fecal excretion of ^{51}Cr-labeled erythrocytes and ^{111}In-labelled neutrophils. Both markers declined significantly during metronidazole treatment, leading the authors to conclude that "these results suggest that the neutrophil is the main damaging effector cell in NSAID induced enteropathy" and that the main chemoattractant "may be a metronidazole sensitive microbe".

The observations from animal studies that NSAID-enteropathy was accompanied by dramatic shifts in numbers and types of intestinal bacteria led to a number of studies of the potential value of probiotics for treatment or prevention of NSAID-enteropathy. In studies in rats, Kinouchi *et al*[95] demonstrated that *Lactobacillus acidophilus* and *Bifidobacteria adolescentis* administration markedly reduced the severity of NSAID-induced ileal ulceration. Syer *et al*[96] also showed a marked protective effect of *Bifidobacteria adolescentis* in a rat model of NSAID-enteropathy. Only two clinical trials of a probiotic for NSAID-enteropathy have been reported to date. Montalto *et al*[115] performed a randomized, double-bind, placebo-controlled trial of VSL#3, a probiotic formulation consisting of 8 different live bacteria. Volunteers received indomethacin daily for 4 d, and fecal calprotectin levels were the endpoint. The placebo-treated volunteers exhibited markedly elevated fecal calprotectin levels during the period of indomethacin treatment, while during treatment with VSL#3 the fecal calprotectin levels remained within the normal range. In a study by Endo *et al*[116], 25 patients with unexplained iron deficiency anemia who had been taking low-dose enteric-coated aspirin plus omeprazole for more than 3 mo were given either *Lactobacillus casei* (*L. casei*) or placebo for 3 mo while continuing the aspirin and omeprazole therapy. VCE at the end of the treatment period showed a significant reduction of mucosal breaks and "capsule endoscopy score" in the group receiving *L. casei*. The results of this small clinical study are consistent with a study of *L. casei* (strain Shirota) in a rat model of indomethacin-induced enteropathy[117].

Lactoferrin has been shown to prevent NSAID-

induced bleeding in rodents[118] and this effect may be related to its ability to promote the growth of *Bifidobacteria* in the small intestine[119]. Oral treatment of healthy volunteers with recombinant lactoferrin was shown to reduce indomethacin-induced changes in small intestinal permeability[120]. However, in this short-term study, only a very modest increase in intestinal permeability was seen, with only a single administration of lactoferrin that would have been unlikely to have significantly affected the intestinal microbiome.

Rebamipide is a quinolinone derivative that is used to promote the healing of GI ulcers and for mucosal protection. Its mechanism of action is not fully understood, though it appears to stimulate mucus secretion and PG synthesis[121] and to scavenge oxygen-derived free radicals[6]. It has been shown to significantly reduce the severity of NSAID-induced enteropathy in rats[122]. Niwa *et al*[123] performed a pilot study in healthy humans to examine the effectiveness of rebamipride in preventing NSAID-enteropathy. The volunteers received placebo or rebamipride together with diclofenac for 7 d. The small intestine was examined at the end of the study by VCE. Damage was observed in 8 of the 10 of placebo-treated group (2 ulcers, 1 bleed), but in only 2 of the 10 of rebamipide-treated group (no ulcers or bleeding). However, a larger study of healthy volunteers treated for 14 d with an NSAID (diclofenac), a PPI (omeprazole) and either rebamipide or placebo, failed to a detect a significant benefit of rebamipide in terms of reducing the incidence of intestinal mucosal injury[124]. Larger studies of rebamipide, ideally in patients receiving NSAID therapy for an inflammatory disorder, are needed to clarify if this drug will have benefit in reducing the incidence and/or severity of NSAID-enteropathy.

Studies in animal models have suggested other possible approaches to prevention of NSAID-enteropathy, but have not yet been assessed in humans. For example, in a mouse model of acute indomethacin-induced intestinal damage, Yasuda *et al*[125] found that dopamine D2 receptor antagonists reduced the severity of damage, and these effects were mediated through the activation of endogenous anti-inflammatory pathways mediated by *via* α7-nicotinic acetylcholine receptors, as had been observed previously[126]. Using the same model, Kato *et al*[127] demonstrated that certain 5-HT receptors could modulate susceptibility to NSAID-enteropathy. They reported that antagonists of the 5-HT3 receptor (ondansetron and ramosetron) dose-dependently reduced intestinal damage, while a 5-HT4 antagonist (GR113808) aggravated damage. A 5-HT4 agonist (mosapride) significantly reduced damage. As in the case of protection with dopamine D2 receptor antagonists, the authors suggested that the beneficial effects the 5HT4 agonist may be mediated through activation of α7-nicotinic acetylcholine receptors. There have also been studies demonstrating a significant increase in intestinal motor activity after administration of NSAIDs, and have suggested that this contributes to the generation of injury, but pharmacological approaches targeting this 5-HT/α7-nicotinic acetylcholine receptor axis have not yet been evaluated in human NSAID-enteropathy.

NSAID pro-drugs: The enteropathy remains

Pro-drugs have been defined as "bioreversible derivatives of drug molecules that undergo an enzymatic and/or chemical transformation *in vivo* to release the active parent drug, which can then exert the desired pharmacological effect"[128,129]. A number of NSAID pro-drugs have been developed, based on the premise that if the drug can pass through the stomach in an inactive form, it will not inhibit PG synthesis in the stomach, and therefore will not be ulcerogenic. In essence, an NSAID pro-drug of this design does not differ significantly from an enteric coated NSAID, and the problems associated with the latter are well documented[8,36]. Moreover, there are several problems with the premise upon which NSAID pro-drugs are based. First, once the drug is absorbed and transformed to release the parent drug, that drug will produce "the desired pharmacological effect". That effect, reduction of pain and inflammation, is attributable to systemic inhibition of COX activity. In the absence of any "protective" intervention, systemic inhibition of COX activity will result in damage and bleeding in the upper GI tract. Thus, NSAIDs administered systemically induce significant gastrointestinal ulceration and bleeding[130-132]. If a pro-drug is formulated such that it produces a marked delay in the release of the parent drug, there will be a similar delay in the onset of the desired activity. Second, the pro-drug approach is focused entirely on sparing the upper GI tract of injury, ignoring the potential of the drug to cause small intestinal injury, particularly if it undergoes enterohepatic recirculation. Once the pro-drug is metabolized to release the parent drug, the parent drug will behave, pharmacokinetically and pharmacodynamically, in the same way as if the parent drug itself had been administered. These are points that have been acknowledged on the website of a company that is developing a naproxen pro-drug: "the pro-drug approach will not address the GI damage associated with the systemic inhibition of COX after release of the parent drug, nor will it address the toxic effects of metabolites delivered into the gut lumen with bile"[133].

Clinical trials of pro-drugs have often produced data that are very favourable to the pro-drug. However, this is largely because such studies have typically focused on acute gastric or gastroduodenal damage (erosions and "endoscopic ulcers")[134] that are of questionable clinical significance, since they do not necessarily predict the incidence of true ulcers[135]. Indeed, the same is true for most of the trials of selective COX-2 inhibitors and of PPIs, which gave a false signal of the GI safety of those classes of drugs because of reliance on inappropriate endpoints for upper GI damage and lack of consideration of the potential damaging effects of these drugs on the small intestine. When examined in "real world" scenarios, using clinically meaningful endpoints[135], there is little, if any, evidence of significant benefit of NSAID pro-drugs over

the parent drugs or over other NSAIDs. This topic has been very well reviewed by Graham[135]. Thus, while the pro-drug sulindac rarely caused erosions or "endoscopic ulcers" in short-term studies of human volunteers[136,137], longer term studies in at-risk patients showed this drug to offer no upper GI safety benefit as compared to other NSAIDs[138]. Likewise, nabumetone was purported to be a GI-safe pro-drug, and acute upper GI studies suggested that this was the case[139], but in at-risk patients the drug did not offer any benefit over other NSAIDs[140]. Neither sulindac nor nabumetone have been specifically examined with respect to their propensity to cause small intestinal ulceration and bleeding. Moreover, there are suggestions in the literature[132,141], supported by animal studies[130,131], that systemic administration of NSAIDs, which completely avoids contact of the drug with the lining of the stomach and duodenum, does not offer significant benefit in terms of reducing the incidence of significant GI ulceration and bleeding.

Novel intestinal-sparing NSAIDs

The advances that have been made in understanding the pathogenesis of NSAID-enteropathy provide important clues for designing novel NSAIDs that will not damage in the small intestine (or the stomach). Several approaches have been taken that show promise, mainly using the "co-drug" model of drug design[142]. Co-drugs are somewhat like pro-drugs, with the key difference being that the pro-moiety is not inert; rather, it exerts important pharmacological effects[129]. Two such classes of co-drugs are the nitric oxide (NO)-releasing NSAIDs and the hydrogen sulfide (H_2S)-releasing NSAIDs[143-145]. In each case, the NSAID portion of the co-drug behaves the same as expected (inhibition of COX-1 and COX-2, leading to anti-inflammatory and analgesic effects), while the gaseous mediator portion of the co-drug exerts mucosal protective effects, very similar to the effects of endogenous prostaglandins[146,147]. Both of these gaseous mediators are vasodilators and can inhibit leukocyte adherence to the vascular endothelium[148,149]. Suppression of mucosal synthesis of NO or H_2S reduces the resistance of the stomach to the damaging effects of NSAIDs and other irritants, and impairs the healing of pre-existing damage[148,150-156]. NO and H_2S donors can increase the resistance of the gastric mucosa to injury induced by NSAIDs and other noxious substances[148,151,156,157] and can accelerate healing of ulcers in rodent models[37,150,153,154,158]. Some of the other actions of NO-NSAIDs and H_2S-NSAIDs and their underlying mechanisms have been reviewed previously[143,145,152].

NO-NSAIDs were shown to cause significantly less intestinal damage than the parent drugs[159,160], and to be well tolerated in rats with pre-existing colitis[159]. In a small, short-term clinical trial, an NO-NSAID produced significantly less of an increase in small intestinal permeability than was produced by an equivalent dose of the parent drug (naproxen)[161]. Despite very promising results from clinical trials that demonstrated efficacy and safety

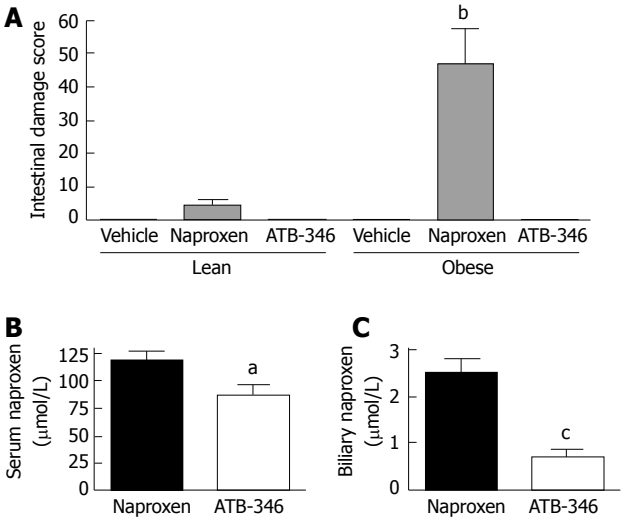


Figure 4 Intestinal safety and altered biliary excretion of ATB-346. A: ATB-346 is a hydrogen sulfide-releasing derivative of naproxen[38].When administered to obese Zucker rats, twice-daily for 4.5 d at 10 mg/kg, naproxen induced small intestinal damage that was significantly more severe in the obese rats ($^bP < 0.01$). However, at an equimolar dose, ATB-346 did not induce intestinal damage in lean or obese rats[175]; B: The serum levels of naproxen in normal rats after 4.5 d of twice-daily administration of ATB-346 were marginally, but significantly ($^aP < 0.05$) lower than those in rats treated with an equimolar dose of naproxen; C: Biliary levels of naproxen in rats treated with ATB-346 (as above) were markedly reduced compared to those in rats treated with an equimolar dose of naproxen ($^cP < 0.001$).The data shown in this graph are from Blackler *et al*[175].

in osteoarthritis[162-166], NO-NSAIDs have not obtained regulatory approval because the safety advantages over the parent drug (naproxen) have not been sufficiently demonstrated. One key GI safety clinical trial fell just short of showing a significant benefit as compared to naproxen ($P = 0.066$)[167].

H_2S-releasing NSAIDs exhibit enhanced anti-inflammatory activity relative to the parent drugs[37,151,155,168,169], presumably attributable to the anti-inflammatory and pro-resolution effects of the H_2S released from these drugs[149,158,170-174]. In addition to sparing the gastric mucosa of damage in several circumstances of impaired mucosal defence[38,175], H_2S-releasing NSAIDs have been shown not to cause damage in the small intestine of rats[38,155,175]. Moreover, when tested in co-morbidity and polypharmacy models, with repeated administration over several days, an H_2S-releasing derivative of naproxen (ATB-346) did not cause small intestinal damage[175] (Figures 2 and 4). For example, obese rats that exhibited markedly greater naproxen-induced enteropathy than was observed in lean rats, but ATB-346 did not elicit damage in lean or obese rats[175]. Interestingly, Zucker obese rats have a microbiota distinct from that of their lean littermates, with a marked reduction in intestinal levels of *Bifidobacteria*[176]. Recall that we observed that PPIs increased the severity of NSAID-enteropathy in rats, and found that this was largely attributable to a decrease in intestinal *Bifidobacteria* levels[37]. ATB-346 retained its favourable profile in the intestine even when co-administered with a PPI and or low-dose aspirin[175] (Figure 2).

A particularly important feature of ATB-346 that may be very important in terms of its lack of damaging effects in the small intestine is that, though metabolized to release naproxen, there are relatively low levels of naproxen in bile after administration of this compound[175] (Figure 4). Moreover, the biliary levels of naproxen-glucuronide were reduced by 72% in the ATB-346 group as compared to the naproxen group[175]. These altered pharmacokinetics of ATB-346 *vs* naproxen did not alter the anti-inflammatory activity of the drug[37], but could contribute significantly to the intestinal-sparing properties of ATB-346.

Recently, a class of drugs was described that consists of an NSAID attached to moieties releasing both NO and H_2S[177]. These compounds show comparable actions as the parent drugs in terms of inhibiting COX activity, but there ares no available data on their GI toxicity.

NSAIDs pre-associated with phospholipids are a unique type of "co-drug".

Surface-active phospholipids have been proposed to constitute an important component of the epithelial "barrier" to acid back-diffusion, and NSAIDs can to disrupt this barrier[178,179]. Lichtenberger *et al*[180] demonstrated that pre-associating an NSAID with a zwitterionic phospholipid prevents the NSAID from disrupting the barrier function of the epithelium. Thus, covalently linking phosphatidylcholine to aspirin, ibuprofen and other NSAIDs results in compounds with equivalent anti-inflammatory properties to the parent drug, but with markedly reduced gastric toxicity[181]. This has been demonstrated in endoscopic clinical trials for an aspirin derivative[181], and also with an ibuprofen derivative[182], though in the latter trial, statistical significance was only seen in an older subset of the patients studied. Recently, Lichtenberger *et al*[78] demonstrated that pre-associating aspirin with phosphatidylcholine greatly reduced the small intestinal damage produced by intraduodenal administration of this compound, as compared to aspirin alone.

CONCLUSION

NSAID-enteropathy has largely been ignored for decades as a result of the focus on NSAID-gastropathy, driven largely by the development of several commercially successful drugs targeting that disorder (H_2RA, PPIs, selective COX-2 inhibitors). Moreover, the difficulty in detecting NSAID-enteropathy and the lack of any proven-effective preventative or treatment options has contributed to an under-appreciation of the magnitude of this significant adverse reaction to a very widely used class of drugs. With the development of video capsule endoscopy, the frequency and severity of NSAID-enteropathy has become more evident. Techniques such as VCE also permit more conclusive studies of the safety of novel NSAIDs and of potential prevention or treatment strategies.

The animal models for NSAID-enteropathy are very good, and they have provided a great deal of information on the pathogenesis of this disorder. Moreover, the animal studies have given some direction as to viable strategies for preventing NSAID-enteropathy, and the models are useful for testing novel therapeutics agents.

As is the case with NSAID-induced injury in the upper GI tract, it is important that studies of NSAID-enteropathy focus on animal models that are most similar to the patients that use these drugs and most often develop serious adverse effects. Thus, future studies should focus on the use of animal models with relevant co-morbidities that display increased susceptibility to NSAID-enteropathy, and on patients most at risk of developing intestinal damage and bleeding.

ACKNOWLEDGMENTS

Dr. Wallace is a founder and shareholder of Antibe Therapeutics Inc., a company developing novel NSAIDs.

REFERENCES

1 **Wallace JL**. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiol Rev* 2008; **88**: 1547-1565 [PMID: 18923189 DOI: 10.1152/physrev.00004.2008]
2 **Adebayo D**, Bjarnason I. Is non-steroidal anti-inflammaory drug (NSAID) enteropathy clinically more important than NSAID gastropathy? *Postgrad Med J* 2006; **82**: 186-191 [PMID: 16517800 DOI: 10.1136/pgmj.2005.039586]
3 **McCarthy DM**. GI bleeding: problems that persist. *Gastrointest Endosc* 2009; **70**: 225-228 [PMID: 19631801 DOI: 10.1016/j.gie.2008.12.247]
4 **Bjarnason I**, Hayllar J, MacPherson AJ, Russell AS. Side effects of nonsteroidal anti-inflammatory drugs on the small and large intestine in humans. *Gastroenterology* 1993; **104**: 1832-1847 [PMID: 8500743]
5 **Graham DY**, Opekun AR, Willingham FF, Qureshi WA. Visible small-intestinal mucosal injury in chronic NSAID users. *Clin Gastroenterol Hepatol* 2005; **3**: 55-59 [PMID: 15645405 DOI: 10.1016/S1542-3565(04)00603-2]
6 **Park SC**, Chun HJ, Kang CD, Sul D. Prevention and management of non-steroidal anti-inflammatory drugs-induced small intestinal injury. *World J Gastroenterol* 2011; **17**: 4647-4653 [PMID: 22180706 DOI: 10.3748/wjg.v17.i42.4647]
7 **Wallace JL**. NSAID gastropathy and enteropathy: distinct pathogenesis likely necessitates distinct prevention strategies. *Br J Pharmacol* 2012; **165**: 67-74 [PMID: 21627632 DOI: 10.1111/j.1476-5381.2011.01509.x]
8 **Davies NM**. Sustained release and enteric coated NSAIDs: are they really GI safe? *J Pharm Pharm Sci* 1999; **2**: 5-14 [PMID: 10951657]
9 **Lanas A**, Sekar MC, Hirschowitz BI. Objective evidence of aspirin use in both ulcer and nonulcer upper and lower gastrointestinal bleeding. *Gastroenterology* 1992; **103**: 862-869 [PMID: 1499936]
10 **Stack WA**, Atherton JC, Hawkey GM, Logan RF, Hawkey CJ. Interactions between Helicobacter pylori and other risk factors for peptic ulcer bleeding. *Aliment Pharmacol Ther* 2002; **16**: 497-506 [PMID: 11876703 DOI: 10.1046/j.1365-2036.2002.01197.x]
11 **Endo H**, Sakai E, Higurashi T, Yamada E, Ohkubo H, Iida H, Koide T, Yoneda M, Abe Y, Inamori M, Hosono K, Takahashi H, Kubota K, Nakajima A. Differences in the severity of small bowel mucosal injury based on the type of aspirin as evaluated by capsule endoscopy. *Dig Liver Dis* 2012; **44**: 833-838 [PMID: 22749649 DOI: 10.1016/j.dld.2012.05.016]
12 **Casado Arroyo R**, Polo-Tomas M, Roncalés MP, Schei-

man J, Lanas A. Lower GI bleeding is more common than upper among patients on dual antiplatelet therapy: long-term follow-up of a cohort of patients commonly using PPI co-therapy. *Heart* 2012; **98**: 718-723 [PMID: 22523056 DOI: 10.1136/heartjnl-2012-301632]

13 **Zhu LL**, Xu LC, Chen Y, Zhou Q, Zeng S. Poor awareness of preventing aspirin-induced gastrointestinal injury with combined protective medications. *World J Gastroenterol* 2012; **18**: 3167-3172 [PMID: 22791953 DOI: 10.3748/wjg.v18.i24.3167]

14 **Bombardier C**, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 2000; **343**: 1520-1528 [PMID: 11087881 DOI: 10.1056/NEJM200011233432103]

15 **Silverstein FE**, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 2000; **284**: 1247-1255 [PMID: 10979111]

16 **Wallace JL**. Selective COX-2 inhibitors: is the water becoming muddy? *Trends Pharmacol Sci* 1999; **20**: 4-6 [PMID: 10101953 DOI: 10.1016/S0165-6147(98)01283-8]

17 **Graham DY**, Jewell NP, Chan FK. Rofecoxib and clinically significant upper and lower gastrointestinal events revisited based on documents from recent litigation. *Am J Med Sci* 2011; **342**: 356-364 [PMID: 21986300 DOI: 10.1097/MAJ.0b013e3182113658]

18 **Bjarnason I**, Price AB, Zanelli G, Smethurst P, Burke M, Gumpel JM, Levi AJ. Clinicopathological features of nonsteroidal antiinflammatory drug-induced small intestinal strictures. *Gastroenterology* 1988; **94**: 1070-1074 [PMID: 3345876]

19 **Chan FK**, Lanas A, Scheiman J, Berger MF, Nguyen H, Goldstein JL. Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR): a randomised trial. *Lancet* 2010; **376**: 173-179 [PMID: 20638563 DOI: 10.1016/S0140-6736(10)60673-3]

20 **Sutherland LR**, Verhoef M, Wallace JL, Van Rosendaal G, Crutcher R, Meddings JB. A simple, non-invasive marker of gastric damage: sucrose permeability. *Lancet* 1994; **343**: 998-1000 [PMID: 7909091 DOI: 10.1016/S0140-6736(94)90125-2]

21 **Bjarnason I**, Peters TJ. Intestinal permeability, non-steroidal anti-inflammatory drug enteropathy and inflammatory bowel disease: an overview. *Gut* 1989; **30** Spec No: 22-28 [PMID: 2691345 DOI: 10.1136/gut.30.Spec_No.22]

22 **Bjarnason I**, Smethurst P, Clark P, Menzies I, Levi J, Peters T. Effect of prostaglandin on indomethacin-induced increased intestinal permeability in man. *Scand J Gastroenterol Suppl* 1989; **164**: 97-102; discussion 102-103 [PMID: 2510288 DOI: 10.3109/00365528909091195]

23 **Hunt RH**, Bowen B, Mortensen ER, Simon TJ, James C, Cagliola A, Quan H, Bolognese JA. A randomized trial measuring fecal blood loss after treatment with rofecoxib, ibuprofen, or placebo in healthy subjects. *Am J Med* 2000; **109**: 201-206 [PMID: 10974182 DOI: 10.1016/S0002-9343(00)00470-8]

24 **Bjarnason I**, Hayllar J, Smethurst P, Price A, Gumpel MJ. Metronidazole reduces intestinal inflammation and blood loss in non-steroidal anti-inflammatory drug induced enteropathy. *Gut* 1992; **33**: 1204-1208 [PMID: 1427372 DOI: 10.1136/gut.33.9.1204]

25 **Maiden L**, Thjodleifsson B, Theodors A, Gonzalez J, Bjarnason I. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. *Gastroenterology* 2005; **128**: 1172-1178 [PMID: 15887101 DOI: 10.1053/j.gastro.2005.03.020]

26 **Endo H**, Hosono K, Inamori M, Kato S, Nozaki Y, Yoneda K, Akiyama T, Fujita K, Takahashi H, Yoneda M, Abe Y, Kirikoshi H, Kobayashi N, Kubota K, Saito S, Matsuhashi N, Nakajima A. Incidence of small bowel injury induced by low-dose aspirin: a crossover study using capsule endoscopy in healthy volunteers. *Digestion* 2009; **79**: 44-51 [PMID: 19246922 DOI: 10.1159/000204465]

27 **Solomon DH**, Gurwitz JH. Toxicity of nonsteroidal anti-inflammatory drugs in the elderly: is advanced age a risk factor? *Am J Med* 1997; **102**: 208-215 [PMID: 9217572 DOI: 10.1016/S0002-9343(96)00380-4]

28 **Hernández-Díaz S**, Rodríguez LA. Incidence of serious upper gastrointestinal bleeding/perforation in the general population: review of epidemiologic studies. *J Clin Epidemiol* 2002; **55**: 157-163 [PMID: 11809354 DOI: 10.1016/S0895-4356(01)00461-9]

29 **Aro P**, Storskrubb T, Ronkainen J, Bolling-Sternevald E, Engstrand L, Vieth M, Stolte M, Talley NJ, Agréus L. Peptic ulcer disease in a general adult population: the Kalixanda study: a random population-based study. *Am J Epidemiol* 2006; **163**: 1025-1034 [PMID: 16554343 DOI: 10.1093/aje/kwj129]

30 **Kearney PM**, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. *BMJ* 2006; **332**: 1302-1308 [PMID: 16740558 DOI: 10.1136/bmj.332.7553.1302]

31 **Laine L**, Maller ES, Yu C, Quan H, Simon T. Ulcer formation with low-dose enteric-coated aspirin and the effect of COX-2 selective inhibition: a double-blind trial. *Gastroenterology* 2004; **127**: 395-402 [PMID: 15300570 DOI: 10.1053/j.gastro.2004.05.001]

32 **Jüni P**, Rutjes AW, Dieppe PA. Are selective COX 2 inhibitors superior to traditional non steroidal anti-inflammatory drugs? *BMJ* 2002; **324**: 1287-1288 [PMID: 12039807 DOI: 10.1136/bmj.324.7349.1287]

33 **Schnitzer TJ**, Burmester GR, Mysler E, Hochberg MC, Doherty M, Ehrsam E, Gitton X, Krammer G, Mellein B, Matchaba P, Gimona A, Hawkey CJ. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. *Lancet* 2004; **364**: 665-674 [PMID: 15325831 DOI: 10.1016/S0140-6736(04)16893-1]

34 **Sostek MB**, Fort JG, Estborn L, Vikman K. Long-term safety of naproxen and esomeprazole magnesium fixed-dose combination: phase III study in patients at risk for NSAID-associated gastric ulcers. *Curr Med Res Opin* 2011; **27**: 847-854 [PMID: 21319944 DOI: 10.1185/03007995.2011.555756]

35 **Wallace JL**. Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. *Gastroenterology* 1997; **112**: 1000-1016 [PMID: 9041264 DOI: 10.1053/gast.1997.v112.pm9041264]

36 **Lanas A**, Scarpignato C. Microbial flora in NSAID-induced intestinal damage: a role for antibiotics? *Digestion* 2006; **73** Suppl 1: 136-150 [PMID: 16498262 DOI: 10.1159/000089789]

37 **Wallace JL**, Syer S, Denou E, de Palma G, Vong L, McKnight W, Jury J, Bolla M, Bercik P, Collins SM, Verdu E, Ongini E. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. *Gastroenterology* 2011; **141**: 1314-1322, 1322.e1-5 [PMID: 21745447 DOI: 10.1053/j.gastro.2011.06.075]

38 **Wallace JL**, Caliendo G, Santagada V, Cirino G. Markedly reduced toxicity of a hydrogen sulphide-releasing derivative of naproxen (ATB-346). *Br J Pharmacol* 2010; **159**: 1236-1246 [PMID: 20128814 DOI: 10.1111/j.1476-5381.2009.00611.x]

39 **Satoh H**, Amagase K, Takeuchi K. Exacerbation of nonsteroidal anti-inflammatory drug-induced small intestinal

lesions by antisecretory drugs in rats: the role of intestinal motility. *J Pharmacol Exp Ther* 2012; **343**: 270-277 [PMID: 22854201 DOI: 10.1124/jpet.112.197475]
40 **Verdu E**, Viani F, Armstrong D, Fraser R, Siegrist HH, Pignatelli B, Idström JP, Cederberg C, Blum AL, Fried M. Effect of omeprazole on intragastric bacterial counts, nitrates, nitrites, and N-nitroso compounds. *Gut* 1994; **35**: 455-460 [PMID: 8174980 DOI: 10.1136/gut.35.4.455]
41 **Williams C**, McColl KE. Review article: proton pump inhibitors and bacterial overgrowth. *Aliment Pharmacol Ther* 2006; **23**: 3-10 [PMID: 16393275 DOI: 10.1111/j.1365-2036.2006.02707.x]
42 **Lombardo L**, Foti M, Ruggia O, Chiecchio A. Increased incidence of small intestinal bacterial overgrowth during proton pump inhibitor therapy. *Clin Gastroenterol Hepatol* 2010; **8**: 504-508 [PMID: 20060064 DOI: 10.1016/j.cgh.2009.12.022]
43 **Daniell HW**. NSAID-PPI enteropathy in humans. *Gastroenterology* 2012; **142**: e20; author reply e20-e21 [PMID: 22374457 DOI: 10.1053/j.gastro.2012.02.004]
44 **Compare D**, Pica L, Rocco A, De Giorgi F, Cuomo R, Sarnelli G, Romano M, Nardone G. Effects of long-term PPI treatment on producing bowel symptoms and SIBO. *Eur J Clin Invest* 2011; **41**: 380-386 [PMID: 21128930 DOI: 10.1111/j.1365-2362.2010.02419.x]
45 **Poullis A**, Foster R, Mendall MA, Shreeve D, Wiener K. Proton pump inhibitors are associated with elevation of faecal calprotectin and may affect specificity. *Eur J Gastroenterol Hepatol* 2003; **15**: 573-574; author reply 574 [PMID: 12702920]
46 **Andréasson K**, Scheja A, Saxne T, Ohlsson B, Hesselstrand R. Faecal calprotectin: a biomarker of gastrointestinal disease in systemic sclerosis. *J Intern Med* 2011; **270**: 50-57 [PMID: 21205026 DOI: 10.1111/j.1365-2796.2010.02340.x]
47 **Wilcox GM**, Mattia AR. Microscopic colitis associated with omeprazole and esomeprazole exposure. *J Clin Gastroenterol* 2009; **43**: 551-553 [PMID: 19142168 DOI: 10.1097/MCG.0b013e31817d3fa1]
48 **Keszthelyi D**, Jansen SV, Schouten GA, de Kort S, Scholtes B, Engels LG, Masclee AA. Proton pump inhibitor use is associated with an increased risk for microscopic colitis: a case-control study. *Aliment Pharmacol Ther* 2010; **32**: 1124-1128 [PMID: 21039674 DOI: 10.1111/j.1365-2036.2010.04453.x]
49 **Pardi DS**, Kelly CP. Microscopic colitis. *Gastroenterology* 2011; **140**: 1155-1165 [PMID: 21303675 DOI: 10.1053/j.gastro.2011.02.003]
50 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141 [PMID: 15704047 DOI: 10.1016/S1542-3565(04)00619-6]
51 **Hawkey CJ**, Ell C, Simon B, Albert J, Keuchel M, McAlindon M, Fortun P, Schumann S, Bolten W, Shonde A, Hugot JL, Yu V, Arulmani U, Krammer G, Rebuli R, Toth E. Less small-bowel injury with lumiracoxib compared with naproxen plus omeprazole. *Clin Gastroenterol Hepatol* 2008; **6**: 536-544 [PMID: 18242145 DOI: 10.1016/j.cgh.2007.12.023]
52 **Reuter BK**, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. *Gastroenterology* 1997; **112**: 109-117 [PMID: 8978349 DOI: 10.1016/S0016-5085(97)70225-7]
53 **Morris AJ**, Madhok R, Sturrock RD, Capell HA, MacKenzie JF. Enteroscopic diagnosis of small bowel ulceration in patients receiving non-steroidal anti-inflammatory drugs. *Lancet* 1991; **337**: 520 [PMID: 1671893]
54 **Fujimori S**, Gudis K, Takahashi Y, Seo T, Yamada Y, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Distribution of small intestinal mucosal injuries as a result of NSAID administration. *Eur J Clin Invest* 2010; **40**: 504-510 [PMID: 20412292 DOI: 10.1111/j.1365-2362.2010.02290.x]
55 **Flower RJ**, Vane JR. Inhibition of prostaglandin synthetase in brain explains the anti-pyretic activity of paracetamol (4-acetamidophenol). *Nature* 1972; **240**: 410-411 [PMID: 4564318 DOI: 10.1038/240410a0]
56 **Xie WL**, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 1991; **88**: 2692-2696 [PMID: 1849272 DOI: 10.1073/pnas.88.7.2692]
57 **Kujubu DA**, Fletcher BS, Varnum BC, Lim RW, Herschman HR. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. *J Biol Chem* 1991; **266**: 12866-12872 [PMID: 1712772]
58 **Wallace JL**, Devchand PR. Emerging roles for cyclooxygenase-2 in gastrointestinal mucosal defense. *Br J Pharmacol* 2005; **145**: 275-282 [PMID: 15778736 DOI: 10.1038/sj.bjp.0706201]
59 **Sigthorsson G**, Simpson RJ, Walley M, Anthony A, Foster R, Hotz-Behoftsitz C, Palizban A, Pombo J, Watts J, Morham SG, Bjarnason I. COX-1 and 2, intestinal integrity, and pathogenesis of nonsteroidal anti-inflammatory drug enteropathy in mice. *Gastroenterology* 2002; **122**: 1913-1923 [PMID: 12055598 DOI: 10.1053/gast.2002.33647]
60 **Wallace JL**, Bak A, McKnight W, Asfaha S, Sharkey KA, MacNaughton WK. Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. *Gastroenterology* 1998; **115**: 101-109 [PMID: 9649464 DOI: 10.1016/S0016-5085(98)70370-1]
61 **Gilroy DW**, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 1999; **5**: 698-701 [PMID: 10371510]
62 **Wallace JL**, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology* 2000; **119**: 706-714 [PMID: 10982765 DOI: 10.1053/gast.2000.16510]
63 **Tanaka A**, Hase S, Miyazawa T, Takeuchi K. Up-regulation of cyclooxygenase-2 by inhibition of cyclooxygenase-1: a key to nonsteroidal anti-inflammatory drug-induced intestinal damage. *J Pharmacol Exp Ther* 2002; **300**: 754-761 [PMID: 11861778 DOI: 10.1124/jpet.300.3.754]
64 **Serhan CN**, Oliw E. Unorthodox routes to prostanoid formation: new twists in cyclooxygenase-initiated pathways. *J Clin Invest* 2001; **107**: 1481-1489 [PMID: 11413151 DOI: 10.1172/JCI13375]
65 **Serhan CN**, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LA, Perretti M, Rossi AG, Wallace JL. Resolution of inflammation: state of the art, definitions and terms. *FASEB J* 2007; **21**: 325-332 [PMID: 17267386 DOI: 10.1096/fj.06-7227rev]
66 **Mizuno H**, Sakamoto C, Matsuda K, Wada K, Uchida T, Noguchi H, Akamatsu T, Kasuga M. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. *Gastroenterology* 1997; **112**: 387-397 [PMID: 9024292 DOI: 10.1053/gast.1997.v112.pm9024292]
67 **Ma L**, del Soldato P, Wallace JL. Divergent effects of new cyclooxygenase inhibitors on gastric ulcer healing: Shifting the angiogenic balance. *Proc Natl Acad Sci USA* 2002; **99**: 13243-13247 [PMID: 12232050 DOI: 10.1073/pnas.202392199]
68 **Schmassmann A**, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi B, Halter F. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. *Br J Pharmacol* 1998; **123**: 795-804 [PMID: 9535006 DOI: 10.1038/sj.bjp.0701672]
69 **Okayama M**, Hayashi S, Aoi Y, Nishio H, Kato S, Takeuchi K. Aggravation by selective COX-1 and COX-2 inhibitors of dextran sulfate sodium (DSS)-induced colon lesions in rats. *Dig Dis Sci* 2007; **52**: 2095-2103 [PMID: 17429720 DOI:

10.1007/s10620-006-9597-z]
70 **Davies NM**, Sharkey KA, Asfaha S, Macnaughton WK, Wallace JL. Aspirin causes rapid up-regulation of cyclooxygenase-2 expression in the stomach of rats. *Aliment Pharmacol Ther* 1997; **11**: 1101-1108 [PMID: 9663836 DOI: 10.1046/j.1365-2036.1997.00247.x]
71 **Fiorucci S**, de Lima OM, Mencarelli A, Palazzetti B, Distrutti E, McKnight W, Dicay M, Ma L, Romano M, Morelli A, Wallace JL. Cyclooxygenase-2-derived lipoxin A4 increases gastric resistance to aspirin-induced damage. *Gastroenterology* 2002; **123**: 1598-1606 [PMID: 12404234 DOI: 10.1053/gast.2002.36558]
72 **Souza MH**, de Lima OM, Zamuner SR, Fiorucci S, Wallace JL. Gastritis increases resistance to aspirin-induced mucosal injury via COX-2-mediated lipoxin synthesis. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G54-G61 [PMID: 12637254]
73 **Maiden L**, Thjodleifsson B, Seigal A, Bjarnason II, Scott D, Birgisson S, Bjarnason I. Long-term effects of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 selective agents on the small bowel: a cross-sectional capsule enteroscopy study. *Clin Gastroenterol Hepatol* 2007; **5**: 1040-1045 [PMID: 17625980 DOI: 10.1016/j.cgh.2007.04.031]
74 **Jones MK**, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, Tarnawski AS. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med* 1999; **5**: 1418-1423 [PMID: 10581086]
75 **Somasundaram S**, Rafi S, Hayllar J, Sigthorsson G, Jacob M, Price AB, Macpherson A, Mahmod T, Scott D, Wrigglesworth JM, Bjarnason I. Mitochondrial damage: a possible mechanism of the "topical" phase of NSAID induced injury to the rat intestine. *Gut* 1997; **41**: 344-353 [PMID: 9378390 DOI: 10.1136/gut.41.3.344]
76 **Wax J**, Clinger WA, Varner P, Bass P, Winder CV. Relationship of the enterohepatic cycle to ulcerogenesis in the rat small bowel with flufenamic acid. *Gastroenterology* 1970; **58**: 772-780 [PMID: 5423889]
77 **Jacob M**, Foster R, Sigthorsson G, Simpson R, Bjarnason I. Role of bile in pathogenesis of indomethacin-induced enteropathy. *Arch Toxicol* 2007; **81**: 291-298 [PMID: 17151867 DOI: 10.1007/s00204-006-0149-2]
78 **Lichtenberger LM**, Phan T, Okabe S. Aspirin's ability to induce intestinal injury in rats is dependent on bile and can be reversed if pre-associated with phosphatidylcholine. *J Physiol Pharmacol* 2011; **62**: 491-496 [PMID: 22100851]
79 **Seitz S**, Boelsterli UA. Diclofenac acyl glucuronide, a major biliary metabolite, is directly involved in small intestinal injury in rats. *Gastroenterology* 1998; **115**: 1476-1482 [PMID: 9834275 DOI: 10.1016/S0016-5085(98)70026-5]
80 **Uchida A**, Yamada T, Hayakawa T, Hoshino M. Taurochenodeoxycholic acid ameliorates and ursodeoxycholic acid exacerbates small intestinal inflammation. *Am J Physiol* 1997; **272**: G1249-G1257 [PMID: 9176237]
81 **Zhou Y**, Dial EJ, Doyen R, Lichtenberger LM. Effect of indomethacin on bile acid-phospholipid interactions: implication for small intestinal injury induced by nonsteroidal anti-inflammatory drugs. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G722-G731 [PMID: 20203063 DOI: 10.1152/ajpgi.00387.2009]
82 **Dial EJ**, Darling RL, Lichtenberger LM. Importance of biliary excretion of indomethacin in gastrointestinal and hepatic injury. *J Gastroenterol Hepatol* 2008; **23**: e384-e389 [PMID: 18086111 DOI: 10.1111/j.1440-1746.2007.05266.x]
83 **Yamada T**, Hoshino M, Hayakawa T, Kamiya Y, Ohhara H, Mizuno K, Yamada H, Nakazawa T, Inagaki T, Uchida A, Miyaji M, Takeuchi T. Bile secretion in rats with indomethacin-induced intestinal inflammation. *Am J Physiol* 1996; **270**: G804-G812 [PMID: 8967492]
84 **Boelsterli UA**, Ramirez-Alcantara V. NSAID acyl glucuronides and enteropathy. *Curr Drug Metab* 2011; **12**: 245-252 [PMID: 21395536 DOI: 10.2174/138920011795101877]
85 **LoGuidice A**, Wallace BD, Bendel L, Redinbo MR, Boelsterli UA. Pharmacologic targeting of bacterial β-glucuronidase alleviates nonsteroidal anti-inflammatory drug-induced enteropathy in mice. *J Pharmacol Exp Ther* 2012; **341**: 447-454 [PMID: 22328575 DOI: 10.1124/jpet.111.191122]
86 **Atchison CR**, West AB, Balakumaran A, Hargus SJ, Pohl LR, Daiker DH, Aronson JF, Hoffmann WE, Shipp BK, Treinen-Moslen M. Drug enterocyte adducts: possible causal factor for diclofenac enteropathy in rats. *Gastroenterology* 2000; **119**: 1537-1547 [PMID: 11113075 DOI: 10.1053/gast.2000.20186]
87 **Robert A**, Asano T. Resistance of germfree rats to indomethacin-induced intestinal lesions. *Prostaglandins* 1977; **14**: 333-341 [PMID: 331401 DOI: 10.1016/0090-6980(77)90178-2]
88 **Uejima M**, Kinouchi T, Kataoka K, Hiraoka I, Ohnishi Y. Role of intestinal bacteria in ileal ulcer formation in rats treated with a nonsteroidal antiinflammatory drug. *Microbiol Immunol* 1996; **40**: 553-560 [PMID: 8887349]
89 **Kent TH**, Cardelli RM, Stamler FW. Small intestinal ulcers and intestinal flora in rats given indomethacin. *Am J Pathol* 1969; **54**: 237-249 [PMID: 5765565]
90 **Dalby AB**, Frank DN, St Amand AL, Bendele AM, Pace NR. Culture-independent analysis of indomethacin-induced alterations in the rat gastrointestinal microbiota. *Appl Environ Microbiol* 2006; **72**: 6707-6715 [PMID: 17021222 DOI: 10.1128/AEM.00378-06]
91 **Hagiwara M**, Kataoka K, Arimochi H, Kuwahara T, Ohnishi Y. Role of unbalanced growth of gram-negative bacteria in ileal ulcer formation in rats treated with a nonsteroidal anti-inflammatory drug. *J Med Invest* 2004; **51**: 43-51 [PMID: 15000255 DOI: 10.2152/jmi.51.43]
92 **Kato N**, Mashita Y, Kato S, Mitsufuji S, Yoshikawa T, Takeuchi K. Sildenafil, an inhibitor of phosphodiesterase subtype 5, prevents indomethacin-induced small-intestinal ulceration in rats via a NO/cGMP-dependent mechanism. *Dig Dis Sci* 2009; **54**: 2346-2356 [PMID: 19101800 DOI: 10.1007/s10620-008-0646-7]
93 **Watanabe T**, Higuchi K, Kobata A, Nishio H, Tanigawa T, Shiba M, Tominaga K, Fujiwara Y, Oshitani N, Asahara T, Nomoto K, Takeuchi K, Arakawa T. Non-steroidal anti-inflammatory drug-induced small intestinal damage is Toll-like receptor 4 dependent. *Gut* 2008; **57**: 181-187 [PMID: 17639086 DOI: 10.1136/gut.2007.125963]
94 **Koga H**, Aoyagi K, Matsumoto T, Iida M, Fujishima M. Experimental enteropathy in athymic and euthymic rats: synergistic role of lipopolysaccharide and indomethacin. *Am J Physiol* 1999; **276**: G576-G582 [PMID: 10070032]
95 **Kinouchi T**, Kataoka K, Bing SR, Nakayama H, Uejima M, Shimono K, Kuwahara T, Akimoto S, Hiraoka I, Ohnishi Y. Culture supernatants of Lactobacillus acidophilus and Bifidobacterium adolescentis repress ileal ulcer formation in rats treated with a nonsteroidal antiinflammatory drug by suppressing unbalanced growth of aerobic bacteria and lipid peroxidation. *Microbiol Immunol* 1998; **42**: 347-355 [PMID: 9654366]
96 **Syer SD**, McKnight W, Aucouturier A, Martin R, Langella P, Wallace JL. Bifidobacteria exert a protective effect against NSAID-induced enteropathy that is dependent on lactate production. *Gastroenterology* 2012; **142**: S-489
97 **Elliott SN**, Buret A, McKnight W, Miller MJ, Wallace JL. Bacteria rapidly colonize and modulate healing of gastric ulcers in rats. *Am J Physiol* 1998; **275**: G425-G432 [PMID: 9724253]
98 **Elliott SN**, Wallace JL, McKnight W, Gall DG, Hardin JA, Olson M, Buret A. Bacterial colonization and healing of gastric ulcers: the effects of epidermal growth factor. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G105-G112 [PMID: 10644568]
99 **Yamada T**, Deitch E, Specian RD, Perry MA, Sartor RB, Grisham MB. Mechanisms of acute and chronic intestinal

inflammation induced by indomethacin. *Inflammation* 1993; **17**: 641-662 [PMID: 7906675 DOI: 10.1007/BF00920471]
100 **Wallace JL**, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990; **259**: G462-G467 [PMID: 2169206]
101 **McCafferty DM**, Granger DN, Wallace JL. Indomethacin-induced gastric injury and leukocyte adherence in arthritic versus healthy rats. *Gastroenterology* 1995; **109**: 1173-1180 [PMID: 7557083 DOI: 10.1016/0016-5085(95)90576-6]
102 **Chmaisse HM**, Antoon JS, Kvietys PR, Grisham MB, Perry MA. Role of leukocytes in indomethacin-induced small bowel injury in the rat. *Am J Physiol* 1994; **266**: G239-G246 [PMID: 8141297]
103 **Konaka A**, Kato S, Tanaka A, Kunikata T, Korolkiewicz R, Takeuchi K. Roles of enterobacteria, nitric oxide and neutrophil in pathogenesis of indomethacin-induced small intestinal lesions in rats. *Pharmacol Res* 1999; **40**: 517-524 [PMID: 10660951 DOI: 10.1006/phrs.1999.0550]
104 **Miura S**, Suematsu M, Tanaka S, Nagata H, Houzawa S, Suzuki M, Kurose I, Serizawa H, Tsuchiya M. Microcirculatory disturbance in indomethacin-induced intestinal ulcer. *Am J Physiol* 1991; **261**: G213-G219 [PMID: 1651657]
105 **Santucci L**, Fiorucci S, Di Matteo FM, Morelli A. Role of tumor necrosis factor alpha release and leukocyte margination in indomethacin-induced gastric injury in rats. *Gastroenterology* 1995; **108**: 393-401 [PMID: 7530670 DOI: 10.1016/0016-5085(95)90065-9]
106 **Reuter BK**, Wallace JL. Phosphodiesterase inhibitors prevent NSAID enteropathy independently of effects on TNF-alpha release. *Am J Physiol* 1999; **277**: G847-G854 [PMID: 10516151]
107 **Bertrand V**, Guimbaud R, Tulliez M, Mauprivez C, Sogni P, Couturier D, Giroud JP, Chaussade S, Chauvelot-Moachon L. Increase in tumor necrosis factor-alpha production linked to the toxicity of indomethacin for the rat small intestine. *Br J Pharmacol* 1998; **124**: 1385-1394 [PMID: 9723949 DOI: 10.1038/sj.bjp.0701968]
108 **Gadelle D**, Raibaud P, Sacquet E. beta-Glucuronidase activities of intestinal bacteria determined both in vitro and in vivo in gnotobiotic rats. *Appl Environ Microbiol* 1985; **49**: 682-685 [PMID: 3994372]
109 **Hayllar J**, Smith T, Macpherson A, Price AB, Gumpel M, Bjarnason I. Nonsteroidal antiinflammatory drug-induced small intestinal inflammation and blood loss. Effects of sulfasalazine and other disease-modifying antirheumatic drugs. *Arthritis Rheum* 1994; **37**: 1146-1150 [PMID: 7914409 DOI: 10.1002/art.1780370806]
110 **Morris AJ**, Murray L, Sturrock RD, Madhok R, Capell HA, Mackenzie JF. Short report: the effect of misoprostol on the anaemia of NSAID enteropathy. *Aliment Pharmacol Ther* 1994; **8**: 343-346 [PMID: 7918931 DOI: 10.1111/j.1365-2036.1994.tb00298.x]
111 **Fujimori S**, Seo T, Gudis K, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Prevention of nonsteroidal anti-inflammatory drug-induced small-intestinal injury by prostaglandin: a pilot randomized controlled trial evaluated by capsule endoscopy. *Gastrointest Endosc* 2009; **69**: 1339-1346 [PMID: 19243767 DOI: 10.1016/j.gie.2008.08.017]
112 **Davies GR**, Wilkie ME, Rampton DS. Effects of metronidazole and misoprostol on indomethacin-induced changes in intestinal permeability. *Dig Dis Sci* 1993; **38**: 417-425 [PMID: 8444070 DOI: 10.1007/BF01316493]
113 **Jenkins RT**, Rooney PJ, Hunt RH. Increased bowel permeability to 51Cr-EDTA in controls caused by naproxen is not prevented by cytoprotection. *Arthritis Rheum* 1998; **31** (Suppl 1): R11
114 **Bjarnason I**, Smethurst P, Fenn CG, Lee CE, Menzies IS, Levi AJ. Misoprostol reduces indomethacin-induced changes in human small intestinal permeability. *Dig Dis Sci* 1989; **34**: 407-411 [PMID: 2493366 DOI: 10.1007/BF01536263]
115 **Montalto M**, Gallo A, Curigliano V, D'Onofrio F, Santoro L, Covino M, Dalvai S, Gasbarrini A, Gasbarrini G. Clinical trial: the effects of a probiotic mixture on non-steroidal anti-inflammatory drug enteropathy - a randomized, double-blind, cross-over, placebo-controlled study. *Aliment Pharmacol Ther* 2010; **32**: 209-214 [PMID: 20384610 DOI: 10.1111/j.1365-2036.2010.04324.x]
116 **Endo H**, Higurashi T, Hosono K, Sakai E, Sekino Y, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, Tokoro C, Inamori M, Abe Y, Nakajima A. Efficacy of Lactobacillus casei treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study. *J Gastroenterol* 2011; **46**: 894-905 [PMID: 21556830 DOI: 10.1007/s00535-011-0410-1]
117 **Watanabe T**, Nishio H, Tanigawa T, Yamagami H, Okazaki H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Asahara T, Nomoto K, Higuchi K, Takeuchi K, Arakawa T. Probiotic Lactobacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G506-G513 [PMID: 19589943 DOI: 10.1152/ajpgi.90553.2008]
118 **Dial EJ**, Dohrman AJ, Romero JJ, Lichtenberger LM. Recombinant human lactoferrin prevents NSAID-induced intestinal bleeding in rodents. *J Pharm Pharmacol* 2005; **57**: 93-99 [PMID: 15651117 DOI: 10.1211/0022357055191]
119 **Liepke C**, Adermann K, Raida M, Mägert HJ, Forssmann WG, Zucht HD. Human milk provides peptides highly stimulating the growth of bifidobacteria. *Eur J Biochem* 2002; **269**: 712-718 [PMID: 11856332 DOI: 10.1046/j.0014-2956.2001.02712.x]
120 **Troost FJ**, Saris WH, Brummer RJ. Recombinant human lactoferrin ingestion attenuates indomethacin-induced enteropathy in vivo in healthy volunteers. *Eur J Clin Nutr* 2003; **57**: 1579-1585 [PMID: 14647223 DOI: 10.1038/sj.ejcn.1601727]
121 **Arakawa T**, Watanabe T, Fukuda T, Yamasaki K, Kobayashi K. Rebamipide, novel prostaglandin-inducer accelerates healing and reduces relapse of acetic acid-induced rat gastric ulcer. Comparison with cimetidine. *Dig Dis Sci* 1995; **40**: 2469-2472 [PMID: 7587834 DOI: 10.1007/BF02063257]
122 **Mizoguchi H**, Ogawa Y, Kanatsu K, Tanaka A, Kato S, Takeuchi K. Protective effect of rebamipide on indomethacin-induced intestinal damage in rats. *J Gastroenterol Hepatol* 2001; **16**: 1112-1119 [PMID: 11686837 DOI: 10.1046/j.1440-1746.2001.02592.x]
123 **Niwa Y**, Nakamura M, Ohmiya N, Maeda O, Ando T, Itoh A, Hirooka Y, Goto H. Efficacy of rebamipide for diclofenac-induced small-intestinal mucosal injuries in healthy subjects: a prospective, randomized, double-blinded, placebo-controlled, cross-over study. *J Gastroenterol* 2008; **43**: 270-276 [PMID: 18458842 DOI: 10.1007/s00535-007-2155-4]
124 **Fujimori S**, Takahashi Y, Gudis K, Seo T, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Rebamipide has the potential to reduce the intensity of NSAID-induced small intestinal injury: a double-blind, randomized, controlled trial evaluated by capsule endoscopy. *J Gastroenterol* 2011; **46**: 57-64 [PMID: 20924615 DOI: 10.1007/s00535-010-0332-3]
125 **Yasuda M**, Kawahara R, Hashimura H, Yamanaka N, Iimori M, Amagase K, Kato S, Takeuchi K. Dopamine D-receptor antagonists ameliorate indomethacin-induced small intestinal ulceration in mice by activating α7 nicotinic acetylcholine receptors. *J Pharmacol Sci* 2011; **116**: 274-282 [PMID: 21691039 DOI: 10.1254/jphs.11037FP]
126 **Kawahara R**, Yasuda M, Hashimura H, Amagase K, Kato S, Takeuchi K. Activation of α7 nicotinic acetylcholine receptors ameliorates indomethacin-induced small intestinal ulceration in mice. *Eur J Pharmacol* 2011; **650**: 411-417 [PMID: 20969854 DOI: 10.1016/j.ejphar.2010.10.031]
127 **Kato S**, Matsuda N, Matsumoto K, Wada M, Onimaru N,

Yasuda M, Amagase K, Horie S, Takeuchi K. Dual role of serotonin in the pathogenesis of indomethacin-induced small intestinal ulceration: pro-ulcerogenic action via 5-HT3 receptors and anti-ulcerogenic action via 5-HT4 receptors. *Pharmacol Res* 2012; **66**: 226-234 [PMID: 22699012 DOI: 10.1016/j.phrs.2012.06.002]

128 **Rautio J**, Kumpulainen H, Heimbach T, Oliyai R, Oh D, Järvinen T, Savolainen J. Prodrugs: design and clinical applications. *Nat Rev Drug Discov* 2008; **7**: 255-270 [PMID: 18219308 DOI: 10.1038/nrd2468]

129 **Qandil AM**. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. *Int J Mol Sci* 2012; **13**: 17244-17274 [PMID: 23247285 DOI: 10.3390/ijms131217244]

130 **Whittle BJ**, Hansen D, Salmon JA. Gastric ulcer formation and cyclo-oxygenase inhibition in cat antrum follows parenteral administration of aspirin but not salicylate. *Eur J Pharmacol* 1985; **116**: 153-157 [PMID: 3932082 DOI: 10.1016/0014-2999(85)90196-7]

131 **Wallace JL**, McKnight GW. Characterization of a simple animal model for nonsteroidal anti-inflammatory drug induced antral ulcer. *Can J Physiol Pharmacol* 1993; **71**: 447-452 [PMID: 8242479 DOI: 10.1139/y93-066]

132 **Estes LL**, Fuhs DW, Heaton AH, Butwinick CS. Gastric ulcer perforation associated with the use of injectable ketorolac. *Ann Pharmacother* 1993; **27**: 42-43 [PMID: 8431619]

133 Available from: URL: http: //www.logicaltx.com/product-pipeline/nsaid-prodrug.php

134 **Goldstein JL**, Jungwirthová A, David J, Spindel E, Bouchner L, Pešek F, Searle S, Skopek J, Grim J, Ulč I, Sewell KL. Clinical trial: endoscopic evaluation of naproxen etemesil, a naproxen prodrug, vs. naproxen - a proof-of-concept, randomized, double-blind, active-comparator study. *Aliment Pharmacol Ther* 2010; **32**: 1091-1101 [PMID: 20804454 DOI: 10.1111/j.1365-2036.2010.04442.x]

135 **Graham DY**. Endoscopic ulcers are neither meaningful nor validated as a surrogate for clinically significant upper gastrointestinal harm. *Clin Gastroenterol Hepatol* 2009; **7**: 1147-1150 [PMID: 19559818 DOI: 10.1016/j.cgh.2009.06.006]

136 **Lanza FL**. Endoscopic studies of gastric and duodenal injury after the use of ibuprofen, aspirin, and other nonsteroidal anti-inflammatory agents. *Am J Med* 1984; **77**: 19-24 [PMID: 6465160 DOI: 10.1016/S0002-9343(84)80014-5]

137 **Graham DY**, Smith JL, Holmes GI, Davies RO. Nonsteroidal anti-inflammatory effect of sulindac sulfoxide and sulfide on gastric mucosa. *Clin Pharmacol Ther* 1985; **38**: 65-70 [PMID: 3874038 DOI: 10.1038/clpt.1985.136]

138 **Carson JL**, Strom BL, Morse ML, West SL, Soper KA, Stolley PD, Jones JK. The relative gastrointestinal toxicity of the nonsteroidal anti-inflammatory drugs. *Arch Intern Med* 1987; **147**: 1054-1059 [PMID: 3496062]

139 **Roth SH**, Tindall EA, Jain AK, McMahon FG, April PA, Bockow BI, Cohen SB, Fleischmann RM. A controlled study comparing the effects of nabumetone, ibuprofen, and ibuprofen plus misoprostol on the upper gastrointestinal tract mucosa. *Arch Intern Med* 1993; **153**: 2565-2571 [PMID: 8239849 DOI: 10.1001/archinte.1993.00410220073008]

140 **Chan FK**, Sung JJ, Ching JY, Wu JC, Lee YT, Leung WK, Hui Y, Chan LY, Lai AC, Chung SC. Randomized trial of low-dose misoprostol and naproxen vs. nabumetone to prevent recurrent upper gastrointestinal haemorrhage in users of non-steroidal anti-inflammatory drugs. *Aliment Pharmacol Ther* 2001; **15**: 19-24 [PMID: 11136274 DOI: 10.1046/j.1365-2036.2001.00890.x]

141 **Henry D**, Dobson A, Turner C. Variability in the risk of major gastrointestinal complications from nonaspirin nonsteroidal anti-inflammatory drugs. *Gastroenterology* 1993; **105**: 1078-1088 [PMID: 8405852]

142 **Wallace JL**, Ferraz JG. New pharmacologic therapies in gastrointestinal disease. *Gastroenterol Clin North Am* 2010; **39**: 709-720 [PMID: 20951926 DOI: 10.1016/j.gtc.2010.08.020]

143 **Wallace JL**, Del Soldato P. The therapeutic potential of NO-NSAIDs. *Fundam Clin Pharmacol* 2003; **17**: 11-20 [PMID: 12588626 DOI: 10.1046/j.1472-8206.2003.00125.x]

144 **Caliendo G**, Cirino G, Santagada V, Wallace JL. Synthesis and biological effects of hydrogen sulfide (H2S): development of H2S-releasing drugs as pharmaceuticals. *J Med Chem* 2010; **53**: 6275-6286 [PMID: 20462257 DOI: 10.1021/jm901638j]

145 **Wallace JL**. Hydrogen sulfide-releasing anti-inflammatory drugs. *Trends Pharmacol Sci* 2007; **28**: 501-505 [PMID: 17884186 DOI: 10.1016/j.tips.2007.09.003]

146 **Fiorucci S**, Distrutti E, Cirino G, Wallace JL. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. *Gastroenterology* 2006; **131**: 259-271 [PMID: 16831608 DOI: 10.1053/j.gastro.2006.02.033]

147 **Wallace JL**, Miller MJ. Nitric oxide in mucosal defense: a little goes a long way. *Gastroenterology* 2000; **119**: 512-520 [PMID: 10930387 DOI: 10.1053/gast.2000.9304]

148 **Fiorucci S**, Antonelli E, Distrutti E, Rizzo G, Mencarelli A, Orlandi S, Zanardo R, Renga B, Di Sante M, Morelli A, Cirino G, Wallace JL. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. *Gastroenterology* 2005; **129**: 1210-1224 [PMID: 16230075 DOI: 10.1053/j.gastro.2005.07.060]

149 **Zanardo RC**, Brancaleone V, Distrutti E, Fiorucci S, Cirino G, Wallace JL. Hydrogen sulfide is an endogenous modulator of leukocyte-mediated inflammation. *FASEB J* 2006; **20**: 2118-2120 [PMID: 16912151 DOI: 10.1096/fj.06-6270fje]

150 **Elliott SN**, McKnight W, Cirino G, Wallace JL. A nitric oxide-releasing nonsteroidal anti-inflammatory drug accelerates gastric ulcer healing in rats. *Gastroenterology* 1995; **109**: 524-530 [PMID: 7615202 DOI: 10.1016/0016-5085(95)90341-0]

151 **Wallace JL**. Physiological and pathophysiological roles of hydrogen sulfide in the gastrointestinal tract. *Antioxid Redox Signal* 2010; **12**: 1125-1133 [PMID: 19769457 DOI: 10.1089/ars.2009.2900]

152 **Wallace JL**, Ignarro LJ, Fiorucci S. Potential cardioprotective actions of no-releasing aspirin. *Nat Rev Drug Discov* 2002; **1**: 375-382 [PMID: 12120413 DOI: 10.1038/nrd794]

153 **Konturek SJ**, Brzozowski T, Majka J, Pytko-Polonczyk J, Stachura J. Inhibition of nitric oxide synthase delays healing of chronic gastric ulcers. *Eur J Pharmacol* 1993; **239**: 215-217 [PMID: 7693490 DOI: 10.1016/0014-2999(93)90997-V]

154 **Ma L**, Wallace JL. Endothelial nitric oxide synthase modulates gastric ulcer healing in rats. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G341-G346 [PMID: 10915643]

155 **Wallace JL**, Caliendo G, Santagada V, Cirino G, Fiorucci S. Gastrointestinal safety and anti-inflammatory effects of a hydrogen sulfide-releasing diclofenac derivative in the rat. *Gastroenterology* 2007; **132**: 261-271 [PMID: 17241876 DOI: 10.1053/j.gastro.2006.11.042]

156 **Whittle BJ**, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; **99**: 607-611 [PMID: 2110019 DOI: 10.1111/j.1476-5381.1990.tb12977.x]

157 **MacNaughton WK**, Cirino G, Wallace JL. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. *Life Sci* 1989; **45**: 1869-1876 [PMID: 2513461 DOI: 10.1016/0024-3205(89)90540-7]

158 **Wallace JL**, Dicay M, McKnight W, Martin GR. Hydrogen sulfide enhances ulcer healing in rats. *FASEB J* 2007; **21**: 4070-4076 [PMID: 17634391 DOI: 10.1096/fj.07-8669com]

159 **Reuter BK**, Cirino G, Wallace JL. Markedly reduced intestinal toxicity of a diclofenac derivative. *Life Sci* 1994; **55**: PL1-PL8 [PMID: 8015340 DOI: 10.1016/0024-3205(94)90083-3]

160 **Davies NM**, Røseth AG, Appleyard CB, McKnight W, Del

Soldato P, Calignano A, Cirino G, Wallace JL. NO-naproxen vs. naproxen: ulcerogenic, analgesic and anti-inflammatory effects. *Aliment Pharmacol Ther* 1997; **11**: 69-79 [PMID: 9042976 DOI: 10.1046/j.1365-2036.1997.115286000.x]

161 **Hawkey CJ**, Jones JI, Atherton CT, Skelly MM, Bebb JR, Fagerholm U, Jonzon B, Karlsson P, Bjarnason IT. Gastrointestinal safety of AZD3582, a cyclooxygenase inhibiting nitric oxide donator: proof of concept study in humans. *Gut* 2003; **52**: 1537-1542 [PMID: 14570719 DOI: 10.1136/gut.52.11.1537]

162 **Schnitzer TJ**, Hochberg MC, Marrero CE, Duquesroix B, Frayssinet H, Beekman M. Efficacy and safety of naproxcinod in patients with osteoarthritis of the knee: a 53-week prospective randomized multicenter study. *Semin Arthritis Rheum* 2011; **40**: 285-297 [PMID: 20828790 DOI: 10.1016/j.semarthrit.2010.06.002]

163 **Karlsson J**, Pivodic A, Aguirre D, Schnitzer TJ. Efficacy, safety, and tolerability of the cyclooxygenase-inhibiting nitric oxide donator naproxcinod in treating osteoarthritis of the hip or knee. *J Rheumatol* 2009; **36**: 1290-1297 [PMID: 19411388 DOI: 10.3899/jrheum.081011]

164 **Baerwald C**, Verdecchia P, Duquesroix B, Frayssinet H, Ferreira T. Efficacy, safety, and effects on blood pressure of naproxcinod 750 mg twice daily compared with placebo and naproxen 500 mg twice daily in patients with osteoarthritis of the hip: a randomized, double-blind, parallel-group, multicenter study. *Arthritis Rheum* 2010; **62**: 3635-3644 [PMID: 20722026 DOI: 10.1002/art.27694]

165 **Schnitzer TJ**, Kivitz A, Frayssinet H, Duquesroix B. Efficacy and safety of naproxcinod in the treatment of patients with osteoarthritis of the knee: a 13-week prospective, randomized, multicenter study. *Osteoarthritis Cartilage* 2010; **18**: 629-639 [PMID: 20202489 DOI: 10.1016/j.joca.2009.12.013]

166 **Wallace JL**, Viappiani S, Bolla M. Cyclooxygenase-inhibiting nitric oxide donators for osteoarthritis. *Trends Pharmacol Sci* 2009; **30**: 112-117 [PMID: 19230986 DOI: 10.1016/j.tips.2009.01.001]

167 **Lohmander LS**, McKeith D, Svensson O, Malmenäs M, Bolin L, Kalla A, Genti G, Szechinski J, Ramos-Remus C. A randomised, placebo controlled, comparative trial of the gastrointestinal safety and efficacy of AZD3582 versus naproxen in osteoarthritis. *Ann Rheum Dis* 2005; **64**: 449-456 [PMID: 15345500 DOI: 10.1136/ard.2004.023572]

168 **Li L**, Rossoni G, Sparatore A, Lee LC, Del Soldato P, Moore PK. Anti-inflammatory and gastrointestinal effects of a novel diclofenac derivative. *Free Radic Biol Med* 2007; **42**: 706-719 [PMID: 17291994 DOI: 10.1016/j.freeradbiomed.2006.12.011]

169 **Sparatore A**, Perrino E, Tazzari V, Giustarini D, Rossi R, Rossoni G, Erdmann K, Schröder H, Del Soldato P. Pharmacological profile of a novel H(2)S-releasing aspirin. *Free Radic Biol Med* 2009; **46**: 586-592 [PMID: 19100325 DOI: 10.1016/j.freeradbiomed.2008.11.013]

170 **Li L**, Bhatia M, Moore PK. Hydrogen sulphide--a novel mediator of inflammation? *Curr Opin Pharmacol* 2006; **6**: 125-129 [PMID: 16487749 DOI: 10.1016/j.coph.2005.10.007]

171 **Whiteman M**, Le Trionnaire S, Chopra M, Fox B, Whatmore J. Emerging role of hydrogen sulfide in health and disease: critical appraisal of biomarkers and pharmacological tools. *Clin Sci* (Lond) 2011; **121**: 459-488 [PMID: 21843150 DOI: 10.1042/CS20110267]

172 **Dufton N**, Natividad J, Verdu EF, Wallace JL. Hydrogen sulfide and resolution of acute inflammation: A comparative study utilizing a novel fluorescent probe. *Sci Rep* 2012; **2**: 499 [PMID: 22787557 DOI: 10.1038/srep00499]

173 **Wallace JL**, Vong L, McKnight W, Dicay M, Martin GR. Endogenous and exogenous hydrogen sulfide promotes resolution of colitis in rats. *Gastroenterology* 2009; **137**: 569-578 [PMID: 19375422 DOI: 10.1053/j.gastro.2009.04.012]

174 **Wallace JL**, Ferraz JG, Muscara MN. Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury. *Antioxid Redox Signal* 2012; **17**: 58-67 [PMID: 22017247 DOI: 10.1089/ars.2011.4351]

175 **Blackler R**, Syer S, Bolla M, Ongini E, Wallace JL. Gastrointestinal-sparing effects of novel NSAIDs in rats with compromised mucosal defence. *PLoS One* 2012; **7**: e35196 [PMID: 22496907 DOI: 10.1371/journal.pone.0035196]

176 **Waldram A**, Holmes E, Wang Y, Rantalainen M, Wilson ID, Tuohy KM, McCartney AL, Gibson GR, Nicholson JK. Top-down systems biology modeling of host metabotype-microbiome associations in obese rodents. *J Proteome Res* 2009; **8**: 2361-2375 [PMID: 19275195 DOI: 10.1021/pr8009885]

177 **Kodela R**, Chattopadhyay M, Kashfi K. NOSH-Aspirin: A Novel Nitric Oxide-Hydrogen Sulfide-Releasing Hybrid: A New Class of Anti-inflammatory Pharmaceuticals. *ACS Med Chem Lett* 2012; **3**: 257-262 [PMID: 22916316 DOI: 10.1021/ml300002m]

178 **Lichtenberger LM**, Zhou Y, Dial EJ, Raphael RM. NSAID injury to the gastrointestinal tract: evidence that NSAIDs interact with phospholipids to weaken the hydrophobic surface barrier and induce the formation of unstable pores in membranes. *J Pharm Pharmacol* 2006; **58**: 1421-1428 [PMID: 17132203 DOI: 10.1211/jpp.58.10.0001]

179 **Lichtenberger LM**, Wang ZM, Romero JJ, Ulloa C, Perez JC, Giraud MN, Barreto JC. Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nat Med* 1995; **1**: 154-158 [PMID: 7585013 DOI: 10.1038/nm0295-154]

180 **Lichtenberger LM**, Barron M, Marathi U. Association of phosphatidylcholine and NSAIDs as a novel strategy to reduce gastrointestinal toxicity. *Drugs Today* (Barc) 2009; **45**: 877-890 [PMID: 20135022]

181 **Cryer B**, Bhatt DL, Lanza FL, Dong JF, Lichtenberger LM, Marathi UK. Low-dose aspirin-induced ulceration is attenuated by aspirin-phosphatidylcholine: a randomized clinical trial. *Am J Gastroenterol* 2011; **106**: 272-277 [PMID: 21081908 DOI: 10.1038/ajg.2010.436]

182 **Lanza FL**, Marathi UK, Anand BS, Lichtenberger LM. Clinical trial: comparison of ibuprofen-phosphatidylcholine and ibuprofen on the gastrointestinal safety and analgesic efficacy in osteoarthritic patients. *Aliment Pharmacol Ther* 2008; **28**: 431-442 [PMID: 18549459 DOI: 10.1111/j.1365-2036.2008.03765.x]

P- Reviewer Tarnawski AS **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1877

World J Gastroenterol 2013 March 28; 19(12): 1877-1889
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Role of T cell death in maintaining immune tolerance during persistent viral hepatitis

Juan Ramón Larrubia, Megha Uttam Lokhande, Silvia García-Garzón, Joaquín Miquel, Dolores Subirá, Eduardo Sanz-de-Villalobos

Juan Ramón Larrubia, Megha Uttam Lokhande, Silvia García-Garzón, Joaquín Miquel, Dolores Subirá, Eduardo Sanz-de-Villalobos, Translational Hepatology Unit, Guadalajara University Hospital, University of Alcalá, 19002 Guadalajara, Spain

Author contributions: Larrubia JR and Lokhande MU contributed equally towards the conception and design of the review; Larrubia JR and Lokhande MU co-wrote and Larrubia JR revised the manuscript; García-Garzón S, Miquel J, Subirá D and Sanz-de-Villalobos E contributed equally to the supportive work and supervision.

Supported by Grants from "Fiscam" JCCM, Ayuda para proyectos de investigación en salud, PI-2010/022; "Fundación de Investigación Médica Mutua Madrileña", Beca Ayudas a la Investigación FMMM, 8922/2011 and A research grant from "Asociación de Hepatología Translacional", No. AHT 2010-01, to Lokhande MU

Correspondence to: Megha Uttam Lokhande, MSc, Translational Hepatology Unit. Guadalajara University Hospital. University of Alcalá. Donante de Sangre st., 19002 Guadalajara, Spain. lokhandemu@gmail.com
Telephone: +34-949-209200 Fax: +34-949-209259

Received: September 14, 2012 Revised: December 20, 2012
Accepted: December 25, 2012
Published online: March 28, 2013

Abstract

Virus-specific T cells play an important role in the resolution of hepatic infection. However, during chronic hepatitis infection these cells lack their effector functions and fail to control the virus. Hepatitis B virus and hepatitis C virus have developed several mechanisms to generate immune tolerance. One of these strategies is the depletion of virus-specific T cells by apoptosis. The immunotolerogenic liver has unique property to retain and activate naïve T cell to avoid the over reactivation of immune response against antigens which is exploited by hepatotropic viruses to persist. The deletion of the virus-specific T cells occurs by intrinsic (passive) apoptotic mechanism. The pro-apoptotic molecule Bcl-2 interacting mediator (Bim) has attracted increasing attention as a pivotal involvement in apoptosis, as a regulator of tissue homeostasis and an enhancer for the viral persistence. Here, we reviewed our current knowledge on the evidence showing critical role of Bim in viral-specific T cell death by apoptotic pathways and helps in the immune tolerance.

Key words: T cell death; Specific cytotoxic T lymphocytes; Hepatitis C virus immune tolerance; Apoptosis; Bcl-2 interacting mediator; Liver tolerance; Apoptotic pathways; Viral hepatitis

Larrubia JR, Lokhande MU, García-Garzón S, Miquel J, Subirá D, Sanz-de-Villalobos E. Role of T cell death in maintaining immune tolerance during persistent viral hepatitis. *World J Gastroenterol* 2013; 19(12): 1877-1889 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1877.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1877

INTRODUCTION

Hepatotropic, non-cytopathic viruses such as hepatitis B virus (HBV) and hepatitis C virus (HCV) behave as intracellular parasites. The activation of cellular immune response by priming of naïve specific CD4+ and CD8+ T cells in the lymph nodes is very important to control viral infection. However, the unique ability of the liver to retain and activate naïve CD8+ T cells leads to liver tolerance, by-passing normal activation in the lymph nodes. The continuous triggering of antigen presenting cells (APCs) in the sinusoids by the antigen-rich blood leads to peripheral tolerance to protect the liver tissue. This physiological feature can be used by hepatotropic viruses as a persistence mechanism. The depletion of liver activated CD8+ T cells is the critical part of the peripheral tolerance in HBV/HCV infection. The anticipated mecha-

nisms for immune tolerance in liver specific pathogens are linked to virus-specific T cells death. The vital role of pro-apoptotic molecule, Bcl-2 interacting mediator (Bim) in the death of the virus-specific T cells has been shown after intrahepatic T cell activation by hepatocytes[1], in chronic HBV and HCV infection[2,3]. Therefore, this review provides glimpse of the recent advances to understand the cellular and molecular mechanism involved on "T cell death" during viral hepatitis as a viral escape mechanism through the induction of a specific-immunotolerant status on the host.

VIRAL HEPATITIS

HBV and HCV viruses are two hepatotropic noncytopathic, human blood-born viruses. HBV is a small, enveloped DNA virus that undergoes a pro-viral state to persist in the host. HCV is an enveloped virus with a plus-strand RNA genome. It has been estimated that more than 350 million for HBV and 170 million people for HCV are infected. Approximately 80% of infections in HCV and > 90% of infected neonates, 5%-10% of infected adults in HBV succeed in establishing a chronic infection, with the potential for developing severe liver diseases such as cirrhosis and hepatocellular carcinoma[4,5].

Highly productive and replicative viruses such as HBV and HCV are associated with ineffective antiviral immunity during persistent viral infections. The complex ineffective immunity involves the functional deterioration of antiviral T cell responses and contraction of the size of this response. In persistent HBV/HCV infections, T cells are continuously challenged by high levels of viral antigens that eventually result in limiting the antiviral T cell response and ultimately leading to T cell exhaustion. This is a progressive process, starting with the deficiency in cytokine production, proliferation and survival[6], to end with physical deletion of specific antiviral T-cell populations[7].

Meticulously, cytotoxic T lymphocytes (CTLs) play a vital role in viral eradication[8] and in the pathogenesis of hepatitis[9-11]. A strong, multi-specific and long-lasting T-cell immune response emerges to be important for control of viral infection[12-14]. Appropriate, polyclonal, vigorous and multi-specific CTL responses can facilitate complete viral clearance, in which long-lasting protective T cell response is observed. However, specific CTL responses are usually not strong enough to eradicate the virus, hence contributing to persistent infection[15,16].

HBV and HCV are hepatotropic viruses that replicate in the liver. This organ features a unique immune tissue, where the deletion of antiviral T cell populations has been shown, being involved in local and systemic immune tolerance.

LIVER AS A FOUNDATION OF IMMUNE TOLERANCE

Liver situates at a hemodynamic convergence, receiving the splanchnic stream, which means an intense contact with exogenous antigens. This fact leads to the development of tolerance mechanisms to avoid inappropriate immune system activation, but it also allows antigen presentation by resident cells. Therefore, the liver is progressively more being recognized as an immune organ[17]. Liver sinusoids, hepatic arteries and portal venous carry blood containing digested nutrients and micro antigens from intestine, and as a primary metabolic organ, the liver produces multiple neo-antigens. All these molecules pass through sinusoids and finally are taken up and metabolized by different hepatic resident cells. The liver has acquired specialized mechanisms of immune tolerance to avoid the over reactivation of immune response against antigens that are metabolized in the liver. In fact, this process may be beneficial for inducing tolerance to liver grafts but also to the liver specific pathogens. Therefore, hepatotropic viruses exploit these immunotolerogenic liver features to persist. It is important to remind that the liver has the ability to retain and activate naïve CD8+ T cells ineffectively, in contrast to other lymphoid tissues. This fact may allow pathogens to escape from T cell mediated immunity and establish a persistent hepatic infection due to immune tolerance induction. This immunotolerant state can be reached by the development of T cell anergy but also by specific T cell deletion.

Uniqueness of the liver

The unique character of the hepatic tissue to tolerate liver allograft across major histocompatibility complex (MHC) mismatch in the pig without immunosupression was described by first time in 1969[17]. Later studies confirmed that this occurred because of the induction of immunological tolerance in the liver[18]. Initially, "graveyard theory" suggested that the exclusive ability of the liver to get rid of activated T cells, programmed to undergo apoptosis, was the root of the hepatic tolerance effect[19]. This theory proposed two functions of the liver as a T cell graveyard: (1) passive killer of the liver cells after their life cycle; and (2) efficient killer of the activated antigen specific T cells. According to this theory, T cell receptor (TCR) triggering by cognate antigen on TCR transgenic T cells leads to activation and accumulation of those cells in the liver and undergoes depletion of mature T cells[20].

The theory was again proved by Mehal *et al*[21] by indicating that the normal liver is a "sink" for activated T cells. The liver was perfused by T cells showing retention of activated, but neither resting nor apoptotic T cells[21]. Liver as a graveyard for activated T cells theory forced to believe that all the immune response in the liver would be silent; in spite of this, the presence of an effective virus specific T cells in patients controlling hepatic viral infections[22,23] could challenge this theory. Nonetheless, the removal of activated T cells by the liver cannot be excluded, as evidenced by the ability of liver allograft to rescue rejecting skin grafts[21], in which lately tolerising capacity of the liver for activated allo-specific T cells occurs. In some cases, the limited capacity of the liver to induce tolerance

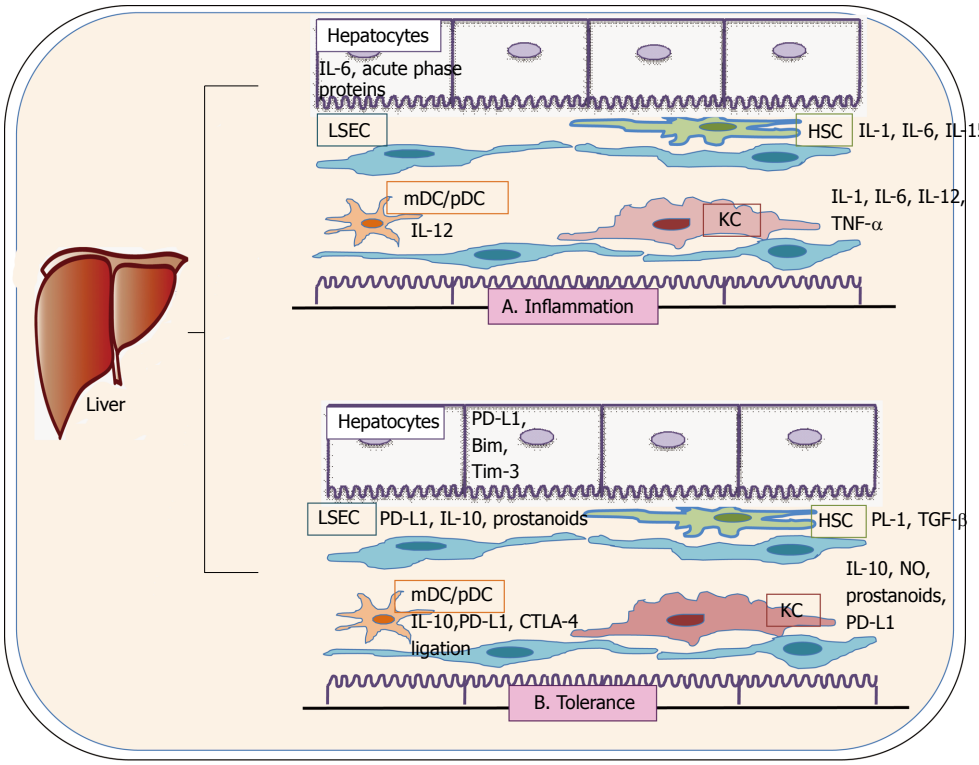


Figure 1 Collective illustration of the hepatic cells with inflammatory and tolerance activities by stimulation of different molecules or receptors. LSEC: Liver sinusoidal endothelial cells; KC: Kuffer cells; DC: Dendritic cells; HSC: Hepatic stellate cells; TNF: Tumor necrosis factor; IL: Interleukin; mDC: Myeloid dendratic cell; pDC: Plasmacytoid dendritic cell; PD-L1: Programmed death ligand-1; Bim: BCL-2 interacting mediator; Tim-3: T cell immunoglobulin mucin-3; CTLA-4: Cytotoxic T-lymphocyte antigen 4; TGF: Transforming growth factor; NO: Nitric oxide.

could be due to large number of activated T cells[24].

Naïve T cells activation in the liver

The site of T cell activation is a determinant of the outcome of an immune response in the liver[22]. Tolerance will occur when T cells are activated in the liver. On the other hand, an effective immune response will be generated, when T cells are activated in the lymph nodes. This model put forward the theory that tolerance during viral hepatitis could be the result of early deletion of antigen-specific T cells from the T cell repertoire in the liver[22]. Usually, naïve T cells are activated in secondary lymphoid organs with consequent up regulation of adhesion molecules and integrins expression, which can bind to endothelial layer of the target organ and ultimately direct T cells to the parenchyma[25]. Moreover, T cells are not able to interact with parenchymal cells easily and thus they are not usually activated in the solid organs. In spite of this, the situation in the liver is slightly different. Fenestrated endothelial layer in the liver makes available interactions between naïve T cells and liver cells[26]. It has been showed by MHC class I -restricted, hepatitis B surface Ag-specific CD8+ polyclonal CTL adoptively transferred into wide-spread antigen expressing transgenic mouse model, leading to retention of those cells within the liver[26]. Moreover, it has been shown that primary antigen-specific T cell can be activated in the liver independently of lymphoid tissues[27].

Liver APCs in tolerance

Retention, activation and tolerance of naïve T cells in the liver is the result of the action of resident liver cells, including liver sinusoidal endothelial cells (LSEC), Kuffer cells (KC), liver dendritic cells (DC), hepatic stellate cells (HSC) and hepatocytes. Their collective function in induction of inflammatory response and tolerance has been illustrated in the Figure 1.

Endocytosis specialist-LSEC can express MHC class Ⅰ and Ⅱ, accessory CD80, CD86 and CD40 molecules. These features enable those cells to behave as potent APCs with the ability to activate both naïve CD4 and CD8 T cells as well as to cross-present exogenous antigen towards CD8 T cells[28]. However, LSEC primed naïve CD4+ T cells produce cytokines typical from Th0 rather than Th1 cells[29]. In addition, LSECs constitutively expressed ICAM-1, which helped in trapping of specific CD8+ T cells in the liver, resulting this process in activated T cell apoptosis[21]. Furthermore, the cross presentation of antigen by LSEC mainly leads to CD8+ T cells tolerance rather than immunity, demonstrating that LSEC-induced tolerance is an active and dynamic process[30].

Bone marrow derived and largest group of liver resident macrophages-KC mediate host resistance to infection. Interleukin (IL)-1, IL-6, IL-12 and tumor necrosis factor-α (TNF-α) pro-inflammatory cytokines released by KC[31] are involved in the inflammatory activities, whereas the nitric oxide, prostaglandin and IL-10 released by KC[29,32] down-regulate the production of pro-inflammatory cytokines and thereby may contribute to induction of hepatic tolerance. Furthermore, DC-induced antigen-specific T cell activation can be inhibited by KCs[29], which could also favor tolerance development. In addition, as in LSECs, KCs expressed ICAM-1 mediated trapping of specific CD8+ T cells in the liver resulting in activated T cell death[21].

Liver DCs are primarily located within periportal areas

and around central veins, which exert tolerogenic properties due to "immature" phenotype. The production of PD-1 and cytotoxic T lymphocyte antigen-4 (CTLA-4) by resting DCs, which are crucial negative co-stimulatory molecules, helps in inducing peripheral CD8+ T cell tolerance by inhibiting proliferation and cytokine production of liver infiltrating effector T cells[33]. In addition, liver generated DCs are more tolerogenic than DC in lymphatic tissue[34].

The role of HSCs in hepatic fibrosis includes stimulation of CD4, CD8+ T cells and NKT cells[35,36]. However, function of HSCs involves not only the inflammatory response[36], but also a tolerogenic role[37,38], which is the result of induction of T cell death[38] by intrinsic mechanism of immune inhibition. The HSCs regulate immune modulation by inducible B7-H1 expression, an inhibitor molecule of B7 family, resulting in T cell apoptosis induction.

Hepatocytes are also capable of activating naïve CD8+ T cells[39,40] and their interactions with CD8+ T cells may occur through LSEC fenestrations[38]. However, hepatocytes fail to promote activated CD8+ T cells survival, leading to an impaired T cell activation[39]. In addition, hepatocyte-activated T cells *in vitro* acquired activity and secrete cytokines but both levels are not constant and T cells consequently appeared to die by passive mechanisms[41]. Furthermore, infiltrating CD4+ T cells differentiate into a less inflammatory phenotype due to the interaction with MHC Ⅱ-expressing hepatocytes, which also helps to abrogate antiviral CD8+ T-cell response and viral clearance[42], which conclude in the tolerance during infection. It has been already proved that T cells activated by hepatocytes undergo premature death[43], whereas naïve CD8+ T cells priming by DC in the lymph nodes acquired effector functions in the liver.

The site of primary T cell activation could also induce emperipolesis of CD8+ T cells in the liver[43], which leads to non-apoptotic, destruction of these CD8+ T cells after degradation by lysosomal proteolytic enzymes. This distinct form of emperipolesis has been termed as "suicidal emperipolesis" (SE)[44]. Benseler *et al*[44] suggested that SE is a significant mechanism by which death of activated naïve CD8+ T cells occur in the liver within the first few hours before T cells are able to divide and expand. It is also involved in maintenance of tolerance, which is reinforced by break of tolerance in immune-mediated liver damage by treatment of wortmannin[44], inhibitor of phosphoinositide 3-kinases that blocks emperipolesis. Therefore, SE is an extremely efficient mechanism, able to rapidly delete T cells.

T cell stimulation in the liver encourages tolerance by using mechanisms such as, immune divergence[45], generation of regulatory T cells[46], T cell anergy[47] and T cell death[1]. Undeniably, hepatic tolerance can explain the elevated frequency of viral persistence during hepatotropic virus infections[1]. Although there are evidences showing that most infectious microorganisms are promptly removed from the liver, a favorable situation for evading immune responses occurs in some viruses, leading to the triumph of certain pathogens such as HBV and HCV. Till date, there are two main mechanisms by which HBV and HCV could successfully escape from CTL action: escape mutant generation, and immunosuppressive effects exertion (effector T cell exhaustion and T cell death by apoptosis)[2,48-50]. Among these mechanisms involved in viral hepatitis persistence, new advances on the role of T cell death induction have been obtained recently and our review in the apoptosis role, paying special attention to the last new insights in this issue will be discussed in the following pages.

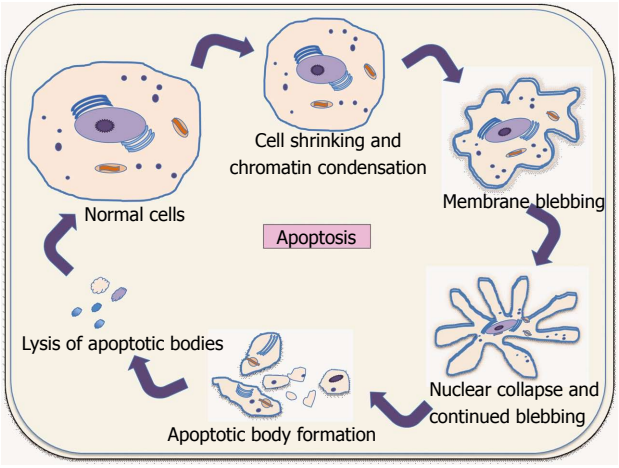


Figure 2 Apoptosis-programmed cell death.

APOPTOSIS

A normal cellular process involving physiologically relevant cell death and deletion of unwanted cells is called apoptosis. Apoptosis is essential for cell selection, tissue homeostasis, morphogenesis, and host defense in multicellular organisms. A cell that undergoes apoptosis dies neatly, without damaging its neighbors. The apoptotic signals give rise to activate various proteins and follow a specific classical caspase chain reaction set activation[51]. Quickly and neatly dismantlement process includes membrane blebbing with shrinking of the cytoplasm and condensation of the nucleus. Phagocytic cells begin to pick up the apoptotic bodies, preventing the release of cellular content and ultimately avoiding inflammation[52] (Figure 2). Apoptosis occurs by two mechanisms: active and passive mechanism. No presence of antigen gives a signal for termination of immune response by passive apoptotic mechanism (intrinsic pathway). On the other hand, the ligation of Fas (CD95) and TNF receptors-"death receptors" triggered apoptosis lead to active mechanism of apoptosis (extrinsic pathway). Briefly, apoptosis mechanisms involve a family of cysteine proteases, called caspases. These molecules are synthesized in the cell as inactive precursors, or pro-caspases for self-protection against accidental death, which are usually activated after receiving proper trigger by cleavage (Figure 3). Structurally, pro-caspases contain three domains: N terminal prodomain, a large subunit

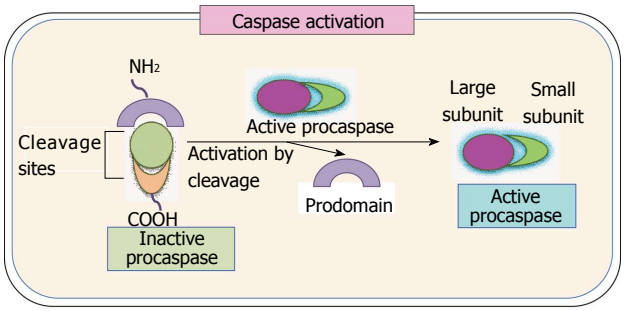


Figure 3 Caspase activation: Inactive proenzyme (procaspase) activated by proteolytic cleavage by another member of caspase family and cleaved two fragments associate to form the active site of the caspase.

and a small subunit. After activation, the active caspase enzyme is formed by heterodimerization of small and large subunits[43]. Moreover, active caspase molecules are ready to cleave target proteins such as structural or signaling proteins and other effector caspases, preventing other proteins cleavage randomly[52].

Extrinsic pathway

The extrinsic pathway initiates from outside the cell through triggering the activation of transmembrane "death receptors" that are members of the TNF receptor gene superfamily. Members of this receptor family bind to extrinsic ligands known as pro-apoptotic ligands[53] and transduce intracellular signals that ultimately result in the destruction of the cell[54,55]. To date the most well characterized ligands of these receptors are FasL, TNF-α, Apo3L and Apo2L and corresponding receptors are FasR, TNFR1, DR3 and DR4/DR5, respectively[55-57]. The signal transduction of active cell death process involves several caspases. Activated caspases have an effect on several cellular functions as part of the process that results in the death of the cells[53].

The signal transduction of mitochondrial-independent active cell death process involves binding of a pro-apoptotic ligand (such as FasL) with its receptors (Fas) on the surface of a target cell. The cytosolic tail of receptors contains a death domain, which when activated, binds to an adaptor protein, which in turn recruits the specific procaspase-8 and -10 and activates them by proteolytic cleavage[58] that finally initiates the proteolytic caspase cascade leading to apoptosis. Activated caspase 8 triggers the caspase cascade *via* two different pathways, leading to cell death. In type 1 apoptosis, such as in lymphocytes, caspase 8 activates caspase 3 whereas in type 2 apoptosis, like in hepatocytes and pancreatic cells, caspase 8 activate the pro-apoptotic molecule Bid and go ahead for apoptosis *via* the disruption of mitochondrial membrane and cytochrome C release[59] (Figure 4). The T cell death by type 1 and type 2 Fas induced apoptosis fate is decided by the ratio between proteolytically activated effector caspases, X-chromosome linked inhibitor of apoptosis protein and proto-typical effector caspase substrate inhibitor of caspase-activated DNase. Interestingly, HCV specific intrahepatic lymphocytes contribute to bystander killing *via* Fas-FasL interaction[60], which support the fact that the liver facilitates liver-trapped activated T cell apoptosis[61].

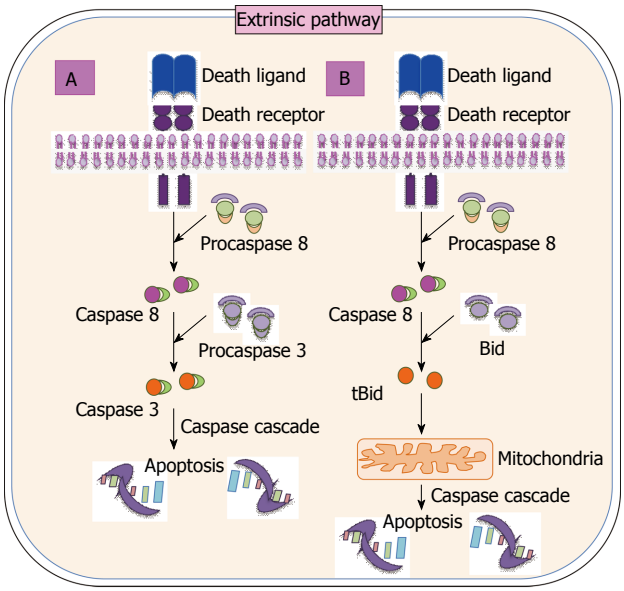


Figure 4 Extrinsic pathway. A: Mitochondria-independent extrinsic pathway: Fas-FasL ligation strikes to recruit pro-caspase 8 activation and induction of caspase cascade by caspase 3 leading to apoptosis; B: Mitochondria-dependent extrinsic pathway: Fas-FasL ligation trigger to activate the pro-caspase 8, which cleave Bid (pro-apoptotic Bcl-2 family molecule) to form truncated Bid (tBid). Then, mitochondrial dependent cell death begins with tBid.

Intrinsic pathway

The intrinsic or mitochondrial pathway is initiated within the cell, involving non-receptor-mediated intracellular signals and inducing activities in the mitochondria that initiate apoptosis. DNA damage, loss of cell-survival factors or other types of severe cell stress causes the induction signal for the intrinsic pathway. This passive death process pivots on the balance of activity between pro- and anti-apoptotic signals of the B cell lymphoma 2 (Bcl-2) family proteins[62]. This balance is maintained by regulation of the permeability of the mitochondrial membrane and by the pro- or anti-apoptotic signal that will be released inside the cell[63]. Following mitochondrial permeabilization, the intrinsic pathway divides into two pathways: Apoptosis protease-activating factor-1 (Apaf-1) dependent and Apaf-1 independent pathway. In Apaf-1 dependent pathway, release of cytochrome c from mitochondria, by triggering the pro-apoptotic Bcl-2 family member[64], and ATP activate monomer inactive Apaf-1 proteins by a conformational change, leading to form a heptamer of Apaf-1 molecules called apoptosome[65]. Apoptosome allows activation of pro-caspase 9, which consequently triggers the caspase cascade[66]. On the other hand, in Apaf-1 independent pathway, permeabilization of mitochondrial membrane release DIABLO like proteins, which activates effector caspases by provoking inhibitors of apoptosis proteins[67] and triggers caspase cascade[68] (Figure 5).

The balance of pro- and anti-apoptotic proteins main-

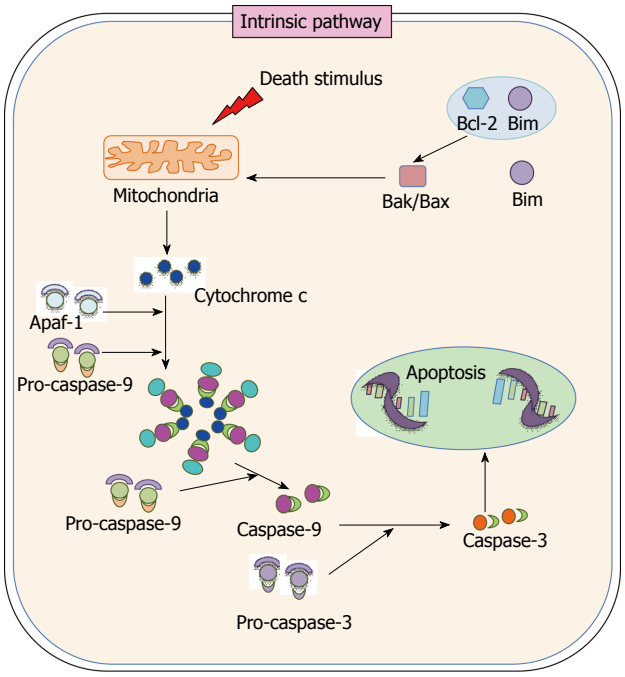


Figure 5 Intrinsic pathway. Death stimulation up regulates Bcl-2 interacting mediator leading to the separation from Bcl-2, favoring the activation of Bax, Bak, which form pores in the mitochondrial membrane leading to release of cytochrome c. Cytochrome c with Apaf-1 and procaspase 9 participate in the formation of apoptosome, which activate caspase 9. Caspase-9 activates caspase 3 after cleavage of pro-caspase-3. That caspase-3 triggers to induction of caspase cascade and cell death. Apaf-1: Apoptosis protease-activating factor-1. Bim: Bcl-2 interacting mediator.

tains the apoptotic activity[69]. The Bcl-2 family members regulate mostly neglect or intrinsic pathway. This family is subdivided into three groups of proteins on the basis of their functions and the number of Bcl-2 homology (BH) motifs included in their primary structure; first group: "anti-apoptotic multidomain" members, such as Bcl-xL, have four BH domains (BH1 to BH4) which inhibits apoptotic process. Other two groups of "pro-apoptotic multidomain" members, which are Bax-like proteins and "BH3-only" proteins[70]. Bax-like proteins possess threee BH domain (BH1 to BH3), including Bax, Bak, and Bok, which are referred as death effector members. BH3-only members contain BH3 domain, including Bim, Bad, Bik, Puma, Noxa and Bid and are known as messengers of death. In addition, C-terminal transmembrane (TM) fragment is thought to confer anchorage to mitochondrial membranes, which is also possessed by most multi-BH members and several BH3-only proteins.

Three models (Figure 6) have been postulated by which the BH3 family promotes passive cell death in which Bax and Bak bind directly or indirectly with cell death sensitizer (*e.g.*, Bad, Bik) and activators of cell death (*e.g.*, Bim, tBid). The direct activation model proposes that sensitizer BH3-only proteins displace the activator BH3-only proteins from the anti-apoptotic proteins to promote apoptosis. Anti-apoptotic proteins inhibit the activator BH3-only proteins but not Bax and Bak to suppress apoptosis. In the displacement model, Bax and Bak are sequestered by anti-apoptotic proteins for cell survival and constitutively active in cells. BH3-only proteins play the sensitizer role and inhibit their respective anti-apoptotic proteins to promote apoptosis. The third model, called embedded together model, highlights the interactions occurring in and on membranes, which were not explained by direct activation and displacement model. In embedded together model, Bcl-2 family proteins insert into and change their conformations according to their functions in membrane[71]. The predominantly studied messenger death molecule, Bcl-2 interacting protein (Bim) will be focused further.

BIM

Bim/Bod is a pro-apoptotic protein belonging to the BH3-only group of Bcl-2 family members and is being called the "ghost" molecule or "suicide" molecule, which enables cells to expire gracefully. Two independent studies discovered Bim as a Bcl-2 binding protein and Mcl1-binding protein in 1998[72,73]. Bim induces apoptosis by binding to and antagonizing anti-apoptotic members of the Bcl-2 family. The Bim interactions have been observed with Bcl-2 family members, such as Bcl-2, Bcl-xL, Mcl-1, Bcl-w, *etc*[72,73].

Bim is a well-known pivotal initiator of apoptosis in thymocyte-negative selection[74]. Bim has 19 Bim isoforms including three major isoforms, which have distinct sizes and pro-apoptotic activities in the mammals, caused by alternative splicing: BimEL (extra long), BimL (long) and BimS (small)[73]. The shortest form, BimS, is the most potent and is generally only transiently expressed during apoptosis[73]. The other two isoforms are sequestered to the dynein motor complex, and apoptotic activity of these longer isoforms is regulated by phosphorylation[75,76], which is triggered by environmental stress, resulting in its dissociation from the dynein complex and increasing apoptotic activity.

Expression of Bim is up regulated in human T cells in response to TCR-triggering by protein kinase C and calcineurin pathways[77]. Nevertheless, there are other mechanisms involved in Bim up-regulation during chronic infection, such as the effect of certain cytokines. In fact, in a persistent viral infection animal model, Bim-mediated apoptosis correlates with low IL-7 receptor expression on specific T cells[78].

The regulation of Bim expression at transcriptional level in growth factor deprivation and in endoplasmic reticulum stress has observed by the class O fork-head box transcription factor (FOX03A) and transcriptional factor CEPB-α respectively[79,80]. Post-transcriptional phosphorylation of Bim can also regulate its function. Phosphorylated Bim is targeted for proteasomal degradation and avoid its interaction with Bax, thus maintaining cell existence[81,82]. The signaling adaptor TNFR-associated factor 1 (TRAF1) negatively correlates with Bim and it contributes to CD8 T cell-mediated control of chronic viral infections. In addition, linking between survival

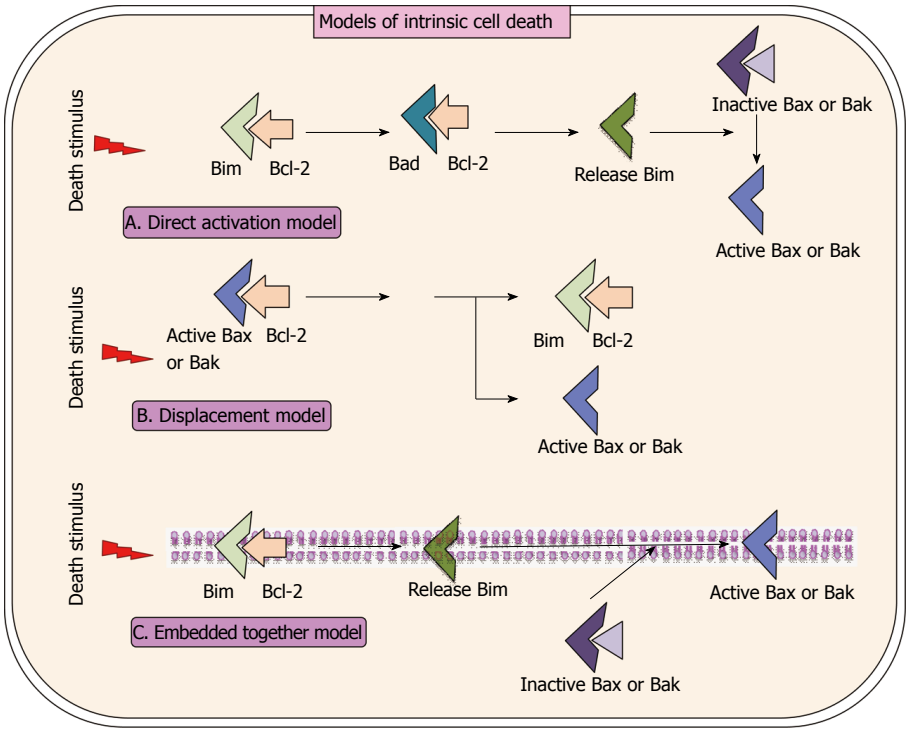


Figure 6 Models for intrinsic cell death. A: Direct activation model postulates Bcl-2 interacting mediator (Bim) is required for activating Bax and Bak. Anti-apoptotic proteins inhibit BH3-only proteins to suppress apoptosis, but not Bax or Bak. Replacement of Bim to sensitizer BH3-proteins from the anti-apoptotic proteins occurs to promote apoptosis; B: The displacement model proposes that anti-apoptotic proteins for cell survival must sequester constitutively active Bax and Bak in cells. Bim inhibits their respective anti-apoptotic proteins by playing sensitizer role to promote apoptosis; C: Embedded together model highlights the active role of the membrane, which is not defined in direct activation model and displacement model. Bcl-2 family proteins insert into and change their conformations that dictate their functions at the membrane. Sensitizer BH3-only proteins relocate the activator BH3-only proteins and Bax/Bak from the anti-apoptotic proteins to endorse apoptosis. Activator BH3-only proteins recruit Bax to the membrane to induce mitochondrial outer membrane permeabilization and apoptosis. These reversible interactions are directed by equilibrium constants that are depended on the concentrations and interactions of the proteins with each other and with membranes.

effects of TRAF1 and TRAF1-dependent Bim down-modulation has been shown in CD8 T cells[83-85]. TRAF1 is particularly vanished from virus-specific CD8 T cells during the chronic human immunodeficiency virus and lymphocytic chorio-meningitis virus (LCMV) infection[86].

Bim plays a vital role in the immune system, in bone biology and in tumor-genesis by inducing apoptosis[87]. Bim in T cells, B cells, neurons and many other cell types can trigger apoptosis[87]. Gene targeting in mice for the important region for apoptosis, BH3 region, uncovered the important physiological role in Bim[88]. In fact, in the absence of Bim leukocytes in blood as well as in LNs, thymus, spleen were high in number[88]. The role of Bim in apoptosis has been revealed in Bim-/- thymocytes, which were more resistant to apoptosis after different apoptotic treatment such as ionomycin, taxol, γ irradiation[88].

DEATH OF ACTIVATED T CELLS BY BIM

The liver is having a property that might explain its role in inducing tolerance due to its recognition as an alternative primary activation of CD8 T cells site. The phenotype of activated CD8 cells in the liver was the same as in lymph nodes. However, liver-activated CD8 T cells displayed poor effector functions and a unique $CD25^{low}$ $CD54^{low}$ phenotype, which was associated with increased expression of the Bim and caspase-3, demonstrating that these cells are programmed to apoptosis following intrahepatic activation. Strikingly, Bim deficient T cells survived following intrahepatic activation[1]. Therefore, the phenotype and fate of naïve CD8 T cells activated by hepatocytes *in vivo* could explain the death penalty role of Bim in chronic hepatotropic viral infection[1]. The distinct phenotype can be due to the lack of co-stimulatory molecule expression on hepatocytes[43]; however the treatment with IL-2 or anti-CD28 antibodies could rescue hepatocyte-activated cells from death[41].

Lymphocyte fate deciding pathways synergize to kill activated T cells in chronic herpes simplex viral immune responses, whereas death of activated T cells in acute immune responses relies only on the mitochondrial pathway involved only Bim with no contribution by Fas, which showed critical overlapping roles for Fas and Bim in T cell death during immune response shutdown, leading to immune tolerance[23].

BIM IN HEPATITIS

Bim has been shown to be important for CD8 T cell viability during chronic LCMV infection in mice[89]. In this study, in Bim mutated mice, Bim mutation almost completely blocked the deletion of cognate antigen specific CD8 T cells in liver during chronic viral infection. Bim has a critical role in maintaining naive and memory T cells in LCMV infection[90]. In another study, it has been shown that a defect in apoptosis dramatically not only enhances the antigen-specific memory T cells but also increased the number of virus-specific CD4+ T cells in the lymph nodes following acute LCMV infection, compared to the parental genotypes or wild type mice[91]. Therefore, the loss of both Bim and Fas caused the increase in memory T cells in acute LCMV infection[91]. The Bim role has been demonstrated in the development of LCMV-induced, T cell-mediated hepatitis by controlling the apoptosis of both T cells and hepatocytes[92].

Bim attrition of virus specific CTLs during HBV

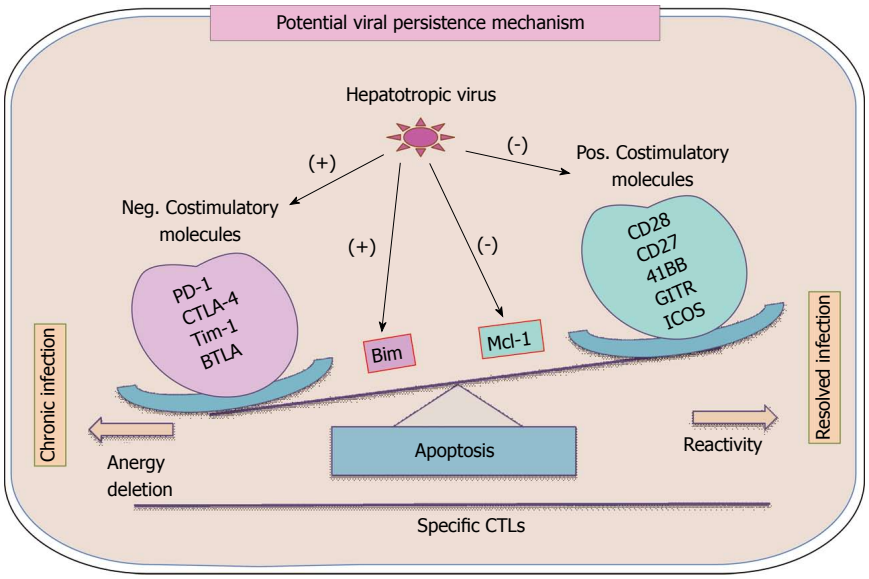


Figure 7 Balance between co-stimulatory/apoptotic molecules and viral-specific cytotoxic T lymphocytes reactivity according to infection outcome. Neg.: Negative; Pos.: Positive; CTLs: Cytotoxic T lymphocytes; (+): Possible molecules induced by viral infection; (-): Possible molecules down-regulated by viral infection; BIM: Bcl-2 interacting mediator; Mcl-1: Myeloid cell leukemia sequence-1.

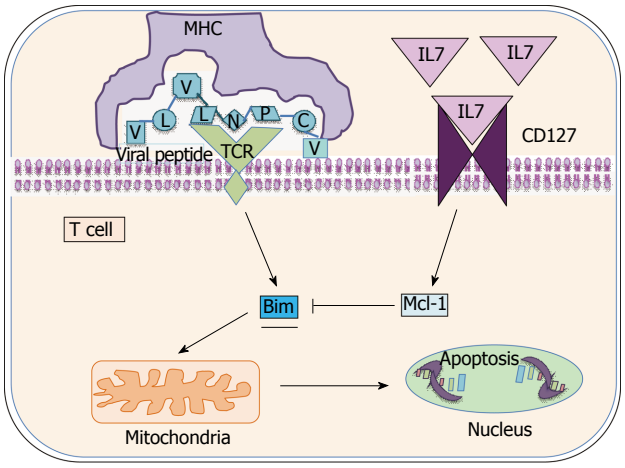


Figure 8 Cell survival marker CD127 modulates Bim and myeloid cell leukemia sequence-1 expression on hepatitis C virus-specific cytotoxic T lymphocytes after cognate antigen stimulation. Misbalance of Mcl-1/Bcl-2 interacting mediator (Bim) triggers to apoptosis of hepatitis C virus specific cytotoxic T lymphocytes. TCR: T cell receptor; Mcl-1: Myeloid cell leukemia sequence-1.

infection has also been confirmed[3,93]. The gene expression profile in HBV infection showed different patterns of gene expression on HBV-specific CD8+ T cells according to viral control. Bim was one of the up-regulated genes in HBV-specific CD8+ T cells from patients with chronic HBV infection. Blocking Bim-mediated apoptosis improved recovery of HBV-specific CD8+ T cell function[3]. Furthermore, the elevated apoptosis has been observed not only with Bim tolerogenic phenotype, but also with co-inhibitory signals through CTLA-4[93] or T cell-intrinsic transforming growth factor-β[94].

As discussed earlier, robust CD8 responses are essential to control HCV infection. However, in HCV chronic infection, HCV specific CD8 are depleted by Bim mediated attrition, and remaining cells are functionally exhausted. The cell survival factor CD127 counteracts the induction of apoptosis after antigen encounter through myeloid cell leukemia sequence-1 (Mcl-1) expression and Bim down-regulation[95] after the cognate antigen recognition by TCR. Similarly, our group has shown in previous work, HCV-specific CTLs displayed a high Bim expression in persistent infection respect to resolved infection patients[2], suggesting a similar apoptotic mechanism to the one described in chronic HBV infection.

The procedure of T cell death during chronic viral infection is determined by a carefully balanced and complex group of pro- and anti-apoptotic proteins of the Bcl-2 family, such as Bim and Mcl-1[96] (Figure 7). Interestingly, persistent hepatotropic viral infection is characterized by continuous TCR triggering and CD127 down-regulation on viral-specific CTLs[97], which could favor Bim up-regulation. In addition, it is well known that Bim is clearly involved in intrahepatic specific-CTL apoptosis in animal models[1]. Furthermore, Bim pro-apoptotic effect is blocked by the action of Bcl-2 family anti-apoptotic proteins such as Mcl-1 and Bcl-2[78,98], clearly pointing out that T cell death also depends on the anti-apoptotic protein expression. Bearing in mind all these facts, recently our group has suggested a model to explain specific CTL deletion during persistent hepatotropic viral infection (Figure 8). This model shows that CD127 phenotype modulates Bim and Mcl-1 expression on virus-specific CTLs, leading to Mcl-1/Bim imbalance during persistent infection, which impairs T cell reactivity and suggesting that restoration of T cell function could occur by correcting the levels of Mcl-1 and Bim expression.

In our work, Bim up-regulation has been observed on $CD127^{low}$-expressing HCV-specific CTLs but not on $CD127^{high}$ cells after antigen encounter, suggesting that TCR triggering can only lead to Bim up-regulation in absence of IL-7 stimulation on HCV-specific CTLs. Nevertheless, Bim level is not enough to lead to T cell apoptosis. Our data also showed the Mcl-1/Bim ratio could decide the fate of the activated T cells by sequestration of experienced $CD127^{low}$/$Mcl\text{-}1^{low}$-expressing T cells to the liver and subsequent Bim up-regulation after antigen encounter due to the absence of IL-7 stimulus[99]. Finally, Bim

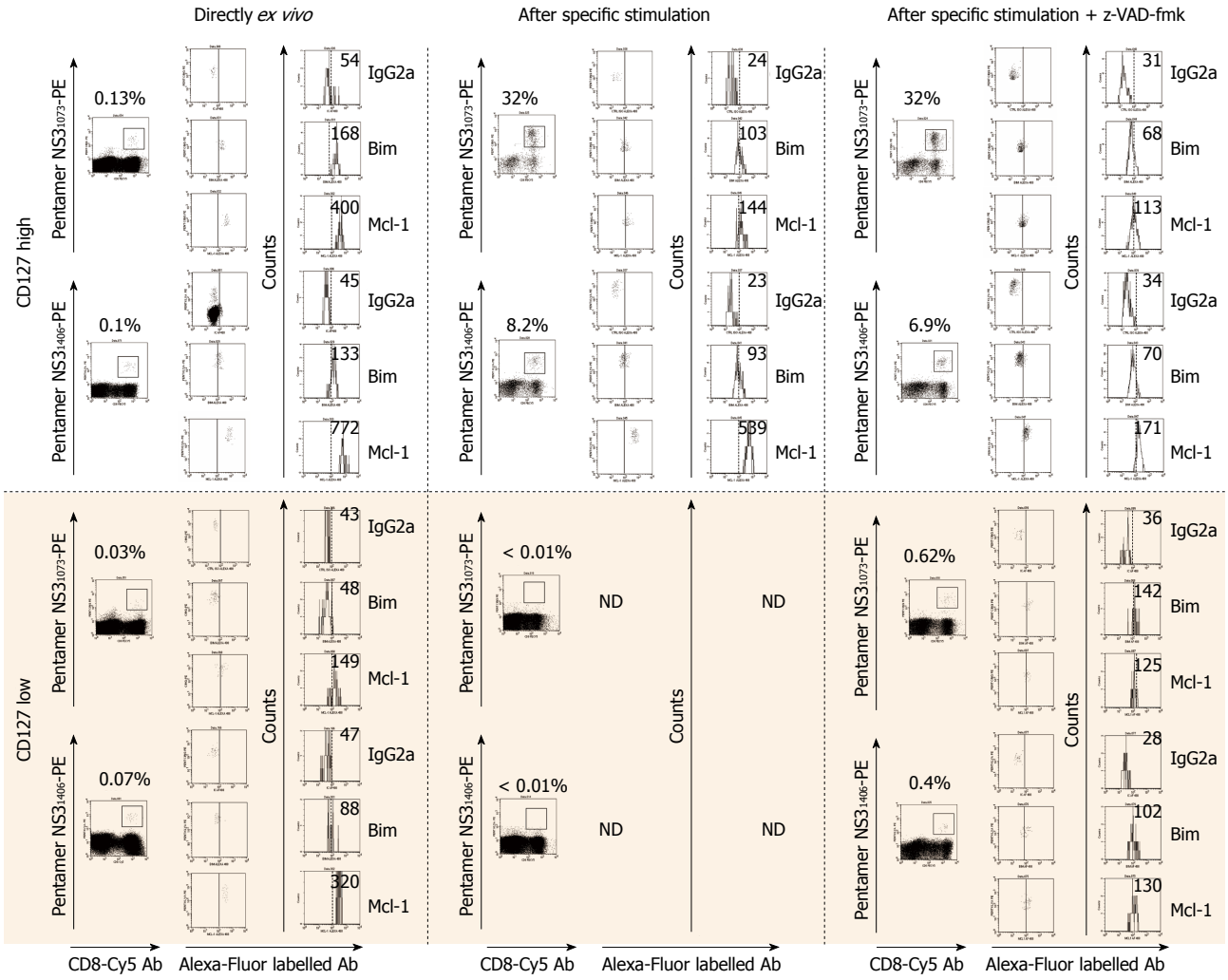


Figure 9 Figure illustrated the FACSan® dot plots and histograms from peripheral blood lymphocytes from two hepatitis C virus patients with different CD127 expression on hepatitis C virus-specific cytotoxic T lymphocytes (CD8+/Pentamer+ cells). The different plots show the Bcl-2 interacting mediator (Bim) and myeloid cell leukemia sequence-1 (Mcl-1) expression directly *ex vivo* and after specific stimulation on peripheral CD8+/pentameter+ cells according to CD127 level. The figure on the top of the dot-plots represents the frequency of penatmer+ cells out of total CD8+ cells. The figure in the upper right corner in the histogram plot represents the MFI for Bim and Mcl-1 staining. The continuous and dashed line in the dot-plots and histograms represents the cut-off point to consider a staining positive according to the negative control. ND: Not done due to lack of pentamer+/CD8+ proliferation after specific stimulation.

would be released freely to activate Bax, due to the low level of the anti-apototic protein Mcl-1 during chronic HCV infection. Consequently, CD127 level play a central role in hepatotropic virus-specific CD8+ T cell apoptosis by regulation of Mcl-1 expression *in vivo* and by Bim modulation after antigen encounter, which is checked by T cell reactivity restoration and Mcl-1/Bim phenotype on CD127^{low} specific CTLs after apoptosis blockade (Figure 9), that suggested a link between apoptosis after TCR triggering and low CD127 expression on experienced specific CTLs during persistent infection that could be related to Mcl-1/Bim imbalance.

Therefore, CD127 phenotype modulates Bim and Mcl-1 expression on specific CTLs and this affect to T cell reactivity through apoptosis regulation. Specifically, during chronic infection, Mcl-1/Bim imbalance could be involved on CD127^{low} specific CTL hyporeactivity, but it could be overcome by blocking apoptosis.

For control of hepatotropic viral infection is essential to develop a robust viral-specific cellular response. However, during chronic infection this response is altered, showing a pro-apoptotic phenotype due to the deprivation of IL-7 secondary to the low expression of CD127. Recently, it has been investigated that TRAF1 is a signal adapter for positive co-stimulatory receptors whose level depends on the action of IL-7 and inhibits the expression of the pro-apoptotic molecule Bim[86]. Therefore, in situations of deprivation of IL-7, action of TRAF1 could be impaired, favoring an imbalance between anti- and pro-apoptotic molecules. On the other hand, in an experimental model, IL-7 deprivation during stressing conditions leads to Mcl-1 down-regulation on T cells, conducting to T cell death that could be avoided by IL-7 treatment[100]. Consequently, strategies directed to block the pro-apoptotic effect of IL-7 deprivation should be designed to increase the effectiveness of CTL response restoration, by enhancing the TRAF1 and Mcl-1 expression level that could restore Bim/Mcl-1 balance. On of those strategies

could be short-term use of cyclosporine-A or FK506 could block the induction of the pro-apoptotic molecule Bim on CD127$^-$ cells[77]. This strategy could favor specific-CTL restoration during anti-viral treatments in combination with the standard of care. Another possible strategy to restore hepatotropic virus-specific CTL reactivity during chronic infection could be the administration of IL-7, in order to increase the stimulation of the reduced number of IL-7R molecules on specific CTLs, to modulate the balance between Bim and Mcl-1. In fact, in an animal model of cytotoxic T cell exhaustion, IL-7 treatment resulted in amplified cytokine production, increased T cell effector function, and viral clearance[101].

CONCLUSION

The deletion of hepatitis virus-specific CD8+ T cells is likely to represent the deregulation of the Bim pro-apoptotic pathway. The balance between pro- and anti-apoptotic molecules is critical for cell survival. The unavailability of appropriate survival marker modulates Bim and Mcl-1 expression on virus hepatitis-specific CTLs and this affect to T cell reactivity through apoptosis regulation. The level of those molecules is regulated by CD127 (IL-7R) expression, which is down-modulated during persistent infection. Consequently, Mcl-1/Bim imbalance could be the reason for the deletion of virus hepatitis-specific T cells, but it could be overcome by interruption of apoptosis. The interruption of this tolerizing mechanism may provide a new strategy to restore the balance between apoptotic molecules in order to achieve viral specific T cell immunity, as a future treatment strategy of chronic viral hepatitis.

REFERENCES

1 **Holz LE**, Benseler V, Bowen DG, Bouillet P, Strasser A, O' Reilly L, d'Avigdor WM, Bishop AG, McCaughan GW, Bertolino P. Intrahepatic murine CD8 T-cell activation associates with a distinct phenotype leading to Bim-dependent death. *Gastroenterology* 2008; **135**: 989-997 [PMID: 18619445 DOI: 10.1053/j.gastro.2008.05.078]

2 **Larrubia JR**, Benito-Martínez S, Miquel J, Calvino M, Sanz-de-Villalobos E, González-Praetorius A, Albertos S, García-Garzón S, Lokhande M, Parra-Cid T. Bim-mediated apoptosis and PD-1/PD-L1 pathway impair reactivity of PD1(+)/CD127(-) HCV-specific CD8(+) cells targeting the virus in chronic hepatitis C virus infection. *Cell Immunol* 2011; **269**: 104-114 [PMID: 21481848 DOI: 10.1016/j.cellimm.2011.03.011]

3 **Lopes AR**, Kellam P, Das A, Dunn C, Kwan A, Turner J, Peppa D, Gilson RJ, Gehring A, Bertoletti A, Maini MK. Bim-mediated deletion of antigen-specific CD8 T cells in patients unable to control HBV infection. *J Clin Invest* 2008; **118**: 1835-1845 [PMID: 18398508 DOI: 10.1172/JCI33402]

4 **Lavanchy D**. The global burden of hepatitis C. *Liver Int* 2009; **29** Suppl 1: 74-81 [PMID: 19207969 DOI: 10.1111/j.1478-3231.2008.01934.x]

5 **Tsukuma H**, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, Fujimoto I, Inoue A, Yamazaki H. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; **328**: 1797-1801 [PMID: 7684822 DOI: 10.1056/NEJM199306243282501]

6 **Wherry EJ**, Barber DL, Kaech SM, Blattman JN, Ahmed R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc Natl Acad Sci USA* 2004; **101**: 16004-16009 [PMID: 15505208 DOI: 10.1073/pnas.0407192101]

7 **Zajac AJ**, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 1998; **188**: 2205-2213 [PMID: 9858507 DOI: 10.1084/jem.188.12.2205]

8 **Gruener NH**, Lechner F, Jung MC, Diepolder H, Gerlach T, Lauer G, Walker B, Sullivan J, Phillips R, Pape GR, Klenerman P. Sustained dysfunction of antiviral CD8+ T lymphocytes after infection with hepatitis C virus. *J Virol* 2001; **75**: 5550-5558 [PMID: 11356962 DOI: 10.1128/JVI.75.12.5550-5558.2001]

9 **Xibing G**, Xiaojuan Y, Zhonghua L, Juanhua W. Alteration in cellular immunity after chronic hepatitis B deteriorated into severe hepatitis and its significance. *Hepat Mon* 2011; **11**: 810-815 [PMID: 22224079]

10 **Zinkernagel RM**, Haenseler E, Leist T, Cerny A, Hengartner H, Althage A. T cell-mediated hepatitis in mice infected with lymphocytic choriomeningitis virus. Liver cell destruction by H-2 class I-restricted virus-specific cytotoxic T cells as a physiological correlate of the 51Cr-release assay? *J Exp Med* 1986; **164**: 1075-1092 [PMID: 3489805 DOI: 10.1084/jem.164.4.1075]

11 **Sewell AK**, Price DA, Oxenius A, Kelleher AD, Phillips RE. Cytotoxic T lymphocyte responses to human immunodeficiency virus: control and escape. *Stem Cells* 2000; **18**: 230-244 [PMID: 10924089 DOI: 10.1634/stemcells.18-4-230]

12 **Dustin LB**, Rice CM. Flying under the radar: the immunobiology of hepatitis C. *Annu Rev Immunol* 2007; **25**: 71-99 [PMID: 17067278 DOI: 10.1146/annurev.immunol.25.022106.141602]

13 **Zhang P**, Zhong L, Struble EB, Watanabe H, Kachko A, Mihalik K, Virata-Theimer ML, Alter HJ, Feinstone S, Major M. Depletion of interfering antibodies in chronic hepatitis C patients and vaccinated chimpanzees reveals broad cross-genotype neutralizing activity. *Proc Natl Acad Sci USA* 2009; **106**: 7537-7541 [PMID: 19380744 DOI: 10.1073/pnas.0902749106]

14 **Maini MK**, Boni C, Lee CK, Larrubia JR, Reignat S, Ogg GS, King AS, Herberg J, Gilson R, Alisa A, Williams R, Vergani D, Naoumov NV, Ferrari C, Bertoletti A. The role of virus-specific CD8(+) cells in liver damage and viral control during persistent hepatitis B virus infection. *J Exp Med* 2000; **191**: 1269-1280 [PMID: 10770795 DOI: 10.1084/jem.191.8.1269]

15 **Fuller MJ**, Khanolkar A, Tebo AE, Zajac AJ. Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. *J Immunol* 2004; **172**: 4204-4214 [PMID: 15034033]

16 **Maini MK**, Boni C, Ogg GS, King AS, Reignat S, Lee CK, Larrubia JR, Webster GJ, McMichael AJ, Ferrari C, Williams R, Vergani D, Bertoletti A. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. *Gastroenterology* 1999; **117**: 1386-1396 [PMID: 10579980 DOI: 10.1016/S0016-5085(99)70289-1]

17 **Calne RY**, Sells RA, Pena JR, Davis DR, Millard PR, Herbertson BM, Binns RM, Davies DA. Induction of immunological tolerance by porcine liver allografts. *Nature* 1969; **223**: 472-476 [PMID: 4894426 DOI: 10.1038/223472a0]

18 **Crispe IN**, Giannandrea M, Klein I, John B, Sampson B, Wuensch S. Cellular and molecular mechanisms of liver tolerance. *Immunol Rev* 2006; **213**: 101-118 [PMID: 16972899 DOI: 10.1111/j.1600-065X.2006.00435.x]

19 **Huang L**, Soldevila G, Leeker M, Flavell R, Crispe IN. The liver eliminates T cells undergoing antigen-triggered apoptosis in vivo. *Immunity* 1994; **1**: 741-749 [PMID: 7895163 DOI: 10.1016/S1074-7613(94)80016-2]

20 **Chase MW**. Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. *Proc Soc Exp Biol Med* 1946; **61**: 257-259 [PMID: 21024163]

21 **Mehal WZ**, Juedes AE, Crispe IN. Selective retention of acti-

vated CD8+ T cells by the normal liver. *J Immunol* 1999; **163**: 3202-3210 [PMID: 10477588]
22 **Bowen DG**, McCaughan GW, Bertolino P. Intrahepatic immunity: a tale of two sites? *Trends Immunol* 2005; **26**: 512-517 [PMID: 16109501 DOI: 10.1016/j.it.2005.08.005]
23 **Hughes PD**, Belz GT, Fortner KA, Budd RC, Strasser A, Bouillet P. Apoptosis regulators Fas and Bim cooperate in shutdown of chronic immune responses and prevention of autoimmunity. *Immunity* 2008; **28**: 197-205 [PMID: 18275830 DOI: 10.1016/j.immuni.2007.12.017]
24 **Qian S**, Demetris AJ, Murase N, Rao AS, Fung JJ, Starzl TE. Murine liver allograft transplantation: tolerance and donor cell chimerism. *Hepatology* 1994; **19**: 916-924 [PMID: 8138266 DOI: 10.1002/hep.1840190418]
25 **Mackay CR**, Marston WL, Dudler L. Naive and memory T cells show distinct pathways of lymphocyte recirculation. *J Exp Med* 1990; **171**: 801-817 [PMID: 2307933 DOI: 10.1084/jem.171.3.801]
26 **Ando K**, Guidotti LG, Cerny A, Ishikawa T, Chisari FV. CTL access to tissue antigen is restricted in vivo. *J Immunol* 1994; **153**: 482-488 [PMID: 8021489]
27 **Bertolino P**, Bowen DG, McCaughan GW, Fazekas de St Groth B. Antigen-specific primary activation of CD8+ T cells within the liver. *J Immunol* 2001; **166**: 5430-5438 [PMID: 11313380]
28 **Limmer A**, Ohl J, Kurts C, Ljunggren HG, Reiss Y, Groettrup M, Momburg F, Arnold B, Knolle PA. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. *Nat Med* 2000; **6**: 1348-1354 [PMID: 11100119 DOI: 10.1038/82161]
29 **Knolle PA**, Schmitt E, Jin S, Germann T, Duchmann R, Hegenbarth S, Gerken G, Lohse AW. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. *Gastroenterology* 1999; **116**: 1428-1440 [PMID:10348827 DOI: 10.1016/S0016-5085(99)70508-1]
30 **Diehl L**, Schurich A, Grochtmann R, Hegenbarth S, Chen L, Knolle PA. Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8+ T cell tolerance. *Hepatology* 2008; **47**: 296-305 [PMID: 17975811 DOI: 10.1002/hep.21965]
31 **Racanelli V**, Rehermann B. The liver as an immunological organ. *Hepatology* 2006; **43**: S54-S62 [PMID: 16447271]
32 **You Q**, Cheng L, Kedl RM, Ju C. Mechanism of T cell tolerance induction by murine hepatic Kupffer cells. *Hepatology* 2008; **48**: 978-990 [PMID: 18712788 DOI: 10.1002/hep.22395]
33 **Probst HC**, McCoy K, Okazaki T, Honjo T, van den Broek M. Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4. *Nat Immunol* 2005; **6**: 280-286 [PMID: 15685176 DOI: 10.1038/ni1165]
34 **Pillarisetty VG**, Shah AB, Miller G, Bleier JI, DeMatteo RP. Liver dendritic cells are less immunogenic than spleen dendritic cells because of differences in subtype composition. *J Immunol* 2004; **172**: 1009-1017 [PMID: 14707074]
35 **Viñas O**, Bataller R, Sancho-Bru P, Ginès P, Berenguer C, Enrich C, Nicolás JM, Ercilla G, Gallart T, Vives J, Arroyo V, Rodés J. Human hepatic stellate cells show features of antigen-presenting cells and stimulate lymphocyte proliferation. *Hepatology* 2003; **38**: 919-929 [PMID: 14512879]
36 **Winau F**, Hegasy G, Weiskirchen R, Weber S, Cassan C, Sieling PA, Modlin RL, Liblau RS, Gressner AM, Kaufmann SH. Ito cells are liver-resident antigen-presenting cells for activating T cell responses. *Immunity* 2007; **26**: 117-129 [PMID: 17239632 DOI: 10.1016/j.immuni.2006.11.011]
37 **Benten D**, Kumaran V, Joseph B, Schattenberg J, Popov Y, Schuppan D, Gupta S. Hepatocyte transplantation activates hepatic stellate cells with beneficial modulation of cell engraftment in the rat. *Hepatology* 2005; **42**: 1072-1081 [PMID: 16250034 DOI: 10.1002/hep.20889]
38 **Chen CH**, Kuo LM, Chang Y, Wu W, Goldbach C, Ross MA, Stolz DB, Chen L, Fung JJ, Lu L, Qian S. In vivo immune modulatory activity of hepatic stellate cells in mice. *Hepatology* 2006; **44**: 1171-1181 [PMID: 17058227 DOI: 10.1002/hep.21379]
39 **Nishimura T**, Ohta A. A critical role for antigen-specific Th1 cells in acute liver injury in mice. *J Immunol* 1999; **162**: 6503-6509 [PMID: 10352265]
40 **Sakaguchi S**, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* 2008; **133**: 775-787 [PMID: 18510923 DOI: 10.1016/j.cell.2008.05.009]
41 **Bertolino P**, Trescol-Biémont MC, Thomas J, Fazekas de St Groth B, Pihlgren M, Marvel J, Rabourdin-Combe C. Death by neglect as a deletional mechanism of peripheral tolerance. *Int Immunol* 1999; **11**: 1225-1238 [PMID: 10421780 DOI: 10.1093/intimm/11.8.1225]
42 **Wiegard C**, Wolint P, Frenzel C, Cheruti U, Schmitt E, Oxenius A, Lohse AW, Herkel J. Defective T helper response of hepatocyte-stimulated CD4 T cells impairs antiviral CD8 response and viral clearance. *Gastroenterology* 2007; **133**: 2010-2018 [PMID: 17967458 DOI: 10.1053/j.gastro.2007.09.007]
43 **Bowen DG**, Zen M, Holz L, Davis T, McCaughan GW, Bertolino P. The site of primary T cell activation is a determinant of the balance between intrahepatic tolerance and immunity. *J Clin Invest* 2004; **114**: 701-712 [PMID: 15343389]
44 **Benseler V**, Warren A, Vo M, Holz LE, Tay SS, Le Couteur DG, Breen E, Allison AC, van Rooijen N, McGuffog C, Schlitt HJ, Bowen DG, McCaughan GW, Bertolino P. Hepatocyte entry leads to degradation of autoreactive CD8 T cells. *Proc Natl Acad Sci USA* 2011; **108**: 16735-16740 [PMID: 21933957 DOI: 10.1073/pnas.1112251108]
45 **Klugewitz K**, Blumenthal-Barby F, Schrage A, Knolle PA, Hamann A, Crispe IN. Immunomodulatory effects of the liver: deletion of activated CD4+ effector cells and suppression of IFN-gamma-producing cells after intravenous protein immunization. *J Immunol* 2002; **169**: 2407-2413 [PMID: 12193708]
46 **Lüth S**, Huber S, Schramm C, Buch T, Zander S, Stadelmann C, Brück W, Wraith DC, Herkel J, Lohse AW. Ectopic expression of neural autoantigen in mouse liver suppresses experimental autoimmune neuroinflammation by inducing antigen-specific Tregs. *J Clin Invest* 2008; **118**: 3403-3410 [PMID: 18802476]
47 **Goubier A**, Dubois B, Gheit H, Joubert G, Villard-Truc F, Asselin-Paturel C, Trinchieri G, Kaiserlian D. Plasmacytoid dendritic cells mediate oral tolerance. *Immunity* 2008; **29**: 464-475 [PMID: 18789731 DOI: 10.1016/j.immuni.2008.06.017]
48 **Bassett SE**, Di Bisceglie AM, Bacon BR, Sharp RM, Govindarajan S, Hubbard GB, Brasky KM, Lanford RE. Effects of iron loading on pathogenicity in hepatitis C virus-infected chimpanzees. *Hepatology* 1999; **29**: 1884-1892 [PMID: 10347134 DOI: 10.1002/hep.510290623]
49 **Thimme R**, Lohmann V, Weber F. A target on the move: innate and adaptive immune escape strategies of hepatitis C virus. *Antiviral Res* 2006; **69**: 129-141 [PMID: 16413618 DOI: 10.1016/j.antiviral.2005.12.001]
50 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-1406 [PMID: 11714747 DOI: 10.1084/jem.194.10.1395]
51 **Akiyama T**, Dass CR, Choong PF. Bim-targeted cancer therapy: a link between drug action and underlying molecular changes. *Mol Cancer Ther* 2009; **8**: 3173-3180 [PMID: 19934277 DOI: 10.1158/1535-7163.MCT-09-0685]
52 **Rathmell JC**, Thompson CB. The central effectors of cell death in the immune system. *Annu Rev Immunol* 1999; **17**: 781-828 [PMID: 10358774 DOI: 10.1146/annurev.immunol.17.1.781]
53 **Ashkenazi A**. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. *Nat Rev Cancer* 2002; **2**: 420-430 [PMID: 12189384 DOI: 10.1038/nrc821]

54 **Bazzoni F**, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996; **334**: 1717-1725 [PMID: 8637518 DOI: 10.1056/NEJM199606273342607]

55 **Elmore S**. Apoptosis: a review of programmed cell death. *Toxicol Pathol* 2007; **35**: 495-516 [PMID: 17562483 DOI: 10.1080/01926230701320337]

56 **Ashkenazi A**, Holland P, Eckhardt SG. Ligand-based targeting of apoptosis in cancer: the potential of recombinant human apoptosis ligand 2/Tumor necrosis factor-related apoptosis-inducing ligand (rhApo2L/TRAIL). *J Clin Oncol* 2008; **26**: 3621-3630 [PMID: 18640940 DOI: 10.1200/JCO.2007.15.7198]

57 **Roy S**, Nicholson DW. Cross-talk in cell death signaling. *J Exp Med* 2000; **192**: F21-F25 [PMID: 11034612 DOI: 10.1084/jem.192.8.F21]

58 **Fernandes-Alnemri T**, Armstrong RC, Krebs J, Srinivasula SM, Wang L, Bullrich F, Fritz LC, Trapani JA, Tomaselli KJ, Litwack G, Alnemri ES. In vitro activation of CPP32 and Mch3 by Mch4, a novel human apoptotic cysteine protease containing two FADD-like domains. *Proc Natl Acad Sci USA* 1996; **93**: 7464-7469 [PMID: 8755496 DOI: 10.1073/pnas.93.15.7464]

59 **Jost PJ**, Grabow S, Gray D, McKenzie MD, Nachbur U, Huang DC, Bouillet P, Thomas HE, Borner C, Silke J, Strasser A, Kaufmann T. XIAP discriminates between type I and type II FAS-induced apoptosis. *Nature* 2009; **460**: 1035-1039 [PMID: 19626005 DOI: 10.1038/nature08229]

60 **Gremion C**, Grabscheid B, Wölk B, Moradpour D, Reichen J, Pichler W, Cerny A. Cytotoxic T lymphocytes derived from patients with chronic hepatitis C virus infection kill bystander cells via Fas-FasL interaction. *J Virol* 2004; **78**: 2152-2157 [PMID: 14747581 DOI: 10.1128/JVI.78.4.2152-2157.2004]

61 **Crispe IN**, Dao T, Klugewitz K, Mehal WZ, Metz DP. The liver as a site of T-cell apoptosis: graveyard, or killing field? *Immunol Rev* 2000; **174**: 47-62 [PMID: 10807506 DOI: 10.1034/j.1600-0528.2002.017412.x]

62 **Fernàndez V**, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. *J Clin Oncol* 2005; **23**: 6364-6369 [PMID: 16155021 DOI: 10.1200/JCO.2005.05.019]

63 **Mayer B**, Oberbauer R. Mitochondrial regulation of apoptosis. *News Physiol Sci* 2003; **18**: 89-94 [PMID: 12750442]

64 **Kluck RM**, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 1997; **275**: 1132-1136 [PMID: 9027315 DOI: 10.1126/science.275.5303.1132]

65 **Acehan D**, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. *Mol Cell* 2002; **9**: 423-432 [PMID: 11864614 DOI: 10.1016/S1097-2765(02)00442-2]

66 **Li P**, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, Wang X. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. *Cell* 1997; **91**: 479-489 [PMID: 9390557 DOI: 10.1016/S0092-8674(00)80434-1]

67 **Hague A**, Paraskeva C. Apoptosis and disease: a matter of cell fate. *Cell Death Differ* 2004; **11**: 1366-1372 [PMID: 15354202 DOI: 10.1038/sj.cdd.4401497]

68 **Okada H**, Suh WK, Jin J, Woo M, Du C, Elia A, Duncan GS, Wakeham A, Itie A, Lowe SW, Wang X, Mak TW. Generation and characterization of Smac/DIABLO-deficient mice. *Mol Cell Biol* 2002; **22**: 3509-3517 [PMID: 11971981 DOI: 10.1128/MCB.22.10.3509-3517.2002]

69 **Oltvai ZN**, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 1993; **74**: 609-619 [PMID: 8358790 DOI: 10.1016/0092-8674(93)90509-O]

70 **Youle RJ**, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 2008; **9**: 47-59 [PMID: 18097445 DOI: 10.1038/nrm2308]

71 **Shamas-Din A**, Brahmbhatt H, Leber B, Andrews DW. BH3-only proteins: Orchestrators of apoptosis. *Biochim Biophys Acta* 2011; **1813**: 508-520 [PMID: 21146563 DOI: 10.1016/j.bbamcr.2010.11.024]

72 **Hsu SY**, Lin P, Hsueh AJ. BOD (Bcl-2-related ovarian death gene) is an ovarian BH3 domain-containing proapoptotic Bcl-2 protein capable of dimerization with diverse antiapoptotic Bcl-2 members. *Mol Endocrinol* 1998; **12**: 1432-1440 [PMID: 9731710 DOI: 10.1210/me.12.9.1432]

73 **O'Connor L**, Strasser A, O'Reilly LA, Hausmann G, Adams JM, Cory S, Huang DC. Bim: a novel member of the Bcl-2 family that promotes apoptosis. *EMBO J* 1998; **17**: 384-395 [PMID: 9430630 DOI: 10.1093/emboj/17.2.384]

74 **Bouillet P**, Purton JF, Godfrey DI, Zhang LC, Coultas L, Puthalakath H, Pellegrini M, Cory S, Adams JM, Strasser A. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. *Nature* 2002; **415**: 922-926 [PMID: 11859372 DOI: 10.1038/415922a]

75 **Lei K**, Davis RJ. JNK phosphorylation of Bim-related members of the Bcl2 family induces Bax-dependent apoptosis. *Proc Natl Acad Sci USA* 2003; **100**: 2432-2437 [PMID: 12591950 DOI: 10.1073/pnas.0438011100]

76 **Putcha GV**, Le S, Frank S, Besirli CG, Clark K, Chu B, Alix S, Youle RJ, LaMarche A, Maroney AC, Johnson EM. JNK-mediated BIM phosphorylation potentiates BAX-dependent apoptosis. *Neuron* 2003; **38**: 899-914 [PMID: 12818176 DOI: 10.1016/S0896-6273(03)00355-6]

77 **Sandalova E**, Wei CH, Masucci MG, Levitsky V. Regulation of expression of Bcl-2 protein family member Bim by T cell receptor triggering. *Proc Natl Acad Sci USA* 2004; **101**: 3011-3016 [PMID: 14970329 DOI: 10.1073/pnas.0400005101]

78 **Wojciechowski S**, Jordan MB, Zhu Y, White J, Zajac AJ, Hildeman DA. Bim mediates apoptosis of CD127(lo) effector T cells and limits T cell memory. *Eur J Immunol* 2006; **36**: 1694-1706 [PMID: 16761315 DOI: 10.1002/eji.200635897]

79 **Dijkers PF**, Medema RH, Lammers JW, Koenderman L, Coffer PJ. Expression of the pro-apoptotic Bcl-2 family member Bim is regulated by the forkhead transcription factor FKHR-L1. *Curr Biol* 2000; **10**: 1201-1204 [PMID: 11050388 DOI: 10.1016/S0960-9822(00)00728-4]

80 **Puthalakath H**, O'Reilly LA, Gunn P, Lee L, Kelly PN, Huntington ND, Hughes PD, Michalak EM, McKimm-Breschkin J, Motoyama N, Gotoh T, Akira S, Bouillet P, Strasser A. ER stress triggers apoptosis by activating BH3-only protein Bim. *Cell* 2007; **129**: 1337-1349 [PMID: 17604722 DOI: 10.1016/j.cell.2007.04.027]

81 **Harada H**, Quearry B, Ruiz-Vela A, Korsmeyer SJ. Survival factor-induced extracellular signal-regulated kinase phosphorylates BIM, inhibiting its association with BAX and proapoptotic activity. *Proc Natl Acad Sci USA* 2004; **101**: 15313-15317 [PMID: 15486085 DOI: 10.1073/pnas.0406837101]

82 **Luciano F**, Jacquel A, Colosetti P, Herrant M, Cagnol S, Pages G, Auberger P. Phosphorylation of Bim-EL by Erk1/2 on serine 69 promotes its degradation via the proteasome pathway and regulates its proapoptotic function. *Oncogene* 2003; **22**: 6785-6793 [PMID: 14555991 DOI: 10.1038/sj.onc.1206792]

83 **Sabbagh L**, Pulle G, Liu Y, Tsitsikov EN, Watts TH. ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival in vivo. *J Immunol* 2008; **180**: 8093-8101 [PMID: 18523273]

84 **Sabbagh L**, Srokowski CC, Pulle G, Snell LM, Sedgmen BJ, Liu Y, Tsitsikov EN, Watts TH. A critical role for TNF receptor-associated factor 1 and Bim down-regulation in CD8 memory T cell survival. *Proc Natl Acad Sci USA* 2006; **103**: 18703-18708 [PMID: 17116875 DOI: 10.1073/pnas.0602919103]

85 **Zhang B**, Wang Z, Li T, Tsitsikov EN, Ding HF. NF-kappaB2 mutation targets TRAF1 to induce lymphomagenesis. *Blood* 2007; **110**: 743-751 [PMID: 17405906 DOI: 10.1182/blood-2006-11-058446]

86 **Wang C**, McPherson AJ, Jones RB, Kawamura KS, Lin GH, Lang PA, Ambagala T, Pellegrini M, Calzascia T, Aidarus N, Elford AR, Yue FY, Kremmer E, Kovacs CM, Benko E, Tremblay C, Routy JP, Bernard NF, Ostrowski MA, Ohashi PS, Watts TH. Loss of the signaling adaptor TRAF1 causes CD8+ T cell dysregulation during human and murine chronic infection. *J Exp Med* 2012; **209**: 77-91 [PMID: 22184633 DOI: 10.1084/jem.20110675]

87 **Akiyama T**, Tanaka S. Bim: guardian of tissue homeostasis and critical regulator of the immune system, tumorigenesis and bone biology. *Arch Immunol Ther Exp* (Warsz) 2011; **59**: 277-287 [PMID: 21633919 DOI: 10.1007/s00005-011-0126-1]

88 **Bouillet P**, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Köntgen F, Adams JM, Strasser A. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999; **286**: 1735-1738 [PMID: 10576740 DOI: 10.1126/science.286.5445.1735]

89 **Grayson JM**, Weant AE, Holbrook BC, Hildeman D. Role of Bim in regulating CD8+ T-cell responses during chronic viral infection. *J Virol* 2006; **80**: 8627-8638 [PMID: 16912311 DOI: 10.1128/JVI.00855-06]

90 **Wojciechowski S**, Tripathi P, Bourdeau T, Acero L, Grimes HL, Katz JD, Finkelman FD, Hildeman DA. Bim/Bcl-2 balance is critical for maintaining naive and memory T cell homeostasis. *J Exp Med* 2007; **204**: 1665-1675 [PMID: 17591857]

91 **Weant AE**, Michalek RD, Crump KE, Liu C, Konopitski AP, Grayson JM. Defects in apoptosis increase memory CD8+ T cells following infection of Bim-/-Faslpr/lpr mice. *Cell Immunol* 2011; **271**: 256-266 [PMID: 21839428 DOI: 10.1016/j.cellimm.2011.07.003]

92 **Lauer C**, Brunner T, Corazza N. The proapoptotic Bcl-2 family member Bim plays a central role during the development of virus-induced hepatitis. *J Immunol* 2012; **188**: 916-922 [PMID: 22156338 DOI: 10.4049/jimmunol.1101864]

93 **Schurich A**, Khanna P, Lopes AR, Han KJ, Peppa D, Micco L, Nebbia G, Kennedy PT, Geretti AM, Dusheiko G, Maini MK. Role of the coinhibitory receptor cytotoxic T lymphocyte antigen-4 on apoptosis-Prone CD8 T cells in persistent hepatitis B virus infection. *Hepatology* 2011; **53**: 1494-1503 [PMID: 21360567 DOI: 10.1002/hep.24249]

94 **Tinoco R**, Alcalde V, Yang Y, Sauer K, Zuniga EI. Cell-intrinsic transforming growth factor-beta signaling mediates virus-specific CD8+ T cell deletion and viral persistence in vivo. *Immunity* 2009; **31**: 145-157 [PMID: 19604493 DOI: 10.1016/j.immuni.2009.06.015]

95 **Opferman JT**, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 2003; **426**: 671-676 [PMID: 14668867 DOI: 10.1038/nature02067]

96 **Holz LE**, Bowen DG, Bertolino P. Mechanisms of T cell death in the liver: to Bim or not to Bim? *Dig Dis* 2010; **28**: 14-24 [PMID: 20460886 DOI: 10.1159/000282060]

97 **Radziewicz H**, Ibegbu CC, Hon H, Osborn MK, Obideen K, Wehbi M, Freeman GJ, Lennox JL, Workowski KA, Hanson HL, Grakoui A. Impaired hepatitis C virus (HCV)-specific effector CD8+ T cells undergo massive apoptosis in the peripheral blood during acute HCV infection and in the liver during the chronic phase of infection. *J Virol* 2008; **82**: 9808-9822 [PMID: 18667503 DOI: 10.1128/JVI.01075-08]

98 **Jamil S**, Wang SW, Bondy L, Mojtabavi S, Duronio V. Prevention of cytokine withdrawal-induced apoptosis by Mcl-1 requires interaction between Mcl-1 and Bim. *Biochem Cell Biol* 2010; **88**: 809-818 [PMID: 20921992 DOI: 10.1139/O10-004]

99 **Larrubia JR**, Lokhande MU, García-Garzón S, Miquel J, González-Praetorius A, Parra-Cid T, Sanz-de-Villalobos E. Persistent hepatitis C virus (HCV) infection impairs HCV-specific cytotoxic T cell reactivity through Mcl-1/Bim imbalance due to CD127 down-regulation. *J Viral Hepat* 2013; **20**: 85-94 [PMID: 23301543 DOI: 10.1111/j.1365-2893.2012.01618.x]

100 **Dzhagalov I**, Dunkle A, He YW. The anti-apoptotic Bcl-2 family member Mcl-1 promotes T lymphocyte survival at multiple stages. *J Immunol* 2008; **181**: 521-528 [PMID: 18566418]

101 **Pellegrini M**, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, Shahinian A, Lang PA, Lang KS, Morre M, Assouline B, Lahl K, Sparwasser T, Tedder TF, Paik JH, DePinho RA, Basta S, Ohashi PS, Mak TW. IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. *Cell* 2011; **144**: 601-613 [PMID: 21295337 DOI: 10.1016/j.cell.2011.01.011]

P- Reviewer Camara NO **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1890

World J Gastroenterol 2013 March 28; 19(12): 1890-1900
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Clinical application of liver stiffness measurement using transient elastography in chronic liver disease from longitudinal perspectives

Beom Kyung Kim, James Fung, Man-Fung Yuen, Seung Up Kim

Beom Kyung Kim, Seung Up Kim, Department of Internal Medicine, Institute of Gastroenterology, Yonsei University College of Medicine, Seoul 120-740, South Korea
Beom Kyung Kim, Seung Up Kim, Liver Cirrhosis Clinical Research Center, Seoul 120-740, South Korea
James Fung, Man-Fung Yuen, Department of Medicine, State Key Laboratory for Liver Research, the University of Hong Kong, Hong Kong, China
Author contributions: Kim BK and Kim SU contributed to the study idea, study design, literature search, manuscript writing and final revision of the article; Fung J and Yuen MF contributed to manuscript writing and final revision of the article.
Supported by Liver Cirrhosis Clinical Research Center, in part by a grant from the Korea Healthcare technology R and D project, Ministry of Health and Welfare, Republic of Korea, No A102065; and by the Yonsei Liver Blood Bank, in part by a grant from sanofi-aventis Korea
Correspondence to: Seung Up Kim, MD, Department of Internal Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul 120-752, South Korea. ksukorea@yuhs.ac
Telephone: +82-2-22281982 Fax: +82-2-3936884
Received: August 10, 2012 Revised: August 29, 2012
Accepted: September 28, 2012
Published online: March 28, 2013

Abstract

Accurate determination of the presence and degree of fibrosis in liver is of great importance, because the prognosis and management strategies for chronic liver disease depend mainly on these factors. To date, liver biopsy (LB) remains the "gold standard" for assessing the severity of liver fibrosis; however, LB is often limited by its invasiveness, sampling error, and intra/inter-observer variability in histological interpretation. Furthermore, repeated LB examinations within a short time interval are indeed ineligible in a real clinical practice. Thus, due to the pressing need for non-invasive surrogates for liver fibrosis, transient elastography (TE), as a novel ultrasound based technology, has allowed a noninvasive measurement of liver stiffness and has gained in popularity over recent years. In the past few years, additional roles for transient TE beyond the initial purpose of a non-invasive surrogate for LB have included the prediction of the most two critical consequences of fibrosis progression: the development of portal hypertension-related complications and hepatocellular carcinoma. This indicates that the role of transient TE is not merely limited to reducing the need for LB, but transient TE can enable the establishment of tailored management strategies by providing more detailed prognostic information. In particular, under the concept in which the clinical course of liver fibrosis is dynamic and bidirectional, especially when appropriate intervention is commenced, transient TE can be used to track the dynamic changes in fibrotic burden during antiviral or antifibrotic treatment. This review discussed extended applications of transient TE in prediction of the development of real clinical endpoints from a longitudinal perspective.

Key words: Liver stiffness; Transient elastography; Fibroscan; Fibrosis; Longitudinal; Outcome

Kim BK, Fung J, Yuen MF, Kim SU. Clinical application of liver stiffness measurement using transient elastography in chronic liver disease from longitudinal perspectives. *World J Gastroenterol* 2013; 19(12): 1890-1900 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1890.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1890

INTRODUCTION

The prognosis and management of chronic liver disease (CLD) depend mainly on the amount and progression

of liver fibrosis, which is defined as the excessive accumulation of extracellular matrix proteins, resulting from chronic liver insults[1,2]. The initiation of its deposition is an important phase of CLD. As liver fibrosis eventually progresses without appropriate intervention, this process will lead to architectural change of the liver, followed by deterioration of liver function and hemodynamics, complications due to portal hypertension, and an increased tendency for hepatocarcinogenesis[3].

Thus, accurate determination of the presence and degree of liver fibrosis is of paramount importance in choosing treatment strategies, evaluating responses to treatment and the risks of developing liver-related complications, and predicting prognosis in patients with CLD. To assess the severity of liver fibrosis, liver biopsy (LB) remains the "gold standard". However, LB is often limited by its invasiveness and rare, but serious, complications, including bleeding, pneumothorax, and procedure-related death[4,5]. Moreover, repeated LB examinations within a short time interval are impractical. Additionally, concerning the reliability of pathological examinations, not only sampling error inherent in the percutaneous approach, but also intra- and inter-observer variability in histological interpretation may still occur[6]. Even if the LB is performed by an experienced physician and interpreted by an expert pathologist, it has an up to 20% error rate in disease staging[7,8].

Ideally, a method of evaluating liver fibrosis should accurately determine the presence of significant fibrosis, and be readily available, highly reproducible, and widely applicable to liver diseases of various etiologies. Although LB does not fulfil all these criteria, it has remained the gold standard, likely due to the absence of a better alternative. Recently, liver stiffness measurement using transient elastography (TE) was introduced as a promising non-invasive method for assessment of liver fibrosis[9-15]. In many studies, TE proved to be a reliable and accurate surrogate for LB in terms of prediction of significant fibrosis or cirrhosis[8,16-19]. In a large-scale meta-analysis including 50 studies, the mean areas under the receiver operating characteristic curves (AUROCs) for the diagnosis of significant fibrosis and cirrhosis were 0.84 and 0.94, respectively, with optimal cutoff values of 7.6 and 13.1 kPa, respectively[20].

Most studies to date have focused on assessing the performance of TE, reflected by AUROC, from a cross-sectional perspective, with reference to histological fibrosis. However, because LB as a reference standard is imperfect, it may have only limited clinical implications in terms of increasing the AUROC of TE to 1 (*i.e.*, perfect concordance with LB). Thus, additional roles for TE, namely prediction of long-term prognosis of the disease and monitoring clinical courses, have recently begun to attract attention. This indicates that the role of TE is not merely limited to lessening the frequency of unnecessary LB, but TE can also enable establishment of tailored management strategies by providing more detailed prognostic information[21]. In this regard, the "classical" end-points of "static" liver fibrosis in recent cross-sectional studies on TE are shifting to the "real and solid" end-points of the development of clinical events related to liver fibrosis progression, including hepatic decompensation, hepatocellular carcinoma (HCC), or liver-related death in a longitudinal study from a prospective cohort with long-term follow-up. Additionally, the performance of non-invasive methods is being judged and compared from this viewpoint.

In this article, we reviewed recent studies that focused on the prognostic value of TE for prediction of clinical end-points related to liver fibrosis progression, such as decompensation events, HCC development, or liver-related death, from a longitudinal perspective.

PREDICTION OF THE DEVELOPMENT OF LIVER-RELATED COMPLICATIONS

Portal hypertension-related complications

The development of portal hypertension is a common consequence of fibrosis progression, leading to the formation of esophageal and gastric varices responsible for variceal bleeding, and other severe complications, such as portosystemic encephalopathy, spontaneous bacterial peritonitis and sepsis[22-24]. Measurement of the hepatic venous pressure gradient (HVPG) is the gold standard for portal hypertension assessment in patients with cirrhosis; however, it is invasive and is routinely available only in experienced centers[25-29]. Although TE was initially proposed for assessment of liver fibrosis, a good correlation between TE values and HVPG has been reported, as well as the presence of esophageal varices, suggesting that it may be a valuable tool for the non-invasive evaluation of portal hypertension[30-32]. Subsequent studies have investigated correlations between TE values and the hepatic decompensation due to increased portal hypertension. A significant correlation between TE values and portal hypertension, expressed as the HVPG, was reported by Vizzutti *et al*[33] suggesting that TE may reflect a progressive rise in portal pressure due primarily to increased hepatic vascular resistance, caused by fibrillar extracellular matrix accumulation. Based on this concept, Foucher *et al*[34] first reported that cutoff values of 27.5, 37.5, 49.1, 53.7 and 62.7 kPa had > 90% negative predictive values for the presence of large esophageal varices (stage 2/3), Child-Pugh score B or C, past history of ascites, HCC and esophageal bleeding, respectively.

As variceal bleeding is a life-threatening complication of portal hypertension, the relationship between TE values and the presence of esophageal varices has been investigated in several studies[35-40]. All demonstrated a significant correlation between TE values and the presence of esophageal varices and that TE values could predict the presence of large varices (more than grade 2)[38,40]. Table 1 summarizes reports of the relationship between TE values and esophageal varices[33,38,40-44].

Although TE can predict the presence of esophageal varices and consequently assist in selection of candidates for endoscopic screening or prophylactic treatment, sever-

Table 1 Diagnostic performance of transient elastography for prediction of esophageal varices or large esophageal varices

Ref.	No. of patients (etiology)	Endpoints	AUROC	Cutoffs (kPa)	Sensitivity	Specificity	PPV	NPV
Vizzutti *et al*[33]	47 (HCV)	EV	0.76	17.6	90%	43%	77%	66%
Castéra *et al*[38]	70 (HCV)	EV	0.84	21.5	76%	78%	68%	84%
		Large EV	0.87	30.5	77%	85%	56%	94%
Kazemi *et al*[40]	165 (CLD)	EV	0.83	13.9	95%	43%	57%	91%
		Large EV	0.84	19.0	91%	60%	48%	95%
Bureau *et al*[41]	89 (CLD)	EV	0.85	21.1	84%	71%	NA	NA
		Large EV	0.76	29.3	81%	61%	NA	NA
Pritchett *et al*[42]	211 (CLD)	EV	0.74	19.5	76%	66%	56%	82%
		Large EV	0.76	19.8	91%	56%	91%	55%
Nguyen-Khac *et al*[43]	183 (CLD)	Large EV	0.76	48.0	73%	73%	44%	90%
	58 (HCV/HBV)	Large EV	0.73	19.8	89%	55%	27%	97%
	103 (alcohol)	Large EV	0.77	47.2	85%	64%	44%	93%
Malik *et al*[44]	124 (CLD)	EV	0.85	20.0	NA	NA	80%	75%

AUROC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value; CLD: Chronic liver disease; EV: Esophageal varix; HCV: Hepatitis C virus; HBV: Hepatitis B virus; NA: Not available.

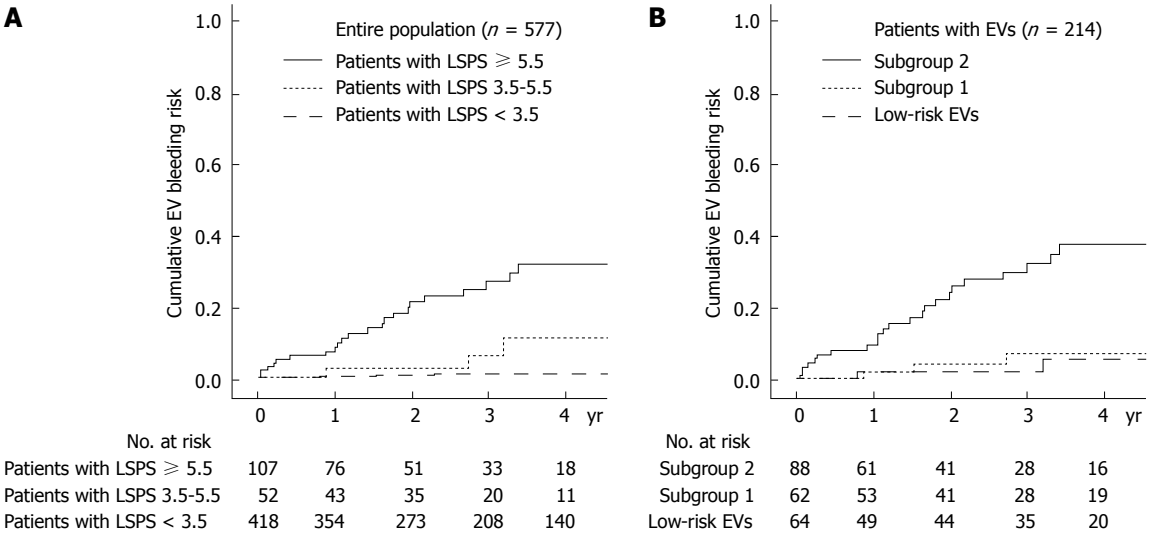


Figure 1 Cumulative incidences of variceal bleeding based on liver stiffness-spleen diameter to platelet ratio score values. A: The incidence of variceal bleeding increased significantly in association with higher liver stiffness-spleen diameter to platelet ratio score (LSPS) values (long-rank test, $P < 0.001$); B: In particular, among patients with high risk esophageal varices (EV), the incidence of variceal bleeding was significantly higher in patient with LSPS 6.5 (subgroup 2) than those with LSPS < 6.5 (subgroup 1).

al issues remain unresolved. First, the cutoff values (range, 13.9-21.5 kPa) and performance of TE varied (AUROC range, 0.76-0.85) among studies[38-40]. Second, from data currently available, diagnostic performances of TE are acceptable for the prediction of esophageal varices, but far from satisfactory for screening cirrhotic patients without endoscopy confidently. Thus, Kim *et al*[45] recently proposed a novel prediction model [liver stiffness-spleen diameter to platelet ratio score (LSPS)] to address this issue, achieving higher accuracy using TE values and other parameters simultaneously that reflect portal hypertension as constituent variables. Overall, this model had excellent diagnostic accuracy for the prediction of high-risk esophageal varices (HEV, AUROC = 0.953; negative predictive value 94.7%, positive predictive value 93.3%).

Beyond this cross-sectional analysis, a subsequent study by the same group recently showed that LSPS can be a reliable predictor of the development of variceal bleeding[20]. In this prospective, longitudinal study analyzing 577 patients with hepatitis B virus-related cirrhosis, those with LSPS ⩾ 5.5 had higher cumulative incidences of esophageal variceal bleeding during the follow-up period and LSPS score ⩾ 6.5 was an independent risk factor of variceal bleeding among those with HEV, indicating that further prophylactic treatment such as endoscopic ligation in addition to a non-selective beta-blocker should be considered in these high-risk patients (Figure 1). In a similar context, Kim *et al*[46] stratified the risk of hepatic decompensation, such as ascites, hepatic encephalopathy, variceal hemorrhage, and deterioration of liver function to Child-Pugh class B or C, based upon three classes of TE values (TE value < 13, 13-18 and ⩾ 18 kPa) in histologically proven hepatitis B virus-related cirrhosis with well-preserved liver function and no history of decompensation. In a multivariate analysis, patients with a TE value of 13-18 kPa [hazard ratio (HR), 4.547; $P = 0.044$] and ⩾ 18

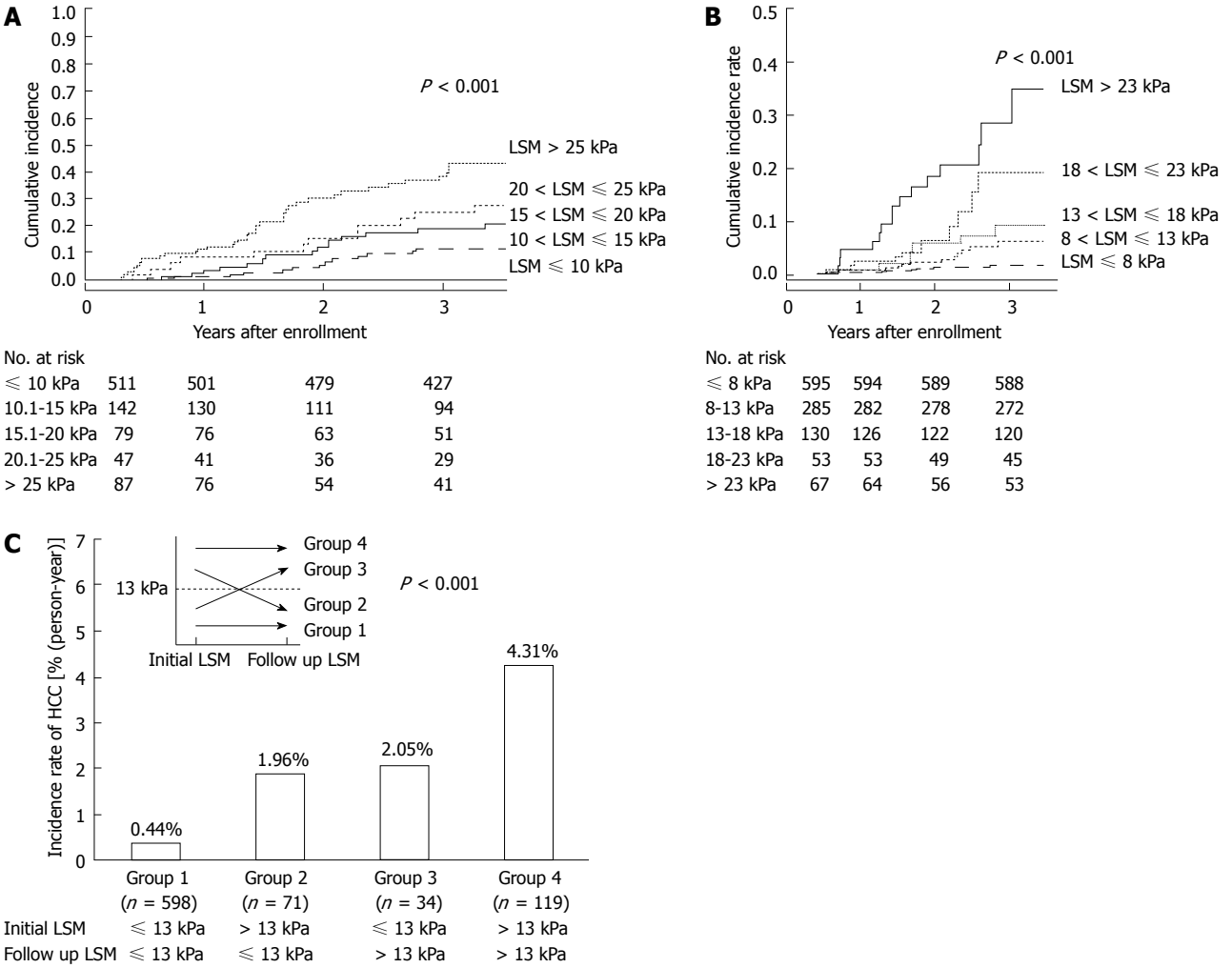


Figure 2 Cumulative incidence of hepatocellular carcinoma development based on stratified transient elastography values in patients with chronic hepatitis C (A, *n* = 866) and those with chronic hepatitis B (B, *n* = 1130). The cumulative incidences increased significantly in association with higher TE values (log-rank test, all $P < 0.001$). In particular, the overall incidence of HCC differed significantly among the four groups (C) (both initial and follow-up TE values ⩽ 13 kPa (group 1), initial TE value > 13 kPa and follow-up TE value ⩽ 13 kPa (group 2), initial TE value ⩽ 13 kPa and follow-up TE value > 13 kPa (group 3), and both initial and follow-up TE values > 13 kPa (group 4) according to changing patterns of TE value during follow-up ($P < 0.001$; Figure 2C). A: Cited from Masuzaki *et al*[52]; B and C: Cited from Jung *et al*[53]. HCC: Hepatocellular carcinoma; TE: Transient elastography; LSM: Liver stiffness measurement.

kPa (HR, 12.446; $P < 0.001$) showed independently higher risks than patients with TE value < 13 kPa.

HCC

Another promising area for the application of TE, other than portal hypertension-related decompensation events, is the prediction of HCC development. Unless HCC is diagnosed at an early stage, a poor prognosis is expected due to the limited treatment options[47-51]. Thus, early prediction of HCC development is of great importance, especially in high-risk patients. Among traditional risk factors, advanced liver fibrosis and cirrhosis is known to have a close association with risk of HCC development[47]. Thus, assessment of the severity of liver fibrosis at a given time point with subsequent monitoring of liver fibrosis progression by serial check-up is essential for effective and optimized surveillance strategies for the early detection of HCC[3].

Recently, several Asian studies have investigated the clinical role of TE in the noninvasive prediction of HCC development[52-56]. The first large prospective cohort study of 866 Japanese patients with chronic hepatitis C (CHC) tested whether TE can predict the future development of HCC[52]. During a mean follow-up of 3 years, 77 patients developed HCC. By multivariate analysis, together with age, male gender, and clinical cirrhosis, stratified TE value was identified as an independent risk factor for HCC development, with relative risks of 16.7, 20.0, 25.6 and 45.5 for TE values of 10-15, 15-20, 20-25 and > 25 kPa, respectively, *vs* an TE value of < 10 kPa as the reference and the cumulative incidence of HCC showed a stepwise increase according to stratified TE value (Figure 2A). Despite there being no histological analysis in relation to TE values and inclusion of patients with high alanine aminotransferase (ALT) levels [> 5× upper limit of normal (ULN)] both of which can attenuate the accuracy of TE, this study confirmed that severity of liver fibrosis, reflected by higher TE values, was closely associated with higher

risk of HCC development and suggested a clinical role for TE in a longitudinal setting using HCC development as a solid clinical endpoint. Interestingly, in this study, even patients with not so high level of TE (10-15 kPa) were still more subject to HCC development with an adjusted HR of 16.7, compared to those with a TE value < 10 kPa.

Another large Korean cohort study with 1130 patients with chronic hepatitis B (CHB) also confirmed the longitudinal role of TE on HCC development[53]. Together with age, male gender, heavy alcohol consumption, lower serum albumin, and HBeAg positivity, stratified TE value was identified as an independent risk factor for HCC development, with relative risks of 3.07, 4.68, 5.55 and 6.60 for liver stiffness measurement (LSM) values of 8-13, 13-18, 18-23 and > 23 kPa, respectively, when compared with a LSM value of < 8 kPa as a reference (Figure 2B). In contrast to the Japanese study[52], several additional issues were further analyzed in this Korean study. First, when the diagnosis of cirrhosis showed discordant results between TE-based and clinical-based criteria, patients with cirrhosis based on TE were at a higher risk of HCC development than those with cirrhosis based on clinical criteria, indicating the superiority of TE for diagnosis of compensated liver cirrhosis. Second, patients with TE values below the cutoff level for cirrhosis, 8-13 kPa, had a higher relative risk of HCC development than those with LSM values < 8 kPa. Although this finding should be validated in large prospective studies, the issue of expansion of the high-risk group for HCC surveillance to include those with significant fibrosis was raised by this study. Furthermore, when patients with available follow-up TE values were analyzed, the risk of HCC development changed according to the pattern of the changes in TE values, suggesting a potential role for serial measurements of TE as a dynamic monitoring tool for risk estimation of HCC development (Figure 2C). However, other confounding factors including lack of histological information, insensitive HBV DNA tests, and heterogeneity in antiviral treatment should be noted when interpreting these results. Recently, Chon *et al*[56] compared the performance of various noninvasive fibrosis prediction methods [aspartate aminotransferase-to-platelet ratio index (ARRI), age-spleen-to-platelet ratio index (ASPRI), TE, LSPS, P2/MS and FIB-4] for prediction of HCC development in patients with CHB and concluded that TE and LSPS showed the best performance (AUROC = 0.789 and 0.788, respectively). Using multivariate analyses, TE and LSPS were identified as independent predictors of HCC development.

In another study[54] from Hong Kong, which followed up 528 patients with HBeAg negative CHB for a median length of 35 mo and identified seven patients with HCC development, the cumulative incidence of HCC was higher in patients with TE values ≥ 10 kPa than those with TE values < 10 kPa (9% *vs* 0%, respectively; $P < 0.001$), and the cumulative liver-related mortality was also higher in patients with TE values < 10 kPa compared with those with TE values ≥ 10 kPa (4% *vs* 0%, respectively; $P < 0.001$). By multivariate analysis, only TE value was significantly associated with HCC development and liver-related mortality.

Similarly, Kim *et al*[55] investigated the prognostic role of TE in predicting the development of overall liver-related events (LREs), defined as development of HCC, hepatic decompensation, or liver-related mortality, among 128 patients with CHB showing histologically advanced liver fibrosis (≥ F3) and high viral loads (HBV DNA ≥ 2000 IU/mL) before starting nucleos(t)ide analogs. When the study population was stratified into two groups using the optimal cutoff value (19 kPa), patients with TE values > 19 kPa were at significantly greater risk for LRE development than those with TE values ≤ 19 kPa (HR, 7.176; $P = 0.001$). Moreover, the incidence of LREs was similar in patients with F3 and F4 (22.2% *vs* 13.6%; $P = 0.472$); however, it differed significantly between patients with TE values ≤ 19 kPa and those with TE values > 19 kPa (6.9% *vs* 44.4%; $P < 0.001$), indicating the superior performance of TE to that of histology in prediction of LRE development.

Apart from predicting HCC development, the application of TE was validated in a study by Vergniol *et al*[57], in which 1457 patients with CHC were followed up; 5-year survival outcomes worsened as TE values increased. The prognostic values of TE were demonstrated to be statistically significant ($P < 0.0001$) after adjustment for other important factors, including treatment response, patient age, and estimates of necroinflammatory grade. For example, the 5-year overall survival was 96% in patients with TE value < 9.5 kPa, and 47% in patients with TE value > 40 kPa.

Overall, TE has shown the potential for a clinical role in predicting the development of portal hypertension-related hepatic decompensation and/or HCC and, in part, demonstrated superior performance to histology and other noninvasive tools[41,58-63]. This is most likely due to the wider dynamic range of TE values in the evaluation of liver cirrhosis. In fact, as the stage of "cirrhosis" has to date been defined by histopathological evidence of one or two qualitative categories (METAVIR stage F4 or ISHAK S5-S6), or more generally by the presence of so-called "regenerative" or "cirrhotic nodules", an interval scale cannot be used in this setting[64-66]. However, the degree of liver fibrosis may vary widely among patients in this category, and the risk of hepatic decompensation and HCC may not be uniform. Thus, in this regard, because TE value, expressed in kPa as a continuous variable, has a wide dynamic range within the cirrhotic stage from the cutoff level from non-cirrhosis (15-17 kPa) to the upper measurement limit of present devices (75 kPa), it would seem to be a more reasonable tool for detailed prognostication.

UTILITY OF TE IN THE SURGICAL SETTING

Because TE values show significant correlations with portal hypertension and HCC development, prediction

of postoperative short-term outcomes, such as hepatic insufficiency, and long-term outcomes, such as recurrence or liver-related death using TE has been tested in several pilot studies[67-69]. Although further studies are required to validate these results, TE may facilitate stratification of patients undergoing curative resection according to different prognoses.

In the first place, Kim *et al*[67] investigated whether preoperative TE values could predict the development of postoperative hepatic insufficiency after curative resection of HCC. In this study, multivariate analyses revealed that a TE value > 25.6 kPa was the only predictor of postoperative insufficiency. The AUROC of 25.6 kPa was higher than that of indocyanine green R15, which is a popular method for assessment of preoperative functional reserve liver function (0.824 *vs* 0.620, respectively). Similar results were obtained in a subsequent investigation by the same group[68]. In this study, the performance of TE was superior to that of diffusion-weighted magnetic resonance imaging, which has also been shown to be a noninvasive fibrosis prediction tool for the assessment of liver fibrosis and the prediction of postoperative hepatic insufficiency.

Another issue is prediction of HCC recurrence after curative resection, that is, *de novo* recurrence in the background liver with fibrotic burden, using preoperative TE. In an analysis of 133 patients who underwent preoperative TE and curative resection (HCC recurred in 62 patients), TE was selected as an independent predictor of recurrence, whereas histological fibrosis status was not[69]. In the study, patients with preoperative TE values > 13.4 kPa were at a greater risk of recurrence, with an HR of 1.925 ($P = 0.010$). More specifically, when recurrence was stratified into early (< 2 years) and late (≥ 2 years), TE values were significantly related to late recurrence, thus supporting the hypothesis. These results suggest that preoperative TE could reveal the potential influence of liver fibrosis on recurrence and explain multicentric carcinogenesis in a fibrotic liver. However, more data are needed to clarify this issue.

ROLE OF TE IN MONITORING FIBROTIC BURDEN DURING ANTIVIRAL THERAPY

Recently, the concept of "cirrhosis" has changed from static and uncompromisingly progressive to rather dynamic and bidirectional, especially when treatment against the causative agent of tissue damage (*i.e.*, antiviral agents against CHB or CHC and antifibrotic agents) can be introduced successfully at this stage of the disease. The ideal approach to evaluate histological outcomes during antiviral therapy, such as fibrosis regression and necroinflammation stabilization, is serial LB examinations. However, this is impractical, primarily due to the inherent invasiveness of LB. Instead, because of the ease, safety, and rapidity of TE, it may be useful for monitoring the dynamic changes in liver fibrosis during antiviral or antifibrotic treatment. Indeed, several studies have reported the clinical usefulness of TE for monitoring potential fibrosis regression during antiviral treatment in patients with CHC and CHB[57,70-77].

Kim *et al*[71] analyzed 41 patients with CHB who received antiviral treatment using nucleos(t)ide analogs. To prevent the confounding effect of high ALT, patients with high ALT levels more than 2× ULN, were excluded. Although ALT levels did not show a statistically significant change during the first 12 or 24 mo of antiviral treatment, TE values decreased significantly, indicating potential fibrosis regression due to prolonged antiviral treatment. Indeed, fibrosis regression and stabilization of necroinflammation was noted in two patients with available paired LBs. Enomoto *et al*[70] reported the changes in LSM values during the first 12 mo of entecavir treatment in 20 patients. Median TE values decreased significantly from 11.2 to 7.8 kPa after 12 mo of treatment, and serum fibrosis markers, such as PIIINP and type Ⅳ collagen 7S domain, also decreased significantly. In one patient with available paired LBs, histological fibrosis regression and stabilization of necroinflammation were noted. Although these studies suggest a role for TE for monitoring fibrosis regression due to prolonged antiviral treatment, the short duration of observation and small sample sizes with paired biopsies are major limitations of these studies.

Recently, data regarding a longer antiviral treatment duration (more than 3 years) have become available[72-74,78]. Fung *et al*[72] reported a significant decline in TE values from baseline after subsequent ALT normalization with 3-year treatment ($n = 110$, 7.8 to 6.1 kPa; $P = 0.002$). In this study, independent factors associated with a significant decline in TE value of ≥ 1 kPa included antiviral therapy and ALT levels at the follow-up time point. Another study by Andersen *et al*[78] also noted significant declines in TE values after a median antiviral treatment duration of 50.5 mo ($n = 66$), and concluded that prolonged antiviral treatment in patients with CHB resulted in significant declines in TE values, suggesting regression of fibrosis in a majority of patients with advanced fibrosis or cirrhosis.

Likewise, for patients with CHC, changes in TE values during antiviral treatment have been investigated in several studies. Two prospective studies by Vergniol *et al*[57] and Ogawa *et al*[75] demonstrated that patients with CHC showing sustained virological responses to pegylated interferon-ribavirin combination therapy had significantly reduced TE values at the end of follow-up. Moreover, Ogawa *et al*[75] reported that patients with non-sustained virological responses, but with a biochemical response, showed a greater reduction in TE values than did those with a non-biochemical response. Subsequent studies reported similar results, suggesting that changes in TE values during antiviral treatment in patients with CHC may represent alterations in the severity of liver fibrosis[76,77]. However, it should be further confirmed whether the favorable changes in LSM values during or after antiviral treatment does have a significant influecne

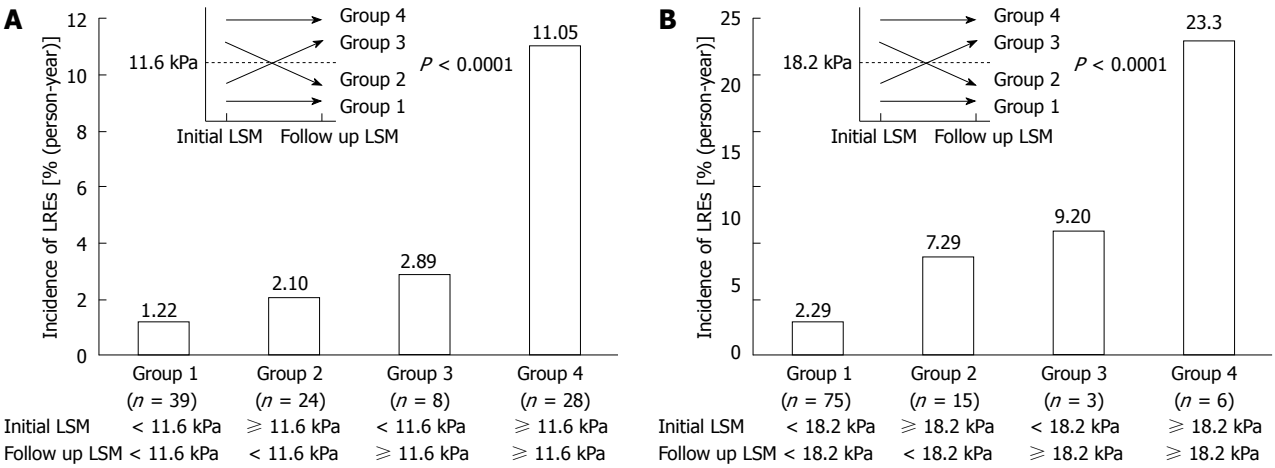


Figure 3 Incidence of liver-related events according to changes in transient elastography values after 6 mo of antiviral therapy. The overall incidence of liver-related events differed significantly among the four groups using TE value cutoffs of 11.6 kPa (A) and 18.2 kPa (B) (both $P < 0.0001$). Adapted from Kim *et al*[79]. TE: Transient elastography; LSM: Liver stiffness measurement; LREs: Liver-related events.

on the long-term prognosis such as disease-specific survival in patients with CHC.

Taken together, TE value seems to decrease during and after antiviral therapy. However, without paired histological results through repeated LB, whether the reduction in TE values is closely correlated with regression of liver fibrosis or improvement in necroinflammatory scores remains unclear. To clarify this, Lim *et al*[73] investigated patterns of TE values among patients who were treated with entecavir. In all subjects, the median TE value at baseline was 15.1 (range, 5.6-75.0) kPa and decreased significantly, to 8.8 (range, 3.0-33.8) kPa after 12 mo of therapy, and a decrease in TE values correlated significantly with increase in albumin, decrease in bilirubin, decrease in ALT level, and decrease in aspartate aminotransferase levels (all $P < 0.05$). However, among 15 patients with available paired LBs, decreases in TE values were correlated significantly with improved necroinflammatory scores, but not with fibrosis regression. Similarly, Wong *et al*[74] insisted that the decline in absolute TE values during antiviral treatment did not reflect the change in histologically assessed liver fibrosis, probably due to the confounding influence of ALT reduction caused by antiviral treatment.

However, regardless of whether TE values during antiviral treatment are due to fibrosis regression, activity stabilization, or both, changes in TE value during antiviral treatment can be translated into the overall response of chronically diseased liver to antiviral treatment from the viewpoint of its long-term clinical implications. Thus, it is more logical to investigate whether the decline in TE value can be used as a favorable predictor of long-term prognosis. Encouraging results were recently published by Jung *et al*[53] suggesting that the change in TE values in patients with CHB showed a significant correlation with differential future risk of HCC development. Additionally, Kim *et al*[79] insisted that changes in TE values were significantly associated with the difference risk of liver-related event occurrence, such as hepatic decompensation, HCC development, and LREs (Figure 3). This would suggest that the assessment of overall background liver status using TE may be an important end-point in the management of CHB and prediction of long-term outcomes. Further research is needed to evaluate the reproducibility of such findings in independent populations.

LIMITATIONS OF TE

Although TE has demonstrated reliable diagnostic accuracy with excellent inter-observer and intra-observer agreement, additional space-occupying tissue abnormalities, such as edema and inflammation, cholestasis, and congestion may interfere with TE, regardless of the degree of liver fibrosis, because the liver is wrapped in a distensible, but non-elastic, envelope (Glisson's capsule)[80].

First, the extent of histological necroinflammatory activity has been shown to influence TE results in patients with viral hepatitis, resulting in an overestimation of TE values that increases in parallel with the degree of necroinflammatory score[81-85]. Consistent with these results, a risk of overestimation of TE values has been reported in cases of ALT flares in patients with acute viral hepatitis or CHB[86-92]. Thus, in such subjects, TE examinations should be delayed until ALT levels have stabilized. In this regard, several studies have investigated the optimal period (3 to 6 mo) for restoration of the reliability of TE values in patients with acute flares[88,91,93,94]. Furthermore, even mild to moderate elevation in ALT can be associated with higher liver stiffness values, and may cause discrepancies between TE results and the actual underlying fibrosis. Apart from necroinflammation, extrahepatic cholestasis[95] and congestive heart failure[96-98] may also contribute to the overestimation of TE.

Additionally, the performance of TE may be limited in patients with a high body mass index (BMI), narrow intercostal space, or ascites[9]. Although TE reproducibility has been shown to be excellent in terms of inter-observer and intra-observer agreement, a high BMI (> 28

Table 2 Proposal of application of transient elastography in each clinical setting

Clinical setting	Role of TE
Prediction of portal hypertension	TE with platelet counts and spleen size complementary to HVPG
Prediction of esophageal varices	TE with platelet counts and spleen size complementary to endoscopy
Prediction of developing esophageal variceal bleeding	TE with platelet counts and spleen size
Prediction of developing portal hypertension-related complications	TE with platelet counts and spleen size
Prediction of developing hepatocellular carcinoma	TE
Prediction of developing postoperative hepatic insufficiency after surgical resection	TE
Prediction of developing recurrence of hepatocellular carcinoma after curative resection	TE
Monitoring of fibrotic burden during antiviral treatment	TE

TE: Transient elastography; HVPG: Hepatic venous pressure gradient.

kg/m^2) and waist circumference were significantly associated with TE failure[99]. These results emphasize the need for adequate operator training and for technological improvements in specific patient populations, such as those with non-alcoholic fatty liver disease. For this, a new TE probe (the XL probe) was recently introduced to lessen the TE failure rate in obese patients; however, its efficacy should be further validated[100].

CONCLUSION

Over the past decade, significant progress has been made in the non-invasive assessment of liver fibrosis in patients with CLD. Of the methods now available, TE appears to be an excellent tool for assessing liver fibrosis, particularly for diagnosis of cirrhosis, and also has prognostic value from a longitudinal perspective. Although TE cannot completely obviate the need for invasive tests, such as LB, endoscopic examination for identification of varices, or HVPG, it represents an important non-invasive tool, enabling more efficient and tailored management strategies for patients with CLD (Table 2). We hope that other researchers will evaluate the usefullness of other similar techniques such as the measurement of spleen stiffness in comparison or in combination with TE in the future.

ACKNOWLEDGMENTS

The authors thank Mr. Dong-Su Jang, Research Assistant, Department of Anatomy, Yonsei University College of Medicine, Seoul, South Korea, for his help with the figures.

REFERENCES

1 **Jang JW**. Current status of liver diseases in Korea: liver cirrhosis. *Korean J Hepatol* 2009; **15** Suppl 6: S40-S49 [PMID: 20037279 DOI: 10.3350/kjhep.2009.15.S6.S40]
2 **Lok AS**. Hepatitis B: liver fibrosis and hepatocellular carcinoma. *Gastroenterol Clin Biol* 2009; **33**: 911-915 [PMID: 19577871 DOI: 10.1016/j.gcb.2009.06.001]
3 **Pungpapong S**, Kim WR, Poterucha JJ. Natural history of hepatitis B virus infection: an update for clinicians. *Mayo Clin Proc* 2007; **82**: 967-975 [PMID: 17673066 DOI: 10.4065/82.8.967]
4 **McGill DB**, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. *Gastroenterology* 1990; **99**: 1396-1400 [PMID: 2101588]
5 **Jin SY**. Role of liver biopsy in the assessment of hepatic fibrosis--its utility and limitations. *Korean J Hepatol* 2007; **13**: 138-145 [PMID: 17585187]
6 **Regev A**, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614-2618 [PMID: 12385448 DOI: 10.1111/j.1572-0241.2002.06038.x]
7 **Abraldes JG**, Araujo IK, Turón F, Berzigotti A. Diagnosing and monitoring cirrhosis: Liver biopsy, hepatic venous pressure gradient and elastography. *Gastroenterol Hepatol* 2012; **35**: 488-495 [PMID: 22560536 DOI: 10.1016/j.gastrohep.2012.02.010]
8 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847 [PMID: 18334275 DOI: 10.1016/j.jhep.2008.02.008]
9 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]
10 **Talwalkar JA**, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2007; **5**: 1214-1220 [PMID: 17916549 DOI: 10.1016/j.cgh.2007.07.020]
11 **Stauber RE**, Lackner C. Noninvasive diagnosis of hepatic fibrosis in chronic hepatitis C. *World J Gastroenterol* 2007; **13**: 4287-4294 [PMID: 17708599]
12 **Kang JK**, Cheong JY, Cho SW, Cho JH, Park JS, Kim YB, Kim DJ, Hwang SG, Yang JM, Park YN. Liver stiffness measurement for the diagnosis of hepatic fibrosis in patients with chronic viral hepatitis. *Korean J Hepatol* 2007; **13**: 521-529 [PMID: 18159150 DOI: 10.3350/kjhep.2007.13.4.521]
13 **Jung HS**, Kim YS, Kwon OS, Ku YS, Kim YK, Choi DJ, Kim JH. Usefulness of liver stiffness measurement for predicting the presence of esophageal varices in patients with liver cirrhosis. *Korean J Hepatol* 2008; **14**: 342-350 [PMID: 18815457 DOI: 10.3350/kjhep.2008.14.3.342]
14 **Kim SG**, Kim YS, Jung SW, Kim HK, Jang JY, Moon JH, Kim HS, Lee JS, Lee MS, Shim CS, Kim BS. The usefulness of transient elastography to diagnose cirrhosis in patients with alcoholic liver disease. *Korean J Hepatol* 2009; **15**: 42-51 [PMID: 19346784 DOI: 10.3350/kjhep.2009.15.1.42]
15 **Kim SM**, Sohn JH, Kim TY, Roh YW, Eun CS, Jeon YC, Han DS, Oh YH. Comparison of various noninvasive serum markers of liver fibrosis in chronic viral liver disease. *Korean J Hepatol* 2009; **15**: 454-463 [PMID: 20037264 DOI: 10.3350/kjhep.2009.15.4.454]
16 **Scott DR**, Levy MT. Liver transient elastography (Fibroscan): a place in the management algorithms of chronic viral hepatitis. *Antivir Ther* 2010; **15**: 1-11 [PMID: 20167986 DOI: 10.3851/IMP1474]
17 **Stebbing J**, Farouk L, Panos G, Anderson M, Jiao LR, Mandalia S, Bower M, Gazzard B, Nelson M. A meta-analysis of transient elastography for the detection of hepatic fibrosis. *J Clin Gastroenterol* 2010; **44**: 214-219 [PMID: 19745758 DOI: 10.1097/MCG.0b013e3181b4af1f]

18 **Han KH**, Yoon KT. New diagnostic method for liver fibrosis and cirrhosis. *Intervirology* 2008; **51** Suppl 1: 11-16 [PMID: 18544943 DOI: 10.1159/000122594]
19 **Smith JO**, Sterling RK. Systematic review: non-invasive methods of fibrosis analysis in chronic hepatitis C. *Aliment Pharmacol Ther* 2009; **30**: 557-576 [PMID: 19519733 DOI: 10.1111/j.1365-2036.2009.04062.x]
20 **Kim BK**, Kim do Y, Han KH, Park JY, Kim JK, Paik YH, Lee KS, Chon CY, Ahn SH. Risk assessment of esophageal variceal bleeding in B-viral liver cirrhosis by a liver stiffness measurement-based model. *Am J Gastroenterol* 2011; **106**: 1654-1662, 1730 [PMID: 21691339]
21 **Kim SU**, Han KH, Ahn SH. Non-invasive assessment of liver fibrosis: time to move from cross-sectional studies to longitudinal ones. *J Gastroenterol Hepatol* 2010; **25**: 1472-1473 [PMID: 20796140 DOI: 10.1111/j.1440-1746.2010.06432.x]
22 **Addley J**, Tham TC, Cash WJ. Use of portal pressure studies in the management of variceal haemorrhage. *World J Gastrointest Endosc* 2012; **4**: 281-289 [PMID: 22816007 DOI: 10.4253/wjge.v4.i7.281]
23 **Bosch J**, García-Pagán JC. Complications of cirrhosis. I. Portal hypertension. *J Hepatol* 2000; **32**: 141-156 [PMID: 10728801]
24 **Suk KT**, Kim CH, Park SH, Sung HT, Choi JY, Han KH, Hong SH, Kim DY, Yoon JH, Kim YS, Baik GH, Kim JB, Kim DJ. Comparison of hepatic venous pressure gradient and two models of end-stage liver disease for predicting the survival in patients with decompensated liver cirrhosis. *J Clin Gastroenterol* 2012; **46**: 880-886 [PMID: 22810110]
25 **Keiding S**, Vilstrup H. Intrahepatic heterogeneity of hepatic venous pressure gradient in human cirrhosis. *Scand J Gastroenterol* 2002; **37**: 960-964 [PMID: 12229973]
26 **Rincón D**, Lo Iacono O, Tejedor M, Hernando A, Ripoll C, Catalina MV, Salcedo M, Matilla A, Senosiain M, Clemente G, Molinero LM, Albillos A, Bañares R. Prognostic value of hepatic venous pressure gradient in patients with compensated chronic hepatitis C-related cirrhosis. *Scand J Gastroenterol* 2012 Aug 8; Epub ahead of print [PMID: 22871085]
27 **Pineda JA**, Recio E, Camacho A, Macías J, Almodóvar C, González-Serrano M, Merino D, Tellez F, Ríos MJ, Rivero A. Liver stiffness as a predictor of esophageal varices requiring therapy in HIV/hepatitis C virus-coinfected patients with cirrhosis. *J Acquir Immune Defic Syndr* 2009; **51**: 445-449 [PMID: 19487952 DOI: 10.1097/QAI.0b013e3181acb675]
28 **Wadhawan M**, Dubey S, Sharma BC, Sarin SK, Sarin SK. Hepatic venous pressure gradient in cirrhosis: correlation with the size of varices, bleeding, ascites, and child's status. *Dig Dis Sci* 2006; **51**: 2264-2269 [PMID: 17080245 DOI: 10.1007/s10620-006-9310-2]
29 **Kim MY**, Baik SK, Suk KT, Yea CJ, Lee IY, Kim JW, Cha SH, Kim YJ, Um SH, Han KH. Measurement of hepatic venous pressure gradient in liver cirrhosis: relationship with the status of cirrhosis, varices, and ascites in Korea. *Korean J Hepatol* 2008; **14**: 150-158 [PMID: 18617762]
30 **Hobolth L**, Bendtsen F, Møller S. Indications for portal pressure measurement in chronic liver disease. *Scand J Gastroenterol* 2012; **8-9**: 887-892 [PMID: 22809270]
31 **Bañares R**, Catalina MV, Ripoll C, Rincón D. Prognostic markers in patients who have recovered from an acute variceal bleeding: role of HVPG measurement. *Dis Markers* 2011; **31**: 165-169 [PMID: 22045402]
32 **Seijo S**, Reverter E, Miquel R, Berzigotti A, Abraldes JG, Bosch J, García-Pagán JC. Role of hepatic vein catheterisation and transient elastography in the diagnosis of idiopathic portal hypertension. *Dig Liver Dis* 2012; **44**: 855-860 [PMID: 22721839]
33 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]
34 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408 [PMID: 16020491 DOI: 10.1136/gut.2005.069153]
35 **Cho SB**, Park KJ, Lee JS, Lee WS, Park CH, Joo YE, Kim HS, Choi SK, Rew JS, Kim SJ. Comparison of terlipressin and octreotide with variceal ligation for controlling acute esophageal variceal bleeding--a randomized prospective study. *Korean J Hepatol* 2006; **12**: 385-393 [PMID: 16998290]
36 **Youn KH**, Kim DJ. Prevention of variceal bleeding and measurement of hepatic vein pressure gradient. *Korean J Hepatol* 2006; **12**: 464-468 [PMID: 16998301]
37 **Seo YS**, Um SH, Hyun JJ, Kim YH, Park S, Keum BR, Kim YS, Jeen YT, Lee HS, Chun HJ, Lee SW, Choi JH, Kim CD, Ryu HS. A prospective study comparing the efficacy of early administration of terlipressin and somatostatin for the control of acute variceal bleeding in patients with cirrhosis. *Korean J Hepatol* 2006; **12**: 373-384 [PMID: 16998289]
38 **Castéra L**, Le Bail B, Roudot-Thoraval F, Bernard PH, Foucher J, Merrouche W, Couzigou P, de Lédinghen V. Early detection in routine clinical practice of cirrhosis and oesophageal varices in chronic hepatitis C: comparison of transient elastography (FibroScan) with standard laboratory tests and non-invasive scores. *J Hepatol* 2009; **50**: 59-68 [PMID: 19013661 DOI: 10.1016/j.jhep.2008.08.018]
39 **Lemoine M**, Katsahian S, Ziol M, Nahon P, Ganne-Carrie N, Kazemi F, Grando-Lemaire V, Trinchet JC, Beaugrand M. Liver stiffness measurement as a predictive tool of clinically significant portal hypertension in patients with compensated hepatitis C virus or alcohol-related cirrhosis. *Aliment Pharmacol Ther* 2008; **28**: 1102-1110 [PMID: 18691352 DOI: 10.1111/j.1365-2036.2008.03825.x]
40 **Kazemi F**, Kettaneh A, N'kontchou G, Pinto E, Ganne-Carrie N, Trinchet JC, Beaugrand M. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* 2006; **45**: 230-235 [PMID: 16797100 DOI: 10.1016/j.jhep.2006.04.006]
41 **Bureau C**, Metivier S, Peron JM, Selves J, Robic MA, Gourraud PA, Rouquet O, Dupuis E, Alric L, Vinel JP. Transient elastography accurately predicts presence of significant portal hypertension in patients with chronic liver disease. *Aliment Pharmacol Ther* 2008; **27**: 1261-1268 [PMID: 18397389 DOI: 10.1111/j.1365-2036.2008.03701.x]
42 **Pritchett S**, Cardenas A, Manning D, Curry M, Afdhal NH. The optimal cut-off for predicting large oesophageal varices using transient elastography is disease specific. *J Viral Hepat* 2011; **18**: e75-e80 [PMID: 21040236 DOI: 10.1111/j.1365-2893.2010.01375.x]
43 **Nguyen-Khac E**, Saint-Leger P, Tramier B, Coevoet H, Capron D, Dupas JL. Noninvasive diagnosis of large esophageal varices by Fibroscan: strong influence of the cirrhosis etiology. *Alcohol Clin Exp Res* 2010; **34**: 1146-1153 [PMID: 20477777]
44 **Malik R**, Lai M, Sadiq A, Farnan R, Mehta S, Nasser I, Challies T, Schuppan D, Afdhal N. Comparison of transient elastography, serum markers and clinical signs for the diagnosis of compensated cirrhosis. *J Gastroenterol Hepatol* 2010; **25**: 1562-1568 [PMID: 20796156 DOI: 10.1111/j.1440-1746.2010.06371.x]
45 **Kim BK**, Han KH, Park JY, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Kim do Y. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010; **105**: 1382-1390 [PMID: 20087336 DOI: 10.1038/ajg.2009.750]
46 **Kim BK**, Park YN, Kim do Y, Park JY, Chon CY, Han KH, Ahn SH. Risk assessment of development of hepatic decompensation in histologically proven hepatitis B viral cirrhosis using liver stiffness measurement. *Digestion* 2012; **85**: 219-227 [PMID: 22414567 DOI: 10.1159/000335430]
47 **Kudo M**. Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. *Oncology* 2008; **75** Suppl 1: 1-12 [PMID: 19092266 DOI: 10.1159/000181865]
48 **El-Serag HB**. Epidemiology of viral hepatitis and hepato-

cellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432]
49 **Frenette C**, Gish R. Targeted systemic therapies for hepatocellular carcinoma: clinical perspectives, challenges and implications. *World J Gastroenterol* 2012; **18**: 498-506 [PMID: 22363115 DOI: 10.3748/wjg.v18.i6.498]
50 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
51 **Maggs JR**, Suddle AR, Aluvihare V, Heneghan MA. Systematic review: the role of liver transplantation in the management of hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 1113-1134 [PMID: 22432733 DOI: 10.1111/j.1365-2036.2012.05072.x]
52 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Imamura J, Goto T, Kanai F, Kato N, Ikeda H, Shiina S, Kawabe T, Omata M. Prospective risk assessment for hepatocellular carcinoma development in patients with chronic hepatitis C by transient elastography. *Hepatology* 2009; **49**: 1954-1961 [PMID: 19434742 DOI: 10.1002/hep.22870]
53 **Jung KS**, Kim SU, Ahn SH, Park YN, Kim do Y, Park JY, Chon CY, Choi EH, Han KH. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). *Hepatology* 2011; **53**: 885-894 [PMID: 21319193 DOI: 10.1002/hep.24121]
54 **Fung J**, Lai CL, Seto WK, Wong DK, Yuen MF. Prognostic significance of liver stiffness for hepatocellular carcinoma and mortality in HBeAg-negative chronic hepatitis B. *J Viral Hepat* 2011; **18**: 738-744 [PMID: 20659306 DOI: 10.1111/j.1365-2893.2010.01355.x]
55 **Kim SU**, Lee JH, Kim do Y, Ahn SH, Jung KS, Choi EH, Park YN, Han KH, Chon CY, Park JY. Prediction of liver-related events using fibroscan in chronic hepatitis B patients showing advanced liver fibrosis. *PLoS One* 2012; **7**: e36676 [PMID: 22574212 DOI: 10.1371/journal.pone.0036676]
56 **Chon YE**, Jung ES, Park JY, Kim do Y, Ahn SH, Han KH, Chon CY, Jung KS, Kim SU. The accuracy of noninvasive methods in predicting the development of hepatocellular carcinoma and hepatic decompensation in patients with chronic hepatitis B. *J Clin Gastroenterol* 2012; **46**: 518-525 [PMID: 22688146 DOI: 10.1097/MCG.0b013e31825079f1]
57 **Vergniol J**, Foucher J, Castéra L, Bernard PH, Tournan R, Terrebonne E, Chanteloup E, Merrouche W, Couzigou P, de Lédinghen V. Changes of non-invasive markers and FibroScan values during HCV treatment. *J Viral Hepat* 2009; **16**: 132-140 [PMID: 19175875 DOI: 10.1111/j.1365-2893.2008.01055.x]
58 **Jung KS**, Kim SU. Clinical applications of transient elastography. *Clin Mol Hepatol* 2012; **18**: 163-173 [PMID: 22893866 DOI: 10.3350/cmh.2012.18.2.163]
59 **Poca M**, Puente A, Graupera I, Villanueva C. Prognostic markers in patients with cirrhosis and portal hypertension who have not bled. *Dis Markers* 2011; **31**: 147-154 [PMID: 22045400]
60 **Robic MA**, Procopet B, Métivier S, Péron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. *J Hepatol* 2011; **55**: 1017-1024 [PMID: 21354450 DOI: 10.1016/j.jhep.2011.01.051]
61 **Pesce A**, Scilletta R, Branca A, Nigro L, Montineri A, Larocca L, Fatuzzo F, Castaing M, Puleo S. Does transient elastography (FibroScan®) have a role in decision making in hepatocellular carcinoma? *HPB* (Oxford) 2012; **14**: 403-408 [PMID: 22568417]
62 **Motosugi U**, Ichikawa T, Koshiishi T, Sano K, Morisaka H, Ichikawa S, Enomoto N, Matsuda M, Fujii H, Araki T. Liver stiffness measured by magnetic resonance elastography as a risk factor for hepatocellular carcinoma: a preliminary case-control study. *Eur Radiol* 2013; **23**: 156-162 [PMID: 22814828]
63 **Masuzaki R**, Tateishi R, Yoshida H, Arano T, Uchino K, Enooku K, Goto E, Nakagawa H, Asaoka Y, Kondo Y, Goto T, Ikeda H, Shiina S, Omata M, Koike K. Assessment of disease progression in patients with transfusion-associated chronic hepatitis C using transient elastography. *World J Gastroenterol* 2012; **18**: 1385-1390 [PMID: 22493553 DOI: 10.3748/wjg.v18.i12.1385]
64 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]
65 **Batts KP**, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995; **19**: 1409-1417 [PMID: 7503362 DOI: 10.1097/00000478-199512000-00007]
66 **Ishak K**, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, Denk H, Desmet V, Korb G, MacSween RN. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696-699 [PMID: 7560864 DOI: 10.1016/0168-8278(95)80226-6]
67 **Kim SU**, Ahn SH, Park JY, Kim do Y, Chon CY, Choi JS, Kim KS, Han KH. Prediction of postoperative hepatic insufficiency by liver stiffness measurement (FibroScan((R))) before curative resection of hepatocellular carcinoma: a pilot study. *Hepatol Int* 2008; **2**: 471-477 [PMID: 19669322 DOI: 10.1007/s12072-008-9091-0]
68 **Kim SU**, Kim YC, Choi JS, Kim KS, Choi GH, Choi JS, Park JY, Kim do Y, Ahn SH, Choi EH, Park YN, Chon CY, Han KH, Kim MJ. Can preoperative diffusion-weighted MRI predict postoperative hepatic insufficiency after curative resection of HBV-related hepatocellular carcinoma? A pilot study. *Magn Reson Imaging* 2010; **28**: 802-811 [PMID: 20395100 DOI: 10.1016/j.mri.2010.03.018]
69 **Jung KS**, Kim SU, Choi GH, Park JY, Park YN, Kim do Y, Ahn SH, Chon CY, Kim KS, Choi EH, Choi JS, Han KH. Prediction of recurrence after curative resection of hepatocellular carcinoma using liver stiffness measurement (FibroScan®). *Ann Surg Oncol* 2012; **19**: 4278-4286 [PMID: 22752370]
70 **Enomoto M**, Mori M, Ogawa T, Fujii H, Kobayashi S, Iwai S, Morikawa H, Tamori A, Sakaguchi H, Sawada A, Takeda S, Habu D, Shiomi S, Kawada N. Usefulness of transient elastography for assessment of liver fibrosis in chronic hepatitis B: Regression of liver stiffness during entecavir therapy. *Hepatol Res* 2010; **40**: 853-861 [PMID: 20887589 DOI: 10.1111/j.1872-034X.2010.00687.x]
71 **Kim SU**, Park JY, Kim do Y, Ahn SH, Choi EH, Seok JY, Lee JM, Park YN, Chon CY, Han KH. Non-invasive assessment of changes in liver fibrosis via liver stiffness measurement in patients with chronic hepatitis B: impact of antiviral treatment on fibrosis regression. *Hepatol Int* 2010; **4**: 673-680 [PMID: 21286337 DOI: 10.1007/s12072-010-9201-7]
72 **Fung J**, Lai CL, Wong DK, Seto WK, Hung I, Yuen MF. Significant changes in liver stiffness measurements in patients with chronic hepatitis B: 3-year follow-up study. *J Viral Hepat* 2011; **18**: e200-e205 [PMID: 21692933 DOI: 10.1111/j.1365-2893.2010.01428.x]
73 **Lim SG**, Cho SW, Lee YC, Jeon SJ, Lee MH, Cho YJ, Kim SS, Kim YB, Seok JY, Cheong JY, Kim JH. Changes in liver stiffness measurement during antiviral therapy in patients with chronic hepatitis B. *Hepatogastroenterology* 2011; **58**: 539-545 [PMID: 21661428]
74 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chu SH, Chan FK, Sung JJ, Chan HL. On-treatment monitoring of liver fibrosis with transient elastography in chronic hepatitis B patients. *Antivir Ther* 2011; **16**: 165-172 [PMID: 21447865 DOI: 10.3851/IMP1726]
75 **Ogawa E**, Furusyo N, Toyoda K, Takeoka H, Maeda S, Hayashi J. The longitudinal quantitative assessment by transient elastography of chronic hepatitis C patients treated with pegylated interferon alpha-2b and ribavirin. *Antiviral Res* 2009; **83**: 127-134 [PMID: 19443053 DOI: 10.1016/j.antiviral.2009.04.002]
76 **Wang JH**, Changchien CS, Hung CH, Tung WC, Kee KM, Chen CH, Hu TH, Lee CM, Lu SN. Liver stiffness decrease after effective antiviral therapy in patients with chronic hepatitis C: Longitudinal study using FibroScan. *J Gastroenterol Hepatol* 2010; **25**: 964-969 [PMID: 20546451 DOI: 10.1111/j.1440-1746.2009.06194.x]
77 **Arima Y**, Kawabe N, Hashimoto S, Harata M, Nitta Y, Murao

M, Nakano T, Shimazaki H, Kobayashi K, Ichino N, Osakabe K, Nishikawa T, Okumura A, Ishikawa T, Yoshioka K. Reduction of liver stiffness by interferon treatment in the patients with chronic hepatitis C. *Hepatol Res* 2010; **40**: 383-392 [PMID: 20236358 DOI: 10.1111/j.1872-034X.2009.00618.x]

78 **Andersen ES**, Weiland O, Leutscher P, Krarup H, Westin J, Moessner B, Konopski Z, Frigstad SO, Kjær M, Christensen PB, Weis N. Low liver stiffness among cirrhotic patients with hepatitis B after prolonged treatment with nucleoside analogs. *Scand J Gastroenterol* 2011; **46**: 760-766 [PMID: 21438780 DOI: 10.3109/00365521.2011.565068]

79 **Kim SU**, Oh HJ, Park YN, Park JY, Kim do Y, Ahn SH, Chon CY, Han KH. Sub-classification of cirrhosis and prediction of liver-related events using fibroscan in patients with HBV-related cirrhosis. *J Hepatol* 2012; **56**: s34 [DOI: 10.1016/S0168-8278(12)60092-0]

80 **Bae RC**, Cho HJ, Oh JT, Lee EK, Heo J, Shin KY, Park SY, Jeong MK, Jeon SW, Cho CM, Tak WY, Kweon YO. Clinical factors influencing liver stiffness as measured by transient elastography (Fibroscan) in patients with chronic liver disease. *Korean J Hepatol* 2010; **16**: 123-130 [PMID: 20606496 DOI: 10.3350/kjhep.2010.16.2.123]

81 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218 DOI: 10.1136/gut.2006.111302]

82 **Arena U**, Vizzutti F, Abraldes JG, Corti G, Stasi C, Moscarella S, Milani S, Lorefice E, Petrarca A, Romanelli RG, Laffi G, Bosch J, Marra F, Pinzani M. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. *Gut* 2008; **57**: 1288-1293 [PMID: 18448567 DOI: 10.1136/gut.2008.149708]

83 **Chan HL**, Wong GL, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Wong VW. Alanine aminotransferase-based algorithms of liver stiffness measurement by transient elastography (Fibroscan) for liver fibrosis in chronic hepatitis B. *J Viral Hepat* 2009; **16**: 36-44 [PMID: 18673426 DOI: 10.1111/j.1365-2893.2008.01037.x]

84 **Taylor-Robinson SD**, Cobbold JF, Thomas HC. Liver stiffness measurements in acute hepatitis B: implications for clinical practice. *Eur J Gastroenterol Hepatol* 2010; **22**: 133-134 [PMID: 20061829 DOI: 10.1097/MEG.0b013e328334250a]

85 **Lee da M**, Moon EJ, Hwang JA, Lee MS, Cheong JY, Cho SW, Kim YB, Kim DJ, Hwang SG, Yang JM. Factors associated with liver stiffness in chronic liver disease. *Korean J Hepatol* 2009; **15**: 464-473 [PMID: 20037265]

86 **Viganò M**, Massironi S, Lampertico P, Iavarone M, Paggi S, Pozzi R, Conte D, Colombo M. Transient elastography assessment of the liver stiffness dynamics during acute hepatitis B. *Eur J Gastroenterol Hepatol* 2010; **22**: 180-184 [PMID: 19855283 DOI: 10.1097/MEG.0b013e328332d2fa]

87 **Sagir A**, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592-595 [PMID: 18098325 DOI: 10.1002/hep.22056]

88 **Wong GL**, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chan FK, Sung JJ, Chan HL. Increased liver stiffness measurement by transient elastography in severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2009; **24**: 1002-1007 [PMID: 19457152 DOI: 10.1111/j.1440-1746.2009.05779.x]

89 **Arena U**, Vizzutti F, Corti G, Ambu S, Stasi C, Bresci S, Moscarella S, Boddi V, Petrarca A, Laffi G, Marra F, Pinzani M. Acute viral hepatitis increases liver stiffness values measured by transient elastography. *Hepatology* 2008; **47**: 380-384 [PMID: 18095306 DOI: 10.1002/hep.22007]

90 **Kim SU**, Kim JK, Park YN, Han KH. Discordance between liver biopsy and Fibroscan® in assessing liver fibrosis in chronic hepatitis b: risk factors and influence of necroinflammation. *PLoS One* 2012; **7**: e32233 [PMID: 22384189 DOI: 10.1371/journal.pone.0032233]

91 **Fung J**, Lai CL, But D, Hsu A, Seto WK, Cheng C, Wong DK, Yuen MF. Reduction of liver stiffness following resolution of acute flares of chronic hepatitis B. *Hepatol Int* 2010; **4**: 716-722 [PMID: 21286342 DOI: 10.1007/s12072-010-9189-z]

92 **Coco B**, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, Bonino F, Brunetto MR. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360-369 [PMID: 17439526 DOI: 10.1111/j.1365-2893.2006.00811.x]

93 **Oliveri F**, Coco B, Ciccorossi P, Colombatto P, Romagnoli V, Cherubini B, Bonino F, Brunetto MR. Liver stiffness in the hepatitis B virus carrier: a non-invasive marker of liver disease influenced by the pattern of transaminases. *World J Gastroenterol* 2008; **14**: 6154-6162 [PMID: 18985805 DOI: 10.3748/wjg.14.6154]

94 **Park H**, Kim SU, Kim D, Kim do Y, Ahn SH, Han KH, Chon CY, Park JY. Optimal time for restoring the reliability of liver stiffness measurement in patients with chronic hepatitis B experiencing acute exacerbation. *J Clin Gastroenterol* 2012; **46**: 602-607 [PMID: 22772739 DOI: 10.1097/MCG.0b013e3182582a31]

95 **Millonig G**, Reimann FM, Friedrich S, Fonouni H, Mehrabi A, Büchler MW, Seitz HK, Mueller S. Extrahepatic cholestasis increases liver stiffness (FibroScan) irrespective of fibrosis. *Hepatology* 2008; **48**: 1718-1723 [PMID: 18836992 DOI: 10.1002/hep.22577]

96 **Colli A**, Pozzoni P, Berzuini A, Gerosa A, Canovi C, Molteni EE, Barbarini M, Bonino F, Prati D. Decompensated chronic heart failure: increased liver stiffness measured by means of transient elastography. *Radiology* 2010; **257**: 872-878 [PMID: 20935077 DOI: 10.1148/radiol.10100013]

97 **Hopper I**, Kemp W, Porapakkham P, Sata Y, Condon E, Skiba M, Farber L, Porapakkham P, Williams TJ, Menahem S, Roberts S, Krum H. Impact of heart failure and changes to volume status on liver stiffness: non-invasive assessment using transient elastography. *Eur J Heart Fail* 2012; **14**: 621-627 [PMID: 22523374 DOI: 10.1093/eurjhf/hfs044]

98 **Millonig G**, Friedrich S, Adolf S, Fonouni H, Golriz M, Mehrabi A, Stiefel P, Pöschl G, Büchler MW, Seitz HK, Mueller S. Liver stiffness is directly influenced by central venous pressure. *J Hepatol* 2010; **52**: 206-210 [PMID: 20022130 DOI: 10.1016/j.jhep.2009.11.018]

99 **Foucher J**, Castéra L, Bernard PH, Adhoute X, Laharie D, Bertet J, Couzigou P, de Lédinghen V. Prevalence and factors associated with failure of liver stiffness measurement using FibroScan in a prospective study of 2114 examinations. *Eur J Gastroenterol Hepatol* 2006; **18**: 411-412 [PMID: 16538113 DOI: 10.1097/00042737-200604000-00015]

100 **de Lédinghen V**, Wong VW, Vergniol J, Wong GL, Foucher J, Chu SH, Le Bail B, Choi PC, Chermak F, Yiu KK, Merrouche W, Chan HL. Diagnosis of liver fibrosis and cirrhosis using liver stiffness measurement: comparison between M and XL probe of FibroScan®. *J Hepatol* 2012; **56**: 833-839 [PMID: 22173167 DOI: 10.1016/j.jhep.2011.10.017]

P- Reviewer Atta HM **S- Editor** Wen LL **L- Editor** A
E- Editor Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1901

World J Gastroenterol 2013 March 28; 19(12): 1901-1911
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Promising effect of Magliasa, a traditional Iranian formula, on experimental colitis on the basis of biochemical and cellular findings

Roja Rahimi, Amir Baghaei, Maryam Baeeri, Gholamreza Amin, Mohammad Reza Shams-Ardekani, Mahnaz Khanavi, Mohammad Abdollahi

Roja Rahimi, Gholamreza Amin, Mohammad Reza Shams-Ardekani, Mahnaz Khanavi, Department of Traditional Pharmacy, Tehran University of Medical Sciences, Tehran 1417653761, Iran
Roja Rahimi, Amir Baghaei, Maryam Baeeri, Mohammad Abdollahi, Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 1417614411, Iran
Amir Baghaei, Mohammad Abdollahi, Department of Pharmacology and Toxicology, Tehran University of Medical Sciences, Tehran 1417614411, Iran
Gholamreza Amin, Mohammad Reza Shams-Ardekani, Mahnaz Khanavi, Department of Pharmacognosy, Tehran University of Medical Sciences, Tehran 1417614411, Iran
Author contributions: Rahimi R designed and performed research, and drafted the paper; Baghaei A contributed the animal studies; Baeeri M contributed the biochemical tests; Amin G contributed botanical authentication and the herbal studies; Khanavi M contributed the chemical drug analysis; Shams-Ardekani MR contributed in finding the appropriate prescription and to the preparation of the formula; Abdollahi M conceived the study and edited the paper.
Supported by The Tehran University of Medical Sciences partly, as outcome of a PhD thesis and Iran National Science foundation
Correspondence to: Mohammad Abdollahi, Professor, Department of Pharmacology and Toxicology, Tehran University of Medical Sciences, Tehran 1417614411, Iran. mohammad.abdollahi@utoronto.ca
Telephone: +98-21-88611663 Fax: +98-21-88611663
Received: June 28, 2012 Revised: September 19, 2012
Accepted: October 30, 2012
Published online: March 28, 2013

Abstract

AIM: To investigate the efficacy of Magliasa, a traditional Iranian formula, on experimental colitis.

METHODS: After botanical authentication of herbal ingredients, formulation of Magliasa, quantitative determination of total glucosinolates and total phenolic content, and analysis of the thin layer chromatography profile were performed. Colitis was then induced in male rats by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in all groups, aside from the Sham group. The experimental groups consisted of: the Sham group that received only normal saline; the Mag-50, Mag-100 and Mag-200 groups, which received 50, 100 and 200 mg/kg per day of Magliasa, respectively; the control group, which received vehicle water orally; the infliximab group, which received infliximab (5 mg/kg per day, subcutaneously); and the Dexa group, which received dexamethasone (1 mg/kg per day, orally). After completing the treatment period (2 wk), the rats were sacrificed, the colon was removed, its macroscopic and microscopic changes were recorded, and tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), total antioxidant capacity, myeloperoxidase (MPO), and lipid peroxidation (LPO) were assessed in colon homogenate.

RESULTS: The mean value of total glucosinolates in one gram of Magliasa was 19 ± 1 μmol. The mean value of the total phenolic content was 293.8 ± 17.6 mg gallic acid equivalents per 100 gram of Magliasa. Macroscopic scores were significantly decreased in Mag-100 (1.80 ± 0.58, P = 0.019) and Mag-200 (1.20 ± 0.20, P = 0.001) compared to the control group (3.40 ± 0.24), although some inflammation and hyperemia were evident. Treatment of rats by dexamethasone (0.33 ± 0.21, P < 0.001) and infliximab (0.83 ± 0.31, P < 0.001) remarkably attenuated scores where mild hyperemia was observed macroscopically. In comparison to the control group (4.00 ± 0.32), only Mag-200 (1.60 ± 0.40) showed a significant decrease in colonic histopathological scores (P = 0.005). Minimal mucosal inflammation was observed in the Dexa group (0.67

± 0.21, $P < 0.001$). The levels of TNF-α, IL-1β and MPO were significantly lower in all groups compared to the controls ($P < 0.05$). A significant decrease in LPO was seen in the Mag-200 (3.27 ± 0.77, $P = 0.01$) and Dexa (3.44 ± 0.22, $P = 0.011$) groups in comparison to the control group (6.43 ± 0.61). Only dexamethasone caused a significant increase in antioxidant power in comparison to the control group (346.73 ± 9.9 *vs* 228.33 ± 2.75, $P < 0.001$). Infliximab and different doses of Magliasa did not show any remarkable increase in antioxidant capacity ($P > 0.05$). The effect of Magliasa in all of mentioned parameters, except antioxidant capacity, was dose dependent.

CONCLUSION: The effects of Magliasa in TNBS-induced colitis are encouraging and warrant clinical trials for further confirmation.

Key words: Magliasa; Traditional Iranian medicine; Colitis; Neutrophil infiltration; Inflammatory cytokines; Oxidative stress

Rahimi R, Baghaei A, Baeeri M, Amin G, Shams-Ardekani MR, Khanavi M, Abdollahi M. Promising effect of Magliasa, a traditional Iranian formula, on experimental colitis on the basis of biochemical and cellular findings. *World J Gastroenterol* 2013; 19(12): 1901-1911 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1901.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1901

INTRODUCTION

Inflammatory bowel disease (IBD) includes two main types (Crohn's disease and ulcerative colitis), and is categorized as one of the chronic disorders of the gastrointestinal tract with an unclear etiology. Related to the involvement of possible pathological factors such as immunological abnormalities[1], oxidative stress[2], gut microflora[3], abnormal epithelial barrier[4], and inflammatory factors[5-9], various drugs are used for the management of IBD, including anti-tumor necrosis factor-alpha (TNF-α) drugs[10-12], immunosuppressants[13,14], antibiotics[15,16], probiotics[17,18], corticosteroids[19], aminosalicylates[20,21], selective cyclooxygenase-2 inhibitors[9], nicotine preparations[22], potassium channel openers[23], adenosine triphosphate donors[24], and phosphodiesterase inhibitors[25-27]. It cannot be ignored that most of the conventional treatments for the management of IBD have serious adverse effects that reduce compliance in patients[28-30], and therefore has led researchers to work on complementary and alternative medicines that can induce remission in disease activity with better safety and tolerability[31-33]. As recently reviewed by Rahimi *et al*[32], there are many plants in traditional Iranian medicine (TIM) that were historically used for the management of IBD. Magliasa is a TIM herbal prescription that has been used to treat tenesmus and diarrhea mixed with blood and mucus for a long time[34]. It consists of 6 components: the seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, the fruit of *Bunium persicum* and *Terminalia chebula*, and the gum resin of *Pistacia lentiscus* (Table 1). Different mechanisms have been described in TIM for the usefulness of these plants in the treatment of colitis, including anti-inflammatory, antiulcer, wound healing, and anti-diarrheal effects[35,36]. Regarding the aforementioned knowledge, the present study was planned to investigate the effect of Magliasa in an experimental model of colitis to determine the involved mechanisms.

MATERIALS AND METHODS

Materials

Plant materials (seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, fruit of *Bunium persicum* and *Terminalia chebula*, and gum resin of *Pistacia lentiscus*), were obtained from the local market at the Tehran bazaar in 2010. After confirmation by a botanist, voucher samples were deposited at the herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences (Tehran, Iran). 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma-Aldrich), ethanol, methanol, ethyl acetate, n-hexane, thiobarbituric acid, trichloroacetic acid, n-butanol, hexadecyl-trimethyl-ammonium bromide, 2,4,6-tri(2-pyridyl)-*S*-triazine (TPTZ), hydrochloric acid, anisaldehyde, malondialdehyde, ethylenediaminetetraacetic acid, Folin-Ciocalteu reagent, toluene, dichloromethane, o-dianisidine hydrochloride, hydrogen peroxide, acetic acid, sodium acetate, Coomassie reagent, bovine serum albumin (BSA), $FeCl_3$-$6H_2O$, Na_2SO_4, H_2SO_4, H_3PO_4, KH_2PO_4, K_2HPO_4, H_2O_2, Na_2CO_3, $NaHCO_3$, Na-K-tartrate, $CuSO_4$-$5H_2O$, Silica gel 60F254 (Merck, Germany), glucose kit (ZistChem, Iran), and rat-specific TNF-α and interleukin-1β (IL-1β) enzyme-linked immunosorbent assay (ELISA) kits (Bender Med Systems, Austria) were used in this study.

Botanical authentication

All 6 herbal ingredients were authenticated macroscopically and microscopically. Macroscopic examinations included measurements of appearance, size, shape, color, texture, odor, taste, fracture, and other characteristics according to pharmacopoeias[36,37]. Microscopic examinations determined the characteristic elements of each ingredient in powder form. For this purpose, each herbal material was mounted on a microscope slide after tissue disintegration with potassium hydroxide and cleared with sodium hypochlorite. The examination protocols followed the World Health Organization's quality control methods for medicinal plant materials[38]. Characteristic elements were photographed *via* a Leitz optical microscope.

Preparation of Magliasa

Bunium persicum fruit (22%), *Linum usitatissimum* seeds (8%), *Allium ampeloprasum* cv. Porrum seeds (8%), *Terminalia che-*

Table 1 Magliasa powder ingredient characteristics

Scientific name	Iranian name	Part	Major constituents	Pharmacological activities	Herbarium No.
Lepidium sativum	Taretizak	Seed	Glucosinolates, imidazole alkaloids, fatty acids, and sterols[60-63]	↓IL-2, TNF-α, leukotriene B4 and nitric oxide in immune cells; anti-inflammatory and analgesic in rats; prokinetic, and laxatives in mice; anti-diarrheal and spasmolytic in rats; anticholinergic and phosphodiesterase inhibitor[64-68]	PMP-716
Bunium persicum	Zire kermani	Fruit	Flavonoids, essential oils, and tannins[69,70]	Antioxidant and radical scavenger; antinociceptive and anti-inflammatory in rats[70-73]	PMP-627
Linum usitatissimum	Katan	Seed	Mucilage, cyanogenic glycoside, lignans, fatty acids, and phenylpropan derivatives[74]	Antiulcer, antioxidant , and protective against intestinal tumors in mice[75-77]	PMP-717
Allium ampeloprasum cv. Porrum	Tare	Seed	Saponins, flavonoids, carotenoids, and sulfur-containing compounds[78-80]	Antioxidant[78]	PMP-718
Terminalia chebula	Halile siah	Fruit	Tannins, anthraquinones, triterpene glycosides, and beta-Sitosterol[81,82]	Antioxidant, anti NF-κB, antiulcer, ↓TNF-α, IL-1β and IL-6, and antibacterial against intestinal bacteria[83-87]	PMP-606
Pistacia lentiscus	Mastaki	Gum resin	Triterpene acids and alcohols, and essential oils[81]	Antioxidant, ↓NO, prostaglandin E2, iNOS, and Cox-2 delayed the onset and progression of colitis and prevented weight loss in mice; ↓Intensity of gastric mucosal damage, ↓TNF-α, CD activity index, plasma IL-6, and ↑total antioxidant potential in CD patients; ulcer healing in patients with duodenal ulcer[88-93]	PMP-811

Cox-2: Cyclooxygenase-2; iNOS: Inducible nitric oxide synthase; CD: Crohn's disease; NO: Nitric oxide; TNF-α: Tumor necrosis factor-α; IL: interleukin; NF-κB: Nuclear factor κB.

bula fruit (8%), and *Pistacia lentiscus* gum resin (4%) were individually powdered by milling, and then mixed. Intact non-milled seed of *Lepidium sativum* (50%, w/w) was added to the powdered material and again mixed.

Quantitative determination of total glucosinolates and total phenols Magliasa

The amount of total glucosinolates as major constituents of *Lepidium sativum* and the amount of total phenolic compounds as major constituents of *Bunium persicum*, *Allium ampeloprasum* cv. Porrum, and *Terminalia chebula* were measured in Magliasa.

Total glucosinolates were determined by the measurement of enzymatically-released glucose[39]. For this purpose, four accurately weighed 1 g samples of Magliasa were transferred into separate loaded ball-mill cups. To three cups 1 mL of water was added (samples), while the last cup had 1 mL of acidified 40% v/v methanol/water added instead (sample blank). All cups were milled side by side for 2 min, allowed to stand for 5 min, and then had 19 mL of acidified 40% v/v methanol added to each cup. After recapping and shaking vigorously, the cup contents were filtered through charcoal-coated papers. Immediately prior to colorimetric assay, each of the filtrates was diluted ten-fold with water, and then 0.2 mL was poured into separate 10 mL tubes. About 0.2 mL of water was added into a fifth tube (water blank), with 0.2 mL of standard glucose solution (1 mg/mL) (ZistChem, Iran) added into a sixth tube. Five mL of buffer/enzyme/chromogen reagent (ZistChem, Iran) was added to all tubes, mixed, and then placed in a water bath at 37 ℃ and read within 10-15 min. The absorbance of each solution against the water blank was measured at 610 nm.

The total phenolic contents in the medicinal plants were determined spectrophotometrically according to the Folin-Ciocalteu method[40]. Gallic acid was used to set up the standard curve. The phenolic compound content of the samples was expressed as gallic acid equivalents (GAE) in mg per 100 g of Magliasa. All the samples were analyzed in triplicate.

Thin layer chromatography profile of Magliasa

Thin layer chromatography (TLC) was performed to obtain preliminary data from essential oils and lipophilic substances. For this purpose, 1 g of Magliasa was extracted by shaking for 15 min in 10 mL of dichloromethane at room temperature. The suspension was filtered, and the clear filtrate evaporated to dryness. The residue was dissolved in 1 mL of toluene. Samples were then applied to the plates, which were developed at room temperature in glass chambers previously saturated for 1 h. The development distance was 5 cm. The mobile phase was n-hexane-ethyl acetate 5:4 (v/v). The spray reagent was anisaldehyde- sulfuric acid[41].

Animals

Male Wistar-albino rats, weighing between 220 and 230 g, were maintained under standard conditions of temperature (23 ℃ ± 1 ℃), relative humidity (55% ± 10%), a 12-h dark and light period, and fed with a standard pellet diet and water *ad libitum*. All ethical themes of the animal studies were considered carefully, and the experimental protocol was approved by the ethical committee of Tehran University of Medical Sciences (code number of 88-04-33-10094).

Interventions

Rats were randomly divided into seven groups containing six individuals in each group. Colitis was induced

by the instillation of TNBS in all groups except group 1. The groups were: (1) Sham which received normal saline; (2) Mag-50 which received 50 mg/kg per day of Magliasa; (3) Mag-100 which received 10 mg/kg per day of Magliasa; (4) Mag-200 which received 200 mg/kg per day of Magliasa; (5) control which received vehicle water orally; (6) Infliximab which received infliximab (5 mg/kg per day, subcutaneously); and (7) Dexa which received dexamethasone (1 mg/kg per day, orally). Magliasa was dissolved in water and administered by gavage. The doses for Magliasa were selected after a pilot study. The effective doses of infliximab and dexamethasone were selected from our previous studies[42].

Induction of colitis

For induction of colitis, 36 h fasted rats were anesthetized with an intraperitoneal administration of 50 mg/kg of pentobarbital sodium, positioned on their right side, and then had 0.3 mL of a mixture containing six volumes of TNBS 5% w/v in H_2O (equal to 15 mg TNBS) plus four volumes of ethanol (99%) instilled *via* the rectum using a rubber cannula (8 cm long)[43]. Following instillation of TNBS, rats were maintained in a supine Trendelenburg position in order to prevent anal leakage of TNBS. Medications were then administered to the animals for 14 d as described above. On the 15th day, the animals were sacrificed by an overdose of ether inhalation. The abdomen was rapidly dissected open and the colon was removed. The colon was cut open in an ice bath, cleansed gently using normal saline, observed normally for macroscopic changes, and scored in a manner described later. Samples were then cut into two pieces; one piece for histopathology assessment (fixed in 5 mL formalin 10%) and one piece for measuring biomarkers weighed and maintained in -20 ℃ for 24 h. The colonic samples were then homogenized in 10 volume ice-cold potassium phosphate buffer (50 mmol/L, pH 7.4), sonicated, and centrifuged for 30 min at 3500 × *g*. The supernatants were transformed into several microtubes for separate biochemical assays, and all were kept at -80 ℃ until analyses[44].

Macroscopic and microscopic assessment of colonic damage

The macroscopic damage was assessed and scored according to criteria as described in our previous work[45,46]. For microscopic analysis, the fixed segments in formalin 10% were embedded in paraffin and stained with hematoxylin and eosin. The scoring was performed by one who was blind to the treated groups.

Determination of TNF-α and IL-1β

Quantitive detection of TNF-α and IL-1β levels in colon tissues were performed using an ELISA kit. The absorbance of the final colored product was measured in 450 nm as the primary wavelength and 620 nm as the reference wavelength. TNF-α and IL-1β levels were expressed as pg/mg protein of tissue, as described in our previous work[44].

Total ferric reducing antioxidant power assay

Total antioxidant power of the colon was evaluated by measuring the ability to reduce Fe^{3+} to Fe^{2+}. Interaction of TPTZ with Fe^{2+} results in the formation of a blue color, with a maximum absorbance at 593 nm. Data were expressed as mmol/L ferric ions reduced to ferrous per mg of protein, as described in our previous work[47].

Myeloperoxidase activity measurement

In this test, supernatant was combined with o-dianisidine and 0.0005% H_2O_2 that resulted in an absorbance at 460 nm that was measured for 3 min. One unit of myeloperoxidase (MPO) activity is described as the change in absorbance per min at room temperature in the final reaction. Details of the procedure have been described in our previous work[48].

Thiobarbituric acid-reactive substances assay

Levels of lipid peroxidation were assessed in colon tissue using thiobarbituric acid reactive substances (TBARS) assay as described in our previous work[49]. Data were reported as μg/mg of protein.

Total protein of colon homogenate

Total protein of the tissue was measured according to the Bradford method, using BSA as the standard[50]. Results were reported as mg of protein per mL of homogenized tissue.

Determination of LD50

In order to determine the acute toxicity (LD50) of Magliasa, doses of 5, 50, 300 and 2000 mg/kg per day were gavaged to rats. The animals were observed for 1 wk and any mortality was recorded at the end of this period[51].

Statistical analysis

Data were analyzed by StatsDirect ver. 2.7.8. One-way analysis of variance followed by a Newman-Keuls *post hoc* test for multiple comparisons were used. *P* values less than 0.05 were considered significant. Results are expressed as mean ± SE.

RESULTS

Botanical authentication

Microscopic characteristics of different herbal components of Magliasa are shown in Figure 1.

Quantitative determination of total glucosinolates and total phenols in Magliasa

The mean value of total glucosinolates in one gram of Magliasa was 19 ± 1 μmol. The mean value of total phenolic content was 293.8 ± 17.6 mg GAE per 100 g of Magliasa.

TLC analysis

Table 2 summarizes the retention value of spots visible in the TLC profile of Magliasa.

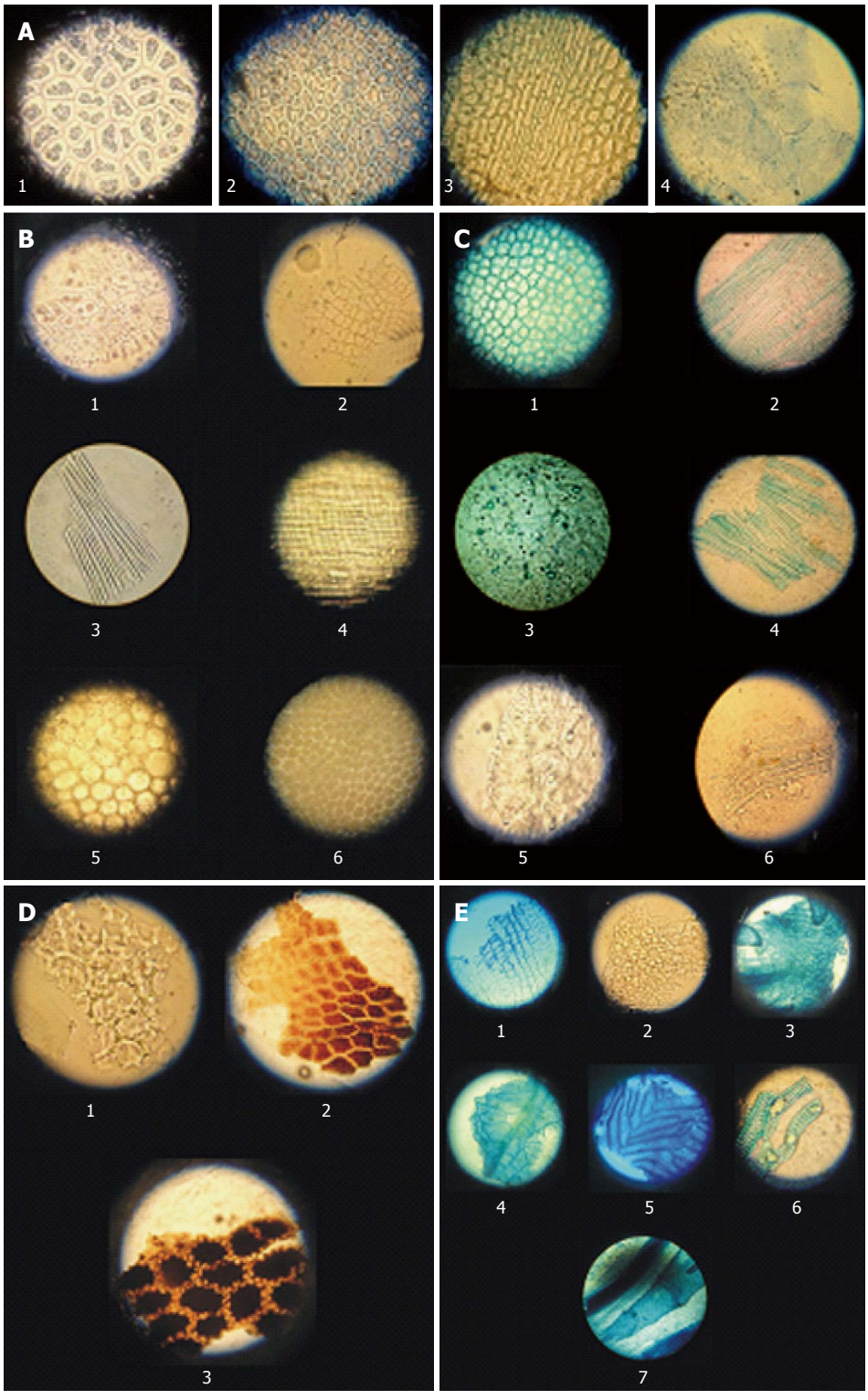


Figure 1 Microscopic characteristics of the herbal ingredients of Magliasa. A: Lepidium sativum seed. A1: Pericarp; A2 and A3: Sclereids of the mesocarp; A4: Parenchyma of the endosperm; B: Linum usitatissimum seed. B1: Endosperm; B2: Epidermis; B3: Fiber; B4: Sclerenchyma; B5: Parenchyma of the testa; B6: Pigment layer of testa; C: Terminalia chebula fruit. C1: Epidermis; C2: Fiber; C3: Parenchyma of the mesocarp; C4 and C5: Sclereids; C6: Vessels; D: Allium ampeloprasum cv. Porrum seed. D1: Endosperm; D2: Mesoderm; D3: Epidermis of the testa; E: Bunium persicum fruit. E1: Endocarp; E2 and E3: Endosperm; E4 and E5: Sclereids of the mesocarp; E6: Vessels; E7: Vittae. Magnification of all images was 40.

Macroscopic and microscopic assessment of colonic damage

Data are shown in Table 3. The colons of the Sham group appeared normal. In contrast, intracolonic administration of TNBS led to mucosal ulceration, inflammation, adhesion, and wall thickening in the control group. Treatment with Mag-50 did not significantly reduce macroscopic scores where linear ulceration and mesenteric inflammation were observed in some samples. Administration of Maglasia reduced the macroscopic score in a dose-dependent manner, and a significant effect was observed in the Mag-100 and Mag-200 groups, although some inflammation and hyperemia were evident. The median effective dose (ED50) value was 104.78 mg/kg. Treatment of rats by dexamethasone and infliximab remarkably attenuated scores where mild hyperemia was observed macroscopically.

Histopathological examination of the control group showed extensive severe transmural inflammation, dif-

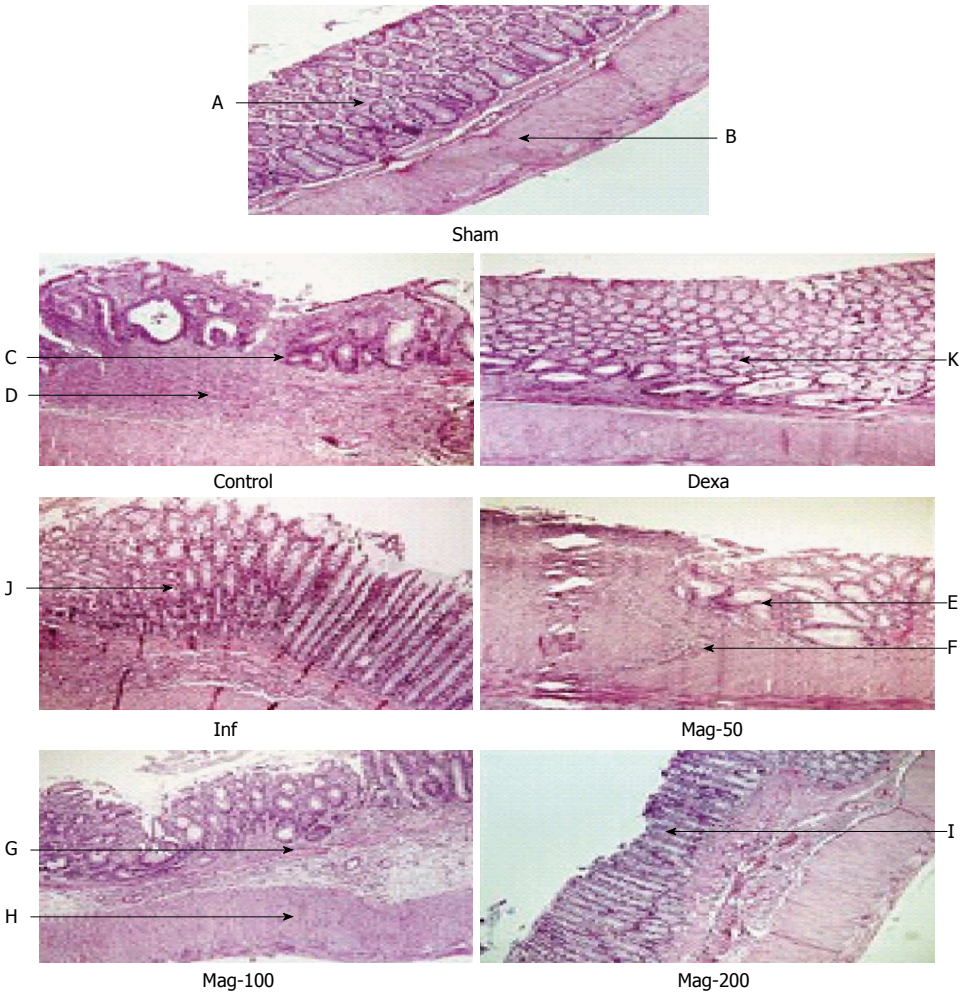


Figure 2 Histological images of colon samples. In the Sham group, colons were within normal limits including crypts (A) and submucosal tissue (B), but intense transmural inflammation (C), and severe crypt destruction (D) were observed in the control group. Moderate crypt distortion (E and G) and inflammatory cell infiltration (F and H) was seen in the Mag-5 and Mag-100 groups. Mild focal inflammation and crypt abscess were observed in the Mag-20 and infliximab groups (I and J). Mild focal inflammation was observed in the Dexa group (K). Magnification of all images was 100. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

Table 2 Thin layer chromatography analysis of Magliasa

Type of extract	Solvent system	RF values	Intensity of spot
Dichloromethane	Hexane: ethyl acetate (5:4 v/v)	0.048	Moderately intense
		0.181	Faint
		0.238	Intense
		0.286	Faint
		0.333	Intense
		0.380	Intense
		0.430	Faint
		0.476	Faint
		0.524	Moderately intense
		0.571	Faint
		0.619	Faint
		0.670	Faint
		0.714	Faint
		0.762	Intense
		0.838	Faint
		0.876	Moderately intense

RF: Retention factor.

Table 3 Macroscopic and microscopic scores as criteria for assessing colonic damage

Group	Macroscopic score		Microscopic score	
	mean ± SE	Median (range)	mean ± SE	Median (range)
Sham	0.00 ± 0.00	0 (0-0)	0.00 ± 0.00	0 (0-0)
Control	3.40 ± 0.24[a]	3 (3-4)	4.00 ± 0.32[a]	4 (3-5)
Dexa	0.33 ± 0.21[c]	0 (0-1)	0.67 ± 0.21[c]	1 (0-1)
Inf	0.83 ± 0.31[c]	1 (0-2)	1.50 ± 0.34[c]	1 (1-3)
Mag-50	2.20 ± 0.37[a,e,g]	2 (1-3)	2.60 ± 0.51[a,e]	3 (1-4)
Mag-100	1.80 ± 0.58[a,c,e]	1 (1-4)	2.40 ± 0.75[a]	2 (1-5)
Mag-200	1.20 ± 0.20[c]	1 (1-2)	1.60 ± 0.40[c]	1 (1-3)

[a]$P < 0.05$ *vs* Sham group; [c]$P < 0.05$ *vs* the control group; [e]$P < 0.05$ *vs* the dexamethasone group; [g]$P < 0.05$ *vs* the infliximab group. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

fused necrosis, mucosal and submucosal polymorphonuclear (PMN) leukocyte infiltration, and crypt destruction, whereas microscopic evaluation of the Sham group showed a normal situation. In the Mag-50 group, microscopic evaluation revealed moderate mucosal and submucosal inflammation, PMN infiltration, and extensive crypt distortion. In the Mag-100 group, moderate inflammation of the mucosa and submucosa, inflammatory cell infiltration, and some crypt abscess and destruction were observed. In the Mag-200 and infliximab groups, mild focal non-hemorrhagic edema and focal submucosal PMN infiltration were observed. Minimal mucosal inflammation was observed in the Dexa group (Figure 2). Administration of Maglasia reduced the microscopic score in a dose-dependent manner, and a significant effect was observed

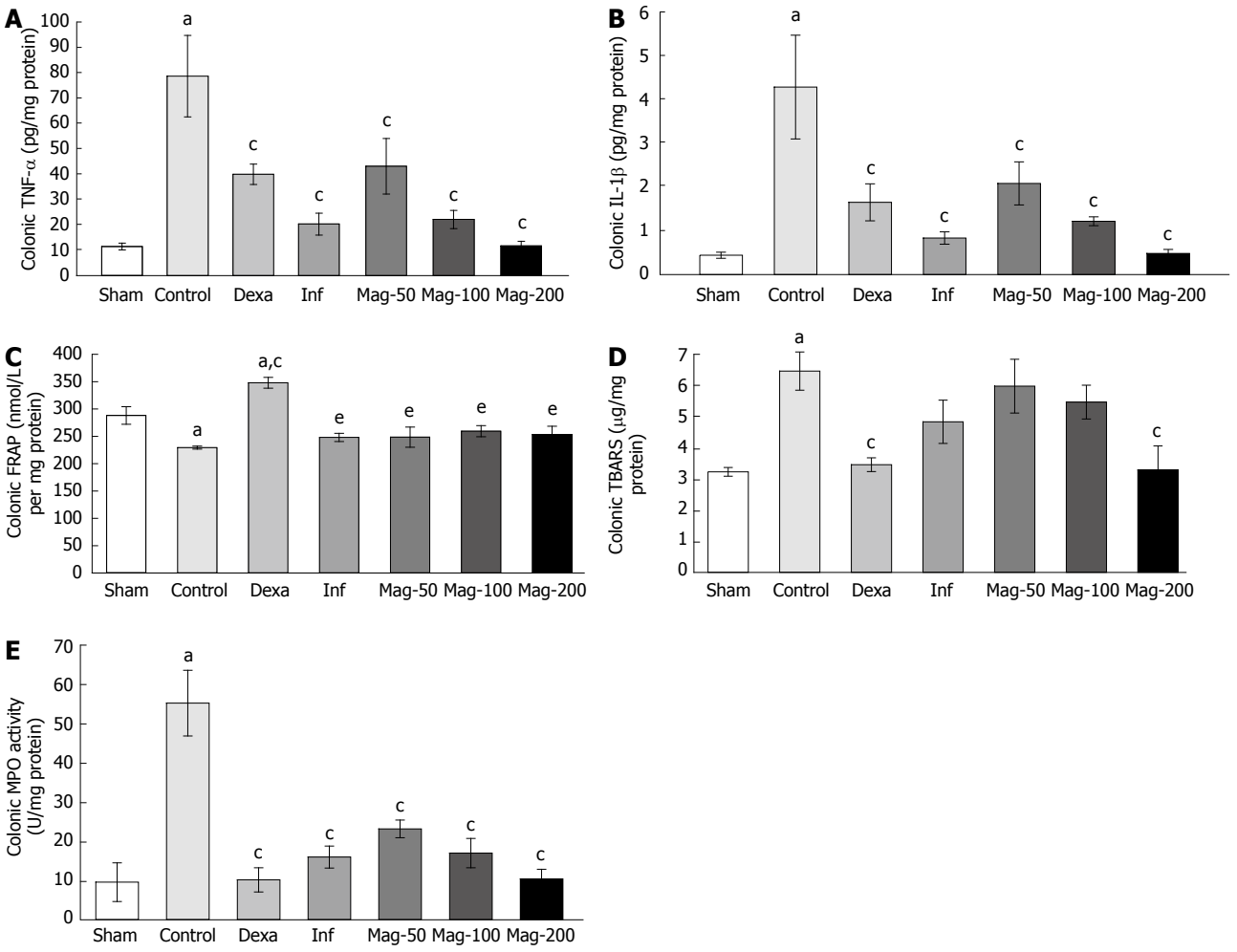


Figure 3 Levels of different biochemical parameters in the colon of rats after 2 wk of treatment. A: Tumor necrosis factor-alpha (TNF-α); B: Interleukin-1 beta (IL-1β); C: Total antioxidant capacity as a ferric reducing antioxidant power (FRAP) level; D: Lipid peroxidation as a thiobarbituric acid reactive substances (TBARS) level; E: Neutrophil infiltration as myeloperoxidase (MPO) activity. Values are mean ± SE. $^{a}P < 0.05$ *vs* the Sham group; $^{c}P < 0.05$ *vs* the control group; $^{e}P < 0.05$ *vs* the dexamethasone group. Dexa: Dexamethasone; Inf: Infliximab; Mag-50: Magliasa at a dose of 50 mg/kg; Mag-100: Magliasa at a dose of 100 mg/kg; Mag-200: Magliasa at a dose of 200 mg/kg.

in the Mag-200 group, with an ED50 of 132.29 mg/kg.

Colonic TNF-α

A significant difference was seen in TNF-α between the control and Sham groups ($P = 0.000$). TNF-α was significantly lower in all groups compared to the control, with an ED50 of 55.36 mg/kg. The level of TNF-α in the Mag-100 group (21.99 ± 3.54) was near to that of the infliximab group (20.18 ± 4.29), and both were lower than that of the Mag-50 (42.85 ± 10.87) and Dexa (39.72 ± 3.97) groups. TNF-α in the Mag-200 group (11.60 ± 1.83) was lower than that of the infliximab group, but the difference was not significant ($P = 0.98$, Figure 3A).

Colonic IL-1β levels

IL-1β was higher in the control group compared to the Sham ($P = 0.000$). IL-1β in all groups was lower than that of the control, with an ED50 of 48.78 mg/kg. IL-1β in the Mag-100 group (1.21 ± 0.10) was near to that of the Dexa group (1.64 ± 0.42), and both were higher than that of the infliximab (0.83 ± 0.14) and Mag-200 (0.48 ± 0.09) groups. IL-1β in the Mag-200 group was near to that of the Sham (0.44 ± 0.07), and was lower than infliximab, but the difference was not significant ($P = 0.998$, Figure 3B).

Colonic total antioxidant power as ferric reducing/antioxidant power

The ferric reducing/antioxidant power (FRAP) value was significantly lower in the control compared to the Sham ($P = 0.008$). Among interventions, only dexamethasone caused a significant increase in FRAP when compared to the control ($P = 0.000$). None of the Mag-50, Mag-100, Mag-200 or infliximab groups showed a significant difference to the control in FRAP (Figure 3C). The effect of Magliasa in FRAP was not dose dependent.

Colonic lipid peroxidation level as TBARS

The TBARS value was significantly higher in the control compared to the Sham ($P = 0.005$), while TBARS in the Mag-200 (3.27 ± 0.77, $P = 0.010$) and Dexa (3.44 ± 0.22, $P = 0.011$) groups was significantly lower than that of the control (6.43 ± 0.61). Other groups did not show a signif-

icant difference from the control in TBARS (Figure 3D). Administration of Maglasia reduced TBARS in a dose-dependent manner, with an ED50 value of 216.4 mg/kg.

Colonic MPO activity

MPO in the control was significantly higher than that of the Sham ($P < 0.002$). Treatment with Magliasa in all groups significantly decreased MPO activity compared to the control. MPO in the Mag-200 group (10.65 ± 2.53) was lowest amongst the Mag groups, and close to the Dexa group (10.42 ± 3.18). MPO in the Mag-200 group was lower than that of the infliximab group (16.41 ± 2.89) (Figure 3E). The ED50 value was 34.38 mg/kg.

LD50

The acute toxicity test (LD50) demonstrated that Magliasa is not lethal up to a dose of 2000 mg/kg after oral administration. In the treated groups, no sign of toxicity was observed. It can therefore be considered as practically non-toxic.

DISCUSSION

There is a strong potential in the traditional and folkloric medicines of various countries, including Iran, for developing new and efficacious drugs for diseases that have a challenging treatment. One such disease is IBD. In this paper, Magliasa, one of the remedies recommended for colitis in TIM, was prepared, and its efficacy and possible mechanisms of action in different doses were evaluated in TNBS-induced colitis and compared with standard drugs. Macroscopic and microscopic scores, as criteria for colonic damage, improved by doses of 100 and 200 mg/kg per day with Magliasa. The microscopic score reduced only in the Mag-200 group, while the Mag-50 group showed no significant benefit against colonic damage. Colonic TNF-α, IL-1β and MPO activities were significantly decreased by all doses of Magliasa. TNF-α and IL-1β have been described as important mediators that contribute to intestinal inflammation in IBD patients[52-54]. Increased TNF-α has been found in the serum and mucosa of patients with IBD[55,56]. Moreover, inhibition of TNF-α by anti-TNF-α drugs, such as infliximab, has been an efficacious strategy in the management of IBD[10,57]. MPO is located in the granules of neutrophils and released upon stimulation by free radicals. The activity of MPO has been known as a marker of neutrophil penetration to the site of inflammation[58,59]. Magliasa did not affect oxidative stress as a factor involved in the pathophysiology of IBD[2]. Lipid peroxidation in the colon decreased only with a high dose of Magliasa (Mag-200). The effects of Magliasa in all investigated parameters were dose-dependent, except in total antioxidant power.

The total phenolic content of Magliasa was determined because phenolic compounds have pharmacological activities (antioxidant, anti-inflammatory, anti-diarrheal, and antimicrobial) that are all useful for the management of IBD, considering its pathogenesis. There is concern about the content uniformity of Magliasa, as intact non-milled seed of *Lepidium sativum* comprises 50% of the product. In addition to a reference marker for the standardization of Magliasa, total glucosinolates can be used for evaluating the content uniformity of the product.

There are some reports on the herbal ingredients of Magliasa that confirm their efficacy in IBD[32]. These reports are summarized in Table 1. Anti-inflammatory, antioxidant, analgesic, spasmolytic, antiulcer, ulcer healing, immunomodulatory, antibacterial, and anti-diarrheal activity are among the pharmacological properties of these ingredients that make them useful for IBD. It seems that the efficacy of Magliasa in IBD is due to the combination of the mentioned activities.

Overall, the results obtained from the efficacy of Magliasa on TNBS-induced colitis of rats are encouraging, although clinical trials are required for confirmation of these results.

COMMENTS

Background

Conventional treatments for the management of inflammatory bowel disease (IBD) have serious adverse effects that reduce patient compliance, and therefore investigators are trying to find useful compounds from complementary and alternative medicines with better safety and tolerability. There are many herbal preparations in traditional Iranian medicine (TIM) that were used for the management of IBD. Magliasa is one of them, and contains 6 components: seeds of *Lepidium sativum*, *Linum usitatissimum*, and *Allium ampeloprasum* cv. Porrum, fruit of *Bunium persicum* and *Terminalia chebula*, and gum resin of *Pistacia lentiscus*. Although, the efficacy of some herbal components of Magliasa in IBD have been confirmed by previous studies, no other study to date has investigated the beneficial effects of this preparation.

Research frontiers

In the present study, after formulation and explanation of the quality control methods of Magliasa, its effects were investigated in trinitrobenzenesulfonic acid-induced colitis of rats to determine the involved mechanisms.

Innovations and breakthroughs

Magliasa demonstrated a significant reduction in macroscopic colonic damage, tumor necrosis factor-alpha, interleukin-1 beta, and neutrophil infiltration. Determination of total glucosinolates and total phenolic contents, as well as performing thin layer chromatography, can be used successfully for quality control of this herbal preparation.

Applications

Since the effects of Magliasa in the experimental model of colitis were encouraging, it could potentially be used as an effective medicine for IBD after confirmation of obtained results by clinical trials. Moreover, this study is a step toward strengthening TIM evidence.

Peer review

Hopefully reviewers are positive to this article and believe that this TIM formula has enough support to go forward future clinical trials.

REFERENCES

1 **Brown SJ**, Mayer L. The immune response in inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 2058-2069 [PMID: 17561966 DOI: 10.1111/j.1572-0241.2007.01343.x]

2 **Rezaie A**, Parker RD, Abdollahi M. Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause? *Dig Dis Sci* 2007; **52**: 2015-2021 [PMID: 17404859 DOI: 10.1007/s10620-006-9622-2]

3 **Reiff C**, Kelly D. Inflammatory bowel disease, gut bacteria and probiotic therapy. *Int J Med Microbiol* 2010; **300**: 25-33 [PMID: 19800289 DOI: 10.1016/j.ijmm.2009.08.004]

4 **Kucharzik T**, Maaser C, Lügering A, Kagnoff M, Mayer L, Targan S, Domschke W. Recent understanding of IBD pathogenesis: implications for future therapies. *Inflamm Bowel Dis* 2006; **12**: 1068-1083 [PMID: 17075348]

5 **Martín MC**, Martinez A, Mendoza JL, Taxonera C, Díaz-Rubio M, Fernández-Arquero M, de la Concha EG, Urcelay E. Influence of the inducible nitric oxide synthase gene (NOS2A) on inflammatory bowel disease susceptibility. *Immunogenetics* 2007; **59**: 833-837 [PMID: 17955236 DOI: 10.1007/s00251-007-0255-1]

6 **Rezaie A**, Khalaj S, Shabihkhani M, Nikfar S, Zamani MJ, Mohammadirad A, Daryani NE, Abdollahi M. Study on the correlations among disease activity index and salivary transforming growth factor-beta 1 and nitric oxide in ulcerative colitis patients. *Ann N Y Acad Sci* 2007; **1095**: 305-314 [PMID: 17404043]

7 **Rezaie A**, Ghorbani F, Eshghtork A, Zamani MJ, Dehghan G, Taghavi B, Nikfar S, Mohammadirad A, Daryani NE, Abdollahi M. Alterations in salivary antioxidants, nitric oxide, and transforming growth factor-beta 1 in relation to disease activity in Crohn's disease patients. *Ann N Y Acad Sci* 2006; **1091**: 110-122 [PMID: 17341608]

8 **Jahanshahi G**, Motavasel V, Rezaie A, Hashtroudi AA, Daryani NE, Abdollahi M. Alterations in antioxidant power and levels of epidermal growth factor and nitric oxide in saliva of patients with inflammatory bowel diseases. *Dig Dis Sci* 2004; **49**: 1752-1757 [PMID: 15628697 DOI: 10.1007/s10620-004-9564-5]

9 **El Miedany Y**, Youssef S, Ahmed I, El Gaafary M. The gastrointestinal safety and effect on disease activity of etoricoxib, a selective cox-2 inhibitor in inflammatory bowel diseases. *Am J Gastroenterol* 2006; **101**: 311-317 [PMID: 16454836 DOI: 10.1111/j.1572-0241.2006.00384.x]

10 **Rahimi R**, Nikfar S, Abdollahi M. Meta-analysis technique confirms the effectiveness of anti-TNF-alpha in the management of active ulcerative colitis when administered in combination with corticosteroids. *Med Sci Monit* 2007; **13**: PI13-PI18 [PMID: 17599035]

11 **Rahimi R**, Nikfar S, Abdollahi M. Do anti-tumor necrosis factors induce response and remission in patients with acute refractory Crohn's disease? A systematic meta-analysis of controlled clinical trials. *Biomed Pharmacother* 2007; **61**: 75-80 [PMID: 17184965 DOI: 10.1016/j.biopha.2006.06.022]

12 **Nikfar S**, Ehteshami-Afshar S, Abdollahi M. A systematic review and meta-analysis of the efficacy and adverse events of infliximab in comparison to corticosteroids and placebo in active ulcerative colitis. *Int J Pharmacol* 2011; **7**: 325-332 [DOI: 10.3923/ijp.2011.325.332]

13 **Khan KJ**, Dubinsky MC, Ford AC, Ullman TA, Talley NJ, Moayyedi P. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 630-642 [PMID: 21407186 DOI: 10.1038/ajg.2011.64]

14 **Nikfar S**, Mirfazaelian H, Abdollahi M. Efficacy and tolerability of immunoregulators and antibiotics in fistulizing Crohn's disease: a systematic review and meta-analysis of placebo-controlled trials. *Curr Pharm Des* 2010; **16**: 3684-3698 [PMID: 21143147 DOI: 10.2174/13816121079407923]

15 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. A meta-analysis of antibiotic therapy for active ulcerative colitis. *Dig Dis Sci* 2007; **52**: 2920-2925 [PMID: 17415632 DOI: 10.1007/s10620-007-9760-1]

16 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. A meta-analysis of broad-spectrum antibiotic therapy in patients with active Crohn's disease. *Clin Ther* 2006; **28**: 1983-1988 [PMID: 17296455 DOI: 10.1016/j.clinthera.2006.12.012]

17 **Rahimi R**, Nikfar S, Abdollahi M. A meta-analysis of the benefit of probiotics in maintaining remission of human ulcerative colitis: evidence for prevention of disease relapse and maintenance of remission. *Arch Med Sci* 2008; **4**: 185-190

18 **Rahimi R**, Nikfar S, Rahimi F, Elahi B, Derakhshani S, Vafaie M, Abdollahi M. A meta-analysis on the efficacy of probiotics for maintenance of remission and prevention of clinical and endoscopic relapse in Crohn's disease. *Dig Dis Sci* 2008; **53**: 2524-2531 [PMID: 18270836 DOI: 10.1007/s10620-007-0171-0]

19 **Triantafillidis JK**, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug Des Devel Ther* 2011; **5**: 185-210 [PMID: 21552489]

20 **Nikfar S**, Rahimi R, Rezaie A, Abdollahi M. A meta-analysis of the efficacy of sulfasalazine in comparison with 5-aminosalicylates in the induction of improvement and maintenance of remission in patients with ulcerative colitis. *Dig Dis Sci* 2009; **54**: 1157-1170 [PMID: 18770034 DOI: 10.1007/s10620-008-0481-x]

21 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Comparison of mesalazine and balsalazide in induction and maintenance of remission in patients with ulcerative colitis: a meta-analysis. *Dig Dis Sci* 2009; **54**: 712-721 [PMID: 18683049 DOI: 10.1007/s10620-008-0428-2]

22 **Nikfar S**, Ehteshami-Ashar S, Rahimi R, Abdollahi M. Systematic review and meta-analysis of the efficacy and tolerability of nicotine preparations in active ulcerative colitis. *Clin Ther* 2010; **32**: 2304-2315 [PMID: 21353102 DOI: 10.1016/j.clinthera.2011.01.004]

23 **Hosseini-Tabatabaei A**, Abdollahi M. Potassium channel openers and improvement of toxic stress: do they have role in the management of inflammatory bowel disease? *Inflamm Allergy Drug Targets* 2008; **7**: 129-135 [PMID: 18782019 DOI: 10.2174/187152808785748164]

24 **Salari P**, Abdollahi M. Current opinion in the pharmaceutical management of irritable and inflammatory bowel diseases: Role of ATP. *Recent Pat Endocr Metab Immune Drug Discov* 2009; **3**: 69-75 [DOI: 10.2174/187221409787002949]

25 **Khoshakhlagh P**, Bahrololoumi-Shapourabadi M, Mohammadirad A, Ashtaral-Nakhai L, Minaie B, Abdollahi M. Beneficial effect of phosphodiesterase-5 inhibitor in experimental inflammatory bowel disease; molecular evidence for involvement of oxidative stress. *Toxicol Mech Methods* 2007; **17**: 281-288 [PMID: 20020951 DOI: 10.1080/15376510601003769]

26 **Salari-Sharif P**, Abdollahi M. Phosphodiesterase 4 inhibitors in inflammatory bowel disease: a comprehensive review. *Curr Pharm Des* 2010; **16**: 3661-3667 [PMID: 21128899]

27 **Rahimi R**, Ghiasi S, Azimi H, Fakhari S, Abdollahi M. A review of the herbal phosphodiesterase inhibitors; future perspective of new drugs. *Cytokine* 2010; **49**: 123-129 [PMID: 20005737]

28 **Stein RB**, Hanauer SB. Comparative tolerability of treatments for inflammatory bowel disease. *Drug Saf* 2000; **23**: 429-448 [PMID: 11085348 DOI: 10.2165/00002018-200023050-00006]

29 **Stallmach A**, Hagel S, Bruns T. Adverse effects of biologics used for treating IBD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 167-182 [PMID: 20227030 DOI: 10.1016/j.bpg.2010.01.002]

30 **Rahimi R**, Nikfar S, Rezaie A, Abdollahi M. Pregnancy outcome in women with inflammatory bowel disease following exposure to 5-aminosalicylic acid drugs: a meta-analysis. *Reprod Toxicol* 2008; **25**: 271-275 [PMID: 18242053 DOI: 10.1016/j.reprotox.2007.11.010]

31 **Rahimi R**, Mozaffari S, Abdollahi M. On the use of herbal medicines in management of inflammatory bowel diseases: a systematic review of animal and human studies. *Dig Dis Sci* 2009; **54**: 471-480 [PMID: 18618255 DOI: 10.1007/s10620-008-0368-x]

32 **Rahimi R**, Shams-Ardekani MR, Abdollahi M. A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. *World J Gastroenterol* 2010; **16**: 4504-4514 [PMID: 20857519 DOI: 10.3748/wjg.v16.i36.4504]

33 **Ardekani MR**, Rahimi R, Javadi B, Abdi L, Khanavi M. Relationship between temperaments of medicinal plants and their major chemical compounds. *J Tradit Chin Med* 2011; **31**: 27-31 [PMID: 21563502 DOI: 10.1016/S0254-6272(11)60006-X]

34 **Arzani MA**. Tebb-e-akbari [in Persian]. Institute of Revival of Natural Medicine, 2007: 778-781
35 **Tonkaboni MM**. Tohfeh al- Momenin [in Persian]. Shahid Beheshti University of Medical Sciences, 2007: 356-542
36 **Aghili MH**. Makhzan-al-Advia [in Persian]. Tehran University of Medical Sciences, 2009: 165-742
37 **Amin G**. Popular medicinal plants of Iran. Tehran University of Medical Sciences, 2008: 104-264
38 **World Health Organization**. Quality control methods for medicinal plant materials. Geneva: World Health Organization, 1998: 5
39 **Smith CA**, Dacombe C. Rapid method for determining total glucosinolates in rapeseed by measurement of enzymatically released glucose. *J Sci Food Agric* 1987; **38**: 141-150 [DOI: 10.1002/jsfa.2740380206]
40 **Singleton VL**, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* 1999; **299**: 152-178 [DOI: 10.1016/S0076-6879(99)99017-1]
41 **Wagner H**, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Springer, 2001: 149-152
42 **Baghaei A**, Esmaily H, Abdolghaffari AH, Baeeri M, Gharibdoost F, Abdollahi M. Efficacy of Setarud (IMod), a novel drug with potent anti-toxic stress potential in rat inflammatory bowel disease and comparison with dexamethasone and infliximab. *Indian J Biochem Biophys* 2010; **47**: 219-226 [PMID: 21174949]
43 **Abdolghaffari AH**, Baghaei A, Moayer F, Esmaily H, Baeeri M, Monsef-Esfahani HR, Hajiaghaee R, Abdollahi M. On the benefit of Teucrium in murine colitis through improvement of toxic inflammatory mediators. *Hum Exp Toxicol* 2010; **29**: 287-295 [PMID: 20144954 DOI: 10.1177/0960327110361754]
44 **Ebrahimi F**, Esmaily H, Baeeri M, Mohammadirad A, Fallah S, Abdollahi M. Molecular evidences on the benefit of N-acetylcysteine in experimental colitis. *Cent Eur J Biol* 2008; **3**: 135-132 [DOI: 10.2478/s11535-008-0005-x]
45 **Ghafari H**, Yasa N, Mohammadirad A, Dehghan G, Zamani MJ, Nikfar S, Khorasani R, Minaie B, Abdollahi M. Protection by Ziziphora clinopoides of acetic acid-induced toxic bowel inflammation through reduction of cellular lipid peroxidation and myeloperoxidase activity. *Hum Exp Toxicol* 2006; **25**: 325-332 [PMID: 16866190 DOI: 10.1191/0960327105ht626oa]
46 **Nakhai LA**, Mohammadirad A, Yasa N, Minaie B, Nikfar S, Ghazanfari G, Zamani MJ, Dehghan G, Jamshidi H, Boushehri VS, Khorasani R, Abdollahi M. Benefits of Zataria multiflora Boiss in Experimental Model of Mouse Inflammatory Bowel Disease. *Evid Based Complement Alternat Med* 2007; **4**: 43-50 [PMID: 17342240 DOI: 10.1093/ecam/nel051]
47 **Hasani P**, Yasa N, Vosough-Ghanbari S, Mohammadirad A, Dehghan G, Abdollahi M. In vivo antioxidant potential of Teucrium polium, as compared to alpha-tocopherol. *Acta Pharm* 2007; **57**: 123-129 [PMID: 19839412 DOI: 10.2478/v10007-007-0010-z]
48 **Ghazanfari G**, Minaie B, Yasa N, Nakhai LA, Mohammadirad A, Nikfar S, Dehghan G, Boushehri VS, Jamshidi H, Khorasani R, Salehnia A, Abdollahi M. Biochemical and histopathological evidences for beneficial effects of satureja khuzestanica jamzad essential oil on the mouse model of inflammatory bowel diseases. *Toxicol Mech Methods* 2006; **16**: 365-372 [PMID: 20021009]
49 **Amini-Shirazi N**, Hoseini A, Ranjbar A, Mohammadirad A, Khoshakhlagh P, Yasa N, Abdollahi M. Inhibition of tumor necrosis factor and nitrosative/oxidative stresses by Ziziphora clinopoides (Kahlioti); a molecular mechanism of protection against dextran sodium sulfate-induced colitis in mice. *Toxicol Mech Methods* 2009; **19**: 183-189 [PMID: 19778264]
50 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051]
51 **Hodgson E**. A textbook of modern toxicology. 3rd ed. New Jersey: John Wily and Sons, 2004 [DOI: 10.1002/0471646776]
52 **Strober W**, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1756-1767 [PMID: 21530742 DOI: 10.1053/j.gastro.2011.02.016]
53 **Cominelli F**, Pizarro TT. Interleukin-1 and interleukin-1 receptor antagonist in inflammatory bowel disease. *Aliment Pharmacol Ther* 1996; **10** Suppl 2: 49-53; discussion 54 [PMID: 8899101]
54 **Esmaily H**, Vaziri-Bami A, Miroliaee AE, Baeeri M, Abdollahi M. The correlation between NF-κB inhibition and disease activity by coadministration of silibinin and ursodeoxycholic acid in experimental colitis. *Fundam Clin Pharmacol* 2011; **25**: 723-733 [PMID: 21077947 DOI: 10.1111/j.1472-8206.2010.00893.x]
55 **Akazawa A**, Sakaida I, Higaki S, Kubo Y, Uchida K, Okita K. Increased expression of tumor necrosis factor-alpha messenger RNA in the intestinal mucosa of inflammatory bowel disease, particularly in patients with disease in the inactive phase. *J Gastroenterol* 2002; **37**: 345-353 [PMID: 12051533]
56 **Komatsu M**, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. *Clin Chem* 2001; **47**: 1297-1301 [PMID: 11427462]
57 **Ford AC**, Sandborn WJ, Khan KJ, Hanauer SB, Talley NJ, Moayyedi P. Efficacy of biological therapies in inflammatory bowel disease: systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 644-659, quiz 660 [PMID: 21407183 DOI: 10.1038/ajg.2011.73]
58 **Grulke S**, Franck T, Gangl M, Péters F, Salciccia A, Deby-Dupont G, Serteyn D. Myeloperoxidase assay in plasma and peritoneal fluid of horses with gastrointestinal disease. *Can J Vet Res* 2008; **72**: 37-42 [PMID: 18214160]
59 **Miroliaee AE**, Esmaily H, Vaziri-Bami A, Baeeri M, Shahverdi AR, Abdollahi M. Amelioration of experimental colitis by a novel nanoselenium-silymarin mixture. *Toxicol Mech Methods* 2011; **21**: 200-208 [PMID: 21247366 DOI: 10.3109/15376516.2010.547887]
60 **Kassie F**, Rabot S, Uhl M, Huber W, Qin HM, Helma C, Schulte-Hermann R, Knasmüller S. Chemoprotective effects of garden cress (Lepidium sativum) and its constituents towards 2-amino-3-methyl-imidazo[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. *Carcinogenesis* 2002; **23**: 1155-1161 [PMID: 12117773 DOI: 10.1093/carcin/23.7.1155]
61 **Datta PK**, Diwakar BT, Viswanatha S, Murthy KN, Naidu KA. Safety evaluation studies on Garden cress (Lepidium sativum L.) seeds in Wistar rats. *Int J Appl Res Nat Prod* 2011; **4**: 37-43
62 **Bafeel SO**, Ali SS. The Potential Liver Toxicity of Lepidium sativum Seeds in Albino Rats. *Res J Biol Sci* 2009; **4**: 1250-1258 [DOI: 10.3923/rjbsci.2009.1250.1258]
63 **Moser BR**, Shah SN, Moser JK, Vaughn SF, Evangelista RL. Composition and Physical Properties of Cress (Lepidium sativum L.) and Field Pennycress (Thlaspi arvense L.) Oils. *Indust Crops Prod* 2009; **30**: 199-205 [DOI: 10.1016/j.indcrop.2009.03.007]
64 **Diwakar BT**, Lokesh BR, Naidu KA. Modulatory effect of α-linolenic acid-rich garden cress (Lepidium sativum L.) seed oil on inflammatory mediators in adult albino rats. *Br J Nutr* 2011; **106**: 530-539 [PMID: 21521541]
65 **Al-Yahya MA**, Mossa JS, Ageel AM, Rafatullah S. Pharmacological and safety evaluation studies on Lepidium sativum L., seeds. *Phytomedicine* 1994; **1**: 155-159 [DOI: 10.1016/S0944-7113(11)80035-8]
66 **Najeeb-Ur-Rehman MH**, Alkharfy KM, Gilani AH. Prokinetic and laxative activities of Lepidium sativum seed extract with species and tissue selective gut stimulatory actions.

J Ethnopharmacol 2011; **134**: 878-883 [PMID: 21296647 DOI: 10.1016/j.jep.2011.01.047]
67 **Rehman NU**, Mehmood MH, Alkharfy KM, Gilani AH. Studies on antidiarrheal and antispasmodic activities of Lepidium sativum crude extract in rats. *Phytother Res* 2012; **26**: 136-141 [PMID: 22006354 DOI: 10.1002/ptr.3642]
68 **Rehman NU**, Khan AU, Alkharfy KM, Gilani AH. Pharmacological Basis for the Medicinal Use of Lepidium sativum in Airways Disorders. *Evid Based Complement Alternat Med* 2012; **2012**: 596524 [PMID: 22291849 DOI: 10.1155/2012/596524]
69 **Azizi M**, Davareenejad G. Essential Oil Content and Constituents of Black Zira (Bunium persicum [Boiss.] B. Fedtsch.) from Iran During Field Cultivation (Domestication). *J Essent Oil Res* 2009; **21**: 78-82 [DOI: 10.1080/10412905.2009.9700117]
70 **Sharififar F**, Yassa N, Mozaffarian V. Bioactivity of major components from the seeds of Bunium persicum (Boiss.) Fedtch. *Pak J Pharm Sci* 2010; **23**: 300-304 [PMID: 20566444]
71 **Nickavar B**, Abolhasani FA. Screening of antioxidant properties of seven Umbelliferae fruits from Iran. *Pak J Pharm Sci* 2009; **22**: 30-35 [PMID: 19168417]
72 **Shahsavari N**, Barzegar M, Sahari MA, Naghdibadi H. Antioxidant activity and chemical characterization of essential oil of Bunium persicum. *Plant Foods Hum Nutr* 2008; **63**: 183-188 [PMID: 18810640]
73 **Hajhashemi V**, Sajjadi SE, Zomorodkia M. Antinociceptive and anti-inflammatory activities of Bunium persicum essential oil, hydroalcoholic and polyphenolic extracts in animal models. *Pharm Biol* 2011; **49**: 146-151 [PMID: 20942602]
74 **Singh KK**, Mridula D, Rehal J, Barnwal P. Flaxseed: a potential source of food, feed and fiber. *Crit Rev Food Sci Nutr* 2011; **51**: 210-222 [PMID: 21390942 DOI: 10.1080/10408390903537241]
75 **Kaithwas G**, Majumdar DK. Evaluation of antiulcer and antisecretory potential of Linum usitatissimum fixed oil and possible mechanism of action. *Inflammopharmacology* 2010; **18**: 137-145 [PMID: 20405222 DOI: 10.1007/s10787-010-0037-5]
76 **Tülüce Y**, Ozkol H, Koyuncu I. Photoprotective effect of flax seed oil (Linum usitatissimum L.) against ultraviolet C-induced apoptosis and oxidative stress in rats. *Toxicol Ind Health* 2012; **28**: 99-107 [PMID: 21665902 DOI: 10.1177/0748233711407239]
77 **Bommareddy A**, Zhang X, Schrader D, Kaushik RS, Zeman D, Matthees DP, Dwivedi C. Effects of dietary flaxseed on intestinal tumorigenesis in Apc(Min) mouse. *Nutr Cancer* 2009; **61**: 276-283 [PMID: 19235044 DOI: 10.1080/01635580802419764]
78 **Fattorusso E**, Lanzotti V, Taglialatela-Scafati O, Cicala C. The flavonoids of leek, Allium porrum. *Phytochemistry* 2001; **57**: 565-569 [PMID: 11394858 DOI: 10.1016/S0031-9422(01)00039-5]
79 **Fattorusso E**, Lanzotti V, Taglialatela-Scafati O, Di Rosa M, Ianaro A. Cytotoxic saponins from bulbs of Allium porrum L. *J Agric Food Chem* 2000; **48**: 3455-3462 [PMID: 10956133]
80 **Heinonen MI**, Ollilainen V, Linkola EK, Varo PT, Koivistoinen PE. Carotenoids in Finnish foods, vegetables, fruits and berries. *J Agric Food Chem* 1989; **37**: 655-659 [DOI: 10.1021/jf00087a017]
81 **Evans WC**. Trease and Evans pharmacognosy. 15th Ed. WB Saunders, 2002
82 **Manosroi A**, Jantrawut P, Akazawa H, Akihisa T, Manosroi J. Biological activities of phenolic compounds isolated from galls of Terminalia chebula Retz. (Combretaceae). *Nat Prod Res* 2010; **24**: 1915-1926 [PMID: 21108118]
83 **Chang CL**, Lin CS. Phytochemical Composition, Antioxidant Activity, and Neuroprotective Effect of Terminalia chebula Retzius Extracts. *Evid Based Complement Alternat Med* 2012; **2012**: 125247 [PMID: 21754945 DOI: 10.1155/2012/125247]
84 **Das ND**, Jung KH, Park JH, Mondol MA, Shin HJ, Lee HS, Park KS, Choi MR, Kim KS, Kim MS, Lee SR, Chai YG. Terminalia chebula extract acts as a potential NF-κB inhibitor in human lymphoblastic T cells. *Phytother Res* 2011; **25**: 927-934 [PMID: 21509843 DOI: 10.1002/ptr.3398]
85 **Sharma P**, Prakash T, Kotresha D, Ansari MA, Sahrm UR, Kumar B, Debnath J, Goli D. Antiulcerogenic activity of Terminalia chebula fruit in experimentally induced ulcer in rats. *Pharm Biol* 2011; **49**: 262-268 [PMID: 21323478]
86 **Nair V**, Singh S, Gupta YK. Anti-arthritic and disease modifying activity of Terminalia chebula Retz. in experimental models. *J Pharm Pharmacol* 2010; **62**: 1801-1806 [PMID: 21054408 DOI: 10.1111/j.2042-7158.2010.01193.x]
87 **Kim HG**, Cho JH, Jeong EY, Lim JH, Lee SH, Lee HS. Growth-inhibiting activity of active component isolated from Terminalia chebula fruits against intestinal bacteria. *J Food Prot* 2006; **69**: 2205-2209 [PMID: 16995525]
88 **Zhou L**, Satoh K, Takahashi K, Watanabe S, Nakamura W, Maki J, Hatano H, Takekawa F, Shimada C, Sakagami H. Re-evaluation of anti-inflammatory activity of mastic using activated macrophages. *In Vivo* 2009; **23**: 583-589 [PMID: 19567394]
89 **Kim HJ**, Neophytou C. Natural anti-inflammatory compounds for the management and adjuvant therapy of inflammatory bowel disease and its drug delivery system. *Arch Pharm Res* 2009; **32**: 997-1004 [PMID: 19641880 DOI: 10.1007/s12272-009-1704-1]
90 **Al-Said MS**, Ageel AM, Parmar NS, Tariq M. Evaluation of mastic, a crude drug obtained from Pistacia lentiscus for gastric and duodenal anti-ulcer activity. *J Ethnopharmacol* 1986; **15**: 271-278 [PMID: 3724207 DOI: 10.1016/0378-8741(86)90165-0]
91 **Kaliora AC**, Stathopoulou MG, Triantafillidis JK, Dedoussis GV, Andrikopoulos NK. Alterations in the function of circulating mononuclear cells derived from patients with Crohn's disease treated with mastic. *World J Gastroenterol* 2007; **13**: 6031-6036 [PMID: 18023095 DOI: 10.3748/wjg.13.6031]
92 **Kaliora AC**, Stathopoulou MG, Triantafillidis JK, Dedoussis GV, Andrikopoulos NK. Chios mastic treatment of patients with active Crohn's disease. *World J Gastroenterol* 2007; **13**: 748-753 [PMID: 17278198]
93 **Al-Habbal MJ**, Al-Habbal Z, Huwez FU. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. *Clin Exp Pharmacol Physiol* 1984; **11**: 541-544 [PMID: 6395994 DOI: 10.1111/j.1440-1681.1984.tb00864.x]

P- Reviewer Bonaz B **S- Editor** Gou SX
L- Editor Rutherford A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1912

World J Gastroenterol 2013 March 28; 19(12): 1912-1918
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Expression and clinical significance of CD73 and hypoxia-inducible factor-1α in gastric carcinoma

Xiao-Xia Lu, Yi-Tian Chen, Bing Feng, Xiao-Bei Mao, Bo Yu, Xiao-Yuan Chu

Xiao-Xia Lu, Yi-Tian Chen, Bing Feng, Xiao-Bei Mao, Xiao-Yuan Chu, Department of Medical Oncology, Nanjing General Hospital of Nanjing Military Command, Medical School of Nanjing University, Nanjing 210002, Jiangsu Province, China
Bo Yu, Department of Pathology, Nanjing General Hospital of Nanjing Military Command, Nanjing 210002, Jiangsu Province, China
Author contributions: Lu XX and Chen YT contributed equally to this work; Lu XX and Chu XY designed the research; Chen YT, Mao XB and Yu B performed the research; Lu XX and Chen YT evaluated data and prepared the manuscript; Lu XX, Feng B and Chu XY were involved in the manuscript writing.
Supported by National Natural Science Foundation of China, No. 81071806
Correspondence to: Xiao-Yuan Chu, Associate Professor, Department of Medical Oncology, Nanjing General Hospital of Nanjing Military Command, Medical School of Nanjing University, No. 305 Zhongshan Road, Nanjing 210002, Jiangsu Province, China. chuxiaoyuan6906@gmail.com
Telephone: +86-25-80860072 Fax: +86-25-80860072
Received: July 15, 2012 Revised: August 15, 2012
Accepted: September 28, 2012
Published online: March 28, 2013

Abstract

AIM: To investigate the expression of CD73 and hypoxia-inducible factor-1α (HIF-1α) in human gastric carcinoma, and explore their clinical significance and prognostic value.

METHODS: CD73 and HIF-1α expressions were detected by immunohistochemistry in consecutive sections of tissue samples from 68 gastric carcinoma patients. The peritumor tissues 2 cm away from the tumor were obtained and served as controls. The presence of CD73 and HIF-1α was analyzed by immunohistochemistry using the Envision technique.

RESULTS: CD73 and HIF-1α expressions in gastric carcinoma were significantly higher than those in gastric mucosal tissues as control ($P < 0.001$) and showed a close correlation (Spearman $r = 0.390$, $P = 0.001$). Overexpression of CD73 was positively correlated with differentiation of tumor ($P = 0.000$), histopathology ($P = 0.041$), depth of invasion ($P < 0.001$), nodal status ($P = 0.003$), metastasis ($P = 0.013$), and the American Joint Committee on Cancer (AJCC) stage ($P < 0.001$). High expression of HIF-1α was positively correlated with tumor diameter ($P = 0.031$), depth of invasion ($P = 0.022$), and AJCC stage ($P = 0.035$). The overall survival rate was low in the patients with high expression of CD73 ($P < 0.001$). Moreover, CD73+/HIF-1α+ patients had the worst prognosis ($P < 0.001$). CD73 expression was proven to be an independent predictor for patients with gastric carcinoma by both multivariate Cox regression analysis ($P = 0.021$) and receiver operating characteristic curves ($P = 0.001$).

CONCLUSION: CD73 expression correlates closely with HIF-1α expression in gastric carcinoma. CD73 could be an independent prognostic indicator for gastric carcinoma.

Key words: CD73; Hypoxia-inducible factor-1α; Gastric carcinoma; Immunohistochemistry; Prognosis

Lu XX, Chen YT, Feng B, Mao XB, Yu B, Chu XY. Expression and clinical significance of CD73 and hypoxia-inducible factor-1α in gastric carcinoma. *World J Gastroenterol* 2013; 19(12): 1912-1918 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1912.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1912

INTRODUCTION

Gastric carcinoma has been the fourth most common cancer in the world since the latter half of the 20th century[1,2]. In spite of the recent advances in diagnostic techniques

for early detection and the improvement in surgical treatment, gastric carcinoma remains the second leading cause of cancer-related deaths[3]. In China, Japan and Korea, the incidence of gastric carcinoma now has reached up to 80 new cases per 100 000 population annually[4]. Changes observed in expression of tumor specific biomarkers in gastric carcinomas may be helpful to understand the transformation of histological heterogeneity and the underlying molecular mechanisms. Searching for specific biomarkers which determine the biological nature and behavior of gastric carcinoma would be of utmost importance to optimize individualized therapy.

Ecto-5'-nucleotidase/CD73 is a homodimer linked to the plasma membrane through a glycosylphosphatidylinositol lipid anchor, which was found in most tissues[5]. It is a part of extracellular ATP metabolism, which dephosphorylates AMP into adenosine rapidly after CD39 catalyzes ATP, ADP and AMP[6]. Recent studies have demonstrated that CD73 could participate in a variety of physiological responses including ischemic preconditioning, platelet function, hypoxia, vascular leak and tissue injury[7,8]. CD73 was up-regulated in various human cancers, including those of lung, colon, breast, pancreas and ovary[9,10]. Importantly, the high expression of CD73 was correlated with tumor neovascularization, invasiveness, metastasis, as well as shorter patient survival[9-13]. These results suggested that CD73 might play a significant role in controlling tumor progression.

Oxygen is only able to diffuse 100-180 μm from a capillary to cells, which makes hypoxia a common feature of rapidly growing solid tumors[14]. Hypoxia-inducible factor-1 (HIF-1) is a heterodimeric basic helix-loop-helix transcription factor composed of HIF-1α and HIF-1β subunits; and HIF-1α determines HIF-1 activity[15]. It is found that HIF-1α is wildly expressed in various types of carcinomas, such as those of brain, breast, lung and colon[16-18]. These results revealed that HIF-1α was correlated with tumor progression, aggressive behavior, and patient prognosis. As is known, pathophysiologic conditions of hypoxia can cause adenine nucleotide metabolic changes. A recent study found that CD73 is transcriptionally regulated by ambient hypoxia and is one of the mechanisms involved HIF-1[19].

The purpose of this study was to ascertain the correlation between CD73 and HIF-1α expressions and their clinicopathological significance in gastric carcinoma, including patient survival. We hypothesized that CD73 expression would be correlated with clinicopathological factors and HIF-1α expression, and the combination of the two molecules would predict recurrence and overall survival.

MATERIALS AND METHODS

Patients

Samples of gastric carcinoma were collected from the resected stomach of 68 patients who were diagnosed histologically as gastric carcinoma and underwent gastrectomy at the Nanjing General Hospital of Nanjing Military Command. None of the patients had previously received radiotherapy, chemotherapy or other medical interventions before surgery. Among them, 43 (63%) patients were male and 25 (37%) were female, with a mean age of 49.86 years. All patients were followed up from the date of surgery until either the date of death or December 2011. For analysis of patient survival, the patients who were lost to follow-up or those who died from causes other than gastric carcinoma were regarded as censored data.

This study was approved by the Institutional Review Board, and informed consent was obtained from each patient.

Immunohistochemistry

The peritumor tissues 2 cm away from the tumor were collected and served as healthy controls. Tumor specimens and healthy control gastric mucosal tissues, which were fixed in 10% buffered formalin and embedded in paraffin, were cut into 4-μm sections and placed on polylysine-coated slides. The staining was conducted by the avidin-biotin-peroxidase complex method. Each paraffin section was deparaffinized and rehydrated though graded alcohols, followed by antigen retrieval with epitope retrieval solution (10 mmol citrate buffer, pH 6.0) in a pre-heated water bath at 98 ℃ for 10 min. Endogenous peroxidase was blocked using 3% hydrogen peroxide. Subsequently, sections were incubated with the primary mouse monoclonal CD73 antibody (1:100, ab71322 Abcam) and mouse monoclonal HIF-1α antibody (1:100, MAB1935 R and D) overnight at 4 ℃, and then were stained with secondary antibody for 30 min. The sections were finally counterstained with haematoxylin (Zymed Laboratories Inc, San Francisco, CA, United States). Negative control was performed by replacing the primary antibody with a normal murine immunoglobulin G. Known immunostaining-positive sections were used as positive controls.

Evaluation of immunohistochemical analysis

We used semi-quantitative method. Five different perspectives were randomly selected under ordinary optical microscope at a magnification of 400. The percentage of positive cells was scored 0 for staining of < 1%, 1 for staining of 2%-25%, 2 for staining of 26%-50%, 3 for staining of 51%-75%, and 4 for staining > 75% of the cells examined. Staining intensity was calculated, no coloring, slightly yellow, brown yellow and tan stains were marked as 0, 1, 2 and 3. Finally, we calculated the product of staining intensity and positive cell percentage: ≤ 5 was defined as negative and ≥ 6 as positive. Two pathologists blinded to the clinical details reviewed the pathological films and staining points.

Statistical analysis

Categorical data were analyzed using the χ^2 or nonparametric test, while measurement data were evaluated with Student's *t* or one-way analysis of variance test. Correlation coefficient between expression of CD73 and HIF-1α was estimated by the Spearman correlation method.

Table 1 Correlation of CD73 and hypoxia-inducible factor-1α expression with clinicopathological characteristics of gastric carcinoma

Clinicopathological data	CD73 expression High	CD73 expression Low	*P* value	HIF-1α expression High	HIF-1α expression Low	*P* value
Gender			0.144			0.136
Male	18	25		21	22	
Female	13	12		15	10	
Age (yr)			0.157			0.107
< 49.82	10	15		11	14	
⩾ 49.82	21	22		25	18	
Tumor diameter (cm)			0.127			0.031
⩽ 5	7	17		9	15	
5-10	10	5		8	7	
> 10	14	15		19	10	
Differentiation			0.000			0.445
Well	1	4		4	1	
Moderate	6	22		13	15	
Poor	24	11		19	16	
Histopathology			0.041			0.168
Tubular adenocarcinoma	3	16		7	12	
Poorly differentiated adenocarcinoma	21	11		21	11	
Signet-ring cell carcinoma	3	3		2	4	
Mucinous adenocarcinoma	4	7		6	5	
Borrmann type			0.140			0.430
Ⅰ	5	19		9	16	
Ⅱ	9	12		9	12	
Ⅲ	15	13		17	11	
Ⅳ	2	2		1	3	
Depth of invasion			0.000			0.036
T1-T2	1	21		8	14	
T3-T4	30	16		28	18	
Nodal status			0.003			0.113
N0	4	17		9	12	
N1/N2	27	20		27	20	
Metastasis			0.013			0.192
M0	20	33		27	26	
M1	11	4		9	6	
AJCC stage			0.000			0.035
Ⅰ / Ⅱ	2	24		10	16	
Ⅲ / Ⅳ	27	15		26	16	

HIF-1α: Hypoxia-inducible factor-1α; AJCC: American Joint Committee on Cancer.

The Kaplan-Meier method was used to estimate the overall survival and the log-rank test was used to analyze the differences between the curves. Multivariate Cox proportional hazard regression model and receiver operating characteristic (ROC) curve analysis were established to assess the prognostic values of protein expression. All statistical analysis were performed using the SPSS software version 16.0 (SPSS, Chicago, IL, United States) and $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The demographic and clinicopathological variables of the 68 patients are shown in Table 1. The ages of the patients ranged from 24 to 59 years with the mean age 49.82 years. Based on the American Joint Committee on Cancer (AJCC) classification, there were 26 stage Ⅰ / Ⅱ patients and 42 stage Ⅲ / Ⅳ patients. The patients were followed up for a period of 1-84 mo. Two were lost to follow-up and 37 patients died during the follow-up.

Immunohistochemical analysis of CD73 and HIF-1α expression

CD73 and HIF-1α were detected on consecutive sections and were found to be mainly expressed in the cytoplasm of gastric carcinoma (Figure 1). We repeated the experiment twice to exclude false positive results. Immunohistochemical analysis showed that in gastric carcinoma, 31 (45.6%) of 68 samples were CD73-positive and 36 (52.9%) of 68 were HIF-1α-positive, while in healthy control gastric mucosa, 8 (11.8%) of 68 were CD73-positive and 12 (17.6%) of 68 were HIF-1α-positive. CD73 ($P < 0.001$) and HIF-1α ($P < 0.001$) expression was significantly higher than in healthy controls. CD73 expression was concordant with HIF-1α expression in 69.1% (47 of 68) of gastric carcinoma cases (Spearman $r = 0.390$, $P = 0.001$). CD73 and HIF-1α were found double-positive in 23 cases, double-negative in 24 cases, and either CD73-positive or HIF-1α-positive only in 21 cases. The Spearman's rank correlation method was used to estimate the expression correlation coefficient ($r = 0.390$, $P = 0.001$), indicating a close correlation between CD73 and HIF-1α expression in gastric carcinomas.

Correlation of CD73 and HIF-1α expression with clinicopathological variables

Chi-squared test was used to investigate the correlation of CD73 and HIF-1α expression with clinicopathological variables. Statistically, CD73 overexpression was significantly correlated with tumor differentiation ($P = 0.000$), histopathology ($P = 0.041$), depth of invasion ($P = 0.000$), nodal status ($P = 0.003$), metastasis ($P = 0.013$), and AJCC stage ($P = 0.000$) (Table 1). In contrast, there was no correlation between the expression of CD73 and age, gender, tumor size, or Borrmann type ($P > 0.05$, Table 1). In addition, HIF-1α expression was significantly correlated with tumor size ($P = 0.031$), depth of invasion ($P = 0.036$) and AJCC stage ($P = 0.035$) (Table 1), but not with age, gender, tumor differentiation, histopathology, depth of invasion, nodal status, metastasis, or Borrmann type ($P > 0.05$, Table 1).

Survival analysis

Further Kaplan-Meier analysis demonstrated that high expression of CD73 (log-rank, $P < 0.001$) had a statistically significant correlation with a poor overall survival (Figure 2). But there was no significant correlation between overexpression of HIF-1α and survival time (log-rank, $P = 0.103$). Moreover, we classified the patients into four groups stratified according to CD73/HIF-1α expression, and a significant difference was observed

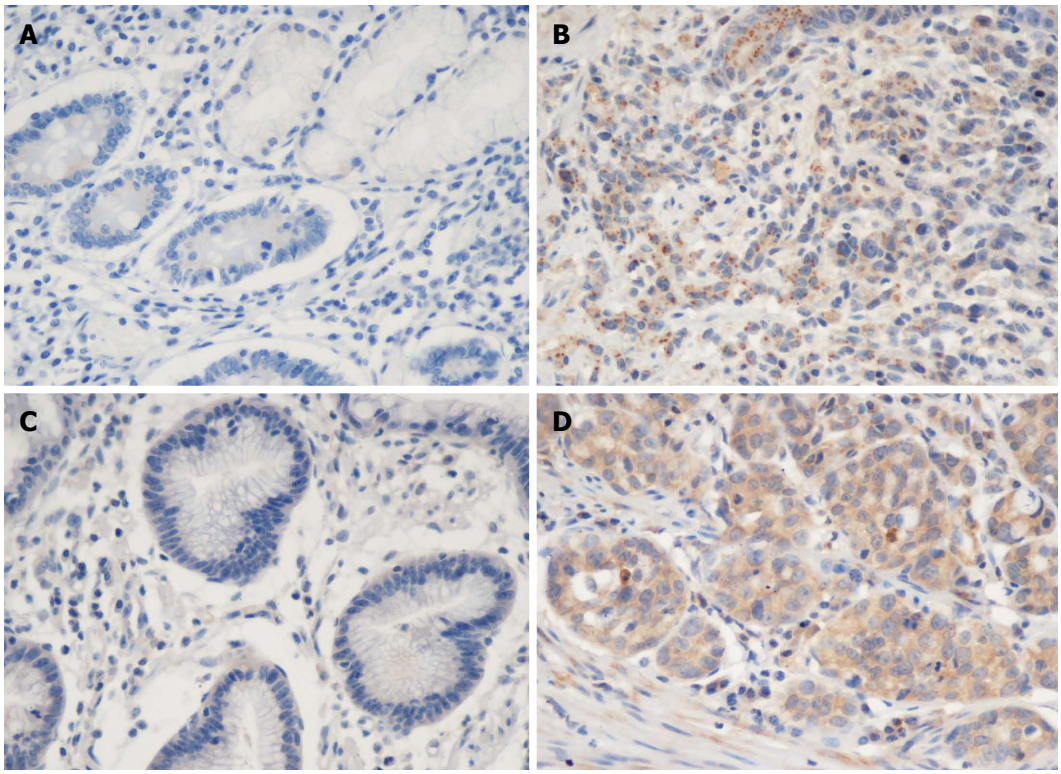


Figure 1 Expression of CD73 and hypoxia-inducible factor-1α in gastric carcinoma (immunohistochemical stain, ×400). A, C: Negative staining for CD73 (A) and hypoxia-inducible factor-1α (HIF-1α) (C) in healthy control gastric mucosa; B, D: Positive staining for CD73 (B) and HIF-1α (D) in gastric carcinoma.

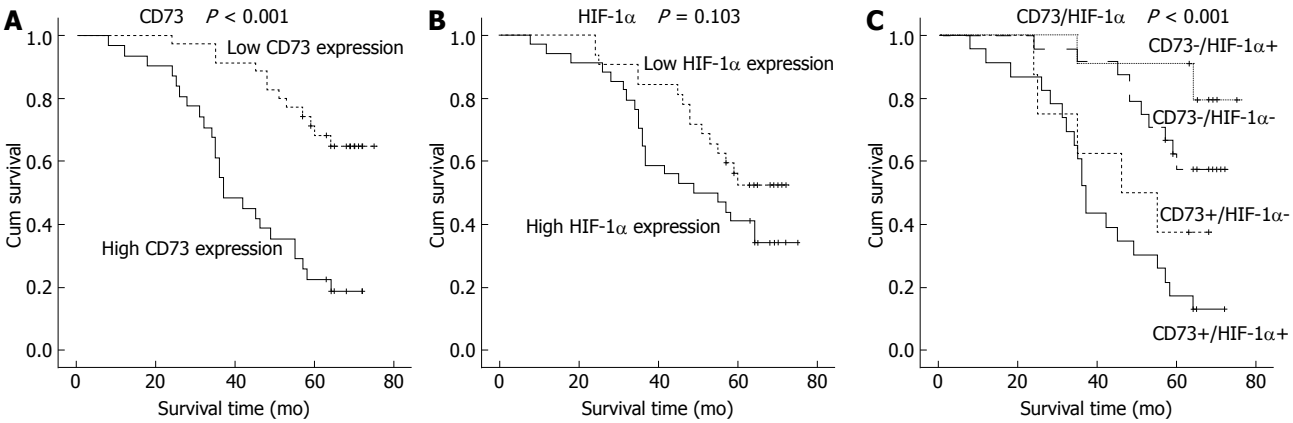


Figure 2 Kaplan-Meier curves for postoperative survival. A: The median survival time of patients with positive CD73 was shorter than that of patients with negative CD73 (log-rank test: $P < 0.001$); B: Hypoxia-inducible factor-1α (HIF-1α) expression had no correlation with the survival time of patients (log-rank test: $P = 0.103$); C: There was a significant difference among groups stratified according to CD73/HIF-1α expression ($P < 0.001$). Patients with CD73+/HIF-1α+ had the worst prognosis.

among the groups (log-rank, $P < 0.001$). The patients with CD73+/HIF-1α+ carcinomas had the worst prognosis. The independent effects of all the significant factors were tested using the Cox proportional hazards model. The exploratory multivariate analyses demonstrated that CD73 [$P = 0.021$, hazard ratio (HR) = 0.385, 95%CI: 0.171-0.865] and AJCC stage ($P = 0.035$, HR = 1.585, 95%CI: 1.032-2.433) were independent prognostic factors, while HIF-1α and others were not independent predictors. ROC curve analysis was also performed to further evaluate the prognostic value of CD73 and HIF-1α expression, which revealed that CD73 expression was encouragingly useful in predicting the overall survival of gastric carcinoma patients (area under the curve = 0.850, $P < 0.001$, Figure 3).

DISCUSSION

Gastric carcinoma is diagnosed with a high frequency throughout the world. In spite of improved diagnostic techniques and therapeutic methods, gastric carcinoma remains a major public health problem. Recently, some investigations showed that biomarkers might be promising predicting factors, and some of them were found

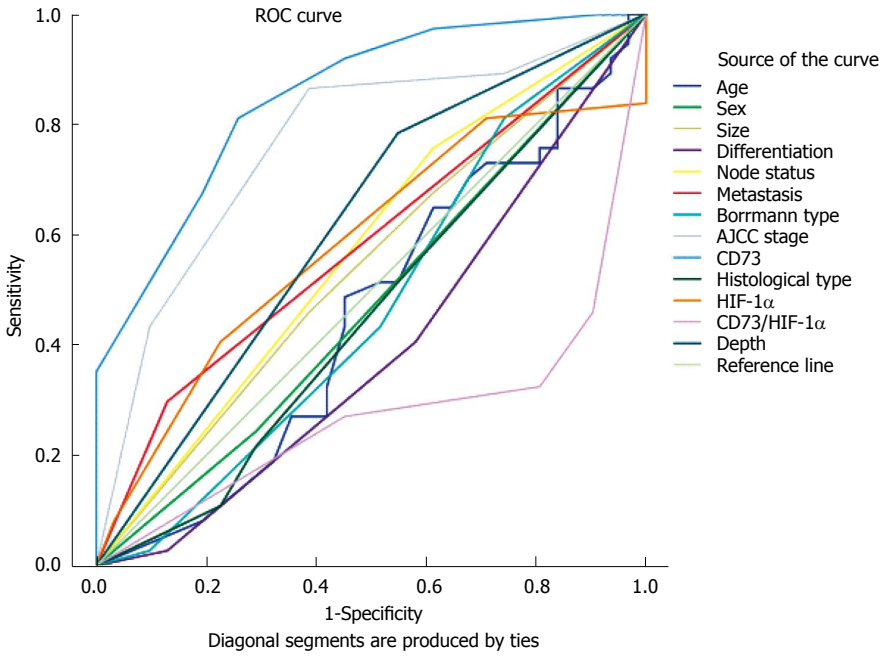


Figure 3 Receiver operating characteristic curves of clinicopathological variables, CD73 expression and hypoxia-inducible factor-1α expression based on outcomes of gastric carcinoma patients. CD73 expression (AUC = 0.850; $P < 0.001$), hypoxia-inducible factor-1α (HIF-1α) (AUC = 0.582; $P = 0.247$), AJCC stage (AUC = 0.765; $P < 0.001$), CD73/HIF-1α (AUC = 0.275; $P = 0.001$), Borrmann type (AUC = 0.472; $P = 0.689$), metastasis (AUC = 0.584; $P = 0.235$), nodal status (AUC = 0.572; $P = 0.310$), differentiation (AUC = 0.394; $P = 0.135$), histopathology (AUC = 0.459; $P = 0.559$), tumor diameter (AUC = 0.541; $P = 0.559$), gender (AUC = 0.476; $P = 0.740$), and age (AUC = 0.456; $P = 0.534$). ROC: Receiver operating characteristic; AJCC: American Joint Committee on Cancer; AUC: Area under the curve.

even superior to the AJCC staging system[20,21]. Increasingly more researches have been conducted to discover the specific biomarkers although some of the results remained conflicting and inconsistent.

CD73, known as a purine salvage enzyme, might play a regulatory role in the immune system response[22,23]. Jin *et al*[22] found that the adenosine generated by tumor-derived CD73 could inhibit both the activation phase and effector phase of the antitumor T cell response and promote T cell apoptosis. Besides, some studies have indicated that CD73 could promote invasion, migration and adhesion of cancer cells[24]. Moreover, the tumor-inhibiting effects of CD73 using siRNA or anti-CD73 antibody could restore efficacy of adoptive T cell therapy, leading to a long-term tumor-free survival[22,25,26]. Host-derived CD73 was also observed in recent years, which provided evidence that CD73 knockdown could significantly delay tumor growth by regulating host immune system[27-29].

The prognostic significance of CD73 has been studied in several cancers such as papillary thyroid carcinoma and breast cancer[9-13]. It was suggested that high expression of CD73 in papillary thyroid carcinomas could be a useful indicator in the differential diagnosis of thyroid tumors. Moreover, strong expression of CD73 was found to be associated with invasiveness, metastasis, and shorter clinical survival in breast cancer. However, few studies have investigated the correlation between CD73 and gastric carcinoma.

CD73 expression of tumor cells may be induced by the selective pressure of the host immune system. Among other influencing factors in tumor microenvironment, hypoxia is the one which has been clearly defined[19]. Hypoxia could induce upregulation of CD73 expression in brain microvessel endothelial cells, which will be reversed by reoxygenation of a short duration[30]. Synnestvedt *et al*[19] reported that hypoxia induced CD73 mRNA, increased protein expression levels and enhanced the CD73 activity in intestinal epithelial cells (T84 cells) and this involved direct binding of HIF-1 to the *Nt5e* gene.

In tumor cells, adaptations to hypoxia are regulated by the activation of specific genes through HIF. And the transcription factor HIF-1α which determines HIF activity is regarded as a hypoxia marker[31]. Overexpression of HIF-1α has been observed in various cancers, such as brain, bladder, lung, breast, esophagus, pancreas, colon, ovary, kidney, and prostate[16-18,32-35]. Furthermore, it was reported that HIF-1α overexpression was significantly correlated with highly aggressive disease, resistance to radiotherapy and chemotherapy, and poor prognosis in various carcinomas[36,37]. Dellas *et al*[38] found that high expression of HIF-1α was associated with tumor progression and metastasis in advanced cervical cancer. Lu *et al*[37] reported that elevated HIF-1α expression was significantly correlated with poor prognosis of rectal adenocarcinoma patients.

In this study, we investigated the relationship between CD73, HIF-1α, clinicalpathological significance, and clinical prognosis in gastric carcinoma. We found that the expression of CD73 was significantly higher in gastric carcinoma than that in normal gastric mucosa, indicating the important role of CD73 in carcinogenesis. Furthermore, CD73 expression was closely correlated with differentiation, histopathology, depth of invasion, nodal status, metastasis, and AJCC stage, but not associated with age, gender, tumor diameter, or Borrmann type. Overexpression of HIF-1α was found to be associated with tumor size, depth of invasion, and AJCC stage. The Spearman's rank correlation analyses indicated a close correlation between CD73 and HIF-1α expressions in gastric carcinoma.

Our data also demonstrated that the overall survival curves in the CD73-negative group were significantly higher than in the CD73-positive group. However, there was no significant correlation between HIF-1α overexpression and the poor overall survival. We classified the

patients into four groups stratified according to CD73/HIF-1α expression, and a significant difference was observed among the groups. The patients with CD73+/HIF-1α+ carcinomas had the worst prognosis. Multivariate analyses showed that only CD73 expression was a prognostic factor independent of certain well-established clinicopathologcial parameters.

In conclusion, CD73 was correlated with the clinicopathological features in gastric carcinoma. High expression of CD73 was an indicator of poor clinical prognosis in patients with gastric carcinoma. Moreover, immunoreactivity of the combined CD73 and HIF-1α could be a useful prognostic marker of gastric carcinoma.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Xiao-Jun Zhou and Xing-Hua Zhang for their technical assistance.

COMMENTS

Background

Gastric carcinoma has been the fourth most common cancer in the world since the latter half of the 20th century. Changes observed in expression of tumor specific biomarkers in gastric carcinomas may be helpful to understand the transformation of histological heterogeneity and the underlying molecular mechanisms. Searching for specific biomarkers which determine the biological nature and behavior of gastric carcinoma would be of utmost importance to optimize individualized therapy.

Research frontiers

Overexpression of CD73 has been observed in various cancers. However, few studies have investigated the correlation between CD73 and gastric carcinoma. Previous studies indicated that hypoxia could induce up-regulation of CD73 expression in different cells, but the correlation between CD73 expression and hypoxia-inducible factor-1α (HIF-1α) expression has not been observed. In this study, the authors demonstrate that CD73 expression is up-regulated in gastric carcinoma and shows close correlation with HIF-1α expression.

Innovations and breakthroughs

In this paper, data firstly shows that there are close correlation between the two biomarkers in gastric carcinoma and the combination of CD73 and HIF-1α could be a useful marker of the prognosis of gastric carcinoma. Moreover, high expression of CD73 was found to be an indicator of poor clinical prognosis in patients with gastric carcinoma.

Applications

Examination of CD73 and HIF-1α expression by immunohistochemistry (IHC) analysis could be used as an additional effective way to identify patients at high risk of tumor progression, thus to optimize individual treatment of patients with gastric carcinoma.

Terminology

Ecto-5'-nucleotidase/CD73 is a homodimer linked to the plasma membrane through a glycosylphosphatidylinositol lipid anchor, which was found in most tissues. It is a part of extracellular ATP metabolism, which dephosphorylates AMP into adenosine rapidly after CD39 catalyzes ATP, ADP and AMP; HIF-1 is a heterodimeric basic helix-loop-helix transcription factor composed of HIF-1α and HIF-1β subunits; and HIF-1α determines HIF-1 activity

Peer review

This manuscript investigate the expression of CD73 and HIF-1α in human gastric carcinoma by IHC. The results showed that CD73 and HIF-1α were higher expressions in gastric carcinoma than that of control and showed close correlation. They concluded that the combination of the two molecules and CD73 expression in gastric cancer tissue is associated with prognosis. The results are interesting and may represent an additional effective way to identify patients at high risk of tumor progression.

REFERENCES

1 **Kelley JR**, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* 2003; **56**: 1-9 [PMID: 12589864 DOI: 10.1016/S0895-4356(02)00534-6]

2 **Fontenot JD**, Rudensky AY. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nat Immunol* 2005; **6**: 331-337 [PMID: 15785758 DOI: 10.1038/ni1179]

3 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

4 **Krejs GJ**. Gastric cancer: epidemiology and risk factors. *Dig Dis* 2010; **28**: 600-603 [PMID: 21088409 DOI: 10.1159/000320277]

5 **Resta R**, Yamashita Y, Thompson LF. Ecto-enzyme and signaling functions of lymphocyte CD73. *Immunol Rev* 1998; **161**: 95-109 [PMID: 9553767 DOI: 10.1111/j.1600-065X.1998.tb01574.x]

6 **Haskó G**, Pacher P. A2A receptors in inflammation and injury: lessons learned from transgenic animals. *J Leukoc Biol* 2008; **83**: 447-455 [PMID: 18160539 DOI: 10.1189/jlb.0607359]

7 **Colgan SP**, Eltzschig HK, Eckle T, Thompson LF. Physiological roles for ecto-5'-nucleotidase (CD73). *Purinergic Signal* 2006; **2**: 351-360 [PMID: 18404475 DOI: 10.1007/s11302-005-5302-5]

8 **Linden J**. Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol* 2001; **41**: 775-787 [PMID: 11264476 DOI: 10.1146/annurev.pharmtox.41.1.775]

9 **Wu XR**, He XS, Chen YF, Yuan RX, Zeng Y, Lian L, Zou YF, Lan N, Wu XJ, Lan P. High expression of CD73 as a poor prognostic biomarker in human colorectal cancer. *J Surg Oncol* 2012; **106**: 130-137 [PMID: 22287455 DOI: 10.1002/jso.23056]

10 **Häusler SF**, Montalbán del Barrio I, Strohschein J, Anoop Chandran P, Engel JB, Hönig A, Ossadnik M, Horn E, Fischer B, Krockenberger M, Heuer S, Seida AA, Junker M, Kneitz H, Kloor D, Klotz KN, Dietl J, Wischhusen J. Ectonucleotidases CD39 and CD73 on OvCA cells are potent adenosine-generating enzymes responsible for adenosine receptor 2A-dependent suppression of T cell function and NK cell cytotoxicity. *Cancer Immunol Immunother* 2011; **60**: 1405-1418 [PMID: 21638125 DOI: 10.1007/s00262-011-1040-4]

11 **Kondo T**, Nakazawa T, Murata SI, Katoh R. Expression of CD73 and its ecto-5'-nucleotidase activity are elevated in papillary thyroid carcinomas. *Histopathology* 2006; **48**: 612-614 [PMID: 16623792 DOI: 10.1111/j.1365-2559.2005.02277.x]

12 **Spychala J**, Lazarowski E, Ostapkowicz A, Ayscue LH, Jin A, Mitchell BS. Role of estrogen receptor in the regulation of ecto-5'-nucleotidase and adenosine in breast cancer. *Clin Cancer Res* 2004; **10**: 708-717 [PMID: 14760094 DOI: 10.1158/1078-0432.ccr-0811-03]

13 **Gocmen E**, Tez M, Ozturk S, Koc M, Demirci S. Activities of adenosine deaminase and 5'-nucleotidase in cancereous and non-cancereous human gastric tissues. *Bratisl Lek Listy* 2009; **110**: 416-418 [PMID: 19711828]

14 **Mizokami K**, Kakeji Y, Oda S, Irie K, Yonemura T, Konishi F, Maehara Y. Clinicopathologic significance of hypoxia-inducible factor 1alpha overexpression in gastric carcinomas. *J Surg Oncol* 2006; **94**: 149-154 [PMID: 16847924 DOI: 10.1002/jso.20568]

15 **Rankin EB**, Giaccia AJ. The role of hypoxia-inducible factors in tumorigenesis. *Cell Death Differ* 2008; **15**: 678-685 [PMID: 18259193 DOI: 10.1038/cdd.2008.21]

16 **Zagzag D**, Zhong H, Scalzitti JM, Laughner E, Simons JW, Semenza GL. Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. *Cancer* 2000; **88**: 2606-2618 [PMID: 10861440]

17 **Bos R**, Zhong H, Hanrahan CF, Mommers EC, Semenza GL, Pinedo HM, Abeloff MD, Simons JW, van Diest PJ, van der Wall E. Levels of hypoxia-inducible factor-1 alpha during breast carcinogenesis. *J Natl Cancer Inst* 2001; **93**: 309-314 [PMID: 11181778 DOI: 10.1093/jnci/93.4.309]

18 **Kuwai T**, Kitadai Y, Tanaka S, Onogawa S, Matsutani N, Kaio E, Ito M, Chayama K. Expression of hypoxia-inducible factor-1alpha is associated with tumor vascularization in human colorectal carcinoma. *Int J Cancer* 2003; **105**: 176-181 [PMID: 12673675 DOI: 10.1002/ijc.11068]

19 **Synnestvedt K**, Furuta GT, Comerford KM, Louis N, Karhausen J, Eltzschig HK, Hansen KR, Thompson LF, Colgan SP. Ecto-5′-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. *J Clin Invest* 2002; **110**: 993-1002 [PMID: 12370277 DOI: 10.1172/jci200215337]

20 **Wang JH**, Huang WS, Hu CR, Guan XX, Zhou HB, Chen LB. Relationship between RGS5 expression and differentiation and angiogenesis of gastric carcinoma. *World J Gastroenterol* 2010; **16**: 5642-5646 [PMID: 21105200 DOI: 10.3748/wjg.v16.i44.5642]

21 **Zhou J**, Zhang L, Gu Y, Li K, Nie Y, Fan D, Feng Y. Dynamic expression of CEACAM7 in precursor lesions of gastric carcinoma and its prognostic value in combination with CEA. *World J Surg Oncol* 2011; **9**: 172 [PMID: 22195770 DOI: 10.1186/1477-7819-9-172]

22 **Jin D**, Fan J, Wang L, Thompson LF, Liu A, Daniel BJ, Shin T, Curiel TJ, Zhang B. CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression. *Cancer Res* 2010; **70**: 2245-2255 [PMID: 20179192 DOI: 10.1158/0008-5472.can-09-3109]

23 **Hoskin DW**, Mader JS, Furlong SJ, Conrad DM, Blay J. Inhibition of T cell and natural killer cell function by adenosine and its contribution to immune evasion by tumor cells (Review). *Int J Oncol* 2008; **32**: 527-535 [PMID: 18292929]

24 **Wang L**, Zhou X, Zhou T, Ma D, Chen S, Zhi X, Yin L, Shao Z, Ou Z, Zhou P. Ecto-5′-nucleotidase promotes invasion, migration and adhesion of human breast cancer cells. *J Cancer Res Clin Oncol* 2008; **134**: 365-372 [PMID: 17671792 DOI: 10.1007/s00432-007-0292-z]

25 **Zhi X**, Wang Y, Zhou X, Yu J, Jian R, Tang S, Yin L, Zhou P. RNAi-mediated CD73 suppression induces apoptosis and cell-cycle arrest in human breast cancer cells. *Cancer Sci* 2010; **101**: 2561-2569 [PMID: 20874842 DOI: 10.1111/j.1349-7006.2010.01733.x]

26 **Stagg J**, Divisekera U, McLaughlin N, Sharkey J, Pommey S, Denoyer D, Dwyer KM, Smyth MJ. Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. *Proc Natl Acad Sci USA* 2010; **107**: 1547-1552 [PMID: 20080644 DOI: 10.1073/pnas.0908801107]

27 **Stagg J**, Divisekera U, Duret H, Sparwasser T, Teng MW, Darcy PK, Smyth MJ. CD73-deficient mice have increased antitumor immunity and are resistant to experimental metastasis. *Cancer Res* 2011; **71**: 2892-2900 [PMID: 21292811 DOI: 10.1158/0008-5472.can-10-4246]

28 **Wang L**, Fan J, Thompson LF, Zhang Y, Shin T, Curiel TJ, Zhang B. CD73 has distinct roles in nonhematopoietic and hematopoietic cells to promote tumor growth in mice. *J Clin Invest* 2011; **121**: 2371-2382 [PMID: 21537079 DOI: 10.1172/jci45559]

29 **Yegutkin GG**, Marttila-Ichihara F, Karikoski M, Niemelä J, Laurila JP, Elima K, Jalkanen S, Salmi M. Altered purinergic signaling in CD73-deficient mice inhibits tumor progression. *Eur J Immunol* 2011; **41**: 1231-1241 [PMID: 21469131 DOI: 10.1002/eji.201041292]

30 **Li X**, Zhou T, Zhi X, Zhao F, Yin L, Zhou P. Effect of hypoxia/reoxygenation on CD73 (ecto-5′-nucleotidase) in mouse microvessel endothelial cell lines. *Microvasc Res* 2006; **72**: 48-53 [PMID: 16828810 DOI: 10.1016/j.mvr.2006.04.005]

31 **Bunn HF**, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. *Physiol Rev* 1996; **76**: 839-885 [PMID: 8757790]

32 **Saramäki OR**, Savinainen KJ, Nupponen NN, Bratt O, Visakorpi T. Amplification of hypoxia-inducible factor 1alpha gene in prostate cancer. *Cancer Genet Cytogenet* 2001; **128**: 31-34 [PMID: 11454426 DOI: 10.1016/s0165-4608(01)00396-x]

33 **Theodoropoulos VE**, Lazaris ACh, Sofras F, Gerzelis I, Tsoukala V, Ghikonti I, Manikas K, Kastriotis I. Hypoxia-inducible factor 1 alpha expression correlates with angiogenesis and unfavorable prognosis in bladder cancer. *Eur Urol* 2004; **46**: 200-208 [PMID: 15245814 DOI: 10.1016/j.eururo.2004.04.008]

34 **Birner P**, Schindl M, Obermair A, Breitenecker G, Oberhuber G. Expression of hypoxia-inducible factor 1alpha in epithelial ovarian tumors: its impact on prognosis and on response to chemotherapy. *Clin Cancer Res* 2001; **7**: 1661-1668 [PMID: 11410504]

35 **Büchler P**, Reber HA, Büchler M, Shrinkante S, Büchler MW, Friess H, Semenza GL, Hines OJ. Hypoxia-inducible factor 1 regulates vascular endothelial growth factor expression in human pancreatic cancer. *Pancreas* 2003; **26**: 56-64 [PMID: 12499918 DOI: 10.1097/00006676-200301000-00010]

36 **Aebersold DM**, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH, Semenza GL. Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Res* 2001; **61**: 2911-2916 [PMID: 11306467]

37 **Lu XG**, Xing CG, Feng YZ, Chen J, Deng C. Clinical significance of immunohistochemical expression of hypoxia-inducible factor-1alpha as a prognostic marker in rectal adenocarcinoma. *Clin Colorectal Cancer* 2006; **5**: 350-353 [PMID: 16512994 DOI: 10.3816/CCC.2006.n.005]

38 **Dellas K**, Bache M, Pigorsch SU, Taubert H, Kappler M, Holzapfel D, Zorn E, Holzhausen HJ, Haensgen G. Prognostic impact of HIF-1alpha expression in patients with definitive radiotherapy for cervical cancer. *Strahlenther Onkol* 2008; **184**: 169-174 [PMID: 18330514 DOI: 10.1007/s00066-008-1764-z]

P- Reviewers Fan H, Kurosaki M, Hahm KB **S- Editor** Jiang L
L- Editor Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1919

World J Gastroenterol 2013 March 28; 19(12): 1919-1929
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*

Danielle A Vieira-Lopes, Nadja L Pinheiro, Armando Sales, Adriana Ventura, Francisco G Araújo, Iracema D Gomes, Aparecida A Nascimento

Danielle A Vieira-Lopes, Nadja L Pinheiro, Armando Sales, Adriana Ventura, Francisco G Araújo, Iracema D Gomes, Aparecida A Nascimento, Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro 23890-000, Brazil
Author contributions: Vieira-Lopes DA, Pinheiro NL, Sales A, Ventura A, Araújo FG, Gomes ID and Nascimento AA designed the research; Vieira-Lopes DA, Ventura A, Gomes ID and Nascimento AA performed the research; Vieira-Lopes DA, Pinheiro NL, Sales A, Araújo FG and Nascimento AA analyzed the data; Vieira-Lopes DA and Nascimento AA wrote the paper.
Supported by Federal Rural University of Rio de Janeiro for the Research Grant under the Internal Scientific Initiation Program, to Vieira-Lopes DA
Correspondence to: Dr. Danielle Vieira-Lopes, Departamento de Biologia Animal, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro 23890-000, Brazil. dmjvieira@hotmail.com
Telephone: +55-21-26821711 Fax: +55-21-26821711
Received: November 8, 2012 Revised: November 26, 2012
Accepted: December 25, 2012
Published online: March 28, 2013

Abstract

AIM: To describe the histology of the digestive tract and to investigate the occurrence of endocrine cells in *Oligosarcus hepsetus* (*O. hepsetus*).

METHODS: The digestive tract (DT) of *O. hepsetus* was divided into esophagus, two stomach regions (glandular and non-glandular) and two intestinal regions (anterior and posterior). These specimens were processed by routine histological techniques and stained with hematoxylin-eosin, Gomori's trichrome, periodic acid Schiff (PAS) and Alcian blue (AB). An immunohistochemical method using avidin-biotin-peroxidase was employed.

RESULTS: The esophagus is lined with a non-keratinized stratified squamous epithelium that is reactive to PAS and AB. The stomach has a mucosa lined with a simple columnar epithelium with mucus-secreting cells that are reactive only to PAS. The intestine has a simple columnar epithelium with a brush border and goblet cells that are reactive to PAS and AB. Somatostatin, serotonin and cholecystokinin immunoreactive cells were identified throughout the DT.

CONCLUSION: This study revealed adaptations for the species' diet and showed that the distribution and relative frequency of immunoreactive cells are similar to those of other fish.

Key words: Fish; Esophagus; Stomach; Intestine; Endocrine cells

Vieira-Lopes DA, Pinheiro NL, Sales A, Ventura A, Araújo FG, Gomes ID, Nascimento AA. Immunohistochemical study of the digestive tract of *Oligosarcus hepsetus*. *World J Gastroenterol* 2013; 19(12): 1919-1929 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1919.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1919

INTRODUCTION

The literature stresses the importance of knowledge of the anatomy of the digestive tract (DT) of fishes, because this structure is highly variable, related to the diversity of feeding habits, type of food and lifestyles[1-3].

The histological architecture of the DT includes a layer of mucus-secreting cells, observed by histochemical techniques in various studies of teleosts. The secretions vary among different fish species and also according to the location in the DT within the same species[4]. These secretions play an important role in lubricating the or-

gan and protecting against proteolytic degeneration and pathogenic microorganisms[5].

Besides this, to control the functions of the different DT segments, endocrine cells compose a complex system disseminated among the epithelial components, with the ability to secrete physiologically active polypeptide hormones and amines[6]. According to Deveney *et al*[7], hormones have important functions in the overall regulation of the digestive process, such as nutrient absorption, the secretion of intestinal and associated glands, gut motility and intestinal blood flow

Oligosarcus hepsetus (*O. hepsetus*), known commonly as the thin dogfish, belongs to the Characidae family. It is carnivorous, with a diet basically composed of small fish. It mainly lives in rivers and reservoirs, generally at the middle to the bottom of the water column. It has a slightly rounded body with small fins and is considered a good swimmer[8]. Many articles have been published about this species' distribution, ecology, feeding habits and diet, but no study has been published reporting the microscopic analysis of the organs of the DT of *O. hepsetus*. Although it does not have economic importance, it is one of the most abundant species in the reservoirs of Rio de Janeiro[9] and the Paraíba do Sul River[10], with distribution in the coastal region of south to south-eastern Brazil between Santa Catarina and Rio de Janeiro[11].

Studies involving microscopic anatomy and histochemistry provide information to characterize the organs of the digestive system, facilitating understanding of the physiology of the DT and the feeding habits of the species under investigation[12]. These studies are essential for efforts to restock native species to improve the condition of ecosystems[13].

The purpose of the present work was to describe histological and histochemical aspects of the DT of *O. hepsetus* and to investigate immunohistochemically the occurrence of the endocrine cells secreting somatostatin (SOM), serotonin (5-hydroxytryptamine, 5-HT), cholecystokinin (CCK), gastrin (GAS), glucagon (GLUC) and insulin (INS) in the DT of this fish, seeking to relate them to its feeding habits, in order to provide data for future studies.

MATERIALS AND METHODS

Collection area

The specimens were collected from two reservoirs: Funil (22°30'S, 44°45'W), and Ribeirão das Lajes (22°43'S, 44° 46'W), as well as two points of the Paraíba do Sul River: Ilha dos Pombos (21°84'S, 42° 58'W) and Santa Cecília (22°48'S; 43°84'W), all located in Rio de Janeiro state, Brazil.

Tissue processing

Fourteen specimens were used in this study without sexual distinction, four collected in Funil Reservoir, four in Ribeirão das Lajes Reservoir, and three each from the Ilha dos Pombos and Santa Cecília sites of the Paraíba do Sul River. The fish were dissected in the field, after being anesthetized with benzocaine hydrochloride (50 mg/L) and killed rapidly by hypothermia for immediate removal of the DT. Fragments of the DT were fixed for 8 h in Bouin's fluid and then placed in 70% alcohol. The esophagus, two stomach regions (glandular and non-glandular) and two intestinal regions (anterior and posterior) were obtained from each specimen (Figure 1). These materials were taken to the Histology and Embryology Laboratory of Rio de Janeiro Federal Rural University where they were processed by routine histological techniques, consisting of: dehydration (in a rising ethanol series - 70° GL to 100° GL), diaphanization in xylol and embedding in Histosec-Paraffin, to obtain 5 μm histological sections.

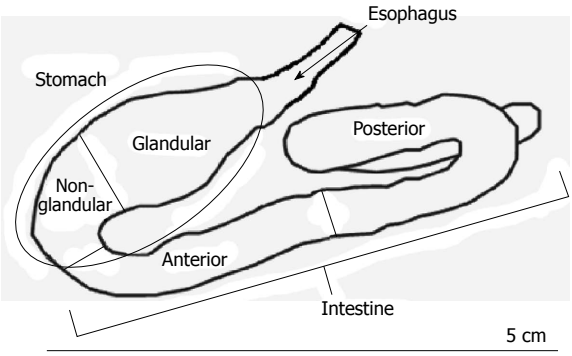


Figure 1 Anatomical diagram of the digestive tract of *Oligosarcus hepsetus*.

Histological and histochemical analysis

The sections obtained from the DT of *O. hepsetus* were stained with hematoxylin-eosin for analysis of the tissue architecture and with Gomori's trichrome for differential visualization of the connective tissue and collagen fibers.

The histochemical analysis involved periodic acid Schiff (PAS) and Alcian blue (AB) pH 2.5 staining to reveal the neutral and acid glycoconjugates (GCs), respectively. Five slides from each specimen were prepared for each protocol, one from each of the five sectioned regions.

Immunohistochemistry

For the immunohistochemical procedure, 5 μm-thick sections were cut by microtome and mounted on glass slides precoated with 0.1% poly-L-lysine, after being dewaxed and dehydrated by the routine protocol. They were incubated in citrate buffer (pH 6.0-0.01 M) and placed in a microwave oven for 15 min to recover the antigen, then they were incubated with a solution of 3% H_2O_2 in methanol for 15 min to block any endogenous peroxidase. Subsequently, the sections were incubated at room temperature in a humid chamber with a 1:100 μL dilution of bovine serum albumin in phosphate buffered saline (PBS) solution for 30 min. The sections were first incubated overnight at 4 ℃ with the primary antisera against the individual gastrointestinal hormones (Table 1). The sections were then incubated with biotinylated "Universal" secondary antibody diluted to 1:200 μL for 30 min at room temperature, then with avidin-biotin-peroxidase complex,

Table 1 Details of primary antisera used in this study

Primary antisera	Donor	Code No.	Working dilution	Source
Somatostatin	Rabbit	A566	1:300	Dako Corp., CA, United States
Serotonin	Rabbit	S5545	1:8.000	Sigma-Aldrich, Inc., United States
Cholecystokinin	Rabbit	C2581	1:8.000	Sigma-Aldrich, Inc., United States
Gastrin	Rabbit	G0785	1:1.000	Sigma-Aldrich, Inc., United States
Glucagon	Mouse	G2654	1:2.000	Sigma-Aldrich, Inc., United States
Insulin	Mouse	I2018	1:1.000	Sigma-Aldrich, Inc., United States

Table 2 Regional distribution and intensity of immunoreaction in endocrine cells in the digestive tract of *Oligosarcus hepsetus*

Antisera	Segment of the esophagus	Segments of the stomach		Segments of the gut	
		GI	NGI	ANT	POST
Somatostatin	+++	++	+	-	-
Serotonin	-	+++	++	-	-
Cholecystokinin	-	-	-	-	++
Glucagon	-	-	-	-	-
Gastrin	-	-	-	-	-
Insulin	-	-	-	-	-

Intesity of immunoreactions: (-), absent; (+), low; (++), medium; (+++), strong. Gl: Glandular; NGl: Non-glandular; ANT: Anterior; POST: Posterior.

diluted at 1:200 μL for 30 min at room temperature. Subsequently, the peroxidase label was revealed by reaction with Stable DAB/Plus, prepared according to the kit's instructions. All dilutions and thorough washes between stages were performed using PBS (pH 7.4). The sections were counterstained with Harris hematoxylin, rinsed with deionized water, dehydrated through a series of ethanol and methylcyclohexane solutions and mounted using Entellan. To investigate the specificity of the reactions, negative and positive controls were used. The negative control was prepared by replacement of the primary antibody with non-immune serum and PBS (pH 7.4). Positive controls were produced using tissue sections for each respective antiserum, as indicated in the product data sheet.

Observation and photomicrography

Photomicrographs of all samples from each of the fourteen specimens were obtained with a digital camera Nikon Coolpix 4300 attached to a microscope Olympus BX41. The number of immunoreactive endocrine cells to each antiserum per analyzed segment was recorded and the intensity of immunoreaction was classified: absent (-) or low (+), medium (++) and strong (+++) immunoreactivity (Table 2).

RESULTS

Histological and histochemical study of digestive tract

The following layers were observed in the DT of *O. hepsetus*: mucosa, submucosa, muscular and adventitia or serosa. The muscularis mucosae is absent in this species.

Esophagus

Histological examination revealed that the mucosa of the esophagus of *O. hepsetus* has many longitudinal folds and is lined with a stratified epithelium with non-keratinized squamous surface cells. The majority of the mucus-secreting cells are interspersed with a smaller number of non-secretory cells (Figure 2A).

The secretory cells reacted positively to PAS and AB staining, indicating the presence of neutral and acid GCs, respectively. The lamina propria is composed of connective tissue and does not have glands. The muscular layer is formed by two sub-layers of striated skeletal muscle, with an internal longitudinal and an external circular layer. Externally, the esophagus is enveloped by an adventitia, composed of connective tissue with some nerve fibers and blood vessels (Figure 2).

Stomach

In the stomach of *O. hepsetus*, the mucosa is lined with a simple epithelium layer composed of columnar mucus-secreting cells with basal nuclei. These were reactive to PAS but not to AB, revealing the presence of only neutral GCs. The stomach epithelium forms crypts along the gastric mucosa. The mucosa layer projects toward the organ's lumen, forming various gastric folds, arranged longitudinally. In the non-glandular region, the submucosa and muscular layers accompany the mucosa in forming these folds, making the lumen very small (Figure 3).

The division of the stomach observed in the present work is in accordance with the structural characteristics of the two regions. The glandular region is characterized by having well-developed tubular gastric glands, composed of oxynticopeptic cells, occupying the entire lamina propria (Figure 3B). These are smaller and less numerous in the initial portion and increase in number and size in the direction of the non-glandular region. The non-glandular region has a well-developed muscular layer (Figure 3D and E). The submucosa layer is composed of connective tissue and blood vessels.

In the stomach, the muscular layer is composed of smooth muscle fibers arranged in two directions: internal circular and external longitudinal (Figure 3E). The glandular region contains myenteric plexuses arranged in sparse groups, composing the enteric nervous system and located between the muscular sub-layers, and there is a serosa layer which surrounds these structures.

Intestine

Just as for the stomach, the adopted division of the intestine follows specific structural patterns in relation to the mucosa layer. The histological analysis of the intestine of *O. hepsetus* revealed that the pattern of folds varies, characterizing two distinct regions: anterior and posterior. The anterior region has numerous thin and elongated folds with villi (Figure 4A). In contrast, in the posterior

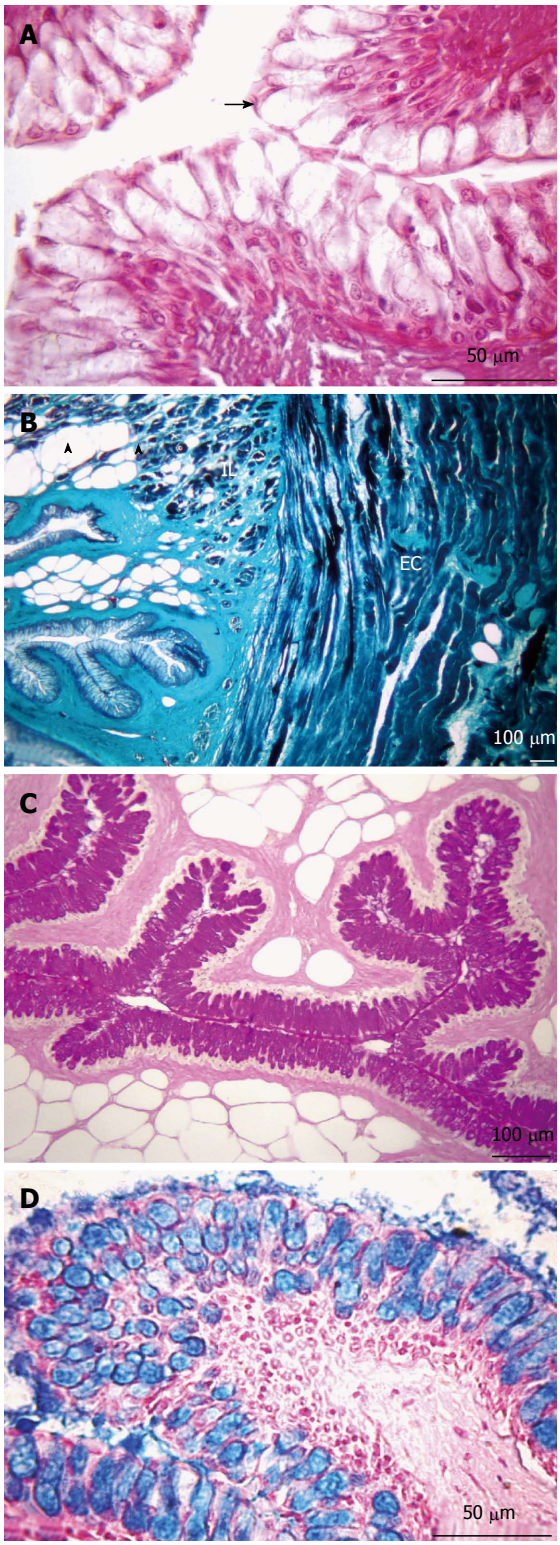


Figure 2 Transversal sections of the esophagus. A: Non-keratinized stratified squamous epithelium (arrow). Hematoxylin and eosin stain; B: Presence of adipose tissue (arrowheads) in the submucosa layer. Muscular layer formed by two sub-layers, internal longitudinal (IL) and external circular (EC). Gomori's trichrome stain; C: Presence of neutral glycoconjugates (GCs). Periodic acid-Schiff stain; D: Acid GCs. Alcian blue stain.

part the folds are less sinuous due to the absence of villi and are thicker, with some being leaf-shaped, and also have a greater number of goblet cells (Figure 4B).

The intestinal mucosa is lined by a simple columnar epithelium with a striated border and goblet cells, which were positive to PAS and AB stainings, with pink (PAS) and blue (AB) coloration (Figure 4), indicating the presence of neutral and acid GCs, respectively.

The limits between the lamina propria and the submucosa layer are not evident, with connective tissue and blood vessels present in both these regions. In both parts of the intestine, the muscular layer has the same organization as in the stomach, with an internal circular layer and an external longitudinal one, observed in the cross-sections, both composed of smooth muscle cells (Figure 4A). The posterior part of the intestine contains a continuous layer of nervous tissue and a myenteric plexus between the muscular sub-layers (Figure 4E). Externally there is a serosa layer.

Immunohistochemical study of digestive tract

SOM-, 5-HT- and CCK-immunorective (IR) cells were identified in the DT of *O. hepsetus*, but GAS-, GLUC- and INS-IR cells were not present (Table 2).

Esophagus

Somatostatin immunoreactivity: In the esophagus, SOM-IR cells were detected in the basal layer of the stratified squamous epithelium (Figure 5). Morphologically, these cells were totally colored by chromogen, making it impossible to visualize a nuclear halo. The nucleus of these cells is very small, occupying a tiny area inside them.

Stomach

Serotonin and somatostatin immunoreactivity: Serotonin (5-HT)-IR cells (Figure 6) and SOM-IR cells (Figure 7) were observed in the lining epithelium and glandular epithelium of the stomach. Regarding the morphology of immunoreactive cells, two types were found in this portion, namely closed-type cells and open-type cells.

Intestine

Cholecystokinin immunoreactivity: CCK-IR cells were only observed in the lining epithelium of the posterior part of the intestine of *O. hepsetus* (Figure 8). Closed-type and open-type immunoreactive cells were found.

DISCUSSION

The stratification of the wall of the DT of *O. hepsetus* has the same organization observed in the majority of other teleosts, with some modifications associated with the species' feeding habits. We observed four layers: mucosa, submucosa, muscular and serosa. The muscularis mucosae is not present in the examined areas of the DT, unlike that observed in *Pimelodus maculates* (*P. maculatus*)[14] and *Semaprochilodus insignis*[15]. These authors assumed that the existence of muscular tissue between the lamina propria and submucosa aids in the elimination of the substances produced by the glands.

The very large longitudinal folds of the mucosa layer

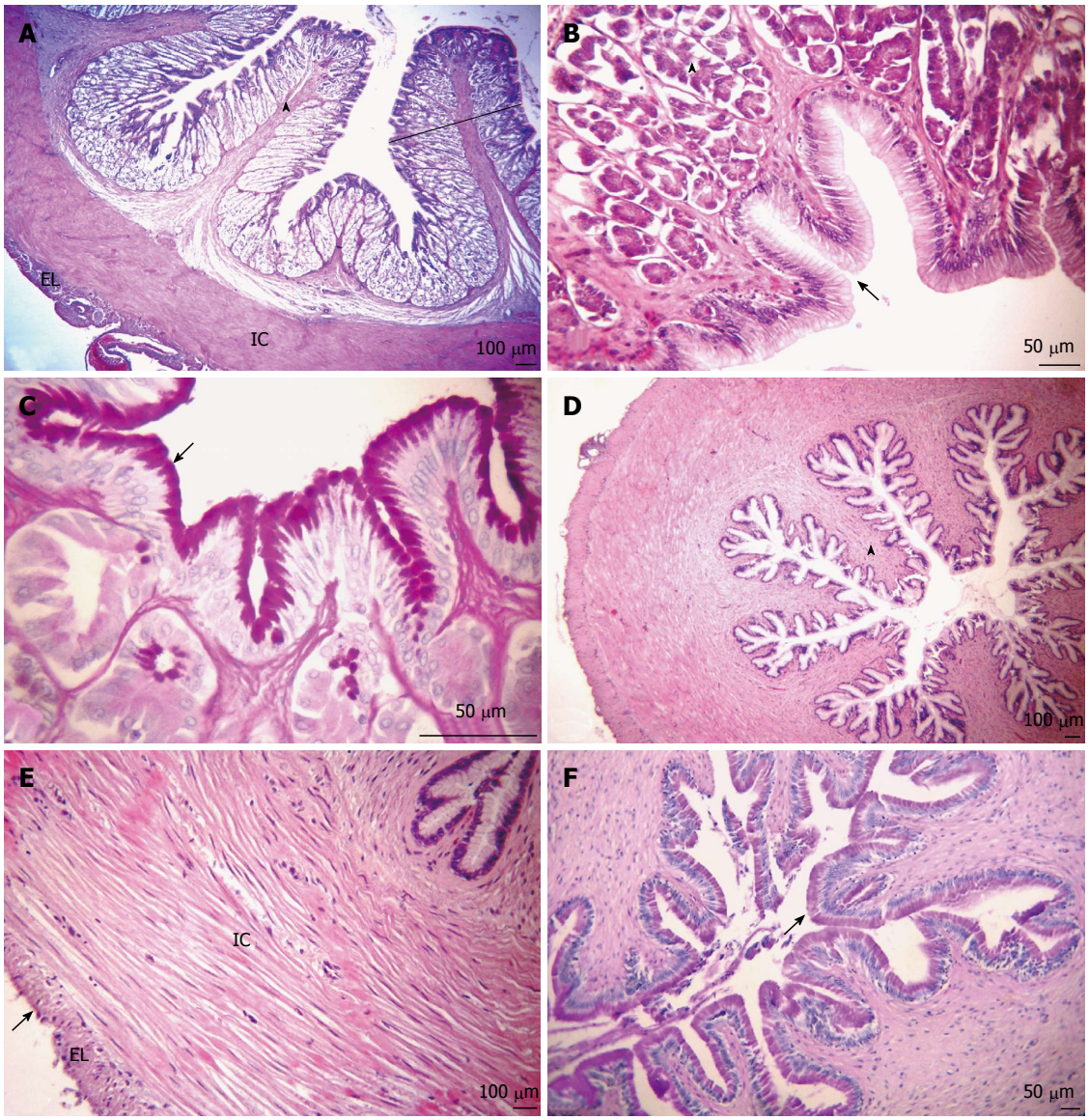


Figure 3 Transversal sections of the stomach. A: Mucosa layer with many high gastric folds (arrowhead), thickening of the lamina propria caused by a large number of tubular gastric glands (outline). Organization of the internal circular (IC) and external longitudinal (EL) muscular layers. Hematoxylin and eosin (HE) stain; B: The simple columnar epithelium with mucus-secreting cells forming faveola gastrica (arrow) and fundic glands composed of oxynticopeptic cells (arrowhead). HE stain; C: Presence of neutral glycoconjugates (GCs) (arrow). Periodic acid Schiff (PAS) stain; D: Mucosa layer, together with the sub-mucosa and internal muscular layers, forming large longitudinal folds (arrowhead). HE stain; E: The muscular layer of smooth muscle fibers, composed of IC and EL sub-layers and serosa (arrow). HE stain; F: Neutral GCs (arrow). PAS stain. (A-C: Glandular region; D-F: Non-glandular region).

of the esophagus of *O. hepsetus* substantially increases the organ's capacity for distension, an effect described by other researchers[16,17]. The digestive capacity is related to the volume of the folds in the mucosa, with a greater number of folds implying more efficient digestion.

The lining of the mucosa by a stratified squamous epithelium, according to Hunbert *et al*[18], acts to protect the fish against mechanical aggression and invasive bacteria. The same pattern was found in *Prionotus carolinus* by Blake[19], but was not observed in *Salmo trutta* by Burnstock[20] or in *P. maculatus* by Santos *et al*[14]. The submucosa contains bundles of adipose tissue and blood vessels; the same was found in *Dentex dentex* (*D. dentex*)[21].

The positive reaction of the epithelium to PAS and AB staining revealed the production of neutral and acid GCs, respectively, in the esophagus. The first type of mucus has low viscosity and is important to assure laminar flow during the lubrication and treatment of particles, to enable digestion to be conducted by the esophagus until the upper region of the stomach. In turn, acid GCs have high viscosity and are fundamental to trap particles[22]. The presence of these GCs was also reported in *Anguilla anguilla*[23], *D. dentex*[21], *P. maculatus*[14], *Cynoscion guatucupa* (*C. guatucupa*)[24], *Pelteobagrus fulvidraco* (*P. fulvidraco*)[25] and *Hyphessobrycon anisitsi* (*H. anisitsi*)[26].

The transition from the esophagus to the stomach is characterized by an abrupt change in the lining epithelium, which starts to present a single layer of columnar cells secreting mucus. This type of stomach lining epithelium has been observed in the majority of other teleosts as

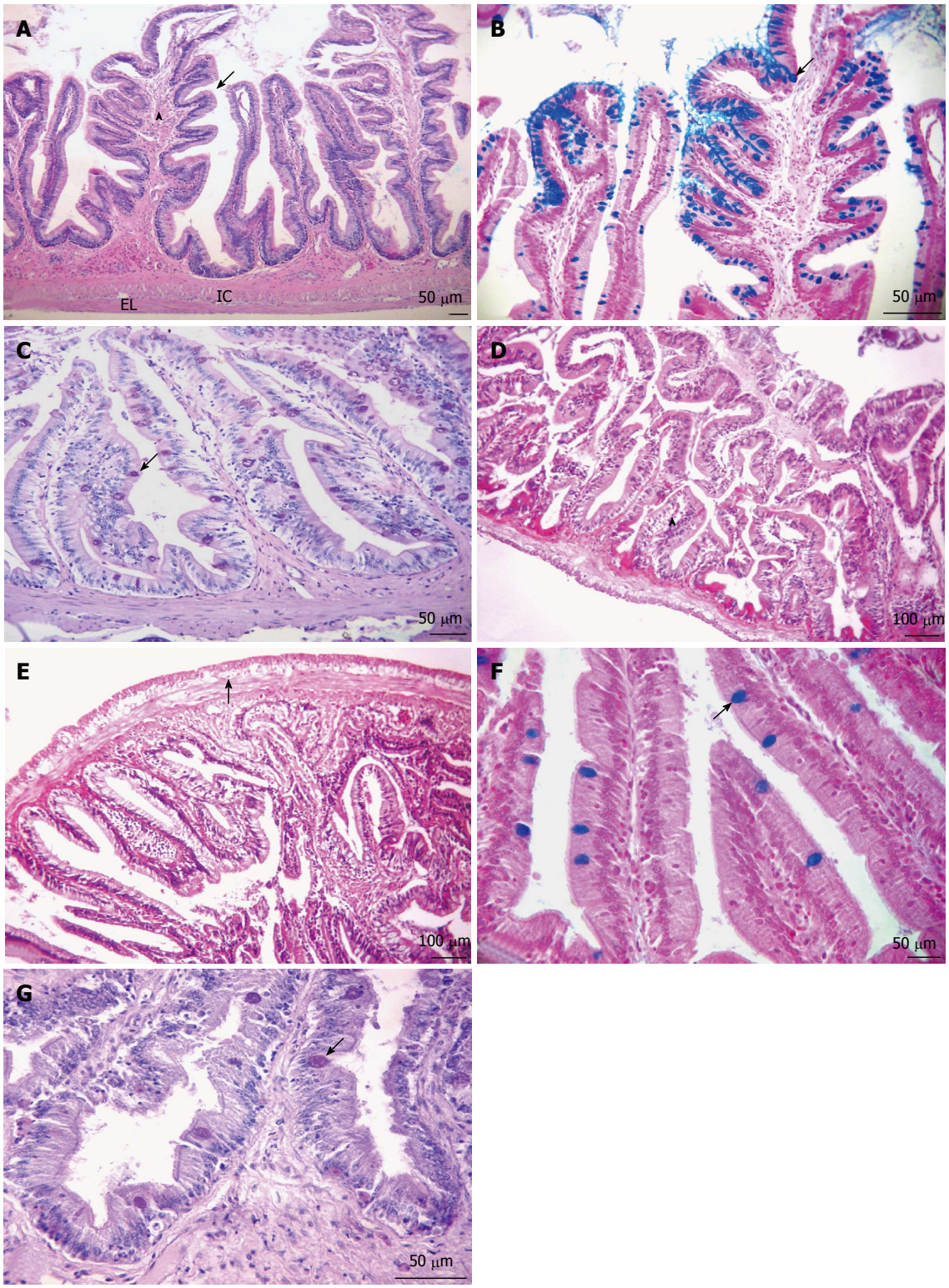


Figure 4 Longitudinal sections of the intestine. A-C: Anterior portion; D-F: Posterior portion; A: Overview of the anterior intestine, showing the arrangement of small folds (arrowhead) presenting villi (arrow). Organization of the internal circular (IC) and external longitudinal (EL) muscular layers. Hematoxylin and eosin (HE) stain; B: Simple columnar epithelium with brush border and goblet cells, indicating the presence of acid glycoconjugates (GCs) (arrow). Alcian blue (AB) stain; C: Neutral GCs (arrows). Periodic acid Schiff (PAS) stain; D: Mucosa layer with thick folds (arrowhead). HE stain; E: Myenteric plexus (arrow). HE stain; F: Simple columnar epithelium with striated border and goblet cells with acid GCs (arrow). AB stain; G: Neutral GCs (arrow). PAS stain.

well[4,14,21,23,24], but in *Plecostomus plecostomus*[27] and *Epinephelus marginatus*[28], the epithelium described at the beginning of the stomach was of the squamous and cubic type, respectively, becoming simple columnar in the posterior regions. The mucus-secreting cells were only reactive to PAS in the two stomach regions; the same was found in *C. guatucupa*[24], *P. fulvidraco*[25] and *H. anisitsi*[26], but was not like that observed in *Anguilla anguilla* (*A. anguilla*)[23] and *Chanos cha-*

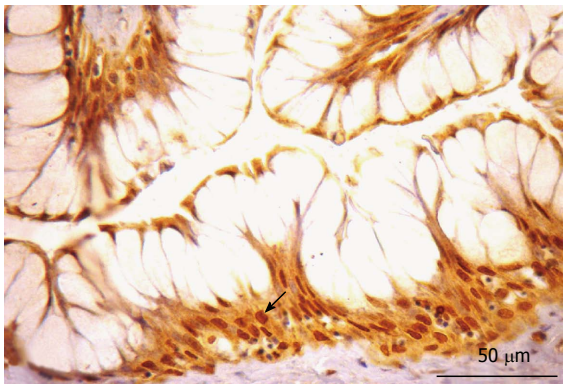


Figure 5 Somatostatin-immunorective cells in the esophagus. Somatostatin-immunorective cell were detected in the basal layer of the stratified squamous epithelium (arrow).

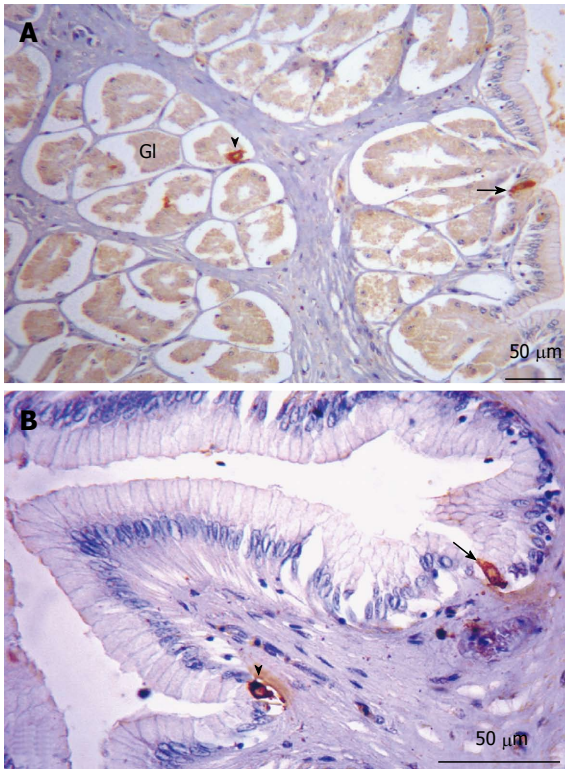


Figure 7 Somatostatin-immunorective cells in the stomach. A: Glandular region. Immunorective (IR) cells along the entire region, of the open (arrow) and closed (arrowhead) types, present both in the lining epithelium and the glandular epithelium; B: Non-glandular region. Indications of IR cells of the open (arrow) and closed (arrowhead) types. Gl: Glandular.

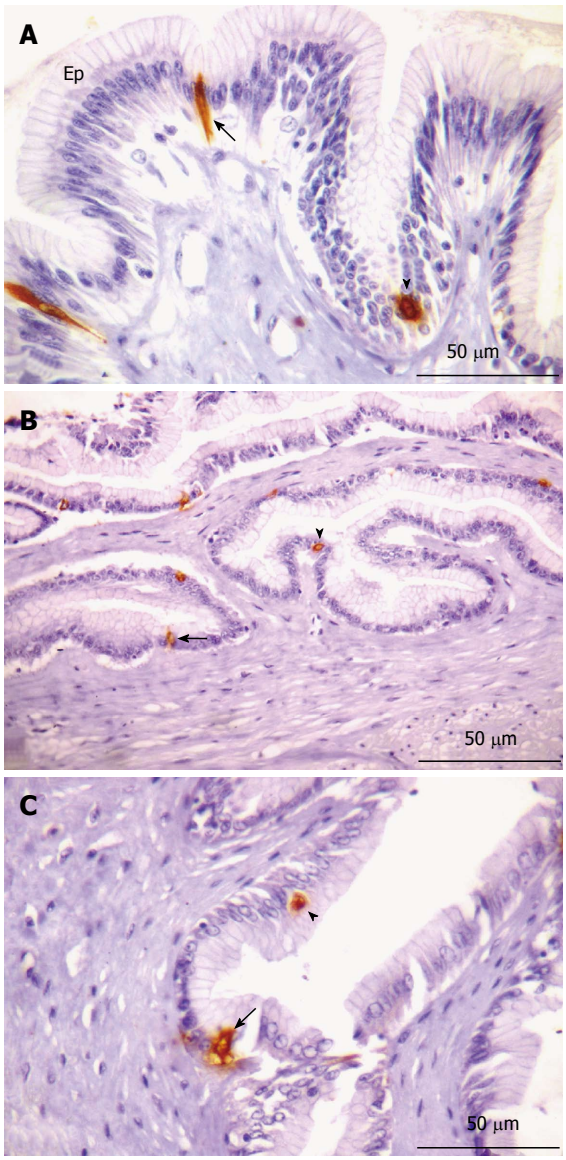


Figure 6 Serotonin-immunorective cells in the stomach. A-B: Glandular region; C: Non-glandular region. A: Presence of open (arrow) and closed (arrowhead) type cells interspersed in the epithelium (Ep); B: Highlight of open (arrow) and closed (arrowhead) type immunorective (IR) cells; C: Indications of open (arrow) and closed (arrowhead) IR cells.

nos[29]. As mentioned, the probable function of this mucus is to promote a flow able to carry the food bolus to the intestine. Besides this, since cells producing hydrochloric acid (HCL), essential for digestion, were identified in the stomach region, this mucus also functions as a layer to protect the epithelial cells.

The invaginations formed by the lining epithelium are called gastric crypts, which in the glandular region communicate with well-developed and branched tubular glands, as also observed by Díaz *et al*[24] in *C. guatucupa* and by Domeneghini *et al*[23] in *A. anguilla*. These are common characteristics of carnivorous fish species[30]. The gastric glands are composed of oxynticopeptic cells, which play a role similar to that of the principal and parietal cells in mammals, by synthesizing HCL and pepsinogen. In this case, we believe the glandular region has digestive functions while the non-glandular region only acts to carry the food to the gut with the epithelial secretions, with the help of the muscular layer, which in this region is thicker[14]. As seen in the esophagus, the stomach regions also contain well-developed longitudinal folds, whose function is to allow expansion of the organ's diameter to store a large volume of food[16,17], another common characteristic of carnivorous fish species[30].

The intestine of *O. hepsetus* has two distinct parts, the same as in *Tilapia spp.*[31]. The anterior part is characterized by a larger number of thin and elongated longitudinal folds,

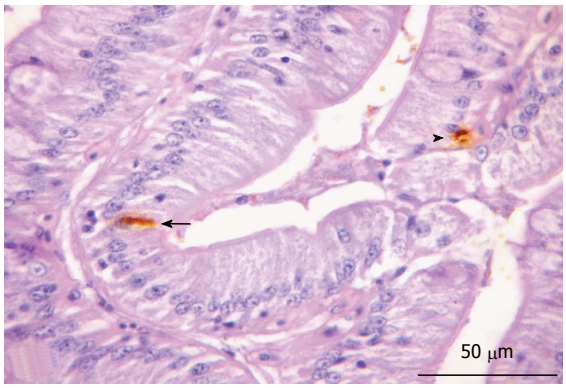


Figure 8 Cholecystokinin-immunorective cells in the posterior region of the intestine. Epithelial marking of open (arrow) and closed (arrowhead) immunorective cells.

which branch out to form villi, making the organ's lumen very small. The posterior region, endowed with thicker and less sinuous longitudinal folds, contains a larger number of goblet cells, similar to the pattern observed in the large intestine of mammals. This pattern of ample folds of the mucosa is common in carnivores, acting to expand the surface absorption area, since carnivores have a relatively short intestine compared to animals with other feeding habits[30]. Sections of the lining epithelium of both regions, when submitted to the PAS and AB histochemical protocols, reacted positively, showing cells with pink (PAS) and blue (AB) coloring, indicating the presence of neutral and acid GCs, respectively, as also observed by Cinar *et al*[32] in *Pseudophoxinus antalyae,* by Carrassón *et al*[21] in *D. dentex* and by Leknes[26] in *H. anisitsi*, but unlike that observed in *P. fulvidraco*[25] and *Chanos chanos*[29].

This allows the hindgut to lubricate the tube and to trap particles to be eliminated, permitting the food bolus to reach this region in dehydrated form[33].

The organization of the muscular layer along the entire DT of *O. hepsetus* is the same as observed in *P. maculatus*[14] and *P. fulvidraco*[25], except in the esophagus, where the pattern resembled that found in mammals. The function of this layer is to promote motility in the DT, carrying and mixing food with the digestive secretions. This motility and also the release of these secretions are favored by the existence of a myenteric plexus between the muscular tissue sub-layers, observed in the glandular region of the stomach as well as in the posterior intestine. Unlike in mammals, the myenteric plexus does not have the form of ganglia, but rather appears in sparse form or in continuous layers, as seen in *Pimelodus maculatus*[34], but unlike that observed in *Salmo trutta*[20] and *P. fulvidraco*[25].

The results obtained in this study demonstrate that the DT of *O. hepsetus* has three types (5-HT-, SOM- and CCK) of endocrine cells, but three other types (GAS-, GLUC- and INS-IR cells) were not present. However, these cells have been observed in other fish, such as: GLU-IR cells in the gastric mucosa of cartilaginous fishes[35]; GAS-IR cells in the stomach pyloric region of *Oncorhynchus mykiss*; and INS-IR cells in the stomach pyloric region of *Monopterus albus* (*M. albus*) and the stomach cardiac and pyloric regions of *Pelteobagrus fulvidraco*[36]. The reason for the absence of these endocrine cells in the DT of *O. hepsetus* may be related to its digestive histophysiology, but further studies should be conducted to confirm this relationship.

The peptide SOM is a component responsible for inhibiting many substances, such as GAS, CCK, GLUC, INS, secretin, motilin and gastric acid[37]. In mammals, it also controls the absorption of amino acids and glucose[38]. It is thus essential in the DT, since it participates in basic mechanisms for efficient food processing. Ku *et al*[39] identified the production of this hormone along the DT of the reptile *Trachemys scripta elegans*, including in the esophagus, where we found SOM-IR cells in *O. hepsetus*. Other studies investigating the presence of this hormone in the DT have been performed by Lee *et al*[40] and Pan *et al*[36], the latter analyzing the presence of endocrine cells in eight fish species: *P. fulvidraco*, *M. albus*, *Siniperca chuatsi* (*S. chuatsi*), *Colossoma brachypomum* and *Tilapia nilotica*, all of which presented SOM-IR cells in the gastric mucosa, as observed in *O. hepsetus*. Although we did not visualize this hormone in the intestinal parts of the thin dogfish, it was reported in the gut of the spiny dogfish *Squalus acanthias*[41], *Oncorhynchus mykiss*[42], *P. fulvidraco, M. albus, S. chuatsi*[36], *Zacco platypus*[43] and *Coreoperca herzi* (*C. herzi*)[44].

5-HT-IR cells have been detected in the DT of various vertebrates: fish[44], amphibians[45], reptiles[46], birds[47] and mammals[48,49]. Researchers state that all vertebrates have this type of endocrine cell in the DT, assuming that these cells' location is based on the evolution of these higher life forms[50]. It is known that in fish, serotonin promotes gastrointestinal motility[51] and blood flow, in the latter case by triggering vasoconstriction[52,53]. In *O. hepsetus,* 5-HT-IR cells were observed only in the regions of the stomach, both in the lining epithelium and the glandular epithelium, as also observed in *C. herzi* by Lee *et al*[44].

As was described for the fish species *Oncorhynchus mykiss*[54], *Salmo trutta*[55], *Odontesthes bonariensis*[56] and *Rhamdia quelen*[57], in *O. hepsetus* we observed CCK-IR cells in the intestine; in this case only in the posterior part, while in *O. bonariensis*[56] these cells were observed throughout the gut, but with greater concentration in the hindgut. There were no CCK-IR cells in the other regions of the DT of *O. hepsetus*. This hormone controls intestinal motility, by stimulating the release of pancreatic juice and inhibiting gastric emptying[58,59]. The existence of these cells has been verified in fish[35,42], reptiles[39], birds[60] and mammals[61].

In conclusion, our histological and histochemical study of the DT of *O. hepsetus* revealed adaptation for the species' feeding habits, to protect the tract and increase the absorptive processes. The immunohistochemical study showed that the DT of this fish species contains different types of endocrine cells similar to those found in other vertebrate species. This study will help comprehension of the digestive physiology of this species and provide a basis for diagnosing diseases that affect the digestive tract of carnivorous teleosts.

ACKNOWLEDGMENTS

We thank Ilza Lucas Coelho Meirelles for her technical assistance.

COMMENTS

Background

Studies indicate the importance of knowledge of the morphology of the digestive tract (DT) because of the different physiological conditions in which animals live and their varied eating habits, which are manifested by adaptations and modifications in the digestive system. Information about the morphological aspects of the DT provides support for research on physiology and nutrition, aiming to improve the diet and management of livestock and support activities for restocking and restoration of natural ecosystems, besides being important for various research areas, including biological systems and conservation.

Research frontiers

The integration of the motor, secretory and absorptive phenomena of the DT is fundamental for the reduction of food into simpler particles that can be absorbed. This association is achieved by actions and interactions of the nervous and endocrine systems, which in the digestive system are represented by the endocrine cells. The diffuse neuroendocrine system (DNS) acts to control the motility and transit speed of the ingesta, the various secretions of the DT, the absorption of nutrients and the blood flow, to assure the activation and action of enzymes at the proper moment. Therefore, the products of the digestive system can be absorbed by the organism and reach the blood and lymphatic circulation systems.

Innovations and breakthroughs

The avidin-biotin-peroxidase complex method was applied to study the endocrine cells in the DT of *Oligosarcus hepsetus* (*O. hepsetus*). This method involves the use of three reagents: the primary antibody, which binds to the receptor of the specific hormone of interest; the secondary antibody, which is produced linked to a molecule of the vitamin biotin (C) and binds to receptors of the primary antibody; and the glycoprotein-avidin complex, produced from biotin and peroxidase, with joins with the previous reagent, the secondary antibody.

Applications

The immunohistochemical study showed that the DT of *O. hepsetus* contains different types of endocrine cells similar to those found in other vertebrate species. This study will help comprehension of the digestive physiology of this species and provide a basis for diagnosing diseases that affect the digestive tube of carnivorous teleosts.

Terminology

The gastrointestinal epithelium is permeated by a set of cells, originating from the DNS, called endocrine cells. The secretions of these cells control the digestion of food to ensure it is efficient, by regulating the digestive processes. Besides controlling the absorption of nutrients, they play an important role in determining secretions from the gut and associated glands and in regulating the intestinal blood flow.

Peer review

In this study, the authors describe the microscopic anatomy of the DT of a carnivorous fish species and analyze the functional components that aid the digestion of food. The results are relevant by enabling comparison with other fish species, contributing to phylogenetic studies.

REFERENCES

1 **Bicca DF**, Queirol E, Braccini MC. Aspectos morfológicos e histológicos do estômago de *Acestrorhynchus pantaneiro* (Menezes, 1992) (Teleostei, Acestrorhynchidae) na Bacia do Rio Uruguai médio. *Biodivers Pampeana* 2006; **4**: 5-10

2 **Winemiller KO**. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan ilanos. *Environ Biol Fishes* 1989; **26**: 177-199 [DOI: 10.1007/BF00004815]

3 **Wainwright PC**, Richard BA. Predicting patterns of prey use from morphology with fishes. *Environ Biol Fishes* 1995; **44**: 97-113 [DOI: 10.1007/BF00005909]

4 **Díaz AO**, García AM, Devincenti CV, Goldemberg AL. Morphological and histochemical characterization of the mucosa of the digestive tract in Engraulis anchoita. *Anat Histol Embryol* 2003; **32**: 341-346 [PMID: 14651481]

5 **Reid PE**, Volz D, Cho KY, Owen DA. A new method for the histochemical demonstration of O-acyl sugars in human colonic epithelial glycoproteins. *Histochem J* 1988; **20**: 510-518 [PMID: 3240952]

6 **Carvalheira AF**, Welsch U, Pearse AG. Cytochemical and ultrastructural observations on the argentaffin and argyrophil cells of the gastro-intestinal tract in mammals, and their place in the APUD series of polypeptide-secreting cells. *Histochemie* 1968; **14**: 33-46 [PMID: 4385780 DOI: 10.1007/BF00268031]

7 **Deveney CW**, Way LW. Regulatory peptides of gut. In: Greenspan FS, Forsham PH. Basic and Clinical Endocrinology. Singapore: Maruzen, 1983: 479-499

8 **Szpilman M**. Peixes marinhos do Brasil. Guia prático de identificação. Rio de Janeiro: Mauad, 2000

9 **Araújo FG**, Santos LN. Distribution of fish assemblages in Lajes reservoir, Rio de Janeiro. *Braz J Biol* 2001; **61**: 563-576 [DOI:10.1590/S1519-69842001000400006]

10 **Teixeira TP**, Terra BF, Estiliano EO, Gracia D, Pinto BCT, Araújo FG. Distribuição da ictiofauna em locais impactados no Rio Paraíba do Sul. *Rev Univ Rural Ser Cien Vida* 2004; **24**: 167-174

11 **Menezes NA**. Três espécies novas de *Oligosarcus* Gunther, 1864 e redefinição taxonômica das demais espécies do gênero (Osteichthyes, Teleostei, Characidae). *Bolm Zool* 1987; **11**: 1-39

12 **Fugi R**, Hahn NS. Espectro alimentar e relações morfológicas com o aparelho digestivo de três espécies de peixes comedores de fundo do Rio Paraná, Brasil. *Rev Bras Biol* 1991; **51**: 873-879

13 **Castro EF**, Fonseca CC, Menin E, Neves MTD. Caracterização histológica e detecção de células endócrinas no estômago de peixes de água doce, com diferentes hábitos alimentares. *Biotemas* 2003; **16**: 105-130

14 **Santos CM**, Duarte S, Souza TGL, Ribeiro TP, Sales A, Araújo FG. Histologia e caracterização histoquímica do tubo gastrintestinal de *Pimelodus maculatus* (Pimelodidae, Siluriformes) no reservatório de Funil, Rio de Janeiro, Brasil. *Iheringia Ser Zool* 2007; **97**: 411-417 [DOI:10.1590/S0073-47212007000400009]

15 **Chaves PTC**, Vazzoler C. Aspectos biológicos de peixes anatômicos. III. Anatomia microscopica do esôfago, estômago e cecos pilóricos de *Semaprochilodus insignis* (Characiformes: Prochilodontidae). *Acta Amaz* 1984; **14**: 343-353

16 **Legler JM**. Morphology and physiology of the Chelonia. In: Glasby CJ, Ross GJB, Beesley PL, editors. Caberra: Fauna of Australia, 1993: 108-119

17 **Zug GR**, Vitt LJ, Caldwell JP. Herpetology: An introduction biology of amphibians and reptiles. 2nd ed. Florida: Academic Press, 2001

18 **Hunbert W**, Kirsch R, Meister MF. Scanning electron microscopic study of the oesophageal mucous layer in the eel, Anguilla anguilla. *J Fish Biol* 1984; **25**: 117-122 [DOI: 10.1111/j.1095-8649.1984.tb04856.x]

19 **Blake IH**. Studies on the comparative histology of the digestive tube of certain teleost fishes. II. A bottom feeding fish, the sea-robin (Prionotus carolinus). *J Morphol* 1936; **60**: 77-102 [DOI: 10.1002/jmor.1050600105]

20 **Burnstock G**. The morphology of the gut of the Brown trout (Salmo trutta). *Q J Microsc Sci* 1959; **100**: 183-198

21 **Carrassón M**, Grau A, Dopazo LR, Crespo S. A histological, histochemical and ultrastructural study of the digestive tract of Dentex dentex (Pisces, Sparidae). *Histol Histopathol* 2006; **21**: 579-593 [PMID: 16528668]

22 **Sibbing FA**, Uribe R. Regional specialization in the oro-pharyngeal wall and food processing in carp (Cyprinus carpio L). *Neth J Zool* 1985; **35**: 377-422 [DOI: 10.1163/002829685X00280]

23 **Domeneghini C**, Arrighi S, Radaelli G, Bosi G, Veggetti A. Histochemical analysis of glycoconjugate secretion in the alimentary canal of Anguilla anguilla L. *Acta Histo-*

chem 2005; **106**: 477-487 [PMID: 15707657 DOI: 10.1016/j.acthis.2004.07.007]
24 **Díaz AO**, García AM, Goldemberg AL. Glycoconjugates in the mucosa of the digestive tract of Cynoscion guatucupa: a histochemical study. *Acta Histochem* 2008; **110**: 76-85 [PMID: 17945334 DOI: 10.1016/j.acthis.2007.08.002]
25 **Cao XJ**, Wang WM. Histology and mucin histochemistry of the digestive tract of yellow catfish, Pelteobagrus fulvidraco. *Anat Histol Embryol* 2009; **38**: 254-261 [PMID: 19426437 DOI: 10.1111/j.1439-0264.2009.00932.x]
26 **Leknes IL**. Histochemical studies on mucin-rich cells in the digestive tract of a teleost, the Buenos Aires tetra (Hyphessobrycon anisitsi). *Acta Histochem* 2011; **113**: 353-357 [PMID: 20144469 DOI: 10.1016/j.acthis.2010.01.010]
27 **Petrini LM**. Sobre a presença de capilares intra-epiteliais na mucosa do estômago do cascudo (*Plecostomus plecostomus Linneu*). *Cien Cult* 1961; **13**: 175
28 **Borges JCS**, Sanches EG, Oliveira MS, Silva JRMC. Anatomia e histologia gastrintestinal da garoupa-verdadeira Epinephelus marginatus (Lowe, 1834) (Teleostei, Serranidae). *Acta Sci Biol Sci* 2010; **32**: 407-414 [DOI: 10.4025/actascibiolsci.v32i4.4462]
29 **Ferraris RP**, Tan JD, De la Cruz MC. Development of the digestive tract of milkfish, Chanos chanos (Forsskal): Histology and histochemistry. *Aquaculture* 1987; **61**: 241-257 [DOI: 10.1016/0044-8486(87)90153-0]
30 **Rotta MA**. Aspectos gerais da fisiologia e estrutura do sistema digestivo dos peixes relacionados à piscicultura. Corumbá: Embrapa Pantanal, 2003
31 **Gargiulo AM**, Ceccarelli P, Dall'Aglio C, Pedini V. Histology and ultrastructure of the gut of the tilapia (Tilapia spp.), a hybrid teleost. *Anat Histol Embryol* 1998; **27**: 89-94 [PMID: 9591370 DOI: 10.1111/j.1439-0264.1998.tb00162.x]
32 **Cinar K**, Senol N. Histological and histochemical characterization of the mucosa of the digestive tract in flower fish (Pseudophoxinus antalyae). *Anat Histol Embryol* 2006; **35**: 147-151 [PMID: 16677207 DOI: 10.1111/j.1439-0264.2005.00629.x]
33 **Young B**, Heath J W. Histologia functional. Rio de Janeiro: Guanabara Koogan, 2000
34 **de Souza RR**, Ferri S, Ferraz de Carvalho CA, Paranhos GS. Myenteric plexus in a freshwater teleost intestine. I. Quantitative study of nerve cells. *Anat Anz* 1982; **152**: 359-362 [PMID: 7165118]
35 **Tagliafierro G**, Farina L, Faraldi G, Rossi GG, Vacchi M. Distribution of somatostatin and glucagon immunoreactive cells in the gastric mucosa of some cartilaginous fishes. *Gen Comp Endocrinol* 1989; **75**: 1-9 [PMID: 2570006 DOI: 10.1016/0016-6480(89)90001-4]
36 **Pan QS**, Fang ZP, Huang FJ. Identification, localization and morphology of APUD cells in gastroenteropancreatic system of stomach-containing teleosts. *World J Gastroenterol* 2000; **6**: 842-847 [PMID: 11819706]
37 **Kong HS**, Lee JH, Park KD, Ku SK, Lee HS. Immunohistochemical study of the endocrine cells in the pancreas of the carp, Cyprinus carpio (Cyprinidae). *J Vet Sci* 2002; **3**: 303-314 [PMID: 12819380]
38 **Brazeau P**, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* 1973; **179**: 77-79 [PMID: 4682131 DOI: 10.1126/science.179.4068.77]
39 **Ku SK**, Lee HS, Lee JH, Park KD. An immunohistochemical study on the endocrine cells in the alimentary tract of the red-eared slider (Trachemys scripta elegans). *Anat Histol Embryol* 2001; **30**: 33-39 [PMID: 11284161]
40 **Lee HS**, Ku SK. An immunohistochemical study of endocrine cells in the alimentary tract of the grass lizard, Takydromus wolteri Fischer (Laceridae). *Acta Histochem* 2004; **106**: 171-178 [PMID: 15147638 DOI: 10.1016/j.acthis.2003.10.008]
41 **El-Salhy M**. Immunocytochemical investigation of the gastro-entero-pancreatic (GEP) neurohormonal peptides in the pancreas and gastrointestinal tract of the dogfish Squalus acanthias. *Histochemistry* 1984; **80**: 193-205 [PMID: 6370932 DOI: 10.1007/BF00679996]
42 **Barrenechea MA**, López J, Martínez A. Regulatory peptides in gastric endocrine cells of the rainbow trout Oncorhynchus mykiss: general distribution and colocalizations. *Tissue Cell* 1994; **26**: 309-321 [PMID: 7915436 DOI: 10.1016/0040-8166(94)90017-5]
43 **Ku SK**, Lee JH, Lee HS. Immunohistochemical study on the endocrine cells in gut of the stomachless teleost, Zacco platypus (Cyprinidae). *Anat Histol Embryol* 2004; **33**: 212-219 [PMID: 15239812 DOI: 10.1111/j.1439-0264.2004.00539.x]
44 **Lee JH**, Ku SK, Park KD, Lee HS. Immunohistochemical study of the gastrointestinal endocrine cells in the Korean aucha perch. *J Fish Biol* 2004; **65**: 170-181 [DOI: 10.1111/j.0022-1112.2004.00442.x]
45 **Pelli-Martins AA**, Machado-Santos C, Sales A, Brito-Gitirana L. Histochemical, immunohistochemical, and ultrastructural observations of the esophagus morphology of Rinnella icterica (Anuran, Bufonidae). *Acta Zool* 2011; **93**: 373-380 [DOI: 10.1111/j.1463-6395.2011.00510.x]
46 **Machado-Santos C**, Zeca SG, Abidu-Figueiredo M, Ribeiro ICA, Sales A. The esophagus of the crocodilian Caiman latirostris (Reptilia, Crocodylia): histological, histochemical and immunohistochemical study. *J Morphol Sci* 2011; **28**: 113-119
47 **Yamanaka Y**, Yamada J, Kitamura N, Yamashita T. An immunohistochemical study on the distribution of endocrine cells in the chicken gastrointestinal tract. *Z Mikrosk Anat Forsch* 1989; **103**: 437-446 [PMID: 2800674]
48 **Agungpriyono S**, Macdonald AA, Leus KY, Kitamura N, Adnyane IK, Goodall GP, Hondo E, Yamada J. Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the babirusa, Babyrousa babyrussa (Suidae). *Anat Histol Embryol* 2000; **29**: 173-178 [PMID: 10916880]
49 **Adnyane IK**, Zuki AB, Noordin MM, Agungpriyono S. Immunohistochemical study of endocrine cells in the gastrointestinal tract of the barking deer, Muntiacus muntjak. *Anat Histol Embryol* 2011; **40**: 365-374 [PMID: 21545643]
50 **El-Salhy M**, Winder E, Lundqvist M. Comparative studies of serotonin-like immunoreactive cells in the digestive tract of vertebrates. *Biomed Res* 1985; **6**: 371-375
51 **Olsson C**, Holmgren S. The control of gut motility. *Comp Biochem Physiol A Mol Integr Physiol* 2001; **128**: 481-503 [PMID: 11246040 DOI: 10.1016/S1095-6433(00)00330-5]
52 **Sundin L**, Davison W, Forster M, Axelsson M. A role of 5-HT2 receptors in the gill vasculature of the antarctic fish Pagothenia borchgrevinki. *J Exp Biol* 1998; **201**: 2129-2138 [PMID: 9639587]
53 **Egginton S**, Forster ME, Davison W. Control of vascular tone in notothenioid fishes is determined by phylogeny, not environmental temperature. *Am J Physiol Regul Integr Comp Physiol* 2001; **280**: R1197-R1205 [PMID: 11247845]
54 **Jensen H**, Rourke IJ, Møller M, Jønson L, Johnsen AH. Identification and distribution of CCK-related peptides and mRNAs in the rainbow trout, Oncorhynchus mykiss. *Biochim Biophys Acta* 2001; **1517**: 190-201 [PMID: 11342099 DOI: 10.1016/S0167-4781(00)00263-3]
55 **Bosi G**, Di Giancamillo A, Arrighi S, Domeneghini C. An immunohistochemical study on the neuroendocrine system in the alimentary canal of the brown trout, Salmo trutta, L., 1758. *Gen Comp Endocrinol* 2004; **138**: 166-181 [PMID: 15302266 DOI: 10.1016/j.ygcen.2004.06.003]
56 **Vigliano FA**, Muñoz L, Hernández D, Cerutti P, Bermúdez R, Quiroga MI. An immunohistochemical study of the gut neuroendocrine system in juvenile pejerrey Odontesthes

bonariensis (Valenciennes). *J Fish Biol* 2011; **78**: 901-911 [PMID: 21366580 DOI: 10.1111/j.1095-8649.2011.02912.x]
57 **Hernández DR**, Vigliano FA, Sánchez S, Bermúdez R, Domitrovic HA, Quiroga MI. Neuroendocrine system of the digestive tract in Rhamdia quelen juvenile: an immunohistochemical study. *Tissue Cell* 2012; **44**: 220-226 [PMID: 22537686 DOI: 10.1016/j.tice.2012.03.005]
58 **Aldman G**, Jönsson AC, Jensen J, Holmgren S. Gastrin/CCK-like peptides in the spiny dogfish, Squalus acanthias; concentrations and actions in the gut. *Comp Biochem Physiol C* 1989; **92**: 103-108 [PMID: 2566429 DOI: 10.1016/0742-8413(89)90210-7]
59 **Olsson C**, Aldman G, Larsson A, Holmgren S. Cholecystokinin affects gastric emptying and stomach motility in the rainbow trout Oncorhynchus mykiss. *J Exp Biol* 1999; **202**: 161-170 [PMID: 9851905]
60 **Rawdon BB**, Andrew A. An immunocytochemical survey of endocrine cells in the gastrointestinal tract of chicks at hatching. *Cell Tissue Res* 1981; **220**: 279-292 [PMID: 6170446 DOI: 10.1007/BF00210509]
61 **Machado-Santos C**, Nascimento AA, Peracchi AL, Mikalauskas JS, Rocha PA, Sales A. Distributions of the endocrine cells in the gastrointestinal tract of nectarivorous and sanguivorous bats: a comparative immunocytochemical study. *Tissue Cell* 2009; **41**: 222-229 [PMID: 19135221 DOI: 10.1016/j.tice.2008.11.004]

P- Reviewer Cinar K **S- Editor** Gou SX **L- Editor** Logan S
E- Editor Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1930

World J Gastroenterol 2013 March 28; 19(12): 1930-1935
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Decreased prevalence of celiac disease among Brazilian elderly

Lucas Malta Almeida, Luiz Claudio Castro, Rosa Harumi Uenishi, Fernanda Coutinho de Almeida, Patricia Maria Fritsch, Lenora Gandolfi, Riccardo Pratesi, Yanna Karla de Medeiros Nóbrega

Lucas Malta Almeida, Fernanda Coutinho de Almeida, Patrícia Maria Fritsch, Graduate Program in Medical Sciences, University of Brasilia School of Medicin, Brasilia DF 70910900, Brazil
Lucas Malta Almeida, Fernanda Coutinho de Almeida, Patrícia Maria Fritsch, Doctoral fellow of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brasilia DF 70910900, Brazil
Luiz Claudio Castro, Rosa Harumi Uenishi, Lenora Gandolfi, Riccardo Pratesi, Graduate Program in Health Sciences, University of Brasilia School of Health Sciences, Brasilia DF 70910900, Brazil
Luiz Claudio Castro, Rosa Harumi Uenishi, Lenora Gandolfi, Riccardo Pratesi, Research Center for Celiac disease, University of Brasilia School of Medicine, Brasilia DF 70910900, Brazil
Yanna Karla de Medeiros Nóbrega, Department of Pharmaceutical Sciences, University of Brasilia School of Health Sciences, Brasilia DF 70910900, Brazil
Yanna Karla de Medeiros Nóbrega, Research Center for Celiac Disease, University of Brasilia School of Medicine, Brasilia DF 70910900, Brazil
Author contributions: Gandolfi L and Pratesi R had the original idea and made substantial contributions to the conception and design of the study; Almeida LM wrote the study protocol and, along with Gandolfi L, wrote the initial version of the paper; Almeida LM, Uenishi RH and de Almeida FC collected the laboratory samples and did the laboratory work; de Medeiros Nóbrega YK supervised the laboratory work, analyzed clinical and laboratory data, performed the statistical analysis, and performed the final critical review of the paper; Castro LC and Pratesi R wrote the final version of the paper; all authors have seen and approved the final version of the paper.
Correspondence to: Yanna Karla de Medeiros Nóbrega, PhD, Professor, Department of Pharmaceutical Sciences, University of Brasilia School of Health Sciences, SQN 314 Bloco E Apt 501 Asa Norte, Brasilia DF 70910900, Brazil. yannanobrega@gmail.com
Telephone: +55-61-31071991 Fax: +55-61-31071991
Received: June 21, 2012 Revised: December 3, 2012
Accepted: December 12, 2012
Published online: March 28, 2013

Abstract

AIM: To evaluate the prevalence of celiac disease in a group of Brazilian individuals over 60 years of age and compare it with the previously known prevalence in a pediatric group living in the same geographical area.

METHODS: The research protocol was approved by the Ethics Committee of the University of Brasilia School of Medicine, Brasilia, Brazil. Blood samples from 946 individuals (295 male and 651 female) aged 60 years or older were collected between May 2010 and July 2011. The study subjects' mean and median ages were 68.1 and 67 years, respectively, ranging from 60 to 92 years. That age distribution closely corresponded to the age distribution of the Brazilian population according to the Brazilian 2010 census. The participants were consecutive and unselected outpatients undergoing blood tests at the University of Brasilia Hospital's Clinical Pathology Laboratory. All sera were tested for immunoglobulin A anti-transglutaminase antibodies (IgA-tTG) by enzyme-linked immunosorbent assay, and those that were positive were further tested for immunoglobulin A anti-endomysium antibodies (IgA-EMA). Human leukocyte antigen (HLA) genotyping was performed for all individuals who exhibited positive serologic results for IgA-tTG and/or IgA-EMA.

RESULTS: Out of the 946 studied patients, only one previously diagnosed case of biopsy-proven celiac disease was detected. For the remaining subjects, nine serum samples tested positive for IgA-tTG antibodies; however, none of them tested positive for IgA-EMA antibodies. The HLA genotyping of those nine subjects revealed that one was carrying DQA1*0501 and two were carrying DQB1*0201 alleles. These data showed that, among those 946 elderly individuals, the prevalence of celiac disease (CD) was 0.1% (95%CI: 0.00-0.59). The prevalence of CD for the elderly group was compared with that observed for the group of 2034 children younger than 15 years (age range, 1-14 years; mean age, 8 years) who took part in our previous CD prevalence screening study. All the children came from the same geographical region and shared a similar ethnic and low-income background. As in the elderly group in

the current study, the younger group was made up of consecutive outpatients who underwent blood evaluation at the University of Brasilia Hospital's Clinical Laboratory. The prevalence of biopsy-proven CD among those children was 0.54% (95%CI: 0.27-0.57). The comparative analysis between the two groups resulted in the following values: odds ratio = 0.19 (95%CI: 0.01-1.45) Fisher test $P = 0.06$.

CONCLUSION: The prevalence of CD among the children of our previous study was 5.4 times higher than that found in the present elderly group.

Key words: Celiac disease; Gluten-sensitive enteropathy; Epidemiology; Elderly; Mortality

Almeida LM, Castro LC, Uenishi RH, de Almeida FC, Fritsch PM, Gandolfi L, Pratesi R, de Medeiros Nóbrega YK. Decreased prevalence of celiac disease among Brazilian elderly. *World J Gastroenterol* 2013; 19(12): 1930-1935 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1930.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1930

INTRODUCTION

Celiac disease (CD) is a chronic autoimmune-mediated disease with both intestinal and systemic manifestations that are induced by the ingestion of gluten in genetically predisposed individuals. CD-related intestinal abnormalities are mainly characterized by villous atrophy, crypt hyperplasia, and lymphocyte infiltration of the small mucosa caused by T-cell responses to the enzyme transglutaminase 2[1] and gluten-derived gliadin peptides[2]. CD is a lifelong disease that can start at any age. As it involves multiple organs or systems, it may manifest in a wide range of clinical pictures. The only effective therapy for CD is strict dietary abstinence from gluten-containing foods.

During the last few decades, the advent of reliable serologic tests has greatly facilitated in the diagnosis of CD, allowing large-scale screening studies to be performed. Worldwide prevalence rates, determined by a similar sequential testing paradigm [*i.e.*, immunoglobulin A anti-transglutaminase (IgA-tTG) antibodies and/or anti-endomysium antibodies (IgA-EMA) tests] averaged 1:160 in the Western world[3]. Recent epidemiological data showed that CD is also a common disease in developing countries (Middle East, South Asia, Africa, and South America), with a prevalence similar to that in Western countries[4]. The prevalence of CD in Brazil has shown significant variation, probably due to the different degrees of miscegenation of the ethnic groups that make up the Brazilian population, especially Amerindians, Afro-descendants, and Europeans. Screening studies performed during the last decade in distinct Brazilian regions showed prevalence rates varying from 1:214 to 1:681 in presumably healthy blood donors[5-8], and from 1:119 to 1:417 in the general population[9,10]. The geographical variation in the prevalence rates may also be due to differences in genetic background, age-related differences of exposure to gluten, and/or to changes in environmental risk factors. Due to the widely variable pattern of its clinical spectrum, the confirmatory diagnosis of CD can be delayed, after being left unrecognized for many years[11,12].

Traditionally, CD has been regarded as a disease of childhood and early adulthood that rarely develops in older people. Nevertheless, during the last few decades, diagnosis of CD among adults and the elderly has increased, including patients 70 years of age and older. CD is considered a lifelong disease and consequently a progressive increase in its prevalence would be expected in older age groups.

Nevertheless, studies focusing on this topic are rather controversial. While some of the studies pointed to a high prevalence of CD in older age groups[13,14], others showed a higher prevalence in children and adolescents[10,15,16].

In a previous study, we found an increased prevalence of CD in children compared with adults and elderly individuals[10]. Following the same line of research, in this study we aimed to determine the prevalence of CD in elderly Brazilians over 60 years of age, all of them living in the same geographic region and belonging to similar socioeconomic and cultural backgrounds as the children evaluated in our previous study.

MATERIALS AND METHODS

The research protocol was approved by the Ethics Committee of the University of Brasilia School of Medicine. All individuals included in the protocol were informed about the objectives, related risks, and benefits of this study, and agreed with the use of the collected specimens for research. Between May 2010 and July 2011, a total of 946 outpatients (295 male and 651 female) aged 60 years or older had a blood sample collected and stored at -20 ℃ until their use. The subjects' mean and median ages were 68.1 and 67 years, respectively, ranging from 60 to 92 years. That age distribution closely corresponded to the age distribution of the Brazilian population according to the 2010 census[17].

The participants were consecutive and unselected outpatients undergoing blood tests at the Clinical Pathology Laboratory of the University of Brasilia Hospital. The most frequent reasons for blood testing were routine health check-up, suspected or recurrent infections, chronic ailments, metabolic disorders, and pre-operative check-up. Patients from the gastroenterology outpatient clinic were excluded to avoid a selection bias. No other exclusion criteria were applied, regardless of the possible existence of symptoms or conditions commonly associated with CD.

The University of Brasilia Hospital is a public reference hospital that predominately serves a low-income population from the city of Brasilia and the surrounding area in the midwest region of Brazil. Such individuals usually depend on the Governmental National Health

System. They exhibit mixed ancestry, with a considerable contribution of European intermixed with variable parcels of other races, mainly Afro-descendants and Amerindians.

The serum samples from the patients were tested for IgA-human anti-tissue-transglutaminase-IgA (htTG) antibodies using an IgA-htTG enzyme- linked immunosorbent assay Kit (QUANTA Lite® h-tTG IgA Inova Diagnostic, Inc. San Diego, CA, United States). The limit of positivity was set at 20 arbitrary units, in accordance with the manufacturer's instructions. As a second step, all IgA-htTG positive samples were further tested for the presence of IgA-EMA antibodies using indirect immunofluorescence on primate distal esophagus cryostatic sections (Inova Diagnostic, Inc. San Diego, CA, United States).

All individuals who exhibited positive serologic results for IgA-tTG and/or IgA-EMA antibodies underwent HLA genotyping. Genomic DNA was extracted from peripheral venous blood samples using the Illustra ™ blood genomicPrep. Mini Spin Kit (GE Healthcare, Buckinghamshire, United Kingdom). HLA-DQA1*0501 (DQ2 α chain), HLA-DQB1*02 (DQ2 β chain), HLA-DQA1*0301 (DQ8 α chain), and DQB1*0302 (DQ8 β chain) genotyping was performed by polymerase chain reaction amplification using sequence-specific primers (PCR-SSP). For internal positive amplification control, each PCR reaction included a primer pair for a conserved region of the *DRB1* gene. The amplified products were separated using 2% agarose gel, stained with ethidium bromide and then visualized under an ultraviolet transilluminator.

RESULTS

Out of the 946 subjects, only a single previously diagnosed case of biopsy-proven CD in a 66-year-old woman was detected. Among the remaining subjects, nine serum samples tested positive for IgA-tTG antibodies. None of the patients tested positive for IgA-EMA antibodies. HLA genotyping disclosed the presence of one CD predisposing allele in three of the IgA-tTG positive elderly. The clinical and laboratory data of the nine patients who tested positive for IgA-tTG antibodies are depicted in Table 1. These data showed that among those 946 elderly individuals, the prevalence of CD (n = 1) was 0.1% (95%CI: 0.00-0.59).

The prevalence of CD for the elderly group was compared with that observed for the group of 2034 children younger than 15 years (age range, 1-14 years; mean age, 8 years) who took part in our previous CD prevalence screening study[10]. All the children came from the same geographical region and presented a similar ethnic and low-income background. As in the elderly group in the current study, the younger group was made up of consecutive outpatients who underwent blood evaluation at the University of Brasilia Hospital's Clinical Laboratory. The prevalence of biopsy-proven CD (n = 11) among those 2034 children was 0.54% (95%CI: 0.27-0.57).

DISCUSSION

Out of the 946 elderly individuals tested in this study, only a single case of previously-detected CD was found. Although nine individuals showed moderately increased levels of anti-tTG antibodies ranging from 30.6 to 52.3, no subjects tested positive for IgA-EMA antibodies. Although IgA-tTG is an effective screening test for CD, occasional anti-tTG false positive results cannot be excluded, especially in the presence of other autoimmune diseases[18,19]. The clinical effectiveness of the IgA-tTG test is improved if its positive results are confirmed with the IgA-EMA test[20] and by the presence of predisposing alleles on HLA PCR-SSP typing. Typing for HLA-DQ2 and HLA-DQ8 is a useful tool for either excluding CD or making its diagnosis unlikely in the case of a negative test result for both markers[21,22]. Predisposing HLA alleles were present in only three of the subjects who tested positive for IgA-tTG antibodies. A jejunal biopsy was suggested and refused by both patients carrying the higher degree of risk allele DQB1*0201, although they agreed in being followed with periodical clinical evaluations and serological testing.

The results of this current screening are in agreement with the result obtained in our previous study, in which an unanticipated variation in the prevalence of CD was found[10]. In this study performed in the same geographical area with a similar population group, most cases of CD were clustered in the younger age group, with the prevalence of CD in children aged 1 to 14 years being 2.6 times higher than the one found for adults and elderly individuals (5.44 *vs* 2.11 per 1000, respectively). This variation in the prevalence of CD between different age groups was actually unexpected, considering that intestinal sensitivity to gluten is a permanent condition. Aside from gluten ingestion, CD might be triggered at any stage of life by additional environmental factors that remain largely unknown. Thus, a progressive increase in prevalence rates towards advanced ages or, at least, a similar rate throughout life would be expected. Recent studies in Finland by Vilppula *et al*[13,14] showed an increase in the prevalence of CD among individuals over 52 years of age compared with the general prevalence in Finnish children (2.13% *vs* 1%). Furthermore, the authors also demonstrated an increasing prevalence throughout a three-year period for the same group, going from 2.13% to 2.34%, and resulting in an annual CD incidence of 0.08% in that population. However, several other studies report contradictory results by showing a higher prevalence of CD in younger populations[15,16,23,24].

Several hypotheses have been suggested to explain this discordance in the prevalence rates among different age groups, although none have been definitely proven. One hypothesis offered to justify a higher prevalence in the younger age group is that the incidence of CD, similarly to other autoimmune diseases, is progressively

Table 1 Clinical and laboratory data of patients who tested positive for immunoglobulin A anti-transglutaminase antibodies by enzyme-linked immunosorbent assay

Patient	Sex	Age (yr)	tTG	EMA	HLA	Symptomatology and associated disorders
1	M	63	39.9	Neg	Negative	Anemia, arthritis
2	M	71	34.5	Neg	DQB1*0201	Hyperthyroidism
3	M	81	31.3	Neg	Negative	No complaints
4	F	60	42.9	Neg	DQB1*0201	No complaints
5	F	60	30.6	Neg	Negative	Osteopenia, arthritis, recurrent abdominal pain, flatulence
6	F	63	52.3	Neg	Negative	Arthritis, osteoporosis, hyperthyroidism
7	F	65	34.0	Neg	Negative	No complaints
8	F	68	45.3	Neg	DQA1*0501	Osteoporosis, weight loss, flatulence
9	F	72	45.2	Neg	Negative	Osteoporosis

M: Male; F: Female; Neg: Negative; tTG: Transglutaminase; EMA: Endomysium; HLA: Human leukocyte antigen.

increasing worldwide. CD was considered a rare disease until the late 1970s, and its prevalence was estimated to be as low as 0.03%[25]. With the advent of highly sensitive and specific serological tests, a dramatic rise in its prevalence was observed during the following decades. However, this increase would not be solely due to better diagnostic methods that enabled extensive screening studies and diagnosis of atypical forms of the disease; consistent with other autoimmune disorders that have shown rising incidence rates over the last few decades[26], CD would also have shown a significant increase in its prevalence, consequently justifying the increased number of cases found among younger age groups[27,28]. Although the causes underlying this increased age-related prevalence remain unknown, likely explanations include environmental influences (such as changes in quantity and quality of cereal processing), changed patterns of early childhood exposure to infectious agents that impair the natural development of the immune system (hygiene hypothesis)[29], and changes in infant dietetic habits[30].

Another possible cause for an increased CD prevalence in the younger population was suggested by Mariné *et al*[15], who proposed that a significant number of CD cases that appear during childhood progress to a latent form or into total gluten tolerance. Those patients would therefore exhibit negative serologic results as they got older. Several other studies support this hypothesis[31-33], although the number of described spontaneous recoveries of normal villous architecture in CD patients on a gluten-containing diet is generally small[34] and it is uncertain as to whether these clinically silent periods accompanied by negative serologic tests can be considered only temporary remissions or actual definitive recoveries. Extended follow-up is therefore required for these patients[32,35].

A third explanation is the postulated existence of an increased mortality rate among CD patients. Publications addressing this issue are numerous, but conflicting[28,36,37]. The reported overall mortality rates among CD patients vary from 1.26%[29] to 3.9%[19] in studies focusing on undiagnosed CD in adults. Despite the differing results, at least two publications point to undiagnosed CD as a major cause of increased morbidity and mortality among individuals with the disease[28,38].

These three hypotheses are not mutually exclusive, and it is both possible and probable that each of these factors contribute, to a greater or lesser degree, to the observed variation in the prevalence of CD in elderly individuals, depending on the different environmental conditions found in distinct regions or countries.

In Brasilia, many of the low-income adult population came from poor regions of the country, where they have had little or no access to medical care during childhood, and awareness of CD was somehow deficient among healthcare professionals. In these regions, CD knowledge several decades ago was not much different from that before effective treatment for CD was established. Even with the current facilities for the proper diagnosis and compliance of CD patients, diet behaviors among individuals of economically disadvantaged backgrounds in Brazil is still far from ideal.

In Finland, the higher prevalence of CD in patients aged over 52 years and the lower CD mortality rate[13,14] are noteworthy. Comparisons between Finland and Brazil are contentious at best, since they occupy different positions in the World Health Organization's health system ranking of countries[39] (31st and 125th, respectively). If this same survey was again performed in Brasilia, but instead focused on a higher socioeconomic group with a higher quality of life, the outcomes would probably have been different.

Our study has potential limitations that should be noted. The screening of the elderly group was conducted in a single geographic setting and the participants were unselected outpatients undergoing routine blood tests, although both groups, children and elderly, were similar with regard to their ethnicity and socioeconomic level. In addition, the number of elderly screened was insufficient to reach statistical significance ($P = 0.06$). In spite of these drawbacks, this study supports our previous findings of an age-related variation in the prevalence rate of CD. Only a single biopsy-proven previously diagnosed case of CD was identified among the elderly group, showing that the prevalence of CD among the children of our previous study was 5.4 times higher than that found in the present elderly group. These findings reinforce that, for the low socioeconomic populations

of our region, prevalence of CD is unexpectedly higher in children compared to older individuals, and that this discrepancy increases towards older ages. We hypothesize that among the plausible explanations for the discrepancy between the CD prevalence rates among children and elderly, the most likely culprit would be an increased mortality rate among undiagnosed celiac disease patients.

COMMENTS

Background

Epidemiological studies during the last few decades have shown an increasing prevalence of celiac disease over time, not only in Europe and in people of European ancestry, but also in developing countries. Socioeconomically disadvantaged population groups in developing countries additionally suffer from a low level of awareness, clinical suspicion of this disorder among physicians, and difficult access by the patient to diagnostic methods, which result in unrecognized or delayed diagnosis of celiac disease (CD). Additionally, patients in whom an appropriate diagnosis is reached have their treatment hampered by an impossibility to use commercial gluten-free products, which are too expensive for these populations, as well as the lack of patients' awareness and information regarding their diet gluten content.

Research frontiers

Overcoming the difficulties of treating CD in the context of developing countries implies the introduction of CD diagnostic laboratory tests as routine in the State Health System, funding research of cheap sources of gluten free foods, and an increased recognition of CD and of its symptoms.

Applications

The results obtained in this study may provide support for future epidemiological studies to map the onset of CD in different age groups in at-risk populations in developing countries. It can additionally contribute to the adoption of public health policies that will allow the socioeconomically disadvantaged access to health services for medical consultations, laboratory tests and, after diagnosis, financial support for a lifelong gluten-free diet.

Peer review

In this interesting survey, the authors report their results on the prevalence of celiac disease in a typical Brazilian population. The manuscript is very well written. The abstract is appropriate in length and content. The results are reported clearly. The discussion is exhaustive and provides an interesting view of this controversial topic.

REFERENCES

1 **Dieterich W**, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997; **3**: 797-801 [PMID: 9212111 DOI: 10.1038/nm0797-797]

2 **Niveloni S**, Sugai E, Cabanne A, Vazquez H, Argonz J, Smecuol E, Moreno ML, Nachman F, Mazure R, Kogan Z, Gomez JC, Mauriño E, Bai JC. Antibodies against synthetic deamidated gliadin peptides as predictors of celiac disease: prospective assessment in an adult population with a high pretest probability of disease. *Clin Chem* 2007; **53**: 2186-2192 [PMID: 17901114 DOI: 10.1373/clinchem.2006.081364]

3 **Biagi F**, Klersy C, Balduzzi D, Corazza GR. Are we not overestimating the prevalence of coeliac disease in the general population? *Ann Med* 2010; **42**: 557-561 [PMID: 20883139 DOI: 10.3109/07853890.2010.523229]

4 **Cataldo F**, Montalto G. Celiac disease in the developing countries: a new and challenging public health problem. *World J Gastroenterol* 2007; **13**: 2153-2159 [PMID: 17465493]

5 **Oliveira RP**, Sdepanian VL, Barreto JA, Cortez AJ, Carvalho FO, Bordin JO, de Camargo Soares MA, da Silva Patrício FR, Kawakami E, de Morais MB, Fagundes-Neto U. High prevalence of celiac disease in Brazilian blood donor volunteers based on screening by IgA antitissue transglutaminase antibody. *Eur J Gastroenterol Hepatol* 2007; **19**: 43-49 [PMID: 17206076 DOI: 10.1097/01.meg.0000250586.61232.a3]

6 **Melo SB**, Fernandes MI, Peres LC, Troncon LE, Galvão LC. Prevalence and demographic characteristics of celiac disease among blood donors in Ribeirão Preto, State of São Paulo, Brazil. *Dig Dis Sci* 2006; **51**: 1020-1025 [PMID: 16758312 DOI: 10.1007/s10620-006-9340-9]

7 **Pereira MA**, Ortiz-Agostinho CL, Nishitokukado I, Sato MN, Damião AO, Alencar ML, Abrantes-Lemos CP, Cançado EL, de Brito T, Ioshii SO, Valarini SB, Sipahi AM. Prevalence of celiac disease in an urban area of Brazil with predominantly European ancestry. *World J Gastroenterol* 2006; **12**: 6546-6550 [PMID: 17072989]

8 **Gandolfi L**, Pratesi R, Cordoba JC, Tauil PL, Gasparin M, Catassi C. Prevalence of celiac disease among blood donors in Brazil. *Am J Gastroenterol* 2000; **95**: 689-692 [PMID: 10710058 DOI: 10.1111/j.1572-0241.2000.01847.x]

9 **Crovella S**, Brandao L, Guimaraes R, Filho JL, Arraes LC, Ventura A, Not T. Speeding up coeliac disease diagnosis in the developing countries. *Dig Liver Dis* 2007; **39**: 900-902 [PMID: 17706474]

10 **Pratesi R**, Gandolfi L, Garcia SG, Modelli IC, Lopes de Almeida P, Bocca AL, Catassi C. Prevalence of coeliac disease: unexplained age-related variation in the same population. *Scand J Gastroenterol* 2003; **38**: 747-750 [PMID: 12889561 DOI: 10.1080/00365520310003255]

11 **Ivarsson A**, Persson LA, Juto P, Peltonen M, Suhr O, Hernell O. High prevalence of undiagnosed coeliac disease in adults: a Swedish population-based study. *J Intern Med* 1999; **245**: 63-68 [PMID: 10095818 DOI: 10.1046/j.1365-2796.1999.00403.x]

12 **Ravikumara M**, Nootigattu VK, Sandhu BK. Ninety percent of celiac disease is being missed. *J Pediatr Gastroenterol Nutr* 2007; **45**: 497-499 [PMID: 18030224 DOI: 10.1097/MPG.0b013e31812e5710]

13 **Vilppula A**, Collin P, Mäki M, Valve R, Luostarinen M, Krekelä I, Patrikainen H, Kaukinen K, Luostarinen L. Undetected coeliac disease in the elderly: a biopsy-proven population-based study. *Dig Liver Dis* 2008; **40**: 809-813 [PMID: 18467196]

14 **Vilppula A**, Kaukinen K, Luostarinen L, Krekelä I, Patrikainen H, Valve R, Mäki M, Collin P. Increasing prevalence and high incidence of celiac disease in elderly people: a population-based study. *BMC Gastroenterol* 2009; **9**: 49 [PMID: 19558729 DOI: 10.1186/1471-230x-9-49]

15 **Mariné M**, Farre C, Alsina M, Vilar P, Cortijo M, Salas A, Fernández-Bañares F, Rosinach M, Santaolalla R, Loras C, Marquès T, Cusí V, Hernández MI, Carrasco A, Ribes J, Viver JM, Esteve M. The prevalence of coeliac disease is significantly higher in children compared with adults. *Aliment Pharmacol Ther* 2011; **33**: 477-486 [PMID: 21166832 DOI: 10.1111/j.1365-2036.2010.04543.x]

16 **Lillemäe K**, Ress K, Harro J, Merenäkk L, Maaroos HI, Uibo R, Uibo O. A 10-year serological follow-up of celiac disease in an Estonian population. *Eur J Gastroenterol Hepatol* 2012; **24**: 55-58 [PMID: 22008627 DOI: 10.1097/MEG.0b013e32834d09b9]

17 IBGE, Sinopse dos Resultados do Censo 2010. [Instituto Brasiléiro de Geografia e Estatística, IBGE Web site]. 2011. Cited 2011-08-17. Available from: URL: http: //www.censo2010.ibge.gov.br/sinopse/webservice/

18 **Tesei N**, Sugai E, Vázquez H, Smecuol E, Niveloni S, Mazure R, Moreno ML, Gomez JC, Mauriño E, Bai JC. Antibodies to human recombinant tissue transglutaminase may detect coeliac disease patients undiagnosed by endomysial antibodies. *Aliment Pharmacol Ther* 2003; **17**: 1415-1423 [PMID: 12786636 DOI: 10.1046/j.1365-2036.2003.01595.x]

19 **Clemente MG**, Musu MP, Frau F, Lucia C, De Virgiliis S. Antitissue transglutaminase antibodies outside celiac disease. *J Pediatr Gastroenterol Nutr* 2002; **34**: 31-34 [PMID: 11753161]

20 **Lock RJ**, Stevens S, Pitcher MC, Unsworth DJ. Is immunoglobulin A anti-tissue transglutaminase antibody a reliable serological marker of coeliac disease? *Eur J Gastroenterol*

Hepatol 2004; **16**: 467-470 [PMID: 15097038]
21 **Karell K**, Louka AS, Moodie SJ, Ascher H, Clot F, Greco L, Ciclitira PJ, Sollid LM, Partanen J. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Hum Immunol* 2003; **64**: 469-477 [PMID: 12651074]
22 **Husby S**, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, Troncone R, Giersiepen K, Branski D, Catassi C, Lelgeman M, Mäki M, Ribes-Koninckx C, Ventura A, Zimmer KP. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr* 2012; **54**: 136-160 [PMID: 22197856 DOI: 10.1097/MPG.0b013e3182a23d0]
23 **Corazza GR**, Andreani ML, Biagi F, Corrao G, Pretolani S, Giulianelli G, Ghironzi G, Gasbarrini G. The smaller size of the 'coeliac iceberg' in adults. *Scand J Gastroenterol* 1997; **32**: 917-919 [PMID: 9299671]
24 **Volta U**, Bellentani S, Bianchi FB, Brandi G, De Franceschi L, Miglioli L, Granito A, Balli F, Tiribelli C. High prevalence of celiac disease in Italian general population. *Dig Dis Sci* 2001; **46**: 1500-1505 [PMID: 11478502]
25 **Green PH**, Jabri B. Coeliac disease. *Lancet* 2003; **362**: 383-391 [PMID: 12907013]
26 **Bach JF**. The effect of infections on susceptibility to autoimmune and allergic diseases. *N Engl J Med* 2002; **347**: 911-920 [PMID: 12239261 DOI: 10.1056/NEJMra020100]
27 **Lohi S**, Mustalahti K, Kaukinen K, Laurila K, Collin P, Rissanen H, Lohi O, Bravi E, Gasparin M, Reunanen A, Mäki M. Increasing prevalence of coeliac disease over time. *Aliment Pharmacol Ther* 2007; **26**: 1217-1225 [PMID: 17944736 DOI: 10.1111/j.1365-2036.2007.03502.x]
28 **Rubio-Tapia A**, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, Brantner TL, Kim WR, Phelps TK, Lahr BD, Zinsmeister AR, Melton LJ, Murray JA. Increased prevalence and mortality in undiagnosed celiac disease. *Gastroenterology* 2009; **137**: 88-93 [PMID: 19362553]
29 **Kondrashova A**, Mustalahti K, Kaukinen K, Viskari H, Volodicheva V, Haapala AM, Ilonen J, Knip M, Mäki M, Hyöty H. Lower economic status and inferior hygienic environment may protect against celiac disease. *Ann Med* 2008; **40**: 223-231 [PMID: 18382888 DOI: 10.1080/07853890701678689]
30 **Ivarsson A**, Persson LA, Nyström L, Ascher H, Cavell B, Danielsson L, Dannaeus A, Lindberg T, Lindquist B, Stenhammar L, Hernell O. Epidemic of coeliac disease in Swedish children. *Acta Paediatr* 2000; **89**: 165-171 [PMID: 10709885 DOI: 10.1111/j.1651.-2227.2000.tb01210.x]
31 **Limbach A**, Hoepffner W, Tannapfel A, Müller DM, Mothes T, Richter T. [Long-term study of patients with coeliac disease in childhood and adolescence: latent and transient coeliac disease]. *Klin Padiatr* 2003; **215**: 76-81 [PMID: 12677547 DOI: 10.1055/s-2003-38504]
32 **Matysiak-Budnik T,** Malamut G, de Serre NP, Grosdidier E, Seguier S, Brousse N, Caillat-Zucman S, Cerf-Bensussan N, Schmitz J, Cellier C. Long-term follow-up of 61 coeliac patients diagnosed in childhood: evolution toward latency is possible on a normal diet. *Gut* 2007; **56**: 1379-1386 [PMID: 17303598 DOI: 10.1136/gut.2006.100511]
33 **Hopman EG,** von Blomberg ME, Batstra MR, Morreau H, Dekker FW, Koning F, Lamers CB, Mearin ML. Gluten tolerance in adult patients with celiac disease 20 years after diagnosis? *Eur J Gastroenterol Hepatol* 2008; **20**: 423-429 [PMID: 18403944 DOI: 10.1097/MEG.0b013e3282f4de6e]
34 **Shmerling DH,** Franckx J. Childhood celiac disease: a long-term analysis of relapses in 91 patients. *J Pediatr Gastroenterol Nutr* 1986; **5**: 565-569 [PMID: 3735007]
35 **Kurppa K,** Collin P, Lindfors K, Mäki M, Kaukinen K. Spontaneous negative seroconversion of endomysial antibodies does not exclude subsequent celiac disease. *J Pediatr Gastroenterol Nutr* 2011; **53**: 576-579 [PMID: 22020541]
36 **Biagi F,** Corazza GR. Mortality in celiac disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 158-162 [PMID: 20125093]
37 **Viljamaa M,** Kaukinen K, Pukkala E, Hervonen K, Reunala T, Collin P. Malignancies and mortality in patients with coeliac disease and dermatitis herpetiformis: 30-year population-based study. *Dig Liver Dis* 2006; **38**: 374-380 [PMID: 16627018]
38 **Metzger MH,** Heier M, Mäki M, Bravi E, Schneider A, Löwel H, Illig T, Schuppan D, Wichmann HE. Mortality excess in individuals with elevated IgA anti-transglutaminase antibodies: the KORA/MONICA Augsburg cohort study 1989-1998. *Eur J Epidemiol* 2006; **21**: 359-365 [PMID: 16649072 DOI: 10.1007/s10654-006-9002-4]
39 **WHO.** Health Systems: Improving Performance (The World Health Reporter 2000, WHO Web site). 2000. Cited 2011-10-09. Available from: URL: http: //www.who.int/whr/2000/en/whr00_en.pdf

P- Reviewers Jadallah K, Day AS **S- Editor** Gou SX
L- Editor Rutherford A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1936

World J Gastroenterol 2013 March 28; 19(12): 1936-1942
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Conventional endoscopic retrograde cholangiopancreaticography *vs* the Olympus V-scope system

Martin Raithel, Andreas Nägel, Jürgen Maiss, Dane Wildner, Alexander Fritzkarl Hagel, Sandra Braun, Hiwot Diebel, Eckhart Georg Hahn

Martin Raithel, Andreas Nägel, Jürgen Maiss, Dane Wildner, Alexander Fritzkarl Hagel, Sandra Braun, Hiwot Diebel, Eckhart Georg Hahn, Department of Medicine 1, Gastroenterology, Endoscopy, University Erlangen-Nuremberg, 91054 Erlangen, Germany

Author contributions: Raithel M, Hagel AF and Hahn EG designed the study; Raithel M, Nägel A, Maiss J, Wildner D, Braun S and Diebel H performed the examinations; Hagel AF, Wildner D and Nägel A analysed the data; Raithel M, Maiss J and Hahn EG wrote the paper.

Correspondence to: Martin Raithel, MD, Professor of Medicine, Department of Medicine 1, Gastroenterology, Functional Tissue Diagnostics, University Erlangen-Nuremberg, Ulmenweg 18, 91054 Erlangen, Germany. martin.raithel@uk-erlangen.de
Telephone: +49-9131-8535151 Fax: +49-9131-8535152
Received: August 29, 2012 Revised: January 11, 2013
Accepted: January 23, 2013
Published online: March 28, 2013

Abstract

AIM: To compare the new Olympus V-scope (VS) to conventional endoscopic retrograde cholangiopancreaticography (ERCP).

METHODS: Forty-nine patients with previous endoscopic papillotomy who were admitted for interventional ERCP for one of several reasons were included in this single-centre, prospective randomized study. Consecutive patients were randomized to either the VS group or to the conventional ERCP group. ERCP-naïve patients who had not undergone papillotomy were excluded. The main study parameters were interventional examination time, X-ray time and dose, and premedication dose (all given below as the median, range) and were investigated in addition to each patient's clinical outcome and complications. Subjective scores to assess each procedure were also provided by the physicians and endoscopy assistants who carried out the procedures. A statistical analysis was carried out using the Wilcoxon rank-sum test.

RESULTS: Twenty-five patients with 50 interventions were examined with the VS ERCP technique, and 24 patients with 47 interventions were examined using the conventional ERCP technique. There were no significant differences between the two groups regarding the age, sex, indications, degree of ERCP difficulty, or interventions performed. The main study parameters in the VS group showed a nonsignificant trend towards a shorter interventional examination time (29 min, 5-50 min *vs* 31 min, 7-90 min, $P = 0.28$), shorter X-ray time (5.8 min, 0.6-14.1 min *vs* 6.1 min, 1.6-18.8 min, $P = 0.48$), and lower X-ray dose (1351 cGy/m^2, 159-5039 cGy/m^2 *vs* 1296 cGy/m^2, 202.2-6421 cGy/m^2, $P = 0.34$). A nonsignificant trend towards fewer adverse events occurred in the VS group as compared with the conventional ERCP group (cholangitis: 12% *vs* 16%, $P = 0.12$; pain: 4% *vs* 12.5%, $P = 0.33$; post-ERCP pancreatitis: 4% *vs* 12.5%, $P = 0.14$). In addition, there were no statistically significant differences in assessment by the physicians and endoscopy assistants using subjective questionnaires.

CONCLUSION: ERCP using the short-guidewire V-system did not significantly improve ERCP performance or patient outcomes, but it may reduce and simplify the ERCP procedure in difficult settings.

Key words: Endoscopic retrograde cholangiopancreaticography; Short guidewire endoscopic retrograde cholangiopancreaticography system; X-ray protection; V-scope; Bile duct stenosis

Raithel M, Nägel A, Maiss J, Wildner D, Hagel AF, Braun S, Diebel H, Hahn EG. Conventional endoscopic retrograde cholangiopancreaticography *vs* the Olympus V-scope system. *World J Gastroenterol* 2013; 19(12): 1936-1942 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1936.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1936

INTRODUCTION

Endoscopic retrograde cholangiopancreaticography (ERCP) is a complex diagnostic and therapeutic approach that is used to identify and treat various hepatobiliary and pancreatic diseases. ERCP is a time-consuming, expensive, and laborious method that requires the patient to be exposed to substantial doses of premedication, contrast medium, and X-rays, especially when difficult cannulation of the papilla, biliary, or pancreatic ducts or strictures occurs and/or difficult interventions are performed[1-5]. Thus, future advances to simplify the technical process of ERCP, reduce known ERCP risks for the patient, and reduce the time and effort of the physician for this procedure, as well as attempts to reduce costs, are important issues given the restricted financial resources of hospitals regarding radiation protection, hygiene, and the need for greater patient safety[5-10].

With the release of a specialised side-viewing endoscope from Olympus [V-scope (VS)], which contains a specialised elevator lever with a V-groove in combination with the use of a specialised short guidewire system and a V-holder, further optimization of the entire ERCP process appears possible. The VS, including the V-groove of the elevator lever and the V-holder and its dedicated guidewires, was constructed to help the endoscopist secure the guidewire at a particular visible length during accessory exchange. This allows the physician to perform a guidewire or accessory exchange by him/herself and may thus lead to quicker instrumentation when working with or without assistance[11-14]. In addition, the availability of a specialised short guidewire (2.6 m in length) and corresponding accessories promises to improve guidewire handling during ERCP and to increase hygienic aspects of ERCP, and it may also reduce the efforts of physicians and assistants[12-14].

To analyse this procedure systematically, we performed a randomized prospective pilot trial to explore and document whether clinical, practical, and subjective improvements occur in daily ERCP practice when using the VS and its dedicated guidewire system as compared with conventional ERCP (without a VS, usually using long guidewires of 4.0-4.5 m in length). The main objectives were to evaluate the parameters of interventional examination time, X-ray time and dose, premedication dose, and interventions. In addition, subjective scores from physicians and endoscopy assistants were obtained to provide information about practical handling, hygienic aspects, and the convenience of this new ERCP method at a tertiary care university medical centre.

MATERIALS AND METHODS

Patient population

From June to October, 2007, 49 consecutive patients who were admitted to the Department of Medicine 1 of the University Erlangen-Nuremberg for interventional ERCP were included in this ERCP pilot trial if they had a previous endoscopic papillotomy and had one or more of the following conditions choledocholithiasis or hepaticolithiasis, malignant or benign bile duct stenosis, and chronic pancreatitis or pancreatic tumour. In addition, patients agreed to participate in this pilot trial with randomization to one ERCP technique, the collection of prospective scientific documentation, and the evaluation of their clinical and ERCP findings. Patient characteristics and indications for interventional ERCP are given in Table 1. All patients gave informed consent to participate and agreed to the collection of scientific documentation of the examination results. This clinical study was carried out in accordance with the Helsinki declaration.

Table 1 Patient characteristics and indications for endoscopic retrograde cholangiopancreaticography

	ERCP with V-scope/V-system	Conventional ERCP
Examinations (*n*)	25	24
Age, median (range), yr	57 (33–83)	57 (19–96)
Sex (male/female)	8/17	8/16
Hepaticolithiasis	4	5
Biliary strictures (benign/malignant)	7 (4/3)	5 (4/1)
Chronic pancreatitis	12	12
Pancreatic tumour	2	2

ERCP: Endoscopic retrograde cholangiopancreaticography.

Because cannulation of native papilla, papillotomy, and potential treatment of its associated risks may require a substantial amount of time, ERCP-naïve patients without papillotomy were excluded from this study, as were patients with coagulation disorders, septic cholangitis, or severe cardiovascular or pulmonary disease or who were pregnant[5,9,15,16].

Informed consent to participate in this practice study was obtained the evening before the scheduled ERCP. Thirty minutes before the scheduled ERCP procedure, patients were randomized either to ERCP using the VS and its dedicated (short) guidewire system (VS group) or to conventional ERCP.

ERCP and interventions

ERCP in the VS group was performed with the commercially available Olympus side-viewing VS (TJF160 VR; Olympus, Hamburg, Germany), which contains a modified elevator lever with a V-groove and a specialised fixable guidewire system. The V-groove of the elevator lever induces an increased angle of articulation in the VS and allows complete locking of a specialised guidewire for use with the VS[13,14,17]. This guidewire consists of a linear guide and a flexible hydrophilic tip (5 cm in length) combined with a long, stiff nitinol wire (0.35 mm in diameter, 2.6 or 4.2 m in length); both the linear guide and the nitinol wire have endoscopically visible markings at 5 cm (Olympus). In addition, a V-holder, which attaches to the working channel of the VS, allows the physician, in conjunction with securing the guidewire with the newly constructed V-elevator, to perform changes of instru-

ments and accessories (*e.g.*, appropriate baskets, balloons, papillotomes, *etc.*; Olympus) with or without assistance. This procedure accelerates instrumentation and may reduce X-ray time because of endoscopically visible control of the fixed guidewire[13,14,17].

ERCP in the conventional ERCP group was performed with a side-viewing duodenoscope (Olympus TJF160) and typical 4.0- to 4.8-m-long guidewires (Terumo radifocus guidewire, flexible hydrophilic; Terumo Corporation, Leuven, Belgium; straight green guidewire, Teflon coated; Dispomedica, Hamburg, Germany; tracer metro wire guide; Aqua Coat Tip, Cook Ireland Ltd., Limerick, Ireland) and corresponding accessories (catheters, bougies, baskets, balloons, *etc.*)[1,2,18-20]. Exchange of instruments was performed conventionally, and only if necessary, using fluoroscopy with the endoscopist pressing down the elevator lever and carefully withdrawing the instrument, while the assistant tried to retain the position of the guidewire in the cannulated area.

In brief, interventional ERCP in both groups was performed using the following steps: first prosthesis extraction (if necessary for exchange), cannulation of the papilla, visualization of the biliary system or pancreatic ducts with contrast medium (Peritrast 300/60%; Dr. Köhler Chemie GmBH, Alsbach Hähnlein, Germany), radiological documentation of pathological findings in two radiological axes, selective cannulation of pathologically changed biliary or pancreatic ducts using appropriate guidewires, performance of one or more interventions (*e.g.*, bougienage, concrement extraction, prosthesis insertion, *etc.*), and radiological and endoscopic documentation of results.

Of note, the interventional examination time for ERCP did not include insertion of the side-viewing duodenoscope down to the papilla or extraction of the endoprosthesis. To appropriately determine the effects of VS-guided ERCP, the interventional examination time was defined as the start of the ERCP once the side-viewing endoscope had been appropriately positioned in front of the papilla. Timing was started with a stopwatch once the cannulation catheter had been introduced to the working channel of the endoscope for the first time. The interventional examination time ended when the last intervention (endoprosthesis insertion, stone extraction, *etc.*) was completed and the final endoscopic photograph for documentation had been taken. Withdrawal of the endoscope from the patient was not included in the interventional examination time.

X-ray time and dose of each ERCP procedure from the first cannulation until the entire procedure and the final intervention had been finished were automatically registered and documented with the multifunctional digital Axiom artis fluoroscope (AXIOM Artis MP, Siemens, Munich, Germany) for each patient.

ERCPs were performed by two experienced investigators with > 10 years experience in gastrointestinal endoscopy, each of whom had performed more than 1500 ERCPs. Before the start of the trial, both investigators and the endoscopy assistants underwent 2 mo of learning and training to become familiar with the VS and its fixable guidewire system. During this learning phase, each investigator performed more than 20 VS ERCPs. From this training phase, it became clear that handling, time requirements, radiological or endoscopic control of intrahepatically or intrapancreatically placed guidewires, and the method of performing instrument exchange had to be learned and require repeated training to improve skills and perhaps to reduce intervention times. From the team of endoscopy assistants, four individuals, each with experience of > 1000 ERCPs, were involved in this prospective randomised study.

Physicians and assistants completed the study documents immediately after the end of the ERCP and independently gave subjective scores (0-10, best to worst) in terms of "overall performance of ERCP", "difficulty of ERCP" and "hygienic performance". "Overall performance of ERCP" concerned global assessment of the course of the entire ERCP process. "Difficulty of ERCP" was described as the degree of interventional technical difficulty of the ERCP. "Hygienic performance" concerned whether the guidewire or accessories exchange was perfectly hygienic and whether guidewires contacted the patient's face or head, were ever outside the covered sterile working area, *etc.*

Premedication was achieved in most patients with midazolam/pethidine and in younger patients with high levels of anxiety or in patients with high consumption of alcohol with propofol/pethidine. Conscious sedation was administered and monitored by a second physician who was responsible for analgo-sedation and documentation of all findings relevant to the study. All patients received continuous measurement of cutaneous oxygen saturation, pulse, blood pressure, and adequate oxygen supply during ERCP, which was performed in the prone position.

Cost analysis was also performed for each ERCP case for all consumables used during the study. The institutional costs for the side-viewing endoscopes and personnel costs for training purposes were not included in this analysis.

Statistical analysis

Statistical analysis was done using SPSS (SPSS for Windows Version 16.0.2, Ehningen, Germany) with descriptive statistics (median and range) for all parameters and performance of the Wilcoxon rank-sum test (*U* test). The statistical hypothesis was that use of the Olympus VS and its fixable guidewire system in the VS group would make ERCP faster (interventional examination time), reduce the X-ray time and dose, and reduce the premedication dose. Additional statistical descriptions are provided for subjective scores describing the convenience and performance of each ERCP procedure given by the endoscopists and the assistants.

RESULTS

Table 2 list all objective and subjective parameters used to

Table 2 Objective and subjective score results from comparison of endoscopic retrograde cholangiopancreaticography using the V-scope with conventional endoscopic retrograde cholangiopancreaticography

	ERCP with V-scope/V-system	Conventional ERCP	*P* value
Objective results			
Total interventions (*n*)	50	47	
Bougienage bile ducts	7	6	
Bougienage pancreas	2	8	
Endoprosthesis insertion	24	16	
Extraction of biliary concrements	10	9	
Extraction of pancreatic concrements	3	3	
Bile duct biopsy	1	0	
Nasobiliary catheter	1	2	
Partial guidewire dislocation	1	3	
Loss of guidewire	1	0	
Examination time (min), median (range)	29 (5–50)	31 (7–90)	0.28
X-ray time (min), median (range)	5.87 (0.6–14.15)	6.12 (1.67–18.85)	0.48
X-ray dose (cGy/m^2), median (range)	1351 (159–5039.2)	1296 (202.3–6421)	0.34
Premedication dose (mg), median (range)			
Midazolam	7 (0–11.5)	6.75 (0–11.5)	0.33
Pethidine	100 (0–200)	100 (0–200)	0.48
Propofol	0 (0–720)	0 (0–490)	0.42
Diazepam	0 (0–10)	0 (0–15)	0.33
Adverse events (*n* patients, % of each group)			
Abdominal pain >24 h without inflammation	1 (4)	3 (12.5)	0.59
Cholangitis[1]	3 (12)	4 (16.7)	0.77
Post-ERCP pancreatitis[2]	1 (4)	3 (12.5)	0.59
Perforation	0	0	
Subjective score results			
Endoscopy assistants (*n* = 4), median (range)			
Overall performance of ERCP	3 (1–8)	2 (1–7)	0.51
Hygienic aspects of ERCP	3 (1–6)	3 (1–7)	0.33
Endoscopists (*n* = 2), median (range)			
Overall performance of ERCP	3 (1–8)	3 (1–7)	0.47
Position to papilla	3 (1–7)	4 (1–7)	0.29
Difficulty of ERCP, median (range)	2 (1–2)	2 (1–3)	0.49

[1]Cholangitis was diagnosed by post-procedural elevation of inflammatory markers in conjunction with an intermittent increase in cholestatic enzymes and/or bilirubin, or subfebrile/febrile temperatures after endoscopic retrograde cholangiopancreaticography (ERCP). Cholangitis was mild, and cases resolved within a median of 8 d (range, 2–10 d); [2]Post-ERCP pancreatitis was diagnosed by elevation of lipase (more than twofold of the upper normal value) and the presence of abdominal pain after ERCP. These patients had mild pancreatitis, and cases resolved after a median of 4 d (range, 2–7 d).

compare ERCP in the VS group and in the conventional group.

Age, indications, and the number and difficulty of interventions were not different between the VS group and the control group. Although the median interventional examination time was 2 min shorter in the VS group (29 min *vs* 31 min), the difference was not statistically significant. Similarly, the median X-ray time and dose, as well as the premedication dose, were nearly the same in both groups. Interestingly, fewer adverse events were seen in the VS group, but this difference was also not statistically significant, perhaps because of the low number of cases (Table 2).

Subjective assessment scores by physicians and endoscopy assistants concerning "overall performance of ERCP" also did not reveal any significant differences between the VS group and the conventional ERCP group. Scores were also the same for "hygienic aspects of the ERCP" as assessed by the assistants.

Individual cost analysis of the ERCP materials and accessories used during the ERCP study revealed no significant difference in consumables used. Accessories used in the VS group amounted to 349 EUR (range, 44-673 EUR), and costs in the conventional ERCP group were 335 EUR (range, 135-604 EUR).

DISCUSSION

ERCP is a resource-intensive, complex, interventional, multi-step endoscopic-radiologic procedure for the treatment of various biliary and pancreatic diseases[1-5,19-22]. However, performance of ERCP, whether it results in therapeutic success or technical failure, harbours a known risk of side effects for the patient (*e.g.*, cholangitis, post-ERCP pancreatitis, analgo-sedation-induced complications, *etc.*), including a radiation risk, which the endoscopy team also experiences. ERCP requires the substantial use of fluoroscopy and expensive materials (balloons, guidewires, *etc.*), and technical success is often accompanied by substantial time and physical efforts on the part of the endoscopist and his/her team of assistants. Thus, further innovations are currently being studied to make ERCP safer for patients, to reduce X-ray dose and premedication, and to simplify the technical ERCP process[2-6,9,17,21].

One possible future approach for a more convenient and perhaps safer, faster, and easier ERCP procedure may be the use of specialized fixable (short) guidewire systems as compared with the use of conventional long guidewires (4.0-4.5 m), which require longer exchange times. Several studies have been published using prototype VSs and prototype linear guidewires that demonstrated shorter accessory exchange times and a reduced need for guidewire adjustments[13,14,17,23]. However, the benefit of the use of this Olympus V-system with respect to the overall ERCP outcome, interventional examination time, and fluoroscopy requirements has not been completely evaluated in daily ERCP practice. Thus, in an effort to optimize ERCP quality and hospital costs in a high-volume ERCP centre, an investigator-driven, prospective, randomized pilot trial was performed to explore whether the use of the Olympus VS and its dedicated guidewire system significantly improves the outcome of patients undergoing ERCP or reduces intervention time, fluoroscopy, or the endoscopists' and assistants' work, handling, and efforts.

This prospective, single-centre study did not, how-

ever, reveal any statistically significant differences between ERCP using the V-system and conventionally performed ERCP in terms of interventional examination time, fluoroscopy, analgo-sedation requirements, or subjective assessments obtained from the endoscopists and the endoscopy assistants. Interestingly, interventional examination time was somewhat shorter in the VS group (29 min) than in the control group (31 min), despite the need to perform additional endoprosthesis insertions in the VS group (Table 2), raising the question of whether the study population was too small to observe significant differences. Alternatively, this result may merely reflect the high quality and training status of the individuals who carry out conventional ERCP at a high-volume tertiary ERCP centre (> 1000 ERCPs per year).

Joyce *et al*[17], in a previous multicentre comparative trial, also did not demonstrate any significant effect of the V-system on ERCP examination time or on fluoroscopy time, although they did demonstrate significant benefits of the V-system when particular individual ERCP working steps were analysed, such as the median exchange time of accessories or the need for guidewire repositioning. Combined with our findings, these data show that a possible improvement in one single working step in ERCP (*e.g.*, exchange of accessories) is not necessarily coupled with an improvement or reduction in the entire examination or fluoroscopy time, especially when varying degrees of case difficulties are being treated by various experienced endoscopists[5,7,11-14,17,24]. However, the real benefit of V-system-guided ERCP may become relevant when the high interindividual variation in ERCP complexity and the different experiences of endoscopists are compensated for in an appropriate follow-up study protocol in which one patient is examined by the same investigator during follow-up interventions, such as endoprosthesis replacement, stone extraction, *etc.*, using both ERCP techniques. Such a stratified study protocol that would compare both ERCP techniques performed by one endoscopist on the same patient with the same therapeutic indication promises to better demonstrate whether a real benefit exists concerning interventional examination time and possibly other ERCP parameters including quality or outcome findings when using the V-system.

This small, prospective, randomized, single-centre pilot ERCP study in routine patients showed that several other uncontrolled factors during ERCP intervention influenced the examination time and radiation requirements more than the proposed time saving that is attributed to the V-system[11-14,17-19,21,25,26]. Thus, from the experience gained during the use of the VS and its short guidewire system, it became apparent that the V-system may be helpful in individual cases with repetitive interventions and several instrument changes (*e.g.*, multiple stenting, numerous stone extractions), but these impressions have not yet been objectively proven with a corresponding interventional ERCP study.

Although previous studies dealing with the use of the V-system have focused primarily on technical aspects and time requirements of single ERCP working steps[14,15,17], this prospective pilot trial also documented all adverse events and analgo-sedation requirements in each group. Interestingly, in the VS group, the frequency of adverse events in terms of abdominal pain lasting longer than 24 h, cholangitis, and post-ERCP pancreatitis was not significantly different as compared with that of the conventional ERCP group. However, the tendency of fewer instances of post-ERCP pancreatitis in the VS group raises the question of whether the completely fixed guidewire within the pancreatic duct reduces mechanical irritation of the pancreatic tissue, which may be a cause of an inflammatory response during conventional ERCP. This unexpected observation warrants further prospective studies, because post-ERCP pancreatitis is still a major adverse event following ERCP procedures[4,16,18,19].

Cost evaluation of the consumables used in each case revealed that both ERCP techniques have nearly the same cost to the hospital according to Germany University prices. This analysis does not favour the exclusive use of only one ERCP technique.

In addition, evaluation of the subjective assessment scores from endoscopists and the endoscopy assistants also demonstrated no advantage of the V-system as compared with the conventional ERCP technique. The overall performance of ERCP and the hygienic aspects of ERCP were similar in the VS group and in the conventional group, although working with shorter guidewires may be more convenient for the personnel than longer guidewires[12-15,17]. However, as discussed above, these results may be related to the high training status of the personnel at our high-volume ERCP centre and to the fact that performing a safe and effective bougienage within the biliary system or pancreas requires the use of the long linear guidewire (4.2 m), which may have influenced the judgement of the personnel. Subjective assessment may vary according to the training status of the endoscopy team, which would preclude the translation of these results to low-volume endoscopy hospitals.

In conclusion, this prospective ERCP trial using the VS and V-system did not show a significant advantage of this dedicated short guidewire system at a high-volume ERCP centre as compared with the conventional ERCP technique. The real value of this V-system in ERCP practice requires further investigation in follow-up interventional studies that compensate for interindividual variation in both patients and endoscopists. Studies should be performed in low-volume endoscopy centres as well.

ACKNOWLEDGMENTS

ERCP materials were provided by Olympus, Hamburg, Germany.

COMMENTS

Background

Endoscopic retrograde cholangiopancreaticography (ERCP) is a complex and cost-intensive diagnostic and therapeutic approach for the identification and

treatment of hepatobiliary and pancreatic disorders. To simplify this method, many new endoscopic techniques and instruments are currently being developed. In this pilot study, authors compared the new Olympus V-Scope (VS) system with the normal Olympus duodenoscope in patients admitted for interventional ERCP.

Research frontiers

The new VS system with its specialized fixable guidewire system (Olympus TJF160 VR) was evaluated and compared to the conventional ERCP technique (Olympus TJF160) with respect to the interventional examination time, X-ray time and dose, premedication dose, frequency of adverse events, handling in daily routine, and cost effectiveness.

Innovations and breakthroughs

Although detailed parameters of the ERCP technique and the outcomes were carefully assessed, no statistically significant differences were found between ERCPs performed with the VS and V-system and those performed using the conventional ERCP technique. Objective parameters such as interventional examination time and X-ray dose, outcome parameters such as adverse events, and subjective assessment scores by the endoscopy personnel were all similar in both ERCP technique groups. However, the study group was small, various levels of ERCP difficulty were included, and the results may have been influenced by the high-level training status of the personnel at our high-volume ERCP centre.

Applications

Regarding the fairly small number of patients, this study was designed as a pilot study to provide preliminary results concerning future study parameters for sample size estimations, outcome parameters and cost assessments. In further studies with a larger number of patients, the potential benefits of the Olympus V-system may be better evaluated when including only one or two defined ERCP indications and by including endoscopists who examine the same patient during follow-up and use both ERCP techniques.

Terminology

ERCP: A diagnostic and therapeutic tool for selective radiographic illustration of the biliary tract and pancreatic ducts that enables important interventions such as endoprosthesis insertion for drainage, stone extraction, or tumour palliation *via* short or long guidewire techniques. The Olympus side-viewing VS contains a modified elevator lever with a V-groove, which allows the use of short guidewires that can be fixed. However, these potential advantages have not yet been shown to improve the technical aspects of ERCP instrumentation, patient outcomes, or adverse events.

Peer review

This pilot study carefully assessed several outcomes and technical and subjective parameters of both ERCP techniques. Perhaps because of the small number of patients, no significant differences were demonstrated between ERCP using the V-system technique and conventional ERCP. The results obtained may be used for sample size calculation for a larger, more definitive study with more homogeneous ERCP indications.

REFERENCES

1 **Mutignani M**, Tringali A, Shah SG, Perri V, Familiari P, Iacopini F, Spada C, Costamagna G. Combined endoscopic stent insertion in malignant biliary and duodenal obstruction. *Endoscopy* 2007; **39**: 440-447 [PMID: 17516351 DOI: 10.1055/s-2007-966327]

2 **Kawakami H**, Maguchi H, Mukai T, Hayashi T, Sasaki T, Isayama H, Nakai Y, Yasuda I, Irisawa A, Niido T, Okabe Y, Ryozawa S, Itoi T, Hanada K, Kikuyama M, Arisaka Y, Kikuchi S. A multicenter, prospective, randomized study of selective bile duct cannulation performed by multiple endoscopists: the BIDMEN study. *Gastrointest Endosc* 2012; **75**: 362-372, 372.e1 [PMID: 22248605 DOI: 10.1016/j.gie.2011.10.012]

3 **Raithel M**, Dormann H, Naegel A, Boxberger F, Hahn EG, Neurath MF, Maiss J. Double-balloon-enteroscopy-based endoscopic retrograde cholangiopancreatography in post-surgical patients. *World J Gastroenterol* 2011; **17**: 2302-2314 [PMID: 21633596 DOI: 10.3748/wjg.v17.i18.2302]

4 **Andriulli A**, Loperfido S, Napolitano G, Niro G, Valvano MR, Spirito F, Pilotto A, Forlano R. Incidence rates of post-ERCP complications: a systematic survey of prospective studies. *Am J Gastroenterol* 2007; **102**: 1781-1788 [PMID: 17509029 DOI: 10.1111/j.1572-0241.2007.01279.x]

5 **Shah JN**, Marson F, Weilert F, Bhat YM, Nguyen-Tang T, Shaw RE, Binmoeller KF. Single-operator, single-session EUS-guided anterograde cholangiopancreatography in failed ERCP or inaccessible papilla. *Gastrointest Endosc* 2012; **75**: 56-64 [PMID: 22018554 DOI: 10.1016/j.gie.2011.08.032]

6 **Chen CC**. The efficacy of endoscopic ultrasound for the diagnosis of common bile duct stones as compared to CT, MRCP, and ERCP. *J Chin Med Assoc* 2012; **75**: 301-302 [PMID: 22824042 DOI: 10.1016/j.jcma.2012.05.002]

7 **Shteyer E**, Wengrower D, Benuri-Silbiger I, Gozal D, Wilschanski M, Goldin E. Endoscopic retrograde cholangiopancreatography in neonatal cholestasis. *J Pediatr Gastroenterol Nutr* 2012; **55**: 142-145 [PMID: 22790901 DOI: 10.1097/MPG.0b013e318259267a]

8 Technology Assessment Status Evaluation: guidewires in gastrointestinal endoscopy. February, 1997. ASGE. American Society for Gastrointestinal Endoscopy. *Gastrointest Endosc* 1998; **47**: 579-583 [PMID: 9647403 DOI: 10.1016/S0016-5107(98)70271-8]

9 **Ross AS**, Roth J, Waxman I. Resource-intensive endoscopic procedures: do the dollars make sense? *Gastrointest Endosc* 2008; **68**: 642-646 [PMID: 18534582 DOI: 10.1016/j.gie.2008.02.056]

10 **Tabibian JH**, Asham EH, Goldstein L, Han SH, Saab S, Tong MJ, Busuttil RW, Durazo FA. Endoscopic treatment with multiple stents for post-liver-transplantation nonanastomotic biliary strictures. *Gastrointest Endosc* 2009; **69**: 1236-1243 [PMID: 19249040 DOI: 10.1016/j.gie.2008.09.057]

11 **Papachristou GI**, Baron TH, Gleeson F, Levy MJ, Topazian MD. Endoscopic retrograde cholangiopancreatography catheter and accessory exchange using a short hydrophilic guide wire: a prospective study. *Endoscopy* 2006; **38**: 1133-1136 [PMID: 17111336 DOI: 10.1055/s-2006-944739]

12 **Reddy SC**, Draganov PV. ERCP wire systems: the long and the short of it. *World J Gastroenterol* 2009; **15**: 55-60 [PMID: 19115468 DOI: 10.3748/wjg.15.55]

13 **Beilstein MC**, Ahmad NA, Kochman ML, Long WB, Shah JN, Ginsberg GG. Initial evaluation of a duodenoscope modified to allow guidewire fixation during ERCP. *Gastrointest Endosc* 2004; **60**: 284-287 [PMID: 15278065 DOI: 10.1016/S0016-5107(04)01686-4]

14 **Shah RJ**, Somogyi L, Petersen BT, Tierney WM, Adler DG, Chand B, Conway JD, Croffie JM, Disario JA, Mishkin DS, Wong Kee Song LM. Short-wire ERCP systems. *Gastrointest Endosc* 2007; **66**: 650-657 [PMID: 17655847 DOI: 10.1016/j.gie.2007.05.030]

15 **Sakai Y**, Ishihara T, Tsuyuguchi T, Tawada K, Saito M, Kurosawa J, Tamura R, Togo S, Mikata R, Tada M, Yokosuka O. New cannulation method for pancreatic duct cannulation-bile duct guidewire-indwelling method. *World J Gastrointest Endosc* 2011; **3**: 231-234 [PMID: 22110840 DOI: 10.4253/wjge.v3.i11.231]

16 **Testoni PA**, Testoni S, Giussani A. Difficult biliary cannulation during ERCP: how to facilitate biliary access and minimize the risk of post-ERCP pancreatitis. *Dig Liver Dis* 2011; **43**: 596-603 [PMID: 21377432 DOI: 10.1016/j.dld.2011.01.019]

17 **Joyce AM**, Ahmad NA, Beilstein MC, Kochman ML, Long WB, Baron T, Sherman S, Fogel E, Lehman GA, McHenry L, Watkins J, Ginsberg GG. Multicenter comparative trial of the V-scope system for therapeutic ERCP. *Endoscopy* 2006; **38**: 713-716 [PMID: 16810594 DOI: 10.1055/s-2006-925446]

18 **Vihervaara H**, Salminen P, Hurme S, Gullichsen R, Laine S, Grönroos JM. Female gender and post-ERCP pancreatitis: is the association caused by difficult cannulation? *Scand J Gastroenterol* 2011; **46**: 1498-1502 [PMID: 21936723 DOI: 10.3109/00365521.2011.619275]

19 **Testoni PA**, Giussani A, Vailati C, Testoni S, Di Leo M, Mariani A. Precut sphincterotomy, repeated cannulation and

post-ERCP pancreatitis in patients with bile duct stone disease. *Dig Liver Dis* 2011; **43**: 792-796 [PMID: 21733768 DOI: 10.1016/j.dld.2011.05.010]

20 **Kim TH**, Oh HJ, Lee JY, Sohn YW. Can a small endoscopic sphincterotomy plus a large-balloon dilation reduce the use of mechanical lithotripsy in patients with large bile duct stones? *Surg Endosc* 2011; **25**: 3330-3337 [PMID: 21533521 DOI: 10.1007/s00464-011-1720-3]

21 **Sulieman A**, Paroutoglou G, Kapsoritakis A, Kapatenakis A, Potamianos S, Vlychou M, Theodorou K. Reduction of radiation doses to patients and staff during endoscopic retrograde cholangiopancreatography. *Saudi J Gastroenterol* 2011; **17**: 23-29 [PMID: 21196649 DOI: 10.4103/1319-3767.74456]

22 **Albrecht H**, Konturek PC, Diebel H, Kraus F, Hahn EG, Raithel M. Successful interventional treatment of postoperative bile duct leakage after Billroth II resection by unusual procedure using double balloon enteroscopy. *Med Sci Monit* 2011; **17**: CS29-CS33 [PMID: 21358608]

23 **Dhir V**, Kwek BE, Bhandari S, Bapat M, Maydeo A.. EUS-guided biliary rendezvous using a short hydrophilic guidewire. *J Interv Gastroenterol* 2011; **1**: 153-159 [PMID: 22586528 DOI: 10.4161/jig.1.4.19967]

24 **López A**, Ferrer I, Villagrasa RA, Ortiz I, Maroto N, Montón C, Hinojosa J, Moreno-Osset E. A new guidewire cannulation technique in ERCP: successful deep biliary access with triple-lumen sphincterotome and guidewire controlled by the endoscopist. *Surg Endosc* 2011; **25**: 1876-1882 [PMID: 21136105 DOI: 10.1007/s00464-010-1479-y]

25 **Meining A**, Shah RJ, Slivka A, Pleskow D, Chuttani R, Stevens PD, Becker V, Chen YK. Classification of probe-based confocal laser endomicroscopy findings in pancreaticobiliary strictures. *Endoscopy* 2012; **44**: 251-257 [PMID: 22261749 DOI: 10.1055/s-0031-1291545]

26 **Halme L**, Arola J, Numminen K, Krogerus L, Mäkisalo H, Färkkilä M. Biliary dysplasia in patients with primary sclerosing cholangitis: additional value of DNA ploidity. *Liver Int* 2012; **32**: 783-789 [PMID: 22098817 DOI: 10.1111/j.1478-3231.2011.02672.x]

P- Reviewers Sherman S, Kumar A **S- Editor** Gou SX
L- Editor A **E- Editor** Yan JL

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1943

World J Gastroenterol 2013 March 28; 19(12): 1943-1952
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Factors associated with early virological response to peginterferon-α-2a/ribavirin in chronic hepatitis C

Javier García-Samaniego, Miriam Romero, Rafael Granados, Remedios Alemán, Miguel Jorge Juan, Dolores Suárez, Ramón Pérez, Gregorio Castellano, Carlos González-Portela

Javier García-Samaniego, Miriam Romero, Liver Unit, Hospital Carlos Ⅲ, Centro de Investigación Biómedica en Red de Enfermedades Hepáticas y Digestivas, 28029 Madrid, Spain
Rafael Granados, Department of Gastroenterology, Hospital Universitario de Gran Canaria Dr. Negrín, 35010 Las Palmas de Gran Canaria, Spain
Remedios Alemán, Gastroenterology Service, Hospital Universitario de Canarias, 38320 Tenerife, Spain
Miguel Jorge Juan, Department of Gastroenterology, Hospital Universitario Insular de Gran Canaria, 35016 Las Palmas de Gran Canaria, Spain
Dolores Suárez, Internal Medicine Service, Hospital Arquitecto Marcide, 15405 Ferrol , Spain
Ramón Pérez, Gastroenterology Service, Hospital Central de Asturias, 33006 Oviedo, Spain
Gregorio Castellano, Gastroenterology Service, Hospital 12 de Octubre, 28041 Madrid, Spain
Carlos González-Portela, Gastroenterology Service, Hospital Policlínico de Vigo, 36211 Vigo, Spain
Author contributions: García-Samaniego J and Romero M designed the study and contributed to the interpretation of data; all authors collected data, revised the article critically and approved the final version of the article.
Supported by Roche Farma SA, Spain
Correspondence to: Javier García-Samaniego, MD, Liver Unit, Hospital Carlos Ⅲ, Centro de Investigación Biómedica en Red de Enfermedades Hepáticas y Digestivas, C/ Sinesio Delgado 10, 28029 Madrid, Spain. javiersamaniego@telefonica.net
Telephone: +34-91-4532510 Fax: +34-91-7336614
Received: September 20, 2012 Revised: December 19, 2012
Accepted: January 5, 2013
Published online: March 28, 2013

Abstract

AIM: To evaluate the impact of sociodemographic/clinical factors on early virological response (EVR) to peginterferon/ribavirin for chronic hepatitis C (CHC) in clinical practice.

METHODS: We conducted a multicenter, cross-sectional, observational study in Hepatology Units of 91 Spanish hospitals. CHC patients treated with peginterferon α-2a plus ribavirin were included. EVR was defined as undetectable hepatitis C virus (HCV)-ribonucleic acid (RNA) or ⩾ 2 log HCV-RNA decrease after 12 wk of treatment. A bivariate analysis of sociodemographic and clinical variables associated with EVR was carried out. Independent factors associated with an EVR were analyzed using a multiple regression analysis that included the following baseline demographic and clinical variables: age (⩽ 40 years *vs* > 40 years), gender, race, educational level, marital status and family status, weight, alcohol and tobacco consumption, source of HCV infection, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, and gamma glutamyl transpeptidase (GGT) (⩽ 85 IU/mL *vs* > 85 IU/mL), serum ferritin, serum HCV-RNA concentration (< 400 000 *vs* ⩾ 400 000), genotype (1/4 *vs* 3/4), cirrhotic status and ribavirin dose (800/1000/1200 mg/d).

RESULTS: A total of 1014 patients were included in the study. Mean age of the patients was 44.3 ± 9.8 years, 70% were male, and 97% were Caucasian. The main sources of HCV infection were intravenous drug abuse (25%) and blood transfusion (23%). Seventy-eight percent were infected with HCV genotype 1/4 (68% had genotype 1) and 22% with genotypes 2/3. The HCV-RNA level was > 400 000 IU/mL in 74% of patients. The mean ALT and AST levels were 88.4 ± 69.7 IU/mL and 73.9 ± 64.4 IU/mL, respectively, and mean GGT level was 82 ± 91.6 IU/mL. The mean ferritin level was 266 ± 284.8 μg/L. Only 6.2% of patients presented with cirrhosis. All patients received 180 mg of peginterferon α-2a. The most frequently used ribavirin doses were 1000 mg/d (41%) and 1200 mg/d (41%). The planned treatment duration was 48 wk for 92% of patients with genotype 2/3 and 24 wk for 97% of those with genotype 1/4 (P < 0.001). Seven percent of patients experienced at least one reduction in ribavi-

rin or peginterferon α-2a dose, respectively. Only 2% of patients required a dose reduction of both drugs. Treatment was continued until week 12 in 99% of patients. Treatment compliance was ⩾ 80% in 98% of patients. EVR was achieved in 87% of cases (96% *vs* 83% of patients with genotype 2/3 and 1/4, respectively; $P < 0.001$). The bivariate analysis showed that patients who failed to achieve EVR were older ($P < 0.005$), had higher ALT ($P < 0.05$), AST ($P < 0.05$), GGT ($P < 0.001$) and ferritin levels ($P < 0.001$), a diagnosis of cirrhosis ($P < 0.001$), and a higher baseline viral load ($P < 0.05$) than patients reaching an EVR. Age < 40 years [odds ratios (OR): 0.543, 95%CI: 0.373-0.790, $P < 0.01$], GGT < 85 IU/mL (OR: 3.301, 95%CI: 0.192-0.471, $P < 0.001$), low ferritin levels (OR: 0.999, 95%CI: 0.998-0.999, $P < 0.01$) and genotype other than 1/4 (OR: 4.716, 95%CI: 2.010-11.063, $P < 0.001$) were identified as independent predictors for EVR in the multivariate analysis.

CONCLUSION: CHC patients treated with peginterferon-α-2a/ribavirin in clinical practice show high EVR. Older age, genotype 1/4, and high GGT were associated with lack of EVR.

Key words: Antiviral therapy; Baseline factors; Early virological response; Peginterferon α-2a; Ribavirin

García-Samaniego J, Romero M, Granados R, Alemán R, Jorge Juan M, Suárez D, Pérez R, Castellano G, González-Portela C. Factors associated with early virological response to peginterferon-α-2a/ribavirin in chronic hepatitis C. *World J Gastroenterol* 2013; 19(12): 1943-1952 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1943.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1943

INTRODUCTION

Until recent approval of the first direct acting antivirals (DAAs) against hepatitis C virus (HCV)[1-4], the combination of pegylated interferon and ribavirin was the standard of care (SOC) for chronic hepatitis C (CHC)[5-8] with the goal of achieving a sustained viral response (SVR) [undetectable hepatitis C virus ribonucleic acid (HCV-RNA) at week 24]. However, the overall SVR rate with standard peginterferon and ribavirin combination does not exceed 56%-63%[5,9,10], and is even lower in some patient subgroups[10]. Indeed, pivotal studies showed that HCV genotype 1 patients achieve a SVR rate of 41%-56%, whereas in those infected with HCV genotype 2/3, a SVR is obtained in 74%-80%[5,9-11]. Variability in virological response depends on diverse patient factors as well as virological and histological factors. Genotype other than 1, low baseline viral load, age less than 40 years, body weight ⩽ 75 kg and absence of advanced fibrosis and/or cirrhosis have been identified as predictive factors of SVR in the studies evaluating peginterferon α-2a plus ribavirin combination[9,12].

Pivotal studies have shown that early virological response (EVR) is highly predictive of SVR[9,13]. Accordingly, patients who do not achieve an EVR have an almost null probability of achieving a SVR[9,13-15]. The study conducted by Fried *et al*[9] showed that only 3% of patients who did not obtain an EVR with peginterferon α-2a plus ribavirin achieved a SVR. The high negative predictive value (PPV) of EVR has great clinical value as it allows us to decide whether to continue or discontinue treatment at week 12, therefore preventing or minimizing the adverse effects related to treatment continuation. Current treatment guidelines for hepatitis C include this decision criteria at week 12[5,7,8,16] and recommend discontinuation of treatment in patients who fail to achieve an EVR. In addition, the clinical utility of EVR has been demonstrated in the routine clinical practice setting, particularly in genotype 1-infected patients[17].

Limited data have been reported regarding the EVR predictive factors in patients receiving peginterferon α-2a plus ribavirin combination therapy. Correct identification of these factors could be a useful strategy to optimize treatment in CHC and improve SVR rates, particularly in patients with genotype 1. On the other hand, treatment adherence is key to achieve successful treatment. The occurrence of adverse effects associated with peginterferon and/or ribavirin is the main reason for dose reduction or treatment discontinuation. As a result, 15%-20% of patients participating in clinical trials and approximately 25% of those in routine clinical practice discontinue treatment[18]. Lack of adherence during the first 12 wk of treatment has been shown to have a particularly negative impact on EVR[13,19]. Thus, the PPV of EVR associated with good treatment compliance is very high, achieving a SVR in 75% of cases with EVR and good treatment adherence[9].

Given that sociodemographic and clinical factors associated with EVR are not well known and considering that treatment adherence is a variable closely related to EVR, characterization of viral and patient factors associated with treatment compliance, and hence EVR, has important clinical implications. The present study was designed to analyze baseline sociodemographic and clinical characteristics associated with EVR and antiviral treatment compliance in CHC patients treated with peginterferon α-2a in the routine clinical practice setting in Spain.

MATERIALS AND METHODS

Study design

This was a national, multicenter, cross-sectional, observational study. The study was carried out in the Hepatology Units of 91 Spanish hospitals. All participating patients gave their written informed consent, and the study was approved by the Clinical Research Ethics Committee of the Hospital Carlos Ⅲ of Madrid. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and their amendments.

The study included adult patients (over 18 years of age) diagnosed with CHC and treated with peginterferon α-2a plus ribavirin under routine clinical practice conditions. Patients with any contraindication to hepatitis C treatment according to the prescribing information and those with HBV and/or human immunodeficiency virus coinfection were excluded.

The main purpose of the study was to analyze EVR in relation to sociodemographic and clinical characteristics. For this purpose, the following variables were recorded at the start of treatment: age, sex, race, nationality, educational level, marital status, occupation and family status, weight, cigarette smoking, alcohol consumption, source of HCV infection, methadone replacement therapy, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT) and ferritin levels, cirrhotic status, viremia and HCV genotype. In addition, ribavirin dose and scheduled duration of treatment, dose reductions of peginterferon α-2a and/or ribavirin up to week 12, data on premature treatment discontinuation (week and reason for discontinuation) were also collected. Furthermore, data on HCV-RNA concentrations at week 12 were recorded and EVR was analyzed (complete/partial). HCV-RNA levels were measured using quantitative polymerase chain reaction assays, mostly the Amplicor HCV Monitor (Roche, Kenilworth NJ, United States), although other commercial tests were used in some centers. A lower limit of detection of 50 IU/mL was considered in all participating hospitals. The secondary objective of the study was to evaluate treatment adherence at week 12.

Virological response criteria

EVR was defined as undetectable levels of HCV-RNA at week 12 (complete EVR) or ⩾ 2 log reduction in HCV viral load from baseline (partial EVR).

Treatment adherence criteria

To evaluate treatment adherence, compliance was recorded according to the 80/80/80 rule[20] and modified according to the study design. Treatment compliant or adherent patients were those receiving 80% or more of the total dose of peginterferon α-2a plus ribavirin during 80% of the time until week 12. Likewise, noncompliant patients included those who received < 80% of the prescribed dose of one or both drugs during < 80% of the expected duration (12 wk).

Statistical analysis

A descriptive statistical analysis was performed on the sociodemographic and clinical variables collected from the medical records at the start of treatment with peginterferon α-2a plus ribavirin. Quantitative variables were described using measures of central tendency and dispersion (mean, median, SD, minimum, maximum, first quartile and third quartile) and the results are expressed as mean ± SD or median (range). Qualitative variables are presented as absolute and relative frequencies. To characterize the population based on patient sex and the influence of sociodemographic and clinical characteristic on EVR, a bivariate analysis was carried out using Student's *t* test for quantitative variables and the chi-square test for the remaining sociodemographic and clinical qualitative independent variables. Similarly, a bivariate analysis of sociodemographic and clinical variables associated with EVR was carried out based on patient race. Variables with statistical significance or with $P < 0.10$ in the bivariate model were analyzed in a multivariate logistic regression model. Some factors that were not statistically significant were retained in the model based on previous clinical evidence. Odds ratios (OR) and 95%CI were calculated for the independent predictive factors of EVR. In the multivariate logistic regression analysis, only patients with available data for all the variables taken into account for the analysis were included. Significance level was set at $P < 0.05$. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL, United States).

RESULTS

Baseline patient characteristics

Baseline patient characteristics are shown in Table 1. A total of 1202 patients were included in the study. Thirty-six were excluded as they met at least one of the following criteria: peginterferon dose other than 180 mg (30 patients), negative HCV-RNA at baseline visit (1 patient), HCV-RNA level not available at week 12 when withdrawal had not occurred (8 patients). The number of evaluable patients was 1166, of which 1014 were analyzed and 152 were excluded for having a detectable viral load at week 12 without specifying the value and/or detection level.

Sociodemographic characteristics

Seven hundred and twelve (70%) patients were male. The vast majority of patients were Spanish (91%) and Caucasian (97%). Fifty percent of patients had completed compulsory education. Of the total number of patients, 580 (57%) were married and 898 (89%) patients lived with another person (Table 1).

The mean age was 43.3 ± 9.0 years in men and 46.6 ± 11.4 years in women ($P < 0.001$). No differences were found in race, educational level or family status based on gender. No significant differences were found in baseline sociodemographic characteristics based on race. The only differences noted were a greater proportion of women over 40 who were Caucasian ($P < 0.005$) and a higher educational level among Caucasian patients ($P < 0.01$).

Clinical characteristics

Alcohol consumption was reported in 154 (15%) patients and 514 (51%) were smokers. The most common source of HCV infection was intravenous drug use (25%) followed by transfusion (23%). Mean ALT and AST levels

Table 1 Patient baseline characteristics *n* (%)

Characteristics	
Patient sociodemographics	
Sex	
Male	712 (70)
Female	302 (30)
Age (yr), mean ± SD	44.3 ± 9.8
Nationality	
Spanish	919 (91)
Other	93 (9)
Race	
Caucasian	980 (97)
Other	27 (3)
Educational level	
Did not complete compulsory education	125 (12)
Compulsory education	506 (50)
Professional training	243 (24)
University	131 (13)
Postgraduate/Master/PhD	7 (1)
Marital status	
Single	280 (28)
Married	580 (58)
Separated	138 (14)
Widowed	11 (1)
Family status	
Lives with another person	898 (89)
Lives alone	97 (10)
Prison inmate	16 (2)
Clinical characteristics	
Weight (kg), mean ± SD	75.7 ± 13.4
Alcohol consumption	154 (15)
Tobacco consumption	514 (51)
Source of HCV infection[1]	
IVDU	256 (25)
Transfusion	234 (23)
Other	55 (6)
Unknown	462 (46)
Methadone replacement therapy	65 (7)
ALT (IU/mL), mean ± SD	88.4 ± 69.7
AST (IU/mL), mean ± SD	73.9 ± 64.4
GGT (IU/mL), mean ± SD	82.0 ± 91.6
Ferritin (μg/L), mean ± SD	266.0 ± 284.8
Cirrhosis	62 (6.2)
HCV genotype	
1/4	784 (78)
2/3	223 (22)
HCV-RNA	
< 400 000 IU/mL	264 (26)
⩾ 400 000 IU/mL	744 (73)

[1]One patient could have more than one presumed source of infection. Data are presented as number (percentage) of patients unless otherwise indicated. Proportions of patients are presented as valid percentages. Percentages may not add up to 100 due to rounding error. Values for age, weight and serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and ferritin are expressed as mean ± SD. IVDUs: Intravenous drug users; HCV RNA: Hepatitis C virus ribonucleic acid.

were 88.4 ± 69.7 and 73.9 ± 64.4 IU/mL, respectively, and the mean GGT level was 82 ± 91.6 IU/mL. The mean ferritin level was 266 ± 284.8 μg/L. Only 62 (6.2%) patients presented with cirrhosis.

Of the total number of patients, 784 (78%) had genotype 1/4, of which 694 (68%) had genotype 1. The HCV-RNA level was greater than 400 000 IU/mL in 744 (73.8%) patients (Table 1). The proportion of patients with tobacco and alcohol consumption habit was greater in men ($P < 0.001$). The diagnosis of cirrhosis was also more common in men ($P < 0.05$). Intravenous drug abuse as the source of HCV infection was more frequent in men and transfusion was more frequent in women ($P < 0.001$). Among the clinical variables analyzed, there were no differences in baseline viral load level or HCV genotype according to gender, but men had higher ALT, AST and GGT values ($P < 0.001$) and higher levels of ferritin ($P < 0.001$). With regard to ribavirin dose, 800 mg/d and 1000 mg/d were the most common doses given to women, whereas men received the 1200 mg/d dose more frequently ($P < 0.001$).

Table 2 Treatment *n* (%)

Treatment data	
Peginterferon α-2a	
Dose: 180 mg	1014 (100)
At least one dose reduction of peginterferon	66 (7)[1]
At least one discontinuation of peginterferon	46 (81)
Ribavirin	
Dose	
800 mg/d	183 (18)
1000 mg/d	413 (41)
1200 mg/d	416 (41)
1400 mg/d	1 (0.1)
At least one dose reduction of ribavirin	66 (7)[1]
At least one discontinuation of ribavirin	56 (89)
Scheduled treatment duration	
24 wk	234 (23)
48 wk	773 (77)

[1]The percentage of patients with dose reduction of peginterferon α-2a and the frequency of patients with dose reduction of ribavirin coincide, but are not exactly the same patients. The frequencies presented correspond to valid percentages. Percentages may not add up to 100 due to rounding error.

Peginterferon α-2a plus ribavirin treatment

All patients received 180 μg of peginterferon α-2a. The most frequently used ribavirin doses were 1000 mg/d (41%) and 1200 mg/d (41%) (Table 2). In accordance with current treatment recommendations, the 1000 mg/d and 1200 mg/d doses of ribavirin were used more often for patients with genotype 1/4 (49% and 45% of patients received doses of 1200 and 1000 mg/d, respectively), whereas more than half of those with genotype 2/3 (58%) received 800 mg/d of ribavirin ($P < 0.001$).

In more than three-quarters of patients, the scheduled duration of treatment was 48 wk (Table 2). For 97% of patients with genotype 1/4, the planned treatment duration was 48 wk and in 92% of those with genotype 2/3 the scheduled duration was 24 wk ($P < 0.001$).

Treatment discontinuation and dose reduction

Sixty-six (7%) patients had their peginterferon α-2a dose reduced on at least one occasion. Of these, 46 (81%) patients had only one dose reduction. Similarly, 66 (7%) patients experienced at least one dose reduction of ribavirin. Of these, 56 (89%) patients had at least one dose

Table 3 Sociodemographics, clinical and pathological characteristics of patients with early virological response and non-early virological response by bivariate analysis *n* (%)

Factors	EVR	Non-EVR	*P* value[1]
Patient sociodemographics			
Age (yr), mean ± SD	43.9 ± 9.7	47.0 ± 10.0	< 0.005
Sex			
Male	602 (86)	101 (14)	NS
Female	264 (89)	33 (11)	
Origin			
Developed country	64 (93)	5 (7)	NS
Developing country	801 (86)	129 (14)	
Race			
Caucasian	836 (87)	130 (14)	NS
Other	24 (89)	3 (11)	
Educational level			
Equivalent to or less than high	537 (86)	85 (14)	NS
Professional training	209 (87)	31 (13)	
University or higher	119 (88)	17 (13)	
Marital status			
Single	235 (85)	41 (15)	NS
Married	491 (86)	81 (14)	
Separated/divorced/widowed	135 (92)	12 (8)	
Family status			
Lives alone	82 (85)	15 (16)	NS
Lives with another person	769 (87)	117 (13)	
Clinical characteristics			
Weight (kg), mean ± SD	75.6 ± 13.4	77.2 ± 13.5	NS
Alcohol consumption			
No	731 (86)	115 (14)	NS
Yes	133 (88)	19 (13)	
Tobacco consumption			
No	414 (85)	75 (15)	NS
Yes	450 (88)	59 (12)	
Source of HCV infection			
Injection drug use	221 (90)	26 (11)	NS
Transfusion	126 (89)	16 (11)	
IV route	79 (87)	12 (13)	
Other	435 (85)	78 (15)	
Methadone replacement therapy			
No	803 (86)	126 (14)	NS
Yes	57 (89)	7 (11)	
ALT (IU/mL), mean ± SD	86.3 ± 69.4	101.7 ± 71.5	< 0.05
AST (IU/mL), mean ± SD	72.1 ± 65.3	84.8 ± 58.5	< 0.05
GGT (IU/mL), mean ± SD	73.6 ± 85.2	134.0 ± 114.4	< 0.001
Ferritin (μg/L), mean ± SD	248.0 ± 268.8	388.8 ± 357.3	< 0.001
Cirrhosis			
No	817 (88)	112 (12)	< 0.001
Yes	41 (67)	20 (33)	
HCV genotype			
1/4	645 (84)	126 (16)	< 0.001
2/3	214 (96)	8 (4)	
HCV-RNA (IU/mL), mean ± SD	3 354 135.6 ± 5 978 359.9	3 781 940.0 ± 4 780 000.6	NS
Baseline viral load			
< 400 000 IU/mL	237 (91)	25 (10)	< 0.05
⩾ 400 000 IU/mL	623 (85)	109 (15)	

Data are presented as number (percentage) of patients unless otherwise indicated. Proportions of patients are presented as valid percentages. Values for age, weight, and alanine aminotransferase/aspartate aminotransferase (ALT/AST), gamma glutamyl transferase (GGT) and ferritin are expressed as mean ± SD. [1]*P* value of bivariate analysis. EVR: early virological response; HCV-RNA: Hepatitis C virus ribonucleic acid; IV route: Intravenous route; NS: Not significant.

reduction before week 12. Only 15 (2%) patients required a dose reduction of both drugs (Table 2).

Early virological response

Of 1014 patients included in the analysis, 866 (87%) achieved an EVR. Of these patients, 699 (70%) had a complete EVR and 176 (18%) achieved a partial EVR. The results showed significant differences in EVR depending on genotype, and the percentage of patients with EVR at week 12 was higher in the group of patients with genotype 2/3 (96% *vs* 83% of patients with genotype 2/3 and genotype 1/4, respectively; $P < 0.001$).

Predictive factors of early virological response

Table 3 shows the sociodemographic and clinical characteristics of the early responders (EVR) and non-responders (non-EVR). According to the results obtained from the bivariate analysis, the only sociodemographic variable associated with EVR was age ($P < 0.005$). The clinical variables associated with EVR were ALT ($P < 0.005$), AST ($P < 0.05$) and GGT values ($P < 0.001$), ferritin levels ($P < 0.001$), presence of cirrhosis ($P < 0.001$), viral genotype ($P < 0.001$), and baseline viral load ($P < 0.05$).

In the multivariate analysis, the only sociodemographic factor identified as a predictor of EVR was age (OR: 0.543, 95%CI: 0.373-0.790, $P < 0.01$) and the clinical factors predictive of EVR were GGT level (OR: 3.301, 95%CI: 0.192-0.471, $P < 0.001$), ferritin level (OR: 0.999, 95%CI: 0.998-0.999, $P < 0.01$) and genotype (OR: 4.716, 95%CI: 2.010-11.063, $P < 0.001$). Age ⩽ 40 years, GGT level ⩽ 85 IU/mL, low ferritin levels and HCV genotype other than 1/4 were independent predictors of EVR (Figure 1).

Adherence to treatment and predictive factors associated with compliance

Treatment compliance was greater than 80% in 971 (97.8%) patients. No significant differences were found between patients with treatment adherence greater than 80% and those whose compliance was less than 80% with regard to their sociodemographic and clinical characteristics (data not shown).

DISCUSSION

The response to treatment with peginterferon plus ribavirin is heterogeneous and non-optimal in several HCV patients, as occurs in those infected with genotype 1, high viral load, advanced fibrosis, metabolic syndrome or non-CC polymorphisms of the interleukin 28b gene (*IL28b*)[12,13,15,21,22].

EVR is highly predictive of SVR[9,23] and provides hepatologists with a valuable tool to decide on continuation and duration of treatment, as well as providing patients with an additional motivation to adhere to treatment. The predictive value of EVR in patients infected with genotype 1 in the clinical practice setting in Spain[17] was previously shown to be comparable to that obtained in pivotal trials[9,23]. However, although identification of both viral and host factors associated with EVR may be very useful to predict SVR and therefore guide thera-

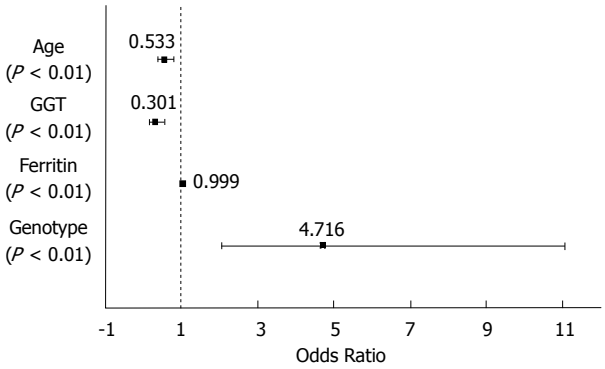


Figure 1 Independent factors associated with an early virologic response according to multiple logistic regression analysis. Baseline demographic factors used in the multiple regression analysis included age (≤ 40 years *vs* > 40 years), gender, race, educational level, marital status and family status. Clinical baseline factors included in the multiple regression analysis were weight, alcohol and tobacco consumption, source of hepatitis C virus (HCV) infection, alanine aminotransferase, aspartate aminotransferase and gamma glutamyl transpeptidase (GGT) levels (≤ 85 *vs* > 85), serum ferritin, serum HCV-RNA concentration (< 400 000 *vs* ≥ 400 000), genotype (1/4 *vs* 3/4), cirrhotic status and ribavirin dose (800/1000/1200 mg/d). An odds ratio equal to 1 (dashed lines) indicates no difference between the subgroups defined according to the given factors. Bars indicate 95%CI. The results demonstrated that age ≤ 40 years ($P < 0.01$), low levels of GGT (≤ 85 IU/L) ($P < 0.001$), low ferritin ($P < 0.01$) and genotype 1/4 ($P < 0.001$) were independent predictors of early virological response.

py[13], to date, there are limited data available addressing this issue.

To our knowledge, the present study is the largest series from a clinical setting to analyze factors associated with EVR in CHC patients treated with peginterferon plus ribavirin under routine clinical practice conditions. The data analysis showed that 87% of patients achieved EVR. This response rate is comparable or even higher than that reported in international pivotal trials with peginterferon α-2a plus ribavirin[9,23] and confirm the results reported in previous studies conducted in Spain, also under routine clinical practice conditions, but with a considerably lower number of patients[17,24]. A complete EVR was observed in 70% of our patients and again this rate was higher than that reported in other previous studies, which ranged from 34% to 64%[9,10,12,25,26].

As expected, the percentage of patients with EVR was higher in the group of patients with genotype 2/3 than in those with genotype 1/4 according to previous studies[9,10,27-29]. However, despite including a high percentage of patients with genotype 1 (the most difficult to cure), our study achieved response rates as high as those in the pivotal trials.

Patients who failed to achieve EVR were older, had higher ALT, AST, GGT and ferritin levels, a more frequent diagnosis of cirrhosis and a higher baseline viral load than patients achieving EVR. Most of the limited available studies on factors associated with EVR have shown that low baseline viral load, younger age, absence of overweight/obesity and lack of cirrhosis are independent factors associated with EVR[30-33]. In our study, age < 40 years, GGT levels < 85 IU/mL, low ferritin levels and genotype non-1/4 were identified as independent predictive factors of EVR in the multivariate analysis.

Interestingly, a recent study has identified high GGT as a predictor of nonresponse to treatment with peginterferon in patients infected with genotype 1[34]. The precise mechanism whereby increased GGT levels may affect treatment response in CHC remains a matter of debate, although it may be related either to a more intense degree of necroinflammatory activity, more advanced fibrosis, or to hepatic steatosis[35,36]. In this regard, a positive correlation between serum GGT levels and the hepatic expression of proinflammatory tumor necrosis factor-α (TNF-α) mRNA in CHC has been suggested[37]. Indeed, hepatic levels of TNF-α mRNA have been found to be significantly higher in nonresponders to IFN-α-based therapy than in those with a SVR. On the other hand, although the multivariate analysis identified high levels of ferritin as an independent predictor of EVR, this association was clearly irrelevant (OR = 0.999). Therefore, ferritin level may lack clinical validity as a predictive factor of EVR in this setting.

Age was found to be an independent factor for EVR in our study, in agreement with previous series where it was shown that age greater than 40 years is an independent predictor of nonresponse to treatment[12,29,34]. Furthermore, older patients have been suggested to be more resistant to peginterferon-based therapies since they are more frequently infected by HCV genotype 1b, have a longer disease duration and exhibit greater liver damage than younger patients.

Consistent with most published studies[10,12,17,38], our findings show that the proportion of women with CHC treated with peginterferon plus ribavirin in routine practice in Spain is much lower in comparison to the proportion of men, in the absence of demographic variables justifying this difference, or known barriers of access to treatment for women. In addition, women are treated at an older age than men. We can speculate that the lower fibrosis severity as well as the higher rate of normal ALT levels in women with CHC can explain the relatively low proportion of female patients treated in our series. Although in some studies women have shown higher response rates than men[31,38], our data did not show differences in EVR based on gender despite the high proportion of male patients. In light of these results, new epidemiological studies on the prevalence of HCV infection, as well as clinical studies including a larger number of women are needed to determine if there is gender inequality in the prescription of antiviral therapy.

Baseline HCV viral load is a well-known independent predictive factor of treatment response[12,29-31,33]. In agreement with previous evidence, the patients in our study who achieved EVR were those who had lower baseline viral loads (< 400 000 IU/mL), although viral load did not constitute a predictive factor for EVR in the multiple logistic regression analysis.

Race has been identified as a predictive factor of EVR in previous studies[12]. The data from our study, despite its large sample size, does not allow us to conclude

that race plays a role in EVR because the vast majority of patients included in the study were Caucasian (97.3%). Furthermore, when the study was designed, genetic analysis of *IL28b* gene polymorphisms, which are related to race[21], was not available. Indeed, one limitation of our study is the lack of information on IL-28B genotype as it has been described as a relevant predictor of treatment response[39,40]. However, the impact of the imbalance of this genetic polymorphism between groups on treatment response was described just after the data from this study were collected.

Our study also revealed that Spanish CHC patients treated in routine clinical practice receive the correct doses of each drug according to current treatment guidelines, and this is critical since both dose optimization and treatment duration are essential to maximize response[17]. The treatment regimen is individualized, according to current guideline recommendations, based on the viral genotype, so that patients with genotype 1/4 are treated with ribavirin in weight-adjusted doses of 1000-1200 mg/d for 48 wk, whereas patients carrying genotypes 2/3 are treated in most cases with ribavirin at a dose of 800 mg/d for 24 wk.

Combination therapy frequently causes adverse effects. Most are mild and controlled by reducing the dose of each drug or with additional treatments including growth factors, but in some cases they require discontinuation of treatment. Various studies have shown that dose reductions and particularly treatment discontinuation are associated with a marked reduction in the EVR rate[20]. Other reports have noted that dose reductions of peginterferon and/or ribavirin are quite frequent during the first 12 wk of treatment[13]. However, in the current analysis, only 7% of patients required dose reduction of peginterferon or ribavirin. Moreover, it should be noted that only 7 patients of the total population analyzed discontinued treatment due to serious adverse effects in the first 12 wk. This percentage (less than 1%) is markedly lower than that reported in other studies[11,41].

Treatment compliance is a variable closely related to EVR[13,19,20]. It has been shown that lack of adherence during the first 12 wk of treatment has a negative impact on EVR. Previous studies have demonstrated that patients who met the 80/80/80 rule have a greater response than those receiving lower doses or for less time[20]. In our series, 98% of patients met the 80/80/80 rule up to week 12. Good treatment compliance is one of the factors explaining the high rate of EVR seen in our study. Treatment modifications and the motivation of patients may have had a significant impact on adherence to treatment. The low dose reductions required for both drugs could have encouraged patients to complete the prescribed course of treatment. Additionally, determination of EVR provides patients and physicians with an early goal and motivates them to adhere to treatment recommendations. Moreover, it is noteworthy that patients were treated by hepatologists belonging to units with wide experience in the care of CHC patients.

The main limitations of this study arise from the occurrence of the major advance in CHC in the last years, the development of DAAs and the recent approval of the triple therapy as the new SOC for CHC. Nevertheless, despite the obvious change in treatment paradigm, therapy based on peginterferon plus ribavirin will continue to play an important role as SOC, especially for non-1 HCV patients, considering that protease inhibitors must be combined with peginterferon plus ribavirin in genotype 1 patients. In addition, while remarkably effective, the recently approved protease inhibitors are also accompanied by frequent serious toxicities and considerable costs. Therefore, some patients who cannot tolerate protease inhibitors will need to be treated with dual combination given that triple combination regimens have a higher side effect burden[3,42,43]. Additionally, the high cost of the DAAs will probably preclude the use of triple-combination therapies in health care systems constrained by rising costs and economically disadvantaged regions. It is well known that patients with rapid virological response to dual combination achieve SVR in higher rates, close to 90%, and therefore, despite the above limitations, our findings may be relevant and applicable at the onset of the DAAs era. Furthermore, our findings provide a meaningful assessment of factors associated with EVR regarding applicability to guide therapy of real-world patients given that our study population comprises a larger, more representative cohort of patients than those included in clinical trials evaluating predictive factors of EVR. However, further studies will be needed to validate whether the predictive factors of EVR to dual therapy remain a predictive tool in the context of new DAA agents in the routine clinical practice setting.

In summary, CHC patients treated with peginterferon α-2a plus ribavirin in routine clinical practice in Spain have high EVR rates, similar to those obtained in pivotal studies, and a high level of treatment compliance. Age > 40 years, genotype 1/4 and GGT ⩾ 85 IU/mL were independent predictive factors of lack of EVR. In the new era of hepatitis C treatment where standard treatment is incorporated with DAAs, identification of predictive factors such as the new definitions of extended rapid viral response will be an essential tool to achieve maximum response rates.

ACKNOWLEDGMENTS

Editorial and medical writing assistance was provided by Cristina Vidal and Antonio Torres from Dynamic Science. The authors would like to acknowledge the investigators and collaborators of MONOTRANS Study Group: Pérez R, Hospital Central de Asturias, Oviedo, Spain; Muñoz R, Hospital 12 de Octubre, Madrid, Spain; Manzano M, Hospital 12 de Octubre, Madrid, Spain; Fernández I, Hospital 12 de Octubre, Madrid, Spain; Domínguez F, Clínica Santa Cristina, Vigo, Spain; Fajardo JM, Hospital Infanta Elena, Huelva, Spain; Pastor E, Hospital Arquitecto Marcide, Ferrol, Spain; Suárez I, Hospital Hospital Infanta Elena, Huelva, Spain; Morano LE, Hospital Meixoeiro, Vigo, Spain; Espinosa MD,

Hospital Virgen de las Nieves, Granada, Spain; Sansó A, Hospital de Manacor, Mallorca, Spain; Montoliú S, Hospital Joan XXIII, Tarragona, Spain; Pardo A, Hospital Joan XXIII, Tarragona, Spain; Sánchez J, Hospital Río Carrión, Palencia, Spain; Amine S, Hospital General de Lanzarote, Las Palmas de Gran Canaria, Spain; Alonso MM, Hospital Universitario de Canarias, Tenerife, Spain; Eraña L, Hospital Santiago Apóstol, Vitoria, Spain; Guerrero J, Hospital Cruz Roja del INGESA, Ceuta, Spain; García E, Hospital General de Fuerteventura, Las Palmas de Gran Canaria, Spain; Sánchez JJ, Virgen de la Salud, Toledo, Spain; Calvo S, Hospital Comarcal de El Escorial, Madrid, Spain; Vázquez E, Complejo Hospitalario Pontevedra, Pontevedra, Spain; Moreno D, Hospital de Móstoles, Madrid, Spain; González R, Hospital de Móstoles, Madrid, Spain; Morán S, Hospital Santa María del Rosell, Cartagena, Spain; Torrente V, Hospital Sant Joan de Reus, Tarragona, Spain; Jiménez E, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain; Lee C, Hospital Dr. Negrín, Las Palmas de Gran Canaria, Spain; Hervias D, Hospital Virgen de Altagracia-Manzanares, Ciudad Real, Madrid, Spain; Rincón JP, Santa María del Rosell, Cartagena, Spain; Gimenez A, Hospital General de Granollers, Barcelona, Spain; Rodriguez R, Complejo Hospitalario Santa María Madre, Orense, Spain; Moreno M, Hospital General de Fuerteventura, Las Palmas de Gran Canaria, Spain; Casanova C, Hospital Universitario de Canarias, Tenerife, Spain; Sánchez H, Hospital La Inmaculada, Almeria, Spain; Juan Carlos Penalva, Hospital de Torrevieja, Alicante, Spain; Compañy L, Hospital General de Alicante, Alicante, Spain; Sáez J, Hospital de Elche, Alicante, Spain.

COMMENTS

Background

Peginterferon plus ribavirin is the standard therapy for chronic hepatitis C (CHC) world-wide. Early virological response (EVR) predicts sustained virological response (SVR) in CHC. Although identification of both viral and host factors associated with EVR may be very useful to predict SVR and therefore guide therapy, to date, there are limited data available addressing this issue in clinical practice.

Research frontiers

The high negative predictive value of EVR has remarkable value in clinical practice since it allows to decide whether to continue or discontinue treatment at week 12, therefore preventing or minimizing the adverse effects related to treatment continuation. Indeed, current treatment guidelines for hepatitis CHC include this decision criterion and recommend discontinuing treatment in patients who fail to achieve an EVR.

Innovations and breakthroughs

The present study is the largest series from a clinical setting to analyze predictive factors of EVR in CHC patients treated with peginterferon α-2a plus ribavirin in routine clinical practice. The remarkably high EVR rates obtained in our study were similar to those reported with this dual therapy in pivotal studies, and confirm the data from previous studies in clinical practice in Spain which included a significantly lower number of patients. Age > 40 years, genotype 1/4 and gamma glutamyl transpeptidase (GGT) $\geq$ 85 IU/mL were identified as independent predictive factors of lack of EVR.

Applications

The results from this study may be useful in guiding treatment decision making. In particular, early prediction of virological response to dual therapy can help to identify candidates who are unlikely to have a SVR before treatment initiation or in the early treatment phase.

Terminology

EVR indicates EVR which is defined as undetectable hepatitis C virus ribonucleic acid (HCV-RNA) or $\geq$ 2 log decrease in HCV-RNA after 12 wk of treatment.

Peer review

This study is well constructed and it is based in a big CHC patient cohort coming from 91 hospitals through all Spanish territory. This is an interesting and relevant study which confirms the results of previous studies with a notably lower number of Spanish cases. Moreover, this study consolidates some basal factors such a GGT levels, age or viral genotype as predictive factors of EVR. This study also highlights the higher rate of ERV in Spain compared with other regions.

REFERENCES

1 **Bacon BR**, Gordon SC, Lawitz E, Marcellin P, Vierling JM, Zeuzem S, Poordad F, Goodman ZD, Sings HL, Boparai N, Burroughs M, Brass CA, Albrecht JK, Esteban R. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1207-1217 [PMID: 21449784 DOI: 10.1056/NEJMoa1009482]

2 **Jacobson IM**, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR, Bzowej NH, Marcellin P, Muir AJ, Ferenci P, Flisiak R, George J, Rizzetto M, Shouval D, Sola R, Terg RA, Yoshida EM, Adda N, Bengtsson L, Sankoh AJ, Kieffer TL, George S, Kauffman RS, Zeuzem S. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011; **364**: 2405-2416 [PMID: 21696307 DOI: 10.1056/NEJMoa1012912]

3 **Kwo PY**, Lawitz EJ, McCone J, Schiff ER, Vierling JM, Pound D, Davis MN, Galati JS, Gordon SC, Ravendhran N, Rossaro L, Anderson FH, Jacobson IM, Rubin R, Koury K, Pedicone LD, Brass CA, Chaudhri E, Albrecht JK. Efficacy of boceprevir, an NS3 protease inhibitor, in combination with peginterferon alfa-2b and ribavirin in treatment-naive patients with genotype 1 hepatitis C infection (SPRINT-1): an open-label, randomised, multicentre phase 2 trial. *Lancet* 2010; **376**: 705-716 [PMID: 20692693 DOI: 10.1016/S0140-673610)60934-8]

4 **Zeuzem S**, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Müllhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011; **364**: 2417-2428 [PMID: 21696308 DOI: 10.1056/NEJMoa1013086]

5 **National Institutes of Health**. National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002--June 10-12, 2002. *Hepatology* 2002; **36**: S3-S20 [PMID: 12407572 DOI: 10.1053/jhep.2002.37117]

6 **Cammà C**, Licata A, Cabibbo G, Latteri F, Craxì A. Treatment of hepatitis C: critical appraisal of the evidence. *Expert Opin Pharmacother* 2005; **6**: 399-408 [PMID: 15794731 DOI: 10.1517/14656566.6.3.399]

7 **Dienstag JL**, McHutchison JG. American Gastroenterological Association medical position statement on the management of hepatitis C. *Gastroenterology* 2006; **130**: 225-230 [PMID: 16401485 DOI: 10.1053/j.gastro.2005.11.011]

8 **Strader DB**, Wright T, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **39**: 1147-1171 [PMID: 15057920 DOI: 10.1002/hep.20119.]

9 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982 [PMID: 12324553 DOI: 10.1056/NEJMoa020047]

10 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M,

Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676]

11 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749]

12 **Rodriguez-Torres M**, Sulkowski MS, Chung RT, Hamzeh FM, Jensen DM. Factors associated with rapid and early virologic response to peginterferon alfa-2a/ribavirin treatment in HCV genotype 1 patients representative of the general chronic hepatitis C population. *J Viral Hepat* 2010; **17**: 139-147 [PMID: 19674282 DOI: 10.1111/j.1365-2893.2009.01157]

13 **Davis GL**, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 645-652 [PMID: 12939591 DOI: 10.1053/jhep.2003.50364]

14 **Husa P**, Oltman M, Ivanovski L, Rehák V, Messinger D, Tietz A, Urbanek P. Efficacy and safety of peginterferon α-2a (40 kD) plus ribavirin among patients with chronic hepatitis C and earlier treatment failure to interferon and ribavirin: an open-label study in central and Eastern Europe. *Eur J Gastroenterol Hepatol* 2011; **23**: 375-381 [PMID: 21502923 DOI: 10.1097/MEG.0b013e3283458407]

15 **Lee SS**, Heathcote EJ, Reddy KR, Zeuzem S, Fried MW, Wright TL, Pockros PJ, Häussinger D, Smith CI, Lin A, Pappas SC. Prognostic factors and early predictability of sustained viral response with peginterferon alfa-2a (40KD). *J Hepatol* 2002; **37**: 500-506 [PMID: 12217604]

16 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

17 **Diago M**, Olveira A, Solá R, Romero-Gómez M, Moreno-Otero R, Pérez R, Salmerón J, Enríquez J, Planas R, Gavilán JC, Del Olmo J, Uribarrena R, Sillero C, Benítez A, Sánchez-Galdón S, Dalmau B, Eraña L, Montoro M, Portu J, Garijo JM, Barniol R, Domínguez A, Rota R, Olcoz JL, Antón M, Pamplona X, Casanovas T, Jiménez E, Huarte M, Díaz F, Sánchez-Ruano J, Orive M, Muñoz-Sánchez M, Roset M. Treatment of chronic he1patitis C genotype 1 with peginterferon-alpha2a (40 kDa) plus ribavirin under routine clinical practice in Spain: early prediction of sustained virological response rate. *Aliment Pharmacol Ther* 2007; **25**: 899-906 [PMID: 17402993 DOI: 10.1111/j.1365-2036.2007.03270]

18 **Cacoub P**, Ouzan D, Melin P, Lang JP, Rotily M, Fontanges T, Varastet M, Chousterman M, Marcellin P. Patient education improves adherence to peg-interferon and ribavirin in chronic genotype 2 or 3 hepatitis C virus infection: a prospective, real-life, observational study. *World J Gastroenterol* 2008; **14**: 6195-6203 [PMID: 18985810]

19 **Manns MP**. Adherence to combination therapy: influence on sustained virologic response and economic impact. *Gastroenterol Clin North Am* 2004; **33**: S11-S24 [PMID: 15081100 DOI: 10.1016/j.gtc.2003.12.003]

20 **McHutchison JG**, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069 [PMID: 12360468]

21 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

22 **Zeuzem S**. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who responds less well? *Ann Intern Med* 2004; **140**: 370-381 [PMID: 14996679]

23 **Ferenci P**, Fried MW, Shiffman ML, Smith CI, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxì A, Chaneac M, Reddy KR. Predicting sustained virological responses in chronic hepatitis C patients treated with peginterferon alfa-2a (40 KD)/ribavirin. *J Hepatol* 2005; **43**: 425-433 [PMID: 15990196 DOI: 10.1016/j.jhep.2005.04.009]

24 **Tural C**, Galeras JA, Planas R, Coll S, Sirera G, Giménez D, Salas A, Rey-Joly C, Cirera I, Márquez C, Tor J, Videla S, García-Retortillo M, Clotet B, Solà R. Differences in virological response to pegylated interferon and ribavirin between hepatitis C virus (HCV)-monoinfected and HCV-HIV-coinfected patients. *Antivir Ther* 2008; **13**: 1047-1055 [PMID: 19195330]

25 **Di Bisceglie AM**, Ghalib RH, Hamzeh FM, Rustgi VK. Early virologic response after peginterferon alpha-2a plus ribavirin or peginterferon alpha-2b plus ribavirin treatment in patients with chronic hepatitis C. *J Viral Hepat* 2007; **14**: 721-729 [PMID: 17875007 DOI: 10.1111/j.1365-2893.2007.00862]

26 **Jeffers LJ**, Cassidy W, Howell CD, Hu S, Reddy KR. Peginterferon alfa-2a (40 kd) and ribavirin for black American patients with chronic HCV genotype 1. *Hepatology* 2004; **39**: 1702-1708 [PMID: 15185312 DOI: 10.1002/hep.20212]

27 **Lindsay KL**, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395-403 [PMID: 11481625 DOI: 10.1053/jhep.2001.26371]

28 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352**: 1426-1432 [PMID: 9807989]

29 **Zeuzem S**, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; **343**: 1666-1672 [PMID: 11106715 DOI: 10.1056/NEJM200012073432301]

30 **Ferenci P**, Laferl H, Scherzer TM, Gschwantler M, Maieron A, Brunner H, Stauber R, Bischof M, Bauer B, Datz C, Löschenberger K, Formann E, Staufer K, Steindl-Munda P. Peginterferon alfa-2a and ribavirin for 24 weeks in hepatitis C type 1 and 4 patients with rapid virological response. *Gastroenterology* 2008; **135**: 451-458 [PMID: 18503773 DOI: 10.1053/j.gastro.2008.04.015]

31 **Jensen DM**, Morgan TR, Marcellin P, Pockros PJ, Reddy KR, Hadziyannis SJ, Ferenci P, Ackrill AM, Willems B. Early identification of HCV genotype 1 patients responding to 24 weeks peginterferon alpha-2a (40 kd)/ribavirin therapy. *Hepatology* 2006; **43**: 954-960 [PMID: 16628671 DOI: 10.1002/hep.21159]

32 **Mangia A**, Minerva N, Bacca D, Cozzolongo R, Ricci GL, Carretta V, Vinelli F, Scotto G, Montalto G, Romano M, Cristofaro G, Mottola L, Spirito F, Andriulli A. Individualized treatment duration for hepatitis C genotype 1 patients: A randomized controlled trial. *Hepatology* 2008; **47**: 43-50 [PMID: 18069698 DOI: 10.1002/hep.22061]

33 **Yu ML**, Dai CY, Huang JF, Chiu CF, Yang YH, Hou NJ, Lee LP, Hsieh MY, Lin ZY, Chen SC, Hsieh MY, Wang LY, Chang WY, Chuang WL. Rapid virological response and treatment duration for chronic hepatitis C genotype 1 patients: a randomized trial. *Hepatology* 2008; **47**: 1884-1893 [PMID: 18508296 DOI: 10.1002/hep.22319]

34 **Weich V**, Herrmann E, Chung TL, Sarrazin C, Hinrichsen H, Buggisch P, Gerlach T, Klinker H, Spengler U, Bergk A, Zeuzem S, Berg T. The determination of GGT is the most reliable predictor of nonresponsiveness to interferon-alpha based therapy in HCV type-1 infection. *J Gastroenterol* 2011; **46**: 1427-1436 [PMID: 21912897 DOI: 10.1007/s00535-011-0458-y]

35 **Hwang SJ**, Luo JC, Chu CW, Lai CR, Lu CL, Tsay SH, Wu JC, Chang FY, Lee SD. Hepatic steatosis in chronic hepatitis C virus infection: prevalence and clinical correlation. *J Gastroenterol Hepatol* 2001; **16**: 190-195 [PMID: 11207900]

36 **Silva IS**, Ferraz ML, Perez RM, Lanzoni VP, Figueiredo VM, Silva AE. Role of gamma-glutamyl transferase activity in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2004; **19**: 314-318 [PMID: 14748879]

37 **Taliani G**, Badolato MC, Nigro G, Biasin M, Boddi V, Pasquazzi C, Clerici M. Serum concentration of gammaGT is a surrogate marker of hepatic TNF-alpha mRNA expression in chronic hepatitis C. *Clin Immunol* 2002; **105**: 279-285 [PMID: 12498809]

38 **Bressler BL**, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; **38**: 639-644 [PMID: 12939590 DOI: 10.1053/jhep.2003.50350]

39 **Iadonato SP**, Katze MG. Genomics: Hepatitis C virus gets personal. *Nature* 2009; **461**: 357-358 [PMID: 19759611]

40 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

41 **Jacobson IM**, Brown RS, Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, Wakil AE, Pound D, Godofsky E, Strauss R, Bernstein D, Flamm S, Pauly MP, Mukhopadhyay P, Griffel LH, Brass CA. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007; **46**: 971-981 [PMID: 17894303 DOI: 10.1002/hep.21932]

42 **Hézode C**, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourlière M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839-1850 [PMID: 19403903 DOI: 10.1056/NEJMoa0807650]

43 **McHutchison JG**, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827-1838 [PMID: 19403902 DOI: 10.1056/NEJMoa0806104]

P- Reviewer Rodriguez-Frias F **S- Editor** Gou SX
L- Editor Webster JR **E- Editor** Yan JL

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1953

World J Gastroenterol 2013 March 28; 19(12): 1953-1961
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Endoscopic submucosal dissection for the treatment of neoplastic lesions in the gastrointestinal tract

Andrzej Białek, Anna Wiechowska-Kozłowska, Jan Pertkiewicz, Katarzyna Karpińska, Wojciech Marlicz, Piotr Milkiewicz, Teresa Starzyńska

Andrzej Białek, Wojciech Marlicz, Teresa Starzyńska, Gastroenterology Department, Pomeranian Medical University, 71-242 Szczecin, Poland
Anna Wiechowska-Kozłowska, Piotr Milkiewicz, Department of Endoscopy, Ministry of Internal Affairs Hospital, 70-382 Szczecin, Poland
Jan Pertkiewicz, Endotherapy Ltd, 02-091 Warsaw, Poland
Katarzyna Karpińska, Cell Pathology Department, Pomeranian Medical University, 71-242 Szczecin, Poland
Piotr Milkiewicz, Liver Unit, Pomeranian Medical University, 71-242 Szczecin, Poland
Author contributions: Białek A, Wiechowska-Kozłowska A, Pertkiewicz J, Milkiewicz P and Starzyńska T designed research; Białek A, Wiechowska-Kozłowska A, Pertkiewicz J, Karpińska K, Milkiewicz P and Starzyńska T performed research; Białek A, Wiechowska-Kozłowska A, Marlicz W and Milkiewicz P analyzed data; Białek A, Wiechowska-Kozłowska A, Milkiewicz P and Starzyńska T wrote the paper.
Correspondence to: Andrzej Bialek, MD, Gastroenterology Department, Pomeranian Medical University, ul. Unii Lubelskiej 1, 71-242 Szczecin, Poland. bialekab@pam.szczecin.pl
Telephone: +48-91-4253211 Fax: +48-91-4253211
Received: October 27, 2012 Revised: December 20, 2012
Accepted: January 11, 2013
Published online: March 28, 2013

Abstract

AIM: To investigate the indications, resection rate, and safety of endoscopic submucosal dissection (ESD) for neoplastic lesions in the gastrointestinal tract at a European referral center.

METHODS: We carried out a retrospective analysis of the ESD procedures performed in our center for mucosal neoplastic and submucosal lesions of the gastrointestinal tract. The duration of the procedure, *en bloc* and complete (R0) resection rates, and complication rates were evaluated. Variables were reported as mean ± SD or simple proportions. Univariate analysis and comparisons of procedure times and resection rates were performed using Mann-Whitney *U* tests, or χ^2 tests for dichotomous variables.

RESULTS: Between 2007 and 2011, ESD was performed in a total of 103 patients (46.7% male, mean age 64.0 ± 12.7 years). The indications for the procedure were epithelial tumor (n = 54), submucosal tumor (n = 42), or other (n = 7). The total *en bloc* resection rate was 90.3% (93/103) and R0 resection rate 80.6% (83/103). The median speed of the procedure was 15.0 min/cm^2. The complete resection rate was lower for submucosal tumors arising from the muscle layer (68%, 15/22, $P < 0.05$). Resection speed was quicker for submucosal tumors localized in the submucosal layer than for lesions arising from the muscularis propria layer (8.1 min/cm^2 *vs* 17.9 min/cm^2, $P < 0.05$). The R0 resection rate and speed were better in the last 24 mo (90.1%, 49/54 and 15.3 min/cm^2) compared to the first 3 years of treatment (73.5%, 36/49, $P < 0.05$ and 22.0 min/cm^2, $P < 0.05$). Complications occurred in 14.6% (n = 15) of patients, including perforation in 5.8% (n = 6), pneumoperitoneum in 3.9% (n = 4), delayed bleeding in 1.9% (n = 2), and other in 2.9% (n = 3). Only one patient with delayed perforation required surgical treatment. During the mean follow-up of 26 ± 15.3 mo, among patients with R0 resection, recurrence occurred in one patient (1.2%).

CONCLUSION: ESD is an effective and safe method for resection of neoplastic lesions with low recurrence. Speed and the R0 resection rate increased after 50 procedures.

Key words: Endoscopic submucosal dissection; Gastrointestinal neoplasms; Gastrointestinal stromal tumors; Treatment

Białek A, Wiechowska-Kozłowska A, Pertkiewicz J, Karpińska K, Marlicz W, Milkiewicz P, Starzyńska T. Endoscopic submucosal dissection for the treatment of neoplastic lesions in the gastrointestinal tract. *World J Gastroenterol* 2013; 19(12): 1953-1961 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1953.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1953

INTRODUCTION

In Japan and South Korea, endoscopic submucosal dissection (ESD) is a commonly accepted method for the resection of early neoplastic lesions in the upper and lower gastrointestinal tract. Good results from and the safety of procedures performed in the last few years have resulted in an increase in the number of procedures. Publications of the results of treatment with this method include more than 1000 patients. Although the dynamic development of this method is visible in Far East countries, this method is still in development in Europe and has not gained in popularity. A few publications describe the results of treatment in more than 50 patients, and some small series of patients or case reports have been published, but there is an overall lack of new European data.

ESD involves the removal of both benign neoplastic (premalignant) and malignant non-invasive lesions, aiming for the highest R0 resection rate (83%-98%) and lowest rate of local recurrence (0%-3%) of all endoscopic techniques[1]. The removal of submucosal lesions is also possible, including those growing out of the proper muscle layer[2].

The papers from Japan and Asia focus on confirming that ESD is a safe method with few complications and a mortality rate of 0%. The time needed to perform the procedure significantly decreases with the number of procedures performed, but it is still longer than the time needed for mucosectomy.

The present paper is one of the few European reports showing the results of treating gastrointestinal malignancies with ESD in a referral center. We present the indications, results, and complications with regard to different parts of the gastrointestinal tract.

MATERIALS AND METHODS

Patients

The procedures were performed between April 2007 and December 2011. Before qualifying for the procedure, patients received both oral and written explanations of the endoscopic examination and possible treatment options, and they signed an informed written consent form.

For each patient with pathology of the upper gastrointestinal tract, endoscopic ultrasound (EUS) was performed before admission for ESD. The sector-scanning echoendoscope (Olympus GF-UM130 or GF-UE160 Olympus Medical Systems Co., Tokyo, Japan) or linear echoendoscope (Olympus GF-UCT140) were used to examine lesion size, EUS layer from which it derives (submucosal tumors, SMTs) or infiltrated layers (other lesions), SMT growth type (inside or outside the walls of the gastrointestinal tract), and lymph node diameter.

Neoplastic lesions of the upper gastrointestinal tract

Neoplastic mucosal tumors were included in the study if > 10 mm with a low risk of lymph node metastases. Lesions in the stomach were included based on the expanded criteria from the Japanese Gastric Cancer Association (JGCA)[3]: well-differentiated carcinoma without ulceration, irrespective of size; well-differentiated carcinoma with ulceration (type Ⅲ) ⩽ 30 mm; or well-differentiated carcinoma with submucosal invasion and no more than 500 μm in size.

Exclusion criteria were lack of consent from the patient for the endoscopic procedure, massive infiltration of the submucosal layer or infiltration of muscle layers assessed in EUS, or enlarged local lymph nodes or metastases found in imaging studies (*i.e.*, EUS, ultrasound, and computed tomography).

Neoplastic lesions of the lower gastrointestinal tract

Patients were qualified for ESD if any of the following were in the previous endoscopic examination: large sessile polyp (LST) (type I s) that could not be removed in one piece, granular type (LST-G) flat polyps with a dominant nodule greater than 2 cm, non-granular type (LST-NG) flat polyps of any size, or scars from previous non-therapeutic tumor resections. Exclusion criteria were a lack of consent from the patient for the endoscopic procedure or deep ulceration apparent in the lesion, suggesting massive submucosal invasion.

Submucosal tumors

Resection of SMT by ESD was performed if the tumor was 1 to 8 cm in size with no growth outside the gastrointestinal tract on EUS. Patients who did not meet these criteria were referred to follow-up (submucosal lesions < 1 cm), mucosectomy (smaller or pedunculated lesions), or surgical treatment (submucosal lesions > 8 cm, epithelial tumors). All cases but one lipoma (bleeding gastric tumor) were excluded from endoscopic treatment.

Procedural technique

Before the treatment of epithelial malignancies by ESD, indigo carmine chromoendoscopy, magnifying endoscopy, NBI, or a combination of these techniques was performed to qualify the patients for the procedure based on the classification of Paris[4] and properly assess the lateral margins of the lesion. ESD in the upper gastrointestinal tract was performed under general anesthesia, whereas analgosedation with midazolam (2.5-5 mg *iv*) and fentanyl (50-100 μg *iv*) was used for procedures in the lower gastrointestinal tract. An Olympus endoscope GIF-T140 with a transparent cap (Olympus D-201-12 704) was used, allowing a better view of the submucosal layer during the procedure. For submucosal injection, 0.9% NaCl solution

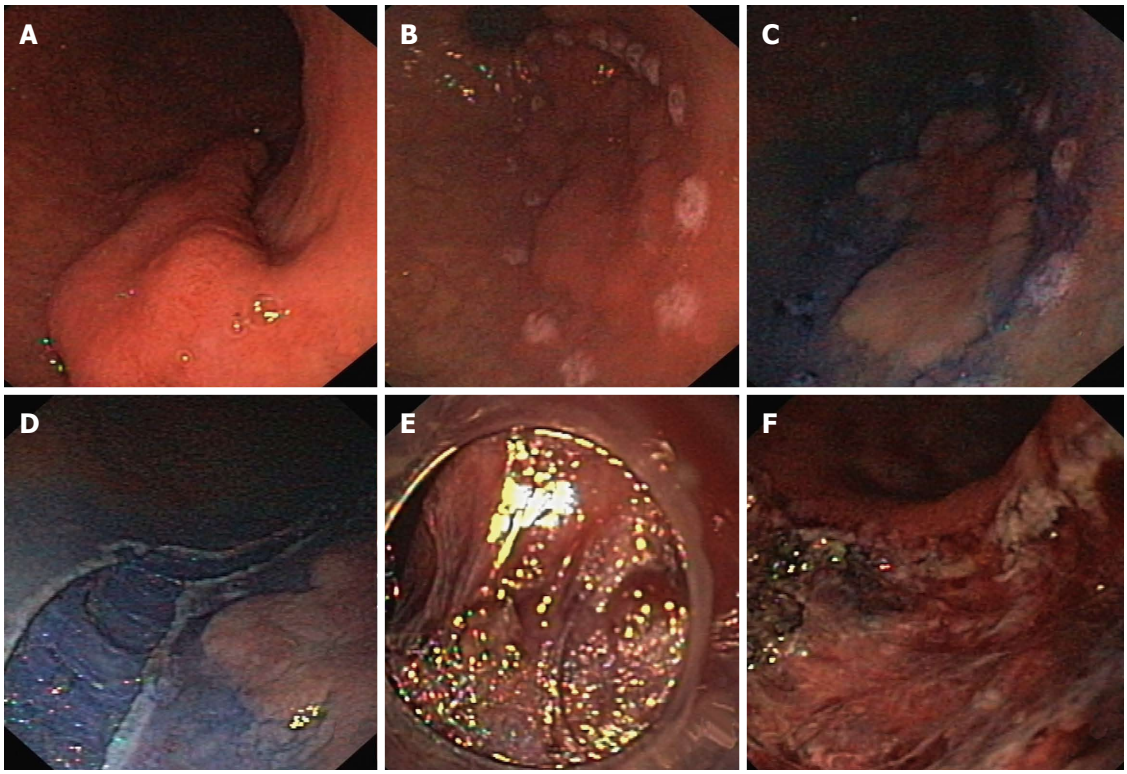


Figure 1 Endoscopic submucosal dissection of gastric neoplastic lesion. A: Lesion type Ⅱa+c localized in the antrum; B: Margins of the lesion border were marked with the needle knife; C: Solution of indigo carmine in saline was injected into the submucosal space; D: An incision was made in the mucosa and submucosa around the lesion with normal mucosal margin; E: Submucosal dissection performed directly under vision control; F: Mucosal defect after the completed procedure.

with epinephrine (1 mg/250 mL NaCl) and indigo carmine dye was used. In some cases in which the elevation of the mucosa after the injection of the solution was too short, a solution of hyaluronic acid was used (Sigmavisc, Hyaltech Ltd, Livingston, United Kingdom).

The resection was started by marking the lesion borders with the needle knife (Olympus KD-441Q) or dual knife (Olympus KD-650L) using forced coagulation of 35 W (Erbe ICC 200, Erbe Elektromedizin GmbH, Tübingen, Germany). The solution was injected submucosally next to the markers. The initial incision, approximately 3-5 mm in length, was made with a needle knife, and then a circular incision was made around the lesion using the isolated-tip knife IT or IT-2 (Olympus KD-611L, EndoCut mode, effect 3, 100 W) or a dual knife (EndoCut mode, effect 3, 35-50 W). The next step was the injection of the same solution directly into the submucosal layer under the lesion. The blue color of the indigo carmine allowed this layer to be distinguished from the proper muscle and the lesion itself. Dissection of the submucosal layer and the tumor was performed using the IT knife, hook-knife (Olympus KD-620LR, EndoCut mode, effect 2-3, 50-90 W), flex-knife (Olympus KD-630L), or dual knife (Figures 1 and 2). Muscular SMTs attached to the muscle layer by muscle fibers or muscle pedicle were cut away from the muscle layer of the wall (Figure 3). Tumors that were fused tightly with muscle and over a larger area were cut away with a loop, or most of the lesion was cut away to obtain enough material for pathological examination. Scar tissue was cut using a needle knife along the muscle layer. Lesions located in the cardia and just behind the anal canal were removed mostly in retroflexion. During the procedure and immediately after the removal of a tumor, all visible blood vessels in the submucosal and muscle layer were coagulated using a coagrasper (Olympus FD-410LR, soft coagulation 35 W) or argon coagulation (Erbe APC 300, 35 W, flow 1.6 L/min), or hemoclips (Olympus HX-610-135) applied. The removed lesion was affixed to a polystyrene substrate, fixed in formalin, and examined morphologically.

Bleeding during the procedure that did not cause hemodynamic disorders or anemia was not considered a complication. All bleeding was treated endoscopically with coagrasper hemostatic forceps, argon beamer coagulation, or hemoclip application. Perforations noticed during the procedure were treated endoscopically by closing the wall defect using hemoclips. After the procedure, antibiotics were administered intravenously (amoxicillin 2 × 1000 mg) for 5 d, as well as proton pump inhibitors if the lesion was resected in the upper gastrointestinal tract (omeprazol 8 mg/h *iv* for 2 d, then 2 × 20 mg orally for 8 wk). After ESD in the lower gastrointestinal tract, patients received ciprofloxacin (2 × 200 mg *iv*) and metronidazol (3 × 500 mg *iv*). On the day of the procedure (day zero) the patients fasted. The first day after the procedure the patients received neutral fluids orally, and normal diet the next day. Colon patients received normal diet from the first day after the procedure. The patients were discharged from the hospital on day 3-5 after the procedure.

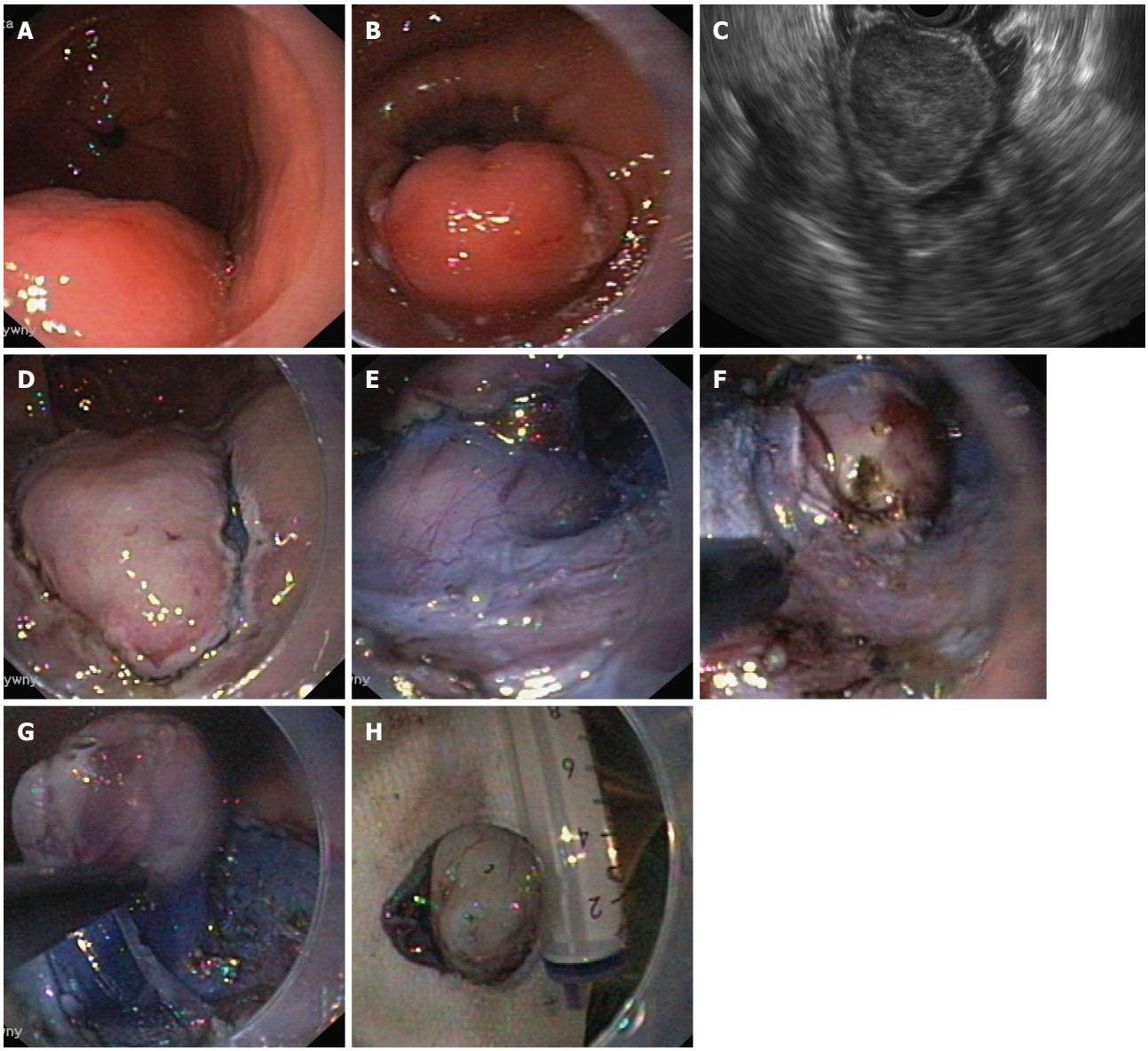


Figure 2 Endoscopic submucosal dissection of gastric submucosal tumor. A, B: Submucosal tumor in the stomach; C: Endosonographic view of the tumor, which is not connected to the muscle layer of the gastric wall; D: An incision was made in the mucosa and submucosa after indigo carmine solution injection into the submucosal layer around the lesion; E-G: Submucosal dissection of the tumor, exposing the tumor in the submucosa and carefully cutting after injecting the solution; H: Resected tumor (GIST, 25 mm, MI 2/50 HPF). GIST: Gastrointestinal stromal tumor; MI: Mitotic index; HPF: High power field.

Pathological examination

The resected specimens were pinned to a mounting board with clearly marked oral and anal orientations and routine formalin fixation performed. The borders of each specimen were colored with ink and sections taken every 2 mm. The pathological reports of the resected specimens included the macroscopic appearance, size, histological type (the most important Lauren classification of gastric cancer), and extent of the tumor depth. The presence of ulceration and lymphovascular involvement, as well as the status of the vertical and lateral resection margins, were reported in detail.

In addition, the SMT preparations were labeled with DAKO antibodies (Dako Polska Sp.z o.o.). Gastrointestinal stromal tumors (GISTs) were characterized by a positive reaction to c-KIT (CD 117) or DOG-1 and CD34 antibodies. Leiomyomas were diagnosed when the mesenchymal tumors had a positive reaction for smooth muscle actin and desmin. A positive reaction for S-100 protein and negative reaction for muscle markers and CD117 indicated nerve tumors. The neoplastic potential of the stromal tumors was determined on the basis of their size and mitotic index (MI, number of mitoses counted in 50 large fields) according to the classification of Miettinen *et al*[5].

The criteria for curative resection of cancer lesions were: the depth of neoplasm infiltration limited to the mucosal or superficial submucosal layer (up to 500 μm in the cardia and stomach and up to 1000 μm in the large intestine as measured from the lower border of the muscle layer of the mucosa), no infiltration or congestion of carcinoma cells in blood vessels and lymph vessels (angioinvasion), lateral and bottom margins free of neoplasm, and

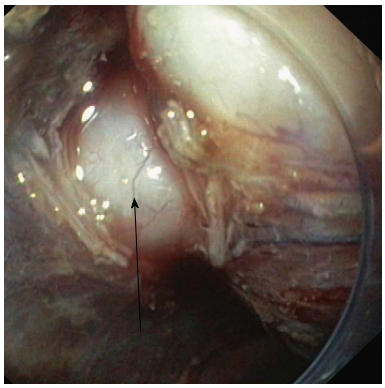


Figure 3 Submucosal tumor (leiomyoma) resected by endoscopic submucosal dissection and connected to the gastric wall with the muscle peduncle (arrow).

well or medium-differentiated cancer. For GIST, the only criterion was confirmed tissue-free margins.

Follow-up

All side effects of the dissection were recorded according to standard procedures[6,7]. The first follow-up visit was 2-3 wk after ESD. The first follow-up endoscopy was performed 3 or 12 mo after surgery using a standard endoscope or EUS. Patients with incompletely resected neoplastic changes were referred for surgery. A control endoscopy after 3 mo was performed in the case of piecemeal resection of the tumor or uncertain histopathological confirmation of the complete tumor resection (Rx). The following cases were qualified for endoscopy after 12 mo: removal of a tumor that fulfilled the criteria for R0, resection of non-neoplastic lesions, and incomplete SMT resection of non-stromal tumors.

Statistical analysis

Variables were reported as mean ± SD or simple proportions. Univariate analysis and comparisons of procedure times and resection rates were performed using Mann-Whitney *U* tests, or χ^2 tests for dichotomous variables. Statistica 9.1 software was used for all data analyses (StatSoft, Inc. 2010; Statistica).

RESULTS

Over a period of 57 mo, ESD was performed in 103 patients (46 males, 44.66%). The mean patient age was 64.0 ± 12.7 years. A total of 69 procedures were performed in the upper gastrointestinal tract, 34 in the colon. The indications for resection were epithelial tumors (n = 54), SMT (n = 42), scars from previous non-therapeutic tumor resection (n = 2), and others (n = 5). All procedures were performed by two physicians (Białek A, Pertkiewicz J).

The total *en bloc* and R0 resection rates were 90.3% (93/103) and 80.6% (83/103), respectively. The rate of *en bloc* and R0 resection for epithelial lesions reached 85.3% (52/61), for overall SMT the rates were 97.6% (41/42) and 73.8% (31/42), respectively, but did not differ significantly. The complete resection rate for SMTs arising from the muscle layer was 68% (15/22), which is significantly lower than that of epithelial lesions ($P < 0.05$).

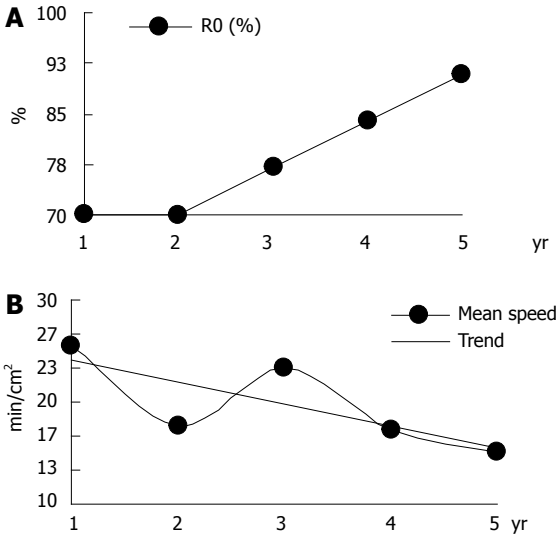


Figure 4 R0 resection rate and mean speed of the procedure in the years following endoscopic submucosal dissection. A: R0 resection rate; B: Mean speed of the procedure.

The mean speed of performing the procedure was 19.0 ± 14.6 min/cm^2. The overall resection speed for SMTs was 15.85 ± 10.99 and 21.17 ± 16.41 min/cm^2 for mucosal lesions, but this difference was not significant. The resection speed was faster for SMTs localized in the submucosal layer compared to lesions arising from the muscularis propria layer (8.1 min/cm^2 *vs* 17.9 min/cm^2, $P < 0.05$).

During the first 3 years following ESD, 49 procedures were performed. Thirty-six of the procedures (73.5%) were R0 resections, and the procedure speed was 22.02 ± 15.33 min/cm^2. In the last 24 mo, the R0 resection rate increased to 90.1% (49/54, $P < 0.05$, Table 1; Figure 4) with a mean speed of 15.3 ± 13.25 min/cm^2 ($P < 0.05$, Table 1; Figure 4). The complication rates did not differ significantly. During a mean follow-up of 26 ± 15.3 mo, recurrence was detected in only one patient who underwent R0 resection (1.2%, 1/83), a 54-year-old female with intestinal type gastric cancer (G1, M2), and recurrence occurred within 6 mo of the procedure. The patient was sent for surgery and middle grade dysplasia diagnosed in the resected specimen.

Esophagus and cardia

ESD procedures were performed in the lower esophagus and cardia in 14 patients. The indications for the procedure were SMTs suspected to be GISTs in 5 patients and mucosal tumors in 9 patients.

Morphologically, tumors were type Ⅰ-1, Ⅱa-2, Ⅱa+c-3, Ⅱb+c-2 and Ⅱb-1 according to the Paris classification. The mean tumor size was 2.37 ± 0.95 cm for all lesions, 2.36 ± 1.0 cm for mucosal lesions, and 2.4 ± 0.96 cm for SMTs. The *en bloc* and R0 resection rates as well as the histological diagnoses of resected tumors are shown in Table 2.

The mean procedure duration was 99 ± 77.2 min and significantly shorter for the resection of SMTs (38

Table 1 Mean speed of endoscopic submucosal dissection and R0 resection rate related to time

Performing ESD (yr)	Procedures (*n*)	Speed (min/cm^2) (mean ± SD)	R0
1	10	25.5 ± 25.54	70.0%
2	17	17.6 ± 25.54	70.0%
3	22	23.4 ± 11.87	77.0%
4	32	17.4 ± 15.33	84.0%
5	22	15.1 ± 12.94	90.9%

ESD: Endoscopic submucosal dissection.

± 13.03 min) than for mucosal lesions (128 ± 80.06 min, $P < 0.05$). Overall, the speed of resection was 24.96 ± 22.71 min/cm^2 and significantly slower for the resection of mucosal lesions (33.29 ± 24.4 min/cm^2) than for SMTs (9.96 ± 6.9 min/cm^2, $P < 0.05$). The overall rate of *en bloc* and R0 resection was 100% (14/14) and 64.3% (9/14), respectively, and it did not differ significantly between mucosal lesions and SMTs.

Both of the incompletely removed SMTs were leiomyomas. The three neoplastic lesions were adenocarcinomas; cancerous infiltration was present in the lower margin (SM3) in two cases and in the lateral margin in one case. One of the patients was treated surgically; the adenocarcinoma was found in neither the cardia nor the lymph nodes of the surgical specimen. The other two patients were not qualified for surgery because of a lack of consent and high risk of surgery due to serious comorbidities.

Stomach

ESD was performed in the stomach in 54 patients, among which 35 were suspected to have GIST (mean size 2.9 ± 1.2 cm) and 19 mucosal neoplastic lesions (mean size 2.6 ± 1.1 cm). According to the Paris classification, the mucosal lesions were type Ⅰ s-2, Ⅱa+c-11, Ⅱa-4, Ⅱb-1 and Ⅱb+c-1. Histological diagnoses and the rates of *en bloc* and R0 resection are shown in Table 2. Four lesions in the stomach qualified according to JGCA classic criteria[7] were removed completely (R0 resection rate 100%). Out of 12 lesions, only 9 (75.0%) reached R0 resection according to expanded criteria.

In two cases of incomplete resection of SMT, GIST was diagnosed. In one of these patients, the tumor was 4 cm, MI = 6 (risk of progression: moderate), and the patient referred for surgery; in the other, the tumor was 1.9 cm, MI = 3 (risk of progression: low), and the patient referred for follow-up examination. In two cases of incomplete resection of adenocarcinoma, one had a lower margin positive for cancer (sm3) and the other a lateral margin positive for cancer (piecemeal resection). The first patient qualified for surgical resection, but the other did not give consent for surgical treatment; in 33 mo of follow-up no tumor recurrence was found.

The mean duration of the endoscopic procedure was 103.8 ± 77.3 min for all lesions, 108.1 ± 88.0 min for epithelial lesions, and 101.4 ± 72.1 min for SMTs. The

Table 2 Endoscopic submucosal dissection resection rate according to histology in esophagus and cardia, stomach and colon *n* (%)

	Diagnosis	Patients	*En bloc*	R0
Esophagus	SMT	5	5 (100.0)	3 (60.0)
	GIST	1	1 (100.0)	1 (100.0)
	Leiomyoma	4	4 (100.0)	2 (50.0)
	Non-SMT	9	9 (100.0)	6 (66.7)
	Early cancer/HGD	6	6 (100.0)	3 (50.0)
	Neoplasia grade min/med	3	3 (100.0)	3 (100.0)
Stomach	SMT	35	34 (97.1)[a]	27 (77.1)
	GIST	18	18 (100.0)	16 (88.9)
	Leiomyoma	6	5 (83.3)	3 (50.0)
	Other	11	11 (100.0)	8 (72.7)
	Non-SMT	19	14 (73.7)[a]	16 (84.2)
	Early cancer/HGD	12	8 (66.7)	10 (83.3)
	Neoplasia grade min/med	4	3 (75.0)	3 (75.0)
	Other	3	3 (100.0)	3 (100.0)
Colon	SMT	2	2 (100.0)	2 (100.0)
	Fibroepithelioma	1	1 (100.0)	1 (100.0)
	Leiomyoma	1	1 (100.0)	1 (100.0)
	Non-SMT	32	28 (87.5)	29 (90.6)
	Early cancer/HGD	19	17 (89.5)	17 (89.5)
	Neoplasia grade min/med	11	10 (90.9)	10 (90.9)
	Other	2	1 (50.0)	2 (100.0)

[a]$P < 0.05$ *vs* non-submucosal tumor (SMT). GIST: Gastrointestinal stromal tumor; HGD: High grade dysplasia.

mean speed of dissection was 18.05 ± 12.1 min/cm^2 for all lesions, 20.1 ± 13.4 min/cm^2 for epithelial lesions, and 16.9 ± 11.4 min/cm^2 for SMTs. The speed of the resection of SMTs connected to the muscle layer ($n = 19$) was 19.7 ± 8.5 min/cm^2 and 14.2 ± 15.2 min/cm^2 for tumors without such a connection ($n = 12$).

Colon

In the large intestine, ESD was performed in 34 patients. The main indications were laterally spreading tumor (LST) type Ⅱa-11 or Ⅱa+c-9 and polyps type Ⅰ s-10. In two cases, ESD was performed for the radicalization of previously incomplete polypectomy procedures and in two cases due to symptomatic SMTs of the rectum. Low or middle grade dysplasia was diagnosed in 11 cases, and high-grade dysplasia or adenocarcinoma in 21 cases (Table 2). Two SMTs were diagnosed as fibroepithelioma and leiomyoma. The majority of lesions were located in the rectum ($n = 28$), followed by the sigmoid colon ($n = 5$) and ascending colon ($n = 1$). The rate of *en bloc* and R0 resection was 87.5% (28/32) and 90.6% (29/32), respectively. Among the lesions with incomplete resection, two adenocarcinomas with infiltration of the lower cut border (sm3) and tubular adenoma with low-grade dysplasia were diagnosed. In both cases of adenocarcinoma, the patients underwent surgical treatment, but cancerous tissue was not found in the surgical specimen. The patient with non-radically resected adenoma was administered follow-up examinations (20 mo) with no recurrence.

The average overall time and speed of treatment was 82.0 ± 56.6 min and 17.9 ± 14.2 min/cm^2, respectively,

Table 3 Complications after endoscopic submucosal dissection according to location

	Patients	Delayed bleeding	Perforation	Other (*n*)	
Cardia	14	0	1	Pneumoperitoneum	2
Stomach	54	2	5		
Upper part	21	1	4	Mucosal tear of lower pharyngal sphincter region, pneumoperitoneum	3
Middle part	7	0	1		
Lower part	26	1	0	Stenosis of the pylorus	2
Colon	34	0	0		0
Duodenum	1	0	0		0
All	103	2	6		7

85.1 ± 56.9 min and 18.2 ± 14.5 min/cm^2, respectively, for epithelial neoplastic lesions, and 32.5 ± 3.5 min/cm^2 and 11.9 ± 9.3 min/cm^2, respectively, for SMTs.

Complications after ESD

Severe complications occurred in two patients in the form of perforations, resulting in prolonged hospitalization for more than 10 d, including surgical treatment in one case. Mild or moderate complications occurred in 13 patients: 2 patients with delayed bleeding treated by transfusion, 1 patient with a mucosal pharyngeal sphincter tear who required prolonged hospitalization for less than 3 d, 4 with pneumoperitoneum who required prolonged hospitalization for less than 3 d, 4 patients with perforation treated conservatively and requiring prolonged hospitalization for 4-10 d, and 2 patients with pyloric stenosis requiring additional endoscopy. The complication rate according to localization is presented in Table 3.

In six patients with perforation, endoscopic closure of the defect was performed using hemoclips, decompression of the peritoneum by puncture, and conservative treatment with fasting, antibiotic therapy, and active suction with a nasogastric tube. In one of the patients, delayed perforation occurred on the fourth day when trying to implement oral feeding.

Bleeding in two patients was controlled by hemoclip application, and patients required the transfusion of two units of blood. In two patients, stenosis occurred within 4 wk after dissection of neoplastic lesions in the pylorus. Both patients were successfully treated with 20-mm balloon dilatation in one and two sessions. In one patient a mucosal tear in the throat sphincter occurred when removing a large, > 3 cm resected SMT. The patient had no symptoms and did not require additional treatment.

DISCUSSION

ESD is a technique aimed at resecting early neoplastic lesions in the gastrointestinal tract without compromising the integrity of the wall. The technique allows R0 resection to be achieved, even for large mucosal and submucosal lesions, by removing them in one piece (*en bloc*), which allows proper pathological evaluation of specimens. The method is the most effective of the endoscopic resection techniques and used by many centers in Japan and the Far East. Many papers, including the multicenter studies and those presenting the results of treatment in over 1000 patients, confirm the technique's efficacy in both the upper and lower parts of the gastrointestinal tract[8-12]. The percentage of *en bloc* resection is estimated to range from 92%-100% in the upper gastrointestinal tract and 81.6%-92.7% in the lower gastrointestinal tract, and the rate of R0 resection is estimated to be 73.6%-94.7% in the upper gastrointestinal tract and 69.7%-89% in the lower gastrointestinal tract, which is significantly higher than that of mucosectomy techniques, in which R0 resection is estimated to be 33%-56%[1,9,13], especially for the resection of large tumors (> 2 cm in size). ESD also allows resection of SMTs, even those growing from the muscularis propria[2,10-18], and allows surgeons to save the organ via a minimally invasive resection of the lesion itself.

Despite such good results of treatment, the method is still rarely used in Europe[2,19-22]. To the best of our knowledge, only two European papers from Augsburg, Germany, present the results of treating more than 100 patients in one center[19,20]. One of the reasons for this low usage could be the time it takes to perform the procedure, especially at the beginning of the learning curve. In the Japanese centers, which have published the results of treating more than 1000 patients, the average time for ESD in the stomach is approximately 37 min[19]. In the present study, both time and speed were worse than in the Japanese studies due to the relatively low volume at the center. The number of ESD procedures necessary to master the method is thought to be approximately 50. In the present study, the speed of performing the procedure increased approximately 30% after the first 49 procedures over 3 years. Similarly, Probst *et al*[20] and Japanese authors noted a significant increase in the speed of the resection after 40-50 procedures[13,19].

The location of the lesions in the upper part of the stomach, their size, and the presence of submucosal fibrosis are associated with longer procedure duration, a lower resection rate, and a higher rate of complications[23-25]. In the present study, the percentage of resection related to lesion localization and timing in the stomach did not differ significantly, probably due to a heterogeneous patient group and a small number of procedures in different locations.

The other factor responsible for the small number of ESD procedures in Europe may be the greater number of complications compared to mucosectomy. The most serious complication of ESD is perforation, which occurs in 1.2%-9.7% of cases, and bleeding, which occurs in 0.1%-15.6% of procedures[1,8,13,20]. In the present study, the percentage of complications was similar, 4.9% for perforation and 1.9% for delayed bleeding, and surgical treatment was required in only one case. Importantly, the mortality rate after endoscopic treatment is 0% in both the present study and most published papers. Factors associated with a higher incidence of complications have

been identified, including location in the upper and middle part of the stomach, lesion size, and the number of procedures performed when less than 50[11-12,25,26]. Also, in the present study, lesions located in the upper and middle third of the stomach were associated with a higher incidence of perforation, as five of six perforations occurred in these locations.

In the present study, the rate of *en bloc* resection and R0 resection was 90.3% and 80.6%, respectively, which is comparable with other European studies but lower than that of studies from Japan and South Korea. This difference is due to the lesser experience of authors in the first years of implementing the procedure. In the last two years, the rate of R0 resection increased from 73.5% to 90.1%, which approached the level of Japanese authors (Table 1; Figure 2). This finding confirms a long learning curve for this technique, which was nearly three years in our study, with 49 treatments completed. A similar tendency has been noticed by other authors, with the rate of total *en bloc* resection beginning at 50%-65.7% and increasing to 72.2%-100%[1,19]. An important factor in achieving R0 resection was a larger proportion of eligible patients fulfilling extended (n = 12), compared to classical (n = 4), indications for endoscopic resection of gastric cancer. The rate of R0 resection for classical indications in the present study was 100% (4/4), which was higher than the rate with the extended criteria (9/12, 75%). A similar tendency was observed by Probst *et al*[20], who reported 90% (9/10) and 68.6% (35/51), respectively, and Japanese authors, who reported 97.1% and 91.1%, respectively[19,27,28].

In the present study, the significant effect on the lower total resection rate comprised a relatively large proportion of patients with SMTs, including tumors arising from the muscularis propria layer, for which the R0 resection rate was significantly lower. Among the patients with confirmed complete R0 resection, only one recurrence occurred within 6 mo of the procedure (1.2%). In the publications from the last few years, the rate of recurrence after R0 resection was 0%-5.1%[29-31].

A limitation of this study is the relatively small number of procedures performed in the reference center and retrospective type of analysis.

In summary, ESD allows endoscopic resection of tumors with high efficacy and low complication rates, even in a low-volume center. Most complications, including perforation, are mild or moderate in severity and can be treated endoscopically or conservatively. Both speed and complete resection rate improved after approximately 50 procedures.

COMMENTS

Background

In Japan and South Korea, endoscopic submucosal dissection (ESD) is a commonly accepted method for the resection of early neoplastic lesions in the upper and lower gastrointestinal tract. Although the dynamic development of this method is visible in Far East countries, ESD is still in development in Europe and has not gained in popularity. A few publications have described the results of treatment in more than 50 patients, and some small series of patients or case reports have been published, but new European data is lacking overall. The present paper is one of the few European reports showing the results of treating gastrointestinal malignancies with ESD in a referral center. Authors present the indications, results, and complications with regard to different parts of the gastrointestinal tract.

Research frontiers

ESD is a technique aimed at resecting early neoplastic lesions in the gastrointestinal tract without compromising the integrity of the wall. The technique allows R0 resection to be achieved, even for large mucosal and submucosal lesions, by removing them in one piece (*en bloc*), which allows for proper pathological evaluation of specimens. The method is the most effective endoscopic resection technique to date and is used by many centers in Japan and the Far East.

Innovations and breakthroughs

One of the reasons for the lack of usage of ESD could be the time required to perform the procedure, especially at the beginning of the learning curve. In the present study, the speed of performing the procedure and R0 resection rate increased approximately 30% after the first 49 procedures over 3 years. Other authors have also noted a significant increase in the speed and R0 resection rate after 40 to 50 procedures. Another factor potentially responsible for the small number of ESD procedures in Europe may be the greater number of complications associated with ESD compared to mucosectomy. The most serious complications of ESD are perforation, which occurs in 1.2%-9.7% of cases, and bleeding, which occurs in 0.1%-15.6% of cases. In this study, the percentage of complications was similar: 4.9% for perforation and 1.9% for delayed bleeding, irrespective of the center experience. Surgical treatment was required in only one case. The significant effect on the total resection rate included a relatively large proportion of patients with submucosal tumors, including tumors arising from the muscularis propria layer, for which the R0 resection rate was significantly lower. Among the patients with confirmed complete R0 resection, only one recurrence occurred within 6 mo of the procedure (1.2%). In publications from the last few years, the rate of recurrence after R0 resection has been 0%-5.1%.

Applications

ESD allows endoscopic resection of tumors with high efficacy and low complication rates, even in a low-volume center. Most complications, including perforations, are mild or moderate in severity and can be treated endoscopically or conservatively. Both speed and the complete resection rate improved after approximately 50 procedures.

Terminology

R0 resection: Complete resection of the lesion confirmed by a pathologist by microscopic examination of the tumor-free borders of resected species.

Peer review

The authors report European experience for ESD. Though the number of cases is small and some points need to be clear, this is valuable study as they mentioned that is European own experience.

REFERENCES

1 **Hulagu S**, Senturk O, Aygun C, Kocaman O, Celebi A, Konduk T, Koc D, Sirin G, Korkmaz U, Duman AE, Bozkurt N, Dindar G, Attila T, Gurbuz Y, Tarcin O, Kalayci C. Endoscopic submucosal dissection for premalignant lesions and noninvasive early gastrointestinal cancers. *World J Gastroenterol* 2011; **17**: 1701-1709 [PMID: 21483630 DOI: 10.3748/wjg.v17.i13.1701]

2 **Białek A**, Wiechowska-Kozłowska A, Pertkiewicz J, Polkowski M, Milkiewicz P, Karpińska K, Ławniczak M, Starzyńska T. Endoscopic submucosal dissection for treatment of gastric subepithelial tumors (with video). *Gastrointest Endosc* 2012; **75**: 276-286 [PMID: 22032850 DOI: 10.1016/j.gie.2011.08.029]

3 **Hermanek P**. The second English edition of the Japanese Classification of Gastric Carcinoma. A Western commentary. *Gastric Cancer* 1999; **2**: 79-82 [PMID: 11957076 DOI: 10.1007/s101209900032]

4 The Paris endoscopic classification of superficial neoplastic lesions: esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest Endosc* 2003; **58**: S3-43 [PMID: 14652541 DOI: 10.1016/S0016-5107(03)02159-X]

5 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70-83 [PMID: 17193820]

6 **Cotton PB**. Outcomes of endoscopy procedures: struggling towards definitions. *Gastrointest Endosc* 1994; **40**: 514-518

[PMID: 7926553]
7 **Cotton PB**, Eisen GM, Aabakken L, Baron TH, Hutter MM, Jacobson BC, Mergener K, Nemcek A, Petersen BT, Petrini JL, Pike IM, Rabeneck L, Romagnuolo J, Vargo JJ. A lexicon for endoscopic adverse events: report of an ASGE workshop. *Gastrointest Endosc* 2010; **71**: 446-454 [PMID: 20189503 DOI: 10.1016/j.gie.2009.10.027]
8 **Sugimoto T**, Okamoto M, Mitsuno Y, Kondo S, Ogura K, Ohmae T, Mizuno H, Yoshida S, Isomura Y, Yamaji Y, Kawabe T, Omata M, Koike K. Endoscopic submucosal dissection is an effective and safe therapy for early gastric neoplasms: a multicenter feasible study. *J Clin Gastroenterol* 2012; **46**: 124-129 [PMID: 21959325 DOI: 10.1097/MCG.0b013e31822f3988]
9 **Saito Y**, Uraoka T, Yamaguchi Y, Hotta K, Sakamoto N, Ikematsu H, Fukuzawa M, Kobayashi N, Nasu J, Michida T, Yoshida S, Ikehara H, Otake Y, Nakajima T, Matsuda T, Saito D. A prospective, multicenter study of 1111 colorectal endoscopic submucosal dissections (with video). *Gastrointest Endosc* 2010; **72**: 1217-1225 [PMID: 21030017 DOI: 10.1016/j.gie.2010.08.004]
10 **Oda I**, Saito D, Tada M, Iishi H, Tanabe S, Oyama T, Doi T, Otani Y, Fujisaki J, Ajioka Y, Hamada T, Inoue H, Gotoda T, Yoshida S. A multicenter retrospective study of endoscopic resection for early gastric cancer. *Gastric Cancer* 2006; **9**: 262-270 [PMID: 17235627 DOI: 10.1007/s10120-006-0389-0]
11 **Chung IK**, Lee JH, Lee SH, Kim SJ, Cho JY, Cho WY, Hwangbo Y, Keum BR, Park JJ, Chun HJ, Kim HJ, Kim JJ, Ji SR, Seol SY. Therapeutic outcomes in 1000 cases of endoscopic submucosal dissection for early gastric neoplasms: Korean ESD Study Group multicenter study. *Gastrointest Endosc* 2009; **69**: 1228-1235 [PMID: 19249769 DOI: 10.1016/j.gie.2008.09.027]
12 **Akasaka T**, Nishida T, Tsutsui S, Michida T, Yamada T, Ogiyama H, Kitamura S, Ichiba M, Komori M, Nishiyama O, Nakanishi F, Zushi S, Nishihara A, Iijima H, Tsujii M, Hayashi N. Short-term outcomes of endoscopic submucosal dissection (ESD) for early gastric neoplasm: multicenter survey by osaka university ESD study group. *Dig Endosc* 2011; **23**: 73-77 [PMID: 21198921 DOI: 10.1111/j.1443-1661.2010.01062.x]
13 **Gotoda T**, Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225 [PMID: 11984739]
14 **Lee IL**, Lin PY, Tung SY, Shen CH, Wei KL, Wu CS. Endoscopic submucosal dissection for the treatment of intraluminal gastric subepithelial tumors originating from the muscularis propria layer. *Endoscopy* 2006; **38**: 1024-1028 [PMID: 17058168 DOI: 10.1055/s-2006-944814]
15 **Shi Q**, Zhong YS, Yao LQ, Zhou PH, Xu MD, Wang P. Endoscopic submucosal dissection for treatment of esophageal submucosal tumors originating from the muscularis propria layer. *Gastrointest Endosc* 2011; **74**: 1194-1200 [PMID: 21963065]
16 **Li QL**, Yao LQ, Zhou PH, Xu MD, Chen SY, Zhong YS, Zhang YQ, Chen WF, Ma LL, Qin WZ. Submucosal tumors of the esophagogastric junction originating from the muscularis propria layer: a large study of endoscopic submucosal dissection (with video). *Gastrointest Endosc* 2012; **75**: 1153-1158 [PMID: 22459663]
17 **Inoue H**, Ikeda H, Hosoya T, Onimaru M, Yoshida A, Eleftheriadis N, Maselli R, Kudo S. Submucosal endoscopic tumor resection for subepithelial tumors in the esophagus and cardia. *Endoscopy* 2012; **44**: 225-230 [PMID: 22354822]
18 **Gong W**, Xiong Y, Zhi F, Liu S, Wang A, Jiang B. Preliminary experience of endoscopic submucosal tunnel dissection for upper gastrointestinal submucosal tumors. *Endoscopy* 2012; **44**: 231-235 [PMID: 22354823]
19 **Hwang JC**, Kim JH, Kim JH, Shin SJ, Cheong JY, Lee KM, Yoo BM, Lee KJ, Cho SW. Endoscopic resection for the treatment of gastric subepithelial tumors originated from the muscularis propria layer. *Hepatogastroenterology* 2009; **56**: 1281-1286 [PMID: 19950778]
20 **Probst A**, Pommer B, Golger D, Anthuber M, Arnholdt H, Messmann H. Endoscopic submucosal dissection in gastric neoplasia - experience from a European center. *Endoscopy* 2010; **42**: 1037-1044 [PMID: 20972955 DOI: 10.1055/s-0030-1255668]
21 **Probst A**, Golger D, Arnholdt H, Messmann H. Endoscopic submucosal dissection of early cancers, flat adenomas, and submucosal tumors in the gastrointestinal tract. *Clin Gastroenterol Hepatol* 2009; **7**: 149-155 [PMID: 19032991 DOI: 10.1016/j.cgh.2008.09.005]
22 **Dinis-Ribeiro M**, Pimentel-Nunes P, Afonso M, Costa N, Lopes C, Moreira-Dias L. A European case series of endoscopic submucosal dissection for gastric superficial lesions. *Gastrointest Endosc* 2009; **69**: 350-355 [PMID: 19185696 DOI: 10.1016/j.gie.2008.08.035]
23 **Ahn JY**, Jung HY, Choi KD, Choi JY, Kim MY, Lee JH, Choi KS, Kim do H, Song HJ, Lee GH, Kim JH, Park YS. Endoscopic and oncologic outcomes after endoscopic resection for early gastric cancer: 1370 cases of absolute and extended indications. *Gastrointest Endosc* 2011; **74**: 485-493 [PMID: 21741645 DOI: 10.1016/j.gie.2011.04.038]
24 **Ahn JY**, Choi KD, Choi JY, Kim MY, Lee JH, Choi KS, Kim do H, Song HJ, Lee GH, Jung HY, Kim JH. Procedure time of endoscopic submucosal dissection according to the size and location of early gastric cancers: analysis of 916 dissections performed by 4 experts. *Gastrointest Endosc* 2011; **73**: 911-916 [PMID: 21296348 DOI: 10.1016/j.gie.2010.11.046]
25 **Imagawa A**, Okada H, Kawahara Y, Takenaka R, Kato J, Kawamoto H, Fujiki S, Takata R, Yoshino T, Shiratori Y. Endoscopic submucosal dissection for early gastric cancer: results and degrees of technical difficulty as well as success. *Endoscopy* 2006; **38**: 987-990 [PMID: 17058162 DOI: 10.1055/s-2006-944716]
26 **Cho KB**, Jeon WJ, Kim JJ. Worldwide experiences of endoscopic submucosal dissection: not just Eastern acrobatics. *World J Gastroenterol* 2011; **17**: 2611-2617 [PMID: 21677828 DOI: 10.3748/wjg.v17.i21.2611]
27 **Farhat S**, Chaussade S, Ponchon T, Coumaros D, Charachon A, Barrioz T, Koch S, Houcke P, Cellier C, Heresbach D, Lepilliez V, Napoleon B, Bauret P, Coron E, Le Rhun M, Bichard P, Vaillant E, Calazel A, Bensoussan E, Bellon S, Mangialavori L, Robin F, Prat F. Endoscopic submucosal dissection in a European setting. A multi-institutional report of a technique in development. *Endoscopy* 2011; **43**: 664-670 [PMID: 21623560 DOI: 10.1055/s-0030-1256413]
28 **Kim ES**, Cho KB, Park KS, Lee KI, Jang BK, Chung WJ, Hwang JS. Factors predictive of perforation during endoscopic submucosal dissection for the treatment of colorectal tumors. *Endoscopy* 2011; **43**: 573-578 [PMID: 21448852 DOI: 10.1055/s-0030-1256339]
29 **Yamaguchi N**, Isomoto H, Fukuda E, Ikeda K, Nishiyama H, Akiyama M, Ozawa E, Ohnita K, Hayashi T, Nakao K, Kohno S, Shikuwa S. Clinical outcomes of endoscopic submucosal dissection for early gastric cancer by indication criteria. *Digestion* 2009; **80**: 173-181 [PMID: 19776581 DOI: 10.1159/000215388]
30 **Lee H**, Yun WK, Min BH, Lee JH, Rhee PL, Kim KM, Rhee JC, Kim JJ. A feasibility study on the expanded indication for endoscopic submucosal dissection of early gastric cancer. *Surg Endosc* 2011; **25**: 1985-1993 [PMID: 21136092 DOI: 10.1007/s00464-010-1499-7]
31 **Jang JS**, Choi SR, Qureshi W, Kim MC, Kim SJ, Jeung JS, Han SY, Noh MH, Lee JH, Lee SW, Baek YH, Kim SH, Choi PJ. Long-term outcomes of endoscopic submucosal dissection in gastric neoplastic lesions at a single institution in South Korea. *Scand J Gastroenterol* 2009; **44**: 1315-1322 [PMID: 19891582 DOI: 10.3109/00365520903254304]

P- Reviewer Kim JC **S- Editor** Gou SX **L- Editor** A
E- Editor Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1962

World J Gastroenterol 2013 March 28; 19(12): 1962-1967
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Curcumin attenuated paracetamol overdose induced hepatitis

Kanjana Somanawat, Duangporn Thong-Ngam, Naruemon Klaikeaw

Kanjana Somanawat, Duangporn Thong-Ngam, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Naruemon Klaikeaw, Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Author contributions: Somanawat K performed the experiments, collected the data and wrote the manuscript; Thong-Ngam D designed the study, performed the experiments, analyzed the data and edited the manuscript; Klaikeaw N coordinated in the pathological examination.
Supported by The 90th Anniversary Fund of Chulalongkorn University, Ratchada Phiseksomphot Endowment Fund and Grant of Ratchadaphiseksomphot, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Correspondence to: Duangporn Thong-Ngam, MD, Associate Professor, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Phyathai Road, Bangkok 10330, Thailand. dr.duangporn@gmail.com
Telephone: +662-256-4267 Fax: +662-256-4267
Received: October 6, 2012 Revised: February 5, 2013
Accepted: February 7, 2013
Published online: March 28, 2013

Abstract

AIM: To investigate whether curcumin could attenuate hepatitis in mice with paracetamol overdose.

METHODS: Male mice were divided into four groups. Group 1 (control, *n* = 8); was fed with distilled water; Group 2 [N-acetyl-P-aminophenol (APAP), *n* = 8]; was fed with a single dose of 400 mg/kg APAP dissolved in distilled water; Group 3 [APAP + curcumin (CUR) 200, *n* = 8], was fed with a single dose of 400 mg/kg APAP and 200 mg/kg CUR; Group 4 (APAP + CUR 600, *n* = 8), was fed with a single dose of 400 mg/kg APAP and 600 mg/kg CUR. Twenty-four hours later, the liver was removed to examine hepatic glutathione (GSH), hepatic malondialdehyde (MDA), and histopathologically. Then whole blood was withdrawn from heart to determine transaminase (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase) and inflammatory cytokines [interleukin (IL)-12 and IL-18] levels by enzyme linked immunosorbent assay.

RESULTS: Serum transaminase, hepatic MDA, and inflammatory cytokines increased significantly in the APAP compared with the control group. Curcumin supplementation in APAP + CUR 200 and APAP + CUR 600 groups significantly decreased these parameters compared with the APAP group. The level of GSH decreased significantly in the APAP compared with the control group. Curcumin supplementation in APAP + CUR 200 and APAP + CUR 600 groups significantly increased these parameters compared with the APAP group. The histological appearance of the liver in the control group showed normal. In the APAP-treated group, the liver showed extensive hemorrhagic hepatic necrosis at all zones. Curcumin supplementation in APAP + CUR 200 and APAP + CUR 600 groups, caused the liver histopathology to improve. In the APAP + CUR 200 group, the liver showed focal necrosis and but the normal architecture was well preserved in APAP + CUR 600 group.

CONCLUSION: APAP overdose can cause liver injury. Results indicate that curcumin prevents APAP-induced hepatitis through the improvement of liver histopathology by decreased oxidative stress, reduced liver inflammation, and restoration of GSH.

Key words: N-acetyl-P-aminophenol; Curcumin; Oxidative stress; Hepatitis; Interleukin-12; Interleukin-18

Somanawat K, Thong-Ngam D, Klaikeaw N. Curcumin attenuated paracetamol overdose induced hepatitis. *World J Gastroenterol* 2013; 19(12): 1962-1967 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1962.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1962

INTRODUCTION

In therapeutic doses, N-acetyl-P-aminophenol (APAP) or paracetamol is mainly metabolized *via* glucuronidation and sulfation pathway and in conjugated forms is excreted from the cells. Besides, APAP is partly metabolized by cytochrome P450 (CYP 2E1), to produce toxic metabolites such as N-acetyl-p-benzoquinone imine. These metabolites are produced in the liver and detoxified by reduced glutathione (GSH) and then removed from cells. In APAP overdose, it is mainly metabolized by CYP 2E1 and causes increase of toxic metabolites and GSH depletion. These metabolites interact with biomolecules such as protein, lipid, and nucleic acid *via* covalent binding, which disrupts hepatocytes function causing hepatic necrosis and liver injury[1-6].

A previous study demonstrated that APAP overdose treatment showed significantly increase in serum transaminase, hepatic malondialdehyde (MDA), and decreased hepatic GSH. Histological examination showed a severe centrilobular hepatic necrosis with fatty changes[7-9].

Curcumin (diferuloymethane), a polyphenol, is an active ingredient of turmeric (*Curcuma longa*) and is pharmacologically safe for humans and animals. Curcumin has many biological activities, including anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, and anti-diabetic activities[10-13]. The hepatoprotection of curcumin has been widely acknowledged and used in traditional medicines for treatment of inflammatory conditions such as hepatitis[14].

A previous study demonstrated that curcumin treatment showed significantly decrease in serum transaminase, hepatic MDA, increase hepatic GSH, and caused improvement of liver histopathology[7-9].

However, it is still unclear whether curcumin has any effect in APAP-induced hepatotoxicity. Therefore, the present study aims to examine the protective effect of curcumin on hepatitis in mice with APAP overdose.

METERIALS AND METHODS

Animal preparation

Male mice (4-5 wk), weighing 25-30 g, were purchased from the National Laboratory Animal Center, Mahidol University (Bangkok, Thailand). They were acclimatized at least 1 wk in a climate-controlled room on a 12-h light-dark cycle and were fed *ad libitum*. The experimental protocol was approved by the Ethical Committee of Faculty of Medicine, Chulalongkorn University, Thailand.

Paracetamol and curcumin preparation

A single dose of 400 mg/kg of APAP (Tylenol®) was dissolved in distilled water that was freshly prepared for the experiment. A single dose of 200 and 600 mg/kg of curcumin (95% purified curcumin, Cayman Chemical Company, Ann Arbor, MI, United States) were dissolved in corn oil that was freshly prepared for the experiment.

Experimental protocol

All mice were fasted, with free access to water *ad libitum*, for 18 h before the experiment. They were randomly divided into four experimental groups.

Group 1 (control, n = 8); mice were fed distilled water orally *via* an intragastric tube; Group 2 (APAP, n = 8); mice were fed a single dose of 400 mg/kg of APAP orally *via* an intragastric tube; Group 3 [APAP + curcumin (CUR) 200, n = 8]; mice were fed a single dose of 400 mg/kg of APAP with a single dose of 200 mg/kg of curcumin orally *via* an intragastric tube; Group 4 (APAP + CUR 600, n = 8); mice were fed a single dose of 400 mg/kg of APAP with a single dose of 600 mg/kg of curcumin orally *via* an intragastric tube.

Twenty-four hours later, the mice were anesthetized with intraperitoneal injection of thiopental (50 mg/kg body weight). The abdominal wall was incised and liver was removed and washed with cold normal saline (4 ℃ -8 ℃). The liver was chopped into small pieces, frozen in liquid nitrogen, and stored at -80 ℃ to examine hepatic MDA and hepatic GSH. The hepatic MDA was quantified by thiobarbituric acid reaction as described by Ohkawa *et al*[15]. The hepatic GSH was quantified by GSH Assay Kit (Cayman Chemical Company, United States). The remaining liver was fixed in 10% formalin solution to examine histologically. Then whole blood was withdrawn from heart. The blood was allowed to coagulate at room temperature (2 h) and centrifuged for 20 min at 3000 × g to obtain serum. The serum was collected to examine transaminase (serum glutamic oxaloacetic transaminase, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase, SGPT) and inflammatory cytokines interleukin (IL)-12 (R and D Systems, Inc., United States) and IL-18 (Medical and Biological Laboratory Co., Ltd, Japan) by enzyme-linked immunosorbent assay method.

Histopathology

Samples of the liver were excised and transferred to formalin and later processed by routine techniques prior to embedding in paraffin. Sections were cut at the thickness of 5 μm and stained with hematoxylin and eosin (HE). An experienced pathologist blinded to the experiment evaluated all samples. All histopathological changes were observed under light microscope. Hepatic necroinflammation score in each section was graded according to the criteria described by Brunt *et al*[16] from 0 to 3 as follow; Score 0 = No hepatocyte injury/inflammation; Score 1 = Sparse or mild focal zone 3 hepatocyte injury/inflammation; Score 2 = Noticeable zone 3 hepatocyte injury/inflammation; Score 3 = Severe zone 3 hepatocyte injury/inflammation.

Statistical analysis

The data were expressed as mean ± SD. For comparison among all groups of animals, one-way analysis of variance (one-way ANOVA) and Tukey PostHoc comparisons were employed. P-value at less than 0.05 was considered statistically significant. The data were analyzed using the SPSS software version 17.0 for windows.

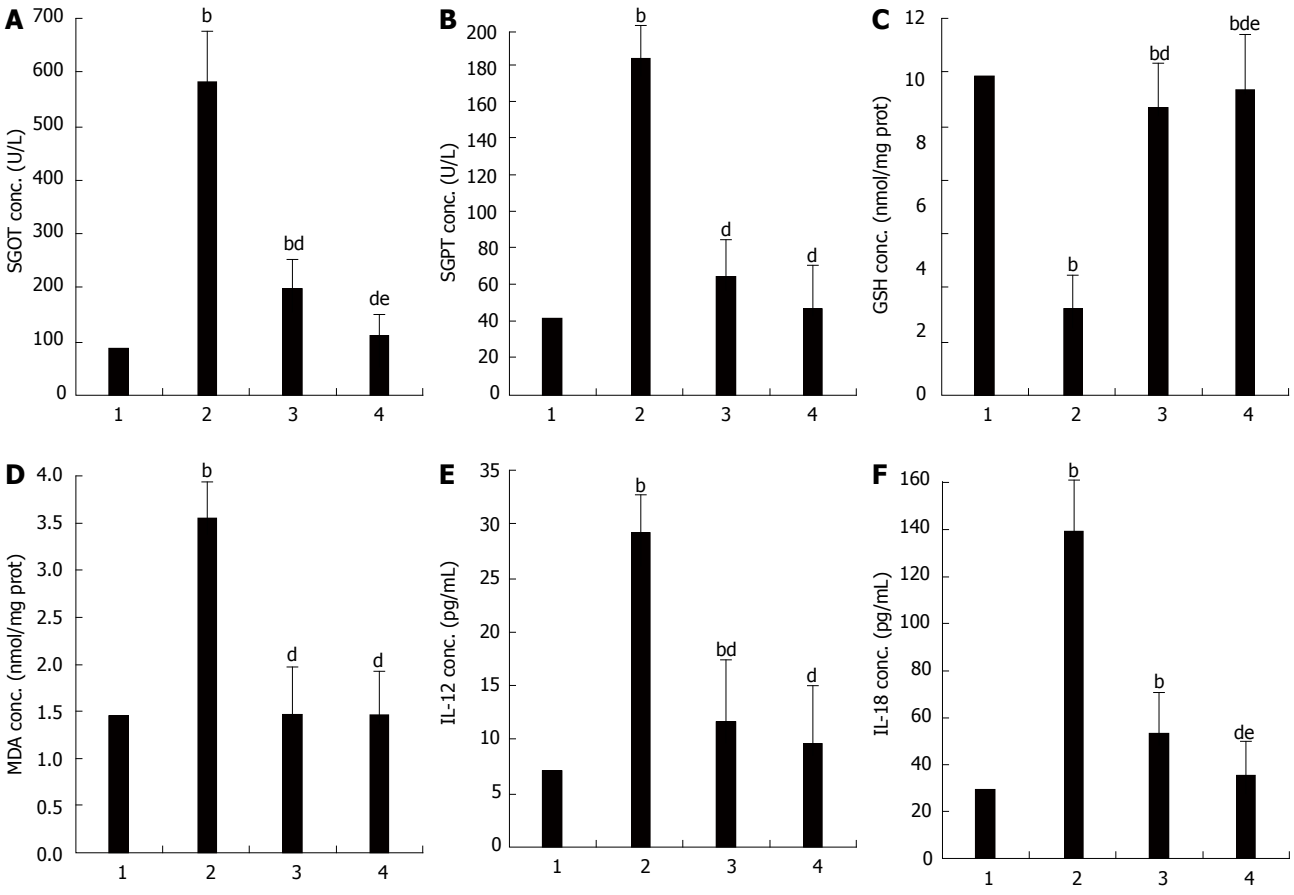


Figure 1 Effects of curcumin on serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, hepatic glutathione, hepatic malondialdehyde, interleukin-12, interleukin-18 in mice with paracetamol overdose. A: Serum glutamic oxaloacetic transaminase (SGOT); B: Serum glutamic pyruvic transaminase (SGPT); C: Hepatic glutathione (GSH); D: Hepatic malondialdehyde (MDA); E: Interleukin (IL)-12; F: IL-18. $^{b}P < 0.01$ *vs* control group; $^{d}P < 0.01$ *vs* N-acetyl-P-aminophenol (APAP) group; $^{e}P < 0.05$ *vs* APAP + curcumin (CUR) 200 group. 1: Control; 2: APAP; 3: APAP + CUR 200; 4: APAP + CUR 600.

RESULTS

Effects of curcumin on serum transaminase in mice with paracetamol overdose

Serum SGOT increased significantly in the APAP when compared to the control group (SGOT, 583.25 ± 118.30 U/L *vs* 86.13 ± 6.90 U/L, $P < 0.001$). These were significantly lower in the APAP + CUR 200 and APAP + CUR 600 groups than that in the APAP group (197.38 ± 14.39 U/L *vs* 538.25 ± 118.30 U/L and 111.38 ± 8.33 U/L *vs* 583.25 ± 118.30 U/L, $P < 0.001$, respectively). There was statistically significant difference in serum SGOT in APAP + CUR 200 and APAP + CUR 600 groups (197.38 ± 14.39 U/L *vs* 111.38 ± 8.33 U/L, $P < 0.05$) (Figure 1A).

Serum SGPT increased significantly in the APAP group when compared to the control group (186.00 ± 43.73 U/L *vs* 42.63 ± 6.95 U/L, $P < 0.001$). They were significantly lower in the APAP + CUR 200 and APAP + CUR 600 groups than in the APAP group (65.25 ± 3.11 U/L *vs* 186.00 ± 43.73 U/L and 47.50 ± 4.72 U/L *vs* 186.00 ± 43.73 U/L, $P < 0.001$, respectively). There was no statistically significant difference in serum SGPT in APAP + CUR 200 and APAP + CUR 600 groups (65.25 ± 3.11 U/L *vs* 47.50 ± 4.72 U/L, $P > 0.05$) (Figure 1B).

Effects of curcumin on hepatic GSH in mice with paracetamol overdose

Hepatic GSH were significantly lower in the APAP group compared to the control group (2.75 ± 0.16 nmol/mg protein *vs* 10.17 ± 0.11 nmol/mg protein, $P < 0.001$). Hepatic GSH in APAP + CUR 200 and APAP + CUR 600 groups were significantly higher than in the APAP group (9.16 ± 0.49 nmol/mg protein *vs* 2.75 ± 0.16 nmol/mg protein and 9.72 ± 0.22 nmol/mg protein *vs* 2.75 ± 0.16 nmol/mg protein, $P < 0.001$, respectively). There was statistically significant difference in hepatic GSH in APAP + CUR 200 and APAP + CUR 600 groups (9.16 ± 0.49 nmol/mg protein *vs* 9.72 ± 0.22 nmol/mg protein, $P < 0.05$) (Figure 1C).

Effects of curcumin on hepatic MDA in mice with paracetamol overdose

Hepatic MDA was elevated significantly in the APAP group when compared to the control group (3.55 ± 0.05 nmol/mg protein *vs* 1.45 ± 0.01 nmol/mg protein, $P < 0.001$). Hepatic MDA in APAP + CUR 200 and APAP + CUR 600 groups were significantly lower than in the APAP group (1.47 ± 0.01 nmol/mg protein *vs* 3.55 ± 0.05 nmol/mg protein and 1.46 ± 0.01 nmol/mg protein *vs* 3.55 ± 0.05 nmol/mg protein, $P < 0.001$, respectively). There

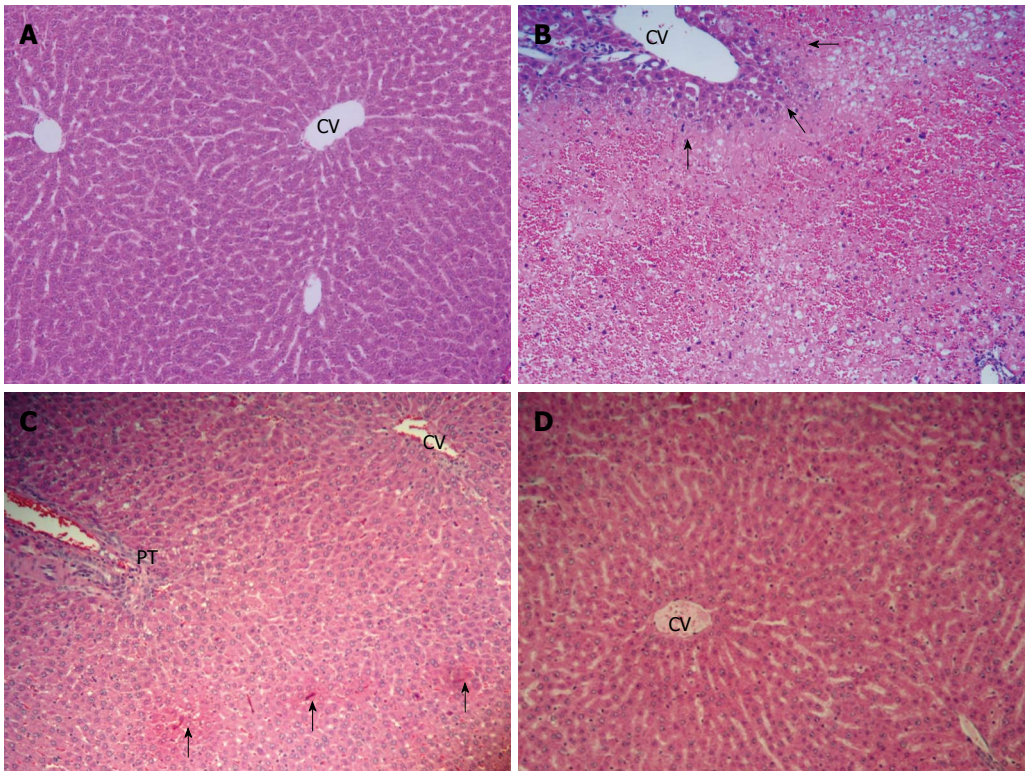


Figure 2 Effects of curcumin improved liver histopathology (hematoxylin and eosin stain, ×10). A: Control group showed normal; B: N-acetyl-P-aminophenol (APAP) group showed extensive hemorrhagic hepatic necrosis at all zones; C: APAP + curcumin (CUR) 200 group showed mild focal necrosis and hepatic lobule was preserved; D: APAP + CUR 600 group showed the hepatic lobule was preserved with limited hepatic change. Arrows indicate hepatic necrosis, CV indicate central vein, and PT indicate portal system.

was no statistically significant difference in hepatic MDA in APAP + CUR 200 and APAP + CUR 600 groups (1.47 ± 0.01 nmol/mg protein *vs* 1.46 ± 0.01 nmol/mg protein, $P > 0.05$) (Figure 1D).

Effects of curcumin on serum IL-12 and IL-18 in mice with paracetamol overdose

The level of serum IL-12 increased significantly in the APAP group compared with the control group (29.16 ± 3.34 pg/mL *vs* 7.08 ± 1.40 pg/mL, $P < 0.001$). Serum IL-12 significantly decreased in the APAP + CUR 200 and APAP + CUR 600 groups compared with the APAP group (11.60 ± 1.68 pg/mL *vs* 29.16 ± 3.34 pg/mL and 9.63 ± 1.38 pg/mL *vs* 29.16 ± 3.34 pg/mL, $P < 0.001$, respectively). There was no statistically significant difference in serum IL-12 in APAP + CUR 200 and APAP + CUR 600 groups (11.60 ± 1.68 pg/mL *vs* 9.63 ± 1.38 pg/mL, $P > 0.05$) (Figure 1E).

The level of serum IL-18 increased significantly in the APAP group compared with the control group (139.52 ± 15.59 pg/mL *vs* 29.17 ± 3.72 pg/mL, $P < 0.001$). Serum IL-18 significantly decreased in the APAP + CUR 200 and APAP + CUR 600 groups compared with the APAP group (53.48 ± 18.19 pg/mL *vs* 139.52 ± 15.59 pg/mL and 35.21 ± 2.18 pg/mL *vs* 139.52 ± 15.59 pg/mL, $P < 0.001$, respectively). There was statistically significant difference in serum IL-18 in APAP + CUR 200 and APAP + CUR 600 groups (53.48 ± 18.19 pg/mL *vs* 35.21 ± 2.18 pg/mL, $P < 0.05$) (Figure 1F).

Effects of curcumin on histopathology in mice with paracetamol overdose

The histological appearance of the liver in the control group showed normal (Figure 2A). In the APAP group, the liver showed extensive hemorrhagic hepatic necrosis of all zones (Figure 2B). In APAP + CUR 200 and APAP + CUR 600 groups, the liver histopathology improved. The APAP + CUR 200 group showed focal necrosis (Figure 2C), whereas the majority of hepatic lobules were preserved as normal architecture with limited hepatic change in the APAP + CUR 600 groups (Figure 2D). The summary of hepatic necroinflammation score in the control and experimental groups are shown in Table 1.

DISCUSSION

This study demonstrated that treatment of APAP overdose induced hepatitis in mice and could be attenuated by treatment with curcumin. This result corresponds to previous observations studied in mice and rat models[7-9].

Chemoprevention is promising as a preventive approach for hepatitis. Curcumin (diferuloylmethane), a polyphenol compound, is an active ingredient of turmeric (*Curcuma longa*)[10]. The phenolic and methoxy groups on the benzene rings of curcumin are important structural features that contribute to its anti-oxidant properties and its ability to reduce the amount of free radicals[17-20]. Curcumin shows beneficial effects in inflammatory conditions including hepatitis[14].

To assess lipid peroxidation we used MDA as a marker[21] and reduced GSH as indicator of hepatoprotectivity for cells. In this study the oxidative damage caused by APAP overdose was significantly attenuated by administrating curcumin. It was conceivable that curcumin could protect against free radical mediated oxidative stress by scavenging for free radicals that limits lipid peroxidation

Table 1 Summary of the hepatic necroinflammation score in all groups

Group	*n*	Hepatic necroinflammation scores			
		0	1	2	3
Control	8	8	-	-	-
APAP	8	-	1	1	6
APAP + CUR 200	8	5	2	1	-
APAP + CUR 600	8	5	3	-	-

Data are expressed as the number of mice exhibiting each score of hepatic necroinflammation (n = 8). Hepatic necroinflammation score in each section was graded according to the criteria described by Brunt *et al*[16] from 0 to 3 as follow; Score 0: No hepatocyte injury/inflammation; Score 1: Sparse or mild focal zone 3 hepatocyte injury/inflammation; Score 2: Noticeable zone 3 hepatocyte injury/inflammation; Score 3: Severe zone 3 hepatocyte injury/inflammation. 0 = None; 1 = Mild; 2 = Moderate; 3 = Severe; APAP: N-acetyl-P-aminophenol; CUR: Curcumin.

involved in membrane damage. In this study the GSH depletion caused by APAP overdose was significantly attenuated by administrating curcumin. The protective action of curcumin can be explained by GSH inducer through induction of glutathione reductase enzyme system.

Serum transaminase, SGOT and SGPT, are often used as markers as their increase indicates liver damage[22,23]. There is a significant increase of both SGOT and SGPT in APAP overdose. We demonstrated that this increase was reduced by the administration of curcumin.

It has been reported that IL-12 is produced by dendritic cells, monocytes, Langerhans cells, neutrophils, and keratinocytes[24]. IL-18 is produced by activated macrophages, keratinocytes, intestinal epithelial cells, oeteoblasts, adrenal cortex cells, and the murine diencephalon. IL-18 is synthesized as a precursor 24 kilodalton molecule without a signal peptide and must be cleaved to produce an active molecule. IL-1 converting enzyme (or caspase-1) cleaves pro-IL-18, producing the mature, bioactive peptide that is readily released from cells[25-29]. Importantly, both of these cytokines are produced from Kupffer cells or hepatic macrophages in the liver. It may be possible that inflammation is due to hepatocytes releasing cytokines into the blood stream. Furthermore, IL-12, in combination with IL-18, causes inflammation *via* the activity of interferon-gamma, which is produced from T-lymphocyte and NK cells.

Curcumin is modulates the inflammatory response by down-regulating the activity of COX-2, inducible NO synthase, tumor necrosis factor-α, IL-1, IL-2, IL-6 and IL-8[30]. This shows that curcumin is an anti-inflammatory substance. These cytokines are required for the expression of many cells linked with the inflammatory response. However, there are no experiments which study the effect of curcumin in preventing hepatitis resulting from APAP overdose and influence the levels of cytokines IL-12 and IL-18. Therefore, we studied the effect of curcumin on decreasing hepatitis resulting from APAP overdose. We showed that curcumin could prevent hepatitis resulting from APAP overdose and cause decrease in IL-12 and IL-18 levels. It may be possible that curcumin can inhibit caspase-1 enzyme, which is cleaved pro-IL-18 into bioactive peptide or active-IL-18[26]. This could explain that another pathway may also reduce liver inflammation that is indirectly mediated by oxidative stress inhibition.

In the present study, APAP overdose caused extensively hepatic necrosis. In curcumin supplementation, liver histopathology improved and showed only a focal hepatic necrosis of lobules.

In conclusion, APAP overdose can cause liver injury. Our results show curcumin could attenuate APAP-induced liver injury by decrease oxidative stress, reduce liver inflammation, restore hepatic GSH, and improve liver histopathology. In addition, curcumin at the dose of 600 mg/kg tends to be more potent than 200 mg/kg in preventing the effects of APAP hepatotoxicity. Hence, curcumin might be a novel therapeutic strategy against hepatitis caused by APAP overdose.

COMMENTS

Background

N-acetyl-P-aminophenol (APAP) overdose induced liver damage is one of the most widespread drug-induced side-effects. Although the exact mechanism of APAP remains largely unknown, it appears to involve two pathways: direct hepatotoxicity and adverse immune reactions. This impairment of liver functions can culminate in cell death, leading to a variety of pathological conditions, including acute hepatitis. Curcumin has been shown to possess a wide spectrum of biological actions. These include anti-inflammatory and anti-oxidant activities. Authors postulated that curcumin, acting through the oxidative stress inhibition, could reduce the production of inflammatory cytokines; thus resulting in the attenuation of liver injury in APAP-induced hepatitis in mice.

Research frontiers

Curcumin (diferuloymethane) is the main yellow bioactive component of turmeric (*Curcuma longa*). It has been shown to possess a wide spectrum of biological actions by the inhibition of oxidative stress and reduction of inflammatory cytokines. APAP can cause liver injury through the increase in oxidative stress and release of inflammatory cytokines leading to liver injury. The hallmark of this study was that we showed an attenuation of liver damage and decrease in oxidative stress, reduced liver inflammation, restoration of hepatic glutathione (GSH), and improved liver histopathology after curcumin administration in APAP-treated animals.

Innovations and breakthroughs

The previous study showed that curcumin is an anti-inflammatory and anti-oxidant agent in an *in vivo* study. However, it is unclear whether curcumin has any effect in APAP-induced hepatitis *in vivo*. Therefore, in this study, authors examined the protective effects of curcumin in APAP-induced liver damage in mice and authors found that curcumin could attenuate APAP-induced liver injury through the decrease in oxidative stress, reduce liver inflammation, restoration of hepatic GSH, and improve liver histopathology.

Applications

Curcumin might be a novel therapeutic strategy against hepatitis caused by APAP overdose.

Peer review

This is an interesting study of the effects of curcumin on APAP-induced hepatitis in mice. This study showed the effects of curcumin in attenuation of APAP-induced hepatitis reflected in attenuated levels of hepatic malondialdehyde, transaminase, inflammatory cytokines, hepatic GSH, and improved liver histopathology.

REFERENCES

1 **Hart SG,** Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD. Acetaminophen nephrotoxicity in CD-1 mice.

I. Evidence of a role for in situ activation in selective covalent binding and toxicity. *Toxicol Appl Pharmacol* 1994; **126**: 267-275 [PMID: 8209379 DOI: 10.1006/taap.1994.1116]
2 **Bessems JG,** Vermeulen NP. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. *Crit Rev Toxicol* 2001; **31**: 55-138 [PMID: 11215692 DOI: 10.1080/20014091111677]
3 **Masubuchi Y,** Suda C, Horie T. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. *J Hepatol* 2005; **42**: 110-116 [PMID: 15629515 DOI: 10.1016/j.jhep.2004.09.015]
4 **McCuskey RS,** Bethea NW, Wong J, McCuskey MK, Abril ER, Wang X, Ito Y, DeLeve LD. Ethanol binging exacerbates sinusoidal endothelial and parenchymal injury elicited by acetaminophen. *J Hepatol* 2005; **42**: 371-377 [PMID: 15710220 DOI: 10.1016/j.jhep.2004.11.033]
5 **Mladenović D,** Radosavljević T, Ninković M, Vucević D, Jesić-Vukićević R, Todorović V. Liver antioxidant capacity in the early phase of acute paracetamol-induced liver injury in mice. *Food Chem Toxicol* 2009; **47**: 866-870 [PMID: 19271286 DOI: 10.1016/j.fct.2009.01.020]
6 **Ishibe T,** Kimura A, Ishida Y, Takayasu T, Hayashi T, Tsuneyama K, Matsushima K, Sakata I, Mukaida N, Kondo T. Reduced acetaminophen-induced liver injury in mice by genetic disruption of IL-1 receptor antagonist. *Lab Invest* 2009; **89**: 68-79 [PMID: 19002106 DOI: 10.1038/labinvest.2008.110]
7 **Somanawat K,** Thong-Ngam D, Klaikeaw N. Effects of curcumin attenuated hepatitis in mice with paracetamol overdose. *Thai J Gastroenterol* 2012; **13**: 43-49
8 **Kheradpezhouh E,** Panjehshahin MR, Miri R, Javidnia K, Noorafshan A, Monabati A, Dehpour AR. Curcumin protects rats against acetaminophen-induced hepatorenal damages and shows synergistic activity with N-acetyl cysteine. *Eur J Pharmacol* 2010; **628**: 274-281 [PMID: 19919835 DOI: 10.1016/j.ejphar.2009.11.027]
9 **Yousef MI,** Omar SA, El-Guendi MI, Abdelmegid LA. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. *Food Chem Toxicol* 2010; **48**: 3246-3261 [PMID: 20804811 DOI: 10.1016/j.fct.2010.08.034]
10 **Maheshwari RK,** Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. *Life Sci* 2006; **78**: 2081-2087 [PMID: 16413584 DOI: 10.1016/j.lfs.2005.12.007]
11 **Murathanum R,** Thong-Ngam D, Klaikaew N. Curcumin Prevents Indomethacin-induced Acute Gastric Mucosal Damage in Rats. *Thai J Gastroenterol* 2008; **9**: 118-123
12 **Sintara K,** Thong-Ngam D, Patumraj S, Klaikeaw N, Chatsuwan T. Curcumin suppresses gastric NF-kappaB activation and macromolecular leakage in Helicobacter pylori-infected rats. *World J Gastroenterol* 2010; **16**: 4039-4046 [PMID: 20731017 DOI: 10.3748/wjg.v16.i32.4039]
13 **Cerný D,** Lekić N, Váňová K, Muchová L, Hořínek A, Kmoníčková E, Zídek Z, Kameníková L, Farghali H. Hepatoprotective effect of curcumin in lipopolysaccharide/-galactosamine model of liver injury in rats: relationship to HO-1/CO antioxidant system. *Fitoterapia* 2011; **82**: 786-791 [PMID: 21545828 DOI: 10.1016/j.fitote.2011.04.003]
14 **Samuhasaneeto S,** Thong-Ngam D, Kulaputana O, Suyasunanont D, Klaikeaw N. Curcumin decreased oxidative stress, inhibited NF-kappaB activation, and improved liver pathology in ethanol-induced liver injury in rats. *J Biomed Biotechnol* 2009; **2009**: 981963 [PMID: 19606259 DOI: 10.1155/2009/981963]
15 **Ohkawa H,** Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-358 [PMID: 36810 DOI: 10.1016/0003-2697(79)90738-3]
16 **Brunt EM,** Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010 DOI: 10.1111/j.1572-0241.1999.01377.x]
17 **Sreejayan N,** Rao MN. Free radical scavenging activity of curcuminoids. *Arzneimittelforschung* 1996; **46**: 169-171 [PMID: 8720307]
18 **Sreejayan MN.** Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol* 1997; **49**: 105-107 [PMID: 9120760 DOI: 10.1111/j.2042-7158.1997.tb06761.x]
19 **Joe B,** Lokesh BR. Role of capsaicin, curcumin and dietary n-3 fatty acids in lowering the generation of reactive oxygen species in rat peritoneal macrophages. *Biochim Biophys Acta* 1994; **1224**: 255-263 [PMID: 7981240 DOI: 10.1016/0167-4889(94)90198-8]
20 **Brouet I,** Ohshima H. Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem Biophys Res Commun* 1995; **206**: 533-540 [PMID: 7530002]
21 **Del Rio D,** Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutr Metab Cardiovasc Dis* 2005; **15**: 316-328 [PMID: 16054557 DOI: 10.1016/j.numecd.2005.05.003]
22 **Hinson JA,** Roberts DW, James LP. Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol* 2010; (196): 369-405 [PMID: 20020268 DOI: 10.1007/978-3-642-00663-0_12]
23 **Thapa BR,** Walia A. Liver function tests and their interpretation. *Indian J Pediatr* 2007; **74**: 663-671 [PMID: 17699976 DOI: 10.1007/s12098-007-0118-7]
24 **Gately MK,** Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998; **16**: 495-521 [PMID: 9597139 DOI: 10.1146/annurev.immunol.16.1.495]
25 **Nakanishi K,** Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001; **19**: 423-474 [PMID: 11244043 DOI: 10.1146/annurev.immunol.19.1.423]
26 **Fantuzzi G,** Dinarello CA. Interleukin-18 and interleukin-1 beta: two cytokine substrates for ICE (caspase-1). *J Clin Immunol* 1999; **19**: 1-11 [PMID: 10080100 DOI: 10.1023/A:1020506300324]
27 **Thong-Ngam D,** Tangkijvanich P, Lerknimitr R, Mahachai V, Theamboonlers A, Poovorawan Y. Diagnostic role of serum interleukin-18 in gastric cancer patients. *World J Gastroenterol* 2006; **12**: 4473-4477 [PMID: 16874857]
28 **Tangkijvanich P,** Thong-Ngam D, Mahachai V, Theamboonlers A, Poovorawan Y. Role of serum interleukin-18 as a prognostic factor in patients with hepatocellular carcinoma. *World J Gastroenterol* 2007; **13**: 4345-4349 [PMID: 17708609]
29 **Finotto S,** Siebler J, Hausding M, Schipp M, Wirtz S, Klein S, Protschka M, Doganci A, Lehr HA, Trautwein C, Khosravi-Far R, Strand D, Lohse A, Galle PR, Blessing M, Neurath MF. Severe hepatic injury in interleukin 18 (IL-18) transgenic mice: a key role for IL-18 in regulating hepatocyte apoptosis in vivo. *Gut* 2004; **53**: 392-400 [PMID: 14960523 DOI: 10.1136/gut.2003.018572]
30 **Jurenka JS.** Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa: a review of preclinical and clinical research. *Altern Med Rev* 2009; **14**: 141-153 [PMID: 19594223]

P- Reviewer Rege RV **S- Editor** Gou SX **L- Editor** A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1968

World J Gastroenterol 2013 March 28; 19(12): 1968-1974
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Loss of BRCA1 expression leads to worse survival in patients with gastric carcinoma

Zi-Zhen Zhang, Yuan Jie Charles Liu, Xiao-Lu Yin, Ping Zhan, Yi Gu, Xing-Zhi Ni

Zi-Zhen Zhang, Xing-Zhi Ni, Department of General Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China
Yuan Jie Charles Liu, Xiao-Lu Yin, Ping Zhan, Yi Gu, AstraZeneca, Innovation Center China, Shanghai 201203, China
Author contributions: Zhang ZZ and Liu YJ contributed equally to this work; Zhang ZZ, Liu YJ, Yin XL, Gu Y and Ni XZ designed the research; Zhang ZZ and Liu YJ performed the research; Zhang ZZ, Liu YJ, Yin XL and Zhan P analyzed the data; Zhang ZZ and Liu YJ co-wrote the paper.
Supported by AstraZeneca
Correspondence to: Xing-Zhi Ni, MD, Professor of Medicine, Department of General Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200127, China. niyin@yahoo.com
Telephone: +86-21-61092234 Fax: +86-21-61097711
Received: October 22, 2012 Revised: January 16, 2013
Accepted: January 23, 2013
Published online: March 28, 2013

Abstract

AIM: To investigate the expression deficiency of key molecular markers in the homologous recombination pathway.

METHODS: Expression loss of breast cancer type 1 susceptibility protein (BRCA1), ataxia telangiectasia mutated (ATM), ATM-Rad3-related (ATR), mediator of DNA damage checkpoint protein 1 (MDC1) and meiotic recombination 11 (Mre11) were correlated with their clinicopathological parameters in gastric cancer (GC). One hundred and twenty treatment-naive GC samples were formalin-fixed and paraffin-embedded into tissue blocks. Two representative cores from each block were extracted and constructed into tissue microarrays. Expression levels of BRCA1, ATM, ATR, MDC1 and Mre11 were determined using immunohistochemical analysis, and correlated with clinical parameters, including age, gender, Lauren subtype, tumor grades, clinical stage and overall survival.

RESULTS: Expression loss of BRCA1, ATM, ATR, MDC1, and Mre11 was found in 21.4%, 20.2%, 21.0%, 11.1% and 4.6%, respectively, of interpretable cases. BRCA1 loss was significantly associated with patients of diffused subtype (intestinal *vs* diffused, 8.2% *vs* 31.7%, $P = 0.001$), higher tumor grade (Ⅰ/Ⅱ *vs* Ⅲ, 10.7% *vs* 20.5; Ⅰ/Ⅱ *vs* Ⅳ, 10.7% *vs* 54.5%, $P = 0.047$) and advanced clinical stage (Ⅰ/Ⅱ *vs* Ⅲ, 12.9% *vs* 16.9%; Ⅰ/Ⅱ *vs* Ⅳ, 12.9% *vs* 45.5%, $P = 0.006$). MDC1 loss was significantly associated with patients of diffused subtype (intestinal *vs* diffused, 0% *vs* 19.7%, $P = 0.001$) and higher tumor grade (Ⅰ/Ⅱ *vs* Ⅲ, 0% *vs* 12%; Ⅰ/Ⅱ *vs* Ⅳ, 0% *vs* 30.8%, $P = 0.012$). In addition, the survival time of the patients with expression loss of BRCA1 was significantly shorter than those with positive expression of BRCA1 (2-year survival rate, 32.4% *vs* 62.8%, $P = 0.015$). No correlations were found between clinicopathological parameters and expression loss of ATM, ATR and Mre11.

CONCLUSION: Our results support the hypothesis that homologous recombination deficiency plays an important role in the progression of gastric carcinoma. Loss of expression of BRCA1 and MDC1 may serve as predictive factors in tumor development or progression in GC patients.

Key words: Homologous recombination deficiency; Gastric cancer; Breast cancer type 1 susceptibility protein; Mediator of DNA damage checkpoint protein 1; Ataxia telangiectasia mutated; Ataxia telangiectasia mutated-Rad3-related

Zhang ZZ, Liu YJ, Yin XL, Zhan P, Gu Y, Ni XZ. Loss of BRCA1 expression leads to worse survival in patients with gastric carcinoma. *World J Gastroenterol* 2013; 19(12): 1968-1974 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1968.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1968

INTRODUCTION

DNA lesions constantly threaten the integrity of our genome. Of the major DNA lesions, double-strand DNA breaks (DSBs) pose the most dangerous threat[1]. DSBs occur when both complimentary strands of DNA break simultaneously, and failure to repair these DSBs can result in chromosomal aberrations including mutations, deletions, amplifications, translocations, all of which can lead to cancer predispositions. Cells employ two major pathways to repair DSBs: homologous recombination (HR) and non-homologous end joining (NHEJ). HR and NHEJ differ mainly in two aspects. First, they differ in the frequency of errors that occur during DSB repairs. NHEJ employs a direct ligation mechanism that is highly error-prone, while HR utilizes the genomic information stored in homologous strands to proof-read the repair process and thus is essentially error-free. Second, the two pathways differ in the cell cycles in which they are primarily involved. NHEJ is most commonly found in G0 and G1 phases; meanwhile HR predominates in S and G2 phases, which are two critical stages that require high-fidelity transmission of genetic information. Attributed to its error-free mechanism and deployment in key cell-cycle phases, HR plays a central role in the protection against DSBs and hence is crucial in maintaining the genomic stability of the cells[2].

A complex and hierarchical network of proteins is implicated in the HR pathway to detect, signal and repair DSBs. In this network, breast cancer type 1 susceptibility protein (BRCA1), ataxia telangiectasia mutated (ATM), ATM-Rad3-related (ATR), mediator of DNA damage checkpoint protein 1 (MDC1) and meiotic recombination 11 (Mre11) are most important functionally. In brief, ATM/ATR located at the top of the signaling cascades act as the core sensors of DSBs[3] by collaborating with other sensor molecules, including MDC1[4] and the complex of MRE11-Rad50-NBSI[5]. Downstream substrates that are involved in checkpoint activation, among them BRCA1/2[6], are then phosphorylated by ATM and ATR, causing cell cycle arrest until DSBs are repaired[7].

Defects in the HR pathway or homologous recombination deficiency (HRD) directly compromise the genomic stability and predispose to cancer formation[8]. The relationship between HRD and development of many cancer types has been well established[9]. Genetic aberrations of BRCA1/2, the most widely studied markers in the HR pathway, have been found to promote both tumor initiation and progression[10,11]. These genetic abnormalities, together with BRCA1/2 protein loss, were reported in many carcinomas[12], especially in breast cancer (BC) and ovarian cancer (OC). In BC, *BRCA1/BRCA2* mutations are responsible for 3%-8% of all cases and 30%-40% of familial cases. Ten percent of patients with OC have a genetic predisposition. About 80% of families with a history of OC have *BRCA1* mutations, while 15% have *BRCA2* mutations[13]. Aberrations in ATM function are linked with head and neck squamous cell carcinoma[14], chronic lymphocytic leukemia[15], colorectal cancer[16], BC and OC[17]. Genetic alterations of ATR were frequently reported in BC and OC[18-20]. Dysfunctional MDC1 was implicated in BC development[21,22] among other cancer types[23,24]. Abnormal Mre11 signaling is strongly linked with BC, with mutations and protein loss found to be associated with BC pathogenesis[25-28].

These HRD tumors also demonstrated enhanced sensitivity toward DNA-damaging agents, through the so-called "synthetic lethality"[29]. These specific populations of tumor cells, under DNA-damaging agents, such as poly (ADP-ribose) polymerase (PARP) inhibitors[30], are unable to recruit the necessary cellular machinery for the repair of DSBs and will undergo apoptosis. Pre-clinical studies of PARP inhibitors had raised the expectations for this highly selective therapeutic approach in HRD patients although these hypotheses need to be further validated in the clinical studies[31,32]. Therefore, it is useful to understand the status of HRD-specific markers in different tumor types, such as GC.

GC is the second leading cause of cancer-related death worldwide and is particularly prevalent in Asia. Previous reports suggested that HRD could play a role in the carcinogenesis of the stomach[33-37]. Yet, HRD's prognostic perspective in GC has not been fully explored and this study aims to address these questions. In order to assess the involvement of HRD in gastric tumorigenesis, we have analyzed the immunohistochemical expression of BRCA1, ATM, ATR, MDC1 and Mre11 in 120 GC samples and correlated them with clinicopathological parameters.

MATERIALS AND METHODS

Clinical samples and patient information

One hundred and twenty formalin-fixed and paraffin-embedded (FFPE) tissue samples were collected from Shanghai Renji Hospital for the study. All patients underwent radical resection between 2007 and 2010. The median age of the patients (82 males and 38 females) was 61.3 years (range: 22-87 years). All tumor tissues were diagnosed with gastric adenocarcinomas by two qualified pathologists.

Immunohistochemistry

GC tumor tissue and adjacent non-tumor tissue samples were collected after surgery following standard FFPE procedure. Tissue microarray (TMA) was then made with 2 representative cores withdrawn from FFPE block for each case. Four μm-thick tissue sections were cut from TMA for immunohistochemical (IHC) study. The slides were baked at 56 ℃ for 1 h, then de-paraffinized in xylene for 20 min and rehydrated through a graded series of ethanol concentrations (5 min in 100% ethanol first, followed by 5 min in 70% ethanol). Antigen retrieval was done in pressure cooker for 5 min using Target Retrieval Solution (Dako, Copenhagen, Denmark). Endogenous peroxidase activity was blocked by Peroxidase Blocking Reagent (Dako, Copenhagen, Denmark) for 5 min. Primary antibodies (ATM, 1:50, Epitomics, cat. No. 1549-1; ATR,

Table 1 Association between expression loss of homologous recombination markers and clinicopathological parameters in gastric cancer patients *n* (%)

	BRCA1 expression (*n* = 112)		ATM expression (*n* = 114)		ATR expression (*n* = 86)		MDC1 expression (*n* = 117)		Mre11 expression (*n* = 86)	
	BRCA1-negative/ total cases	*P* value	ATM-negative/ total cases	*P* value	ATR-negative/ total cases	*P* value	MDC1-negative/ total cases	*P* value	Mre11-negative/ total cases	*P* value
Age, yr (median)										
< 61.3	15 (25.0)	0.043	9 (16.1)	0.133	8 (19.5)	0.193	8 (14.3)	0.625	2 (4.9)	0.119
⩾ 61.3	9 (17.3)		14 (24.1)		10 (22.2)		5 (8.2)		2 (4.4)	
Gender										
Male	15 (19.7)	0.284	15 (19.5)	0.715	12 (21.1)	0.969	10 (12.7)	0.430	3 (5.0)	0.333
Female	9 (25.0)		8 (21.6)		6 (20.7)		3 (7.9)		1 (3.4)	
Lauren type										
Intestinal	4 (8.2)	0.001	12 (24.0)	0.846	8 (21.6)	0.891	0 (0.0)	0.001	2 (5.0)	0.303
Diffused	20 (31.7)		11 (17.2)		10 (20.4)		13 (19.7)		2 (4.3)	
Tumor grade										
Ⅰ / Ⅱ	3 (10.7)	0.047	7 (24.1)	0.513	5 (29.4)	0.327	0 (0.0)	0.012	1 (4.2)	0.742
Ⅲ	15 (20.5)		15 (20.5)		10 (16.7)		9 (12.0)		3 (5.8)	
Ⅳ	6 (54.5)		1 (8.3)		3 (33.3)		4 (30.8)		0 (0.0)	
Clinical stage										
Ⅰ / Ⅱ	4 (12.9)	0.006	6 (19.4)	0.560	6 (23.1)	0.593	3 (9.4)	0.092	2 (7.7)	0.562
Ⅲ	10 (16.9)		11 (17.7)		7 (16.7)		5 (7.7)		2 (4.2)	
Ⅳ	10 (45.5)		6 (28.6)		5 (27.8)		5 (25.0)		0 (0.0)	

BRCA1: Breast cancer type 1 susceptibility protein; ATM: Ataxia telangiectasia mutated; ATR: ATM-Rad3-related; MDC1: Mediator of DNA damage checkpoint protein 1; Mre11: Meiotic recombination 11.

1:100, Santa-Cruz Technology, cat. No. sc-1887; BRCA1, 1:100, Merck, cat. No. OP92; MDC1, 1:500, Sigma, cat. No. M2444; Mre11, 1:200, Abcam, cat. No. ab214) were then applied to cover the specimen for 1 h at room temperature, followed by incubation with labeled polymer-HRP anti-rabbit or anti-mouse secondary antibody (Dako, Copenhagen, Denmark) for 30 min at room temperature. Thorough rinsing with TBST was done after incubation with each reagent. The slides were visualized using DAB substrate-chromagen (Dako, Copenhagen, Denmark) and washed with deionized water before counterstaining with haematoxylin. The slides were then dehydrated through a graded series of ethanol concentrations, cleared in xylene and coverslipped in DPX mounting medium.

Immunohistochemical scoring

The intensity of the staining in the nuclear of tumor cells was recorded. Scoring was established as follows: 0, if absence of staining was observed; 1+, if the tumor cells had weak staining; 2+, if tumor cells had moderate staining; and 3+, if tumor cells had strong staining. Tumors with 1+, 2+ and 3+ expression were interpreted as positive and tumors with no expression (0 score) were interpreted as expression loss. Given the heterogeneity of protein expression in tumor cells, the highest scoring from either one of TMA cores was counted as the final result.

Statistical analysis

The analysis was conducted with SPSS 16.0 software. Characteristics of the two groups were compared using the χ^2 likelihood ratio test. Logistic regression model was applied to interrogate association of IHC data and individual clinical parameter. The Kaplan-Meier method was used to estimate the survival distributions. The log-rank test was used to compare the survival distributions. Two-sided *P* values < 0.05 were considered statistically significant.

RESULTS

Among the 120 cases, 69.2% of tumors (83/120) involved the ventricular sinuses, 14.1% (17/120) involved the ventricle corpora and 16.7% (20/120) involved the cardia in the stomach. All tumor samples were diagnosed with adenocarcinoma with different tumor grade and Lauren subtypes.

The overall follow-up rate is 87% with a median follow-up time of 32 mo. At the time of analysis, 49.2% (49/120) patients were alive and 50.8% (61/120) patients died. The overall 2-year survival rate was 54.2%. Loss of BRCA1 expression was observed in 21.4% (24/112), ATM in 20.2% (23/114), ATR in 20.9% (18/86), MDC1 in 11.1% (13/117), and Mre11 in 4.7% (4/86) of the GC patients (Figure 1). Clinicopathological parameters and expression of HRD biomarkers in the samples are displayed in Table 1.

Expression loss of each marker and its correlation with clinicopatholofical parameters

The clinicopathological parameters of patients in the study included age, gender, Lauren type, tumor grade and clinical stage according to 2010 World Health Organization tumor-node-metastesis classification. Statistical analysis of IHC data and clinicopathological parameters are shown in Table 1. Loss of ATM, ATR and Mre11 expression was not associated with gender or clinical stage. BRCA1 loss was significantly associated with patients of diffused subtype ($P = 0.001$), higher tumor grade ($P = 0.047$) and advanced clinical stage ($P = 0.006$). MDC1

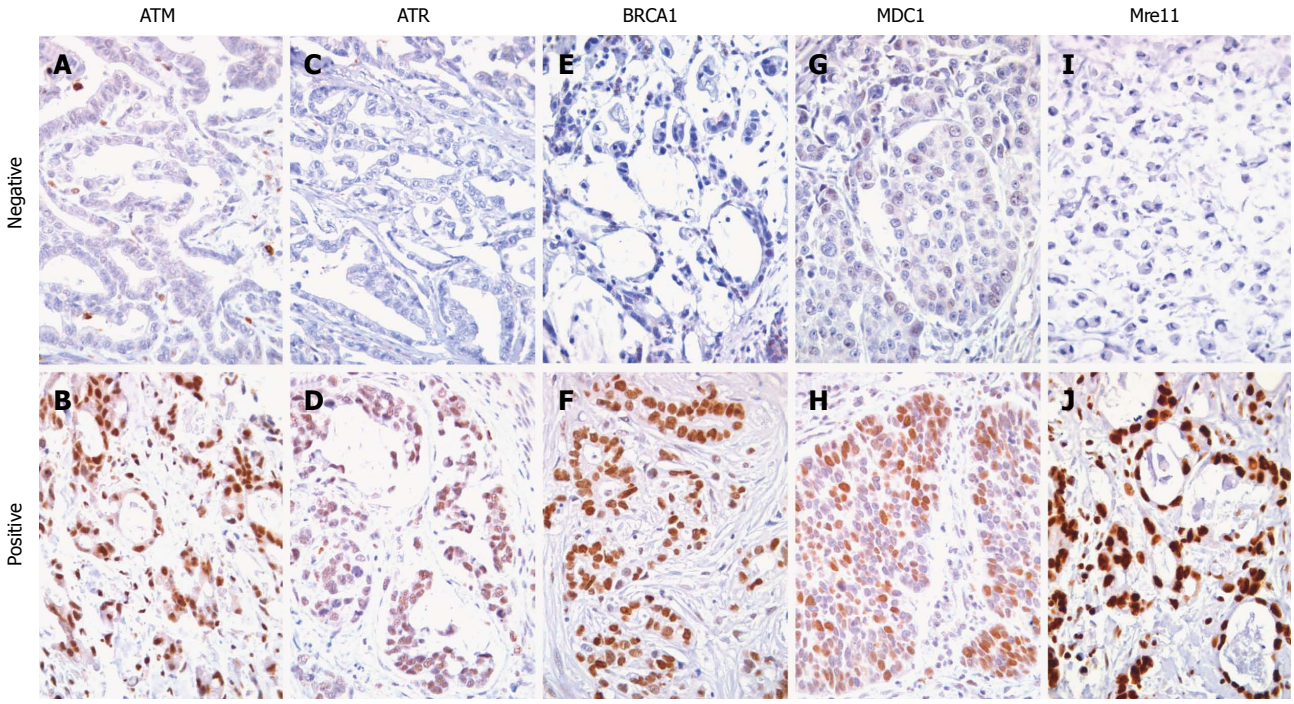


Figure 1 Immunohistochemical expression of ataxia telangiectasia mutated, ataxia telangiectasia mutated-Rad3-related, breast cancer type 1 susceptibility protein, mediator of DNA damage checkpoint protein 1 and meiotic recombination 11 in gastric cancer tissues. ATM: Ataxia telangiectasia mutated; ATR: Ataxia telangiectasia mutated-Rad3-related; BRCA1: Breast cancer type 1 susceptibility protein 1; MDC1: Mediator of DNA damage checkpoint protein 1; Mre11: Meiotic recombination 11. 3,3'-Diaminobenzidine staining, ×200.

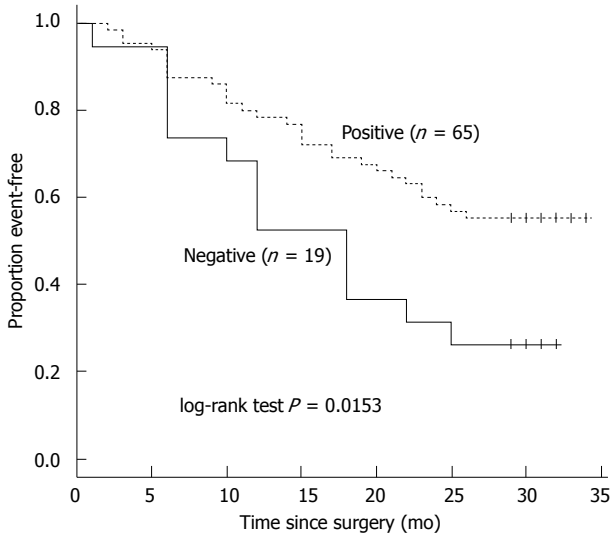


Figure 2 Negative effect of breast cancer type 1 susceptibility protein loss on patient overall survival. The survival time of the patients with positive expression of breast cancer type 1 susceptibility protein (BRCA1) was significantly longer than those with negative expression of BRCA1.

loss was significantly associated with patients of diffused subtype ($P = 0.001$) and higher tumor grade ($P = 0.012$).

Correlation between BRCA1 expression and survival

Expression loss of BRCA1 was significantly associated with the progression of the GC patients. The survival time of the patients with BRCA1 expression loss was significantly shorter than BRCA1-positive patients (2-year survival rate, 32.4% *vs* 62.8%, $P = 0.015$; Figure 2). Expression of the other four markers was not significantly associated with survival ($P > 0.05$).

Combined biomarker analysis

Twenty-seven (51.9%, 27/52) cases had positive expression of all 5 protein kinases (HR+ group) and 25 (48.1%, 25/52) cases had expression loss of at least one protein kinase (HRD group). Significant difference of tumor grade was observed between the two groups, with the HRD group showing significant association with higher tumor grades ($P = 0.013$). But there was no significant difference in gender, Lauren type or clinical stage. Survival analysis also showed no significant difference between the two groups.

DISCUSSION

Gastric cancer is one of the leading causes of cancer-related death worldwide, and although the incidence has decreased in Western countries, Asia remains the specific high-risk area. Various reports have suggested that HRD could play a role in gastric tumorigenesis. However, a systematic analysis of the key markers in the HR pathway is largely missing. In the present study, the expression losses of the five key markers, namely BRCA1, ATM, ATR, MDC1 and Mre11 were correlated with the clinicopathological parameters in a cohort of Chinese GC patients.

Recent studies of the relationship between BRCA1

and tumors mainly focused on BC and OC. The frequency of BRCA1 mutations among breast cancer patients is less than 5%[38], while the loss of BRCA1 protein expression is higher at around 20%[39]. However, BRCA1 expression in gastric cancer has rarely been studied. Our data showed that BRCA1 expression deficiency occurred in 24/112 (21.4%) GC patients. BRCA1 deficiency was significantly associated with patients of diffused Lauren type, higher tumor grades and advanced clinical stage. Patients with BRCA1 deficiency lived significantly shorter ($P = 0.015$) than those patients with positive expression of BRCA1, indicating that loss of BRCA1 can serve as a prognostic marker. Mutations of *BRCA1* in gastric cancer were not found commonly[40]. Rather, microsatellite instability and loss-of-heterozygosity of *BRCA1* gene at locus D17S855 were shown to be the predominant genetic abnormalities found in GC[41]. Both of these genetic instabilities may lead to the reduction or loss of the functional BRCA1 protein. Recently, a high frequency of hypermethylation on the BRCA1 promoter was found in tumor tissues and these epigenetic changes correlated with the loss or reduction of protein expression[42]. These reports together with our data, suggest that BRCA1 protein loss may be a suitable indicator of cancer development in GC.

Lack of reports on *MDC1* mutations suggests that down-regulation of the marker at the protein level may serve as a better prognostic marker. MDC1 protein loss/reduction was previously described[22], although its correlation with survival was not assessed. Patel *et al*[21] addressed this question by profiling MDC1 in subsets of early-stage BC patients who underwent breast-conserving surgery and radiation therapy and found that decreased MDC1 was not related to overall survival. However, they found that MDC1 reduction correlated with nodal failure and concluded the role of MDC1 in early cancer development. To our knowledge, our study is the first to assess MDC1 expression in GCs. The strong association between MDC1 deficiency and diffused subtype indicates that MDC1 plays a major role in this subtype's development. In addition, the association between MDC1 and higher tumor grade also suggests that MDC1 deficiency is implicated in GC pathogenesis. Although MDC1 loss failed to establish a significant correlation with survival, the strong linkage of MDC1 loss with diffused type and higher tumor grades warrants further research into this marker.

Our data suggested ATM, ATR and Mre11 deficiencies were commonly found in GC patients. But there was no significant difference in clinicopathological features between the patients with negative and positive expression for each marker. Mutations of *ATM* have been suggested to play a possible role in the carcinogenesis of other cancer types. The rate of *ATM* mutations in advanced GC has been previously studied and although several variants were found, there were no hot spots. In the same study, decreased level of phosphorylated ATM at Ser1981 significantly correlated with poor differentiation, lymph node metastasis and poor 5-year survival[43]. Mutation of *ATR* was previously reported in BC[19], OC[18] and colon cancers[44], but has never been found in GC. In addition, protein loss of ATR has never been studied in GC and we report here for the first time that protein loss of ATR is a common feature in GC. We investigated whether Mre11 mutation could play a role in GC. In a previous study[45] that correlated MRE11 poly(T)11 mutations with clinicopathological features, a significant association was found only in patients with a family history of GC. In addition, the authors demonstrated that this *MRE11* mutation was associated with absent or strongly reduced Mre11 immunostaining, indicating that protein loss of Mre11 may be a suitable surrogate for the detection of Mre11-related HRD in GC. In our study, the same antibody (Clone 12D7) for the detection of Mre11 was used and the results agreed with those from the previous studies.

In the combined biomarker analysis, we found significant difference in tumor grade between the HR+ and HRD groups, under the assumption that loss of one protein kinase is sufficient to cause a non-functional HR pathway. Our data suggested that HR deficiency played an important role in the GC pathogenesis but is not necessarily crucial in gastric tumor maintenance. Further work will be done to address whether significant association would appear when a larger patient population and a longer follow-up time are available.

These results have made possible the clinical use of DNA-damaging agents in HRD GCs, although finding markers that could predict response is still a daunting challenge[46]. While most of the PARP inhibitors in BC and OC employed *BRCA1/2*-mutation as the patient selection criteria[47,48], this may not be the best strategy in GC, as protein loss is evidently the driver. For the other HRD biomarkers, their prognostic and predictive values need to be further investigated. In our opinion, unless they are validated in both pre-clinical and clinical settings, BRCA1 remains the strongest predictor of response to compounds that are exploiting the HRD pathway.

COMMENTS

Background

DNA lesions constantly threaten the integrity of our genome. Of the major DNA lesions, double-strand DNA breaks (DSBs) pose the most dangerous threat. DSBs occur when both complimentary strands of DNA break simultaneously, and failure to repair these DSBs can result in chromosomal aberrations, including mutations, deletions, amplifications and translocations, all of which can lead to cancer predispositions.

Research frontiers

Various reports have suggested that homologous recombination deficiency (HRD) could play a role in gastric tumorigenesis. However, a systematic analysis of the key markers in the homologous recombination pathway is largely missing. In the present study, the expression losses of the five key markers, namely breast cancer type 1 susceptibility protein (BRCA1), ataxia telangiectasia mutated, ataxia telangiectasia mutated-Rad3-related, mediator of DNA damage checkpoint protein 1 and meiotic recombination 11, were correlated with the clinicopathological parameters in a cohort of Chinese gastric carcinoma (GC) patients.

Innovations and breakthroughs

The results have made possible the clinical use of DNA-damaging agents in HRD GCs, although finding markers that could predict response is still a daunting challenge.

Applications

For the other HRD biomarkers, their prognostic and predictive values need to be further investigated. In author's opinion, unless they are validated in both pre-clinical and clinical settings, BRCA1 remains the strongest predictor of response to compounds that are exploiting the HRD pathway.

Peer review

This is an interesting study in which authors investigated the expression deficiency of key molecular markers in the HR pathway. The results are interesting and suggest that homologous recombination deficiency plays an important role in the progression of GC.

REFERENCES

1 **van Gent DC**, Hoeijmakers JH, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* 2001; **2**: 196-206 [PMID: 11256071 DOI: 10.1038/35056049]

2 **Krejci L**, Altmannova V, Spirek M, Zhao X. Homologous recombination and its regulation. *Nucleic Acids Res* 2012; **40**: 5795-5818 [PMID: 22467216 DOI: 10.1093/nar/gks270]

3 **Tichý A**, Vávrová J, Pejchal J, Rezácová M. Ataxia-telangiectasia mutated kinase (ATM) as a central regulator of radiation-induced DNA damage response. *Acta Medica* (Hradec Kralove) 2010; **53**: 13-17 [PMID: 20608227]

4 **Stewart GS**, Wang B, Bignell CR, Taylor AM, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. *Nature* 2003; **421**: 961-966 [PMID: 12607005 DOI: 10.1038/nature01446]

5 **Stracker TH**, Petrini JH. The MRE11 complex: starting from the ends. *Nat Rev Mol Cell Biol* 2011; **12**: 90-103 [PMID: 21252998 DOI: 10.1038/nrm3047]

6 **Powell SN**, Kachnic LA. Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. *Oncogene* 2003; **22**: 5784-5791 [PMID: 12947386 DOI: 10.1038/sj.onc.1206678]

7 **Shiloh Y**. ATM and related protein kinases: safeguarding genome integrity. *Nat Rev Cancer* 2003; **3**: 155-168 [PMID: 12612651 DOI: 10.1038/nrc1011]

8 **Hoeijmakers JH**. DNA damage, aging, and cancer. *N Engl J Med* 2009; **361**: 1475-1485 [PMID: 19812404 DOI: 10.1056/NEJMra0804615]

9 **Helleday T**. Homologous recombination in cancer development, treatment and development of drug resistance. *Carcinogenesis* 2010; **31**: 955-960 [PMID: 20351092 DOI: 10.1093/carcin/bgq064]

10 **O'Donovan PJ**, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. *Carcinogenesis* 2010; **31**: 961-967 [PMID: 20400477 DOI: 10.1093/carcin/bgq069]

11 **Miki Y**, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 1994; **266**: 66-71 [PMID: 7545954]

12 **Roy R**, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer* 2012; **12**: 68-78 [PMID: 22193408 DOI: 10.1038/nrc3181]

13 **Lux MP**, Fasching PA, Beckmann MW. Hereditary breast and ovarian cancer: review and future perspectives. *J Mol Med* (Berl) 2006; **84**: 16-28 [PMID: 16283147 DOI: 10.1007/s00109-005-0696-7]

14 **Lim AM**, Young RJ, Collins M, Fox SB, McArthur GA, Corry J, Peters L, Rischin D, Solomon B. Correlation of Ataxia-Telangiectasia-Mutated (ATM) gene loss with outcome in head and neck squamous cell carcinoma. *Oral Oncol* 2012; **48**: 698-702 [PMID: 22410096 DOI: 10.1016/j.oraloncology.2012.02.014]

15 **Guarini A**, Marinelli M, Tavolaro S, Bellacchio E, Magliozzi M, Chiaretti S, De Propris MS, Peragine N, Santangelo S, Paoloni F, Nanni M, Del Giudice I, Mauro FR, Torrente I, Foà R. ATM gene alterations in chronic lymphocytic leukemia patients induce a distinct gene expression profile and predict disease progression. *Haematologica* 2012; **97**: 47-55 [PMID: 21993670 DOI: 10.3324/haematol.2011.049270]

16 **Gao Y**, Hayes RB, Huang WY, Caporaso NE, Burdette L, Yeager M, Chanock SJ, Berndt SI. DNA repair gene polymorphisms and tobacco smoking in the risk for colorectal adenomas. *Carcinogenesis* 2011; **32**: 882-887 [PMID: 21504893 DOI: 10.1093/carcin/bgr071]

17 **Thorstenson YR**, Roxas A, Kroiss R, Jenkins MA, Yu KM, Bachrich T, Muhr D, Wayne TL, Chu G, Davis RW, Wagner TM, Oefner PJ. Contributions of ATM mutations to familial breast and ovarian cancer. *Cancer Res* 2003; **63**: 3325-3333 [PMID: 12810666]

18 **Heikkinen K**, Mansikka V, Karppinen SM, Rapakko K, Winqvist R. Mutation analysis of the ATR gene in breast and ovarian cancer families. *Breast Cancer Res* 2005; **7**: R495-R501 [PMID: 15987455 DOI: 10.1186/bcr1037]

19 **Durocher F**, Labrie Y, Soucy P, Sinilnikova O, Labuda D, Bessette P, Chiquette J, Laframboise R, Lépine J, Lespérance B, Ouellette G, Pichette R, Plante M, Tavtigian SV, Simard J. Mutation analysis and characterization of ATR sequence variants in breast cancer cases from high-risk French Canadian breast/ovarian cancer families. *BMC Cancer* 2006; **6**: 230 [PMID: 17010193 DOI: 10.1186/1471-2407-6-230]

20 **Monsees GM**, Kraft P, Chanock SJ, Hunter DJ, Han J. Comprehensive screen of genetic variation in DNA repair pathway genes and postmenopausal breast cancer risk. *Breast Cancer Res Treat* 2011; **125**: 207-214 [PMID: 20496165 DOI: 10.1007/s10549-010-0947-3]

21 **Patel AN**, Goyal S, Wu H, Schiff D, Moran MS, Haffty BG. Mediator of DNA damage checkpoint protein 1 (MDC1) expression as a prognostic marker for nodal recurrence in early-stage breast cancer patients treated with breast-conserving surgery and radiation therapy. *Breast Cancer Res Treat* 2011; **126**: 601-607 [PMID: 20521098 DOI: 10.1007/s10549-010-0960-6]

22 **Bartkova J**, Horejsí Z, Sehested M, Nesland JM, Rajpert-De Meyts E, Skakkebaek NE, Stucki M, Jackson S, Lukas J, Bartek J. DNA damage response mediators MDC1 and 53BP1: constitutive activation and aberrant loss in breast and lung cancer, but not in testicular germ cell tumours. *Oncogene* 2007; **26**: 7414-7422 [PMID: 17546051 DOI: 10.1038/sj.onc.1210553]

23 **Yuan C**, Bu Y, Wang C, Yi F, Yang Z, Huang X, Cheng L, Liu G, Wang Y, Song F. NFBD1/MDC1 is a protein of oncogenic potential in human cervical cancer. *Mol Cell Biochem* 2012; **359**: 333-346 [PMID: 21853275 DOI: 10.1007/s11010-011-1027-7]

24 **Liu ZK**, Zhu SC, Wang YX. Expression of DNA damage checkpoint mediator 1 and p53-binding protein 1 in human esophageal cancer cell lines TE-1, TE-13, and Eca109. *Nanfang Yike Daxue Xuebao* 2007; **27**: 1314-1317 [PMID: 17884766]

25 **Bartkova J**, Tommiska J, Oplustilova L, Aaltonen K, Tamminen A, Heikkinen T, Mistrik M, Aittomäki K, Blomqvist C, Heikkilä P, Lukas J, Nevanlinna H, Bartek J. Aberrations of the MRE11-RAD50-NBS1 DNA damage sensor complex in human breast cancer: MRE11 as a candidate familial cancer-predisposing gene. *Mol Oncol* 2008; **2**: 296-316 [PMID: 19383352 DOI: 10.1016/molonc.2008.09.007]

26 **Dzikiewicz-Krawczyk A**. The importance of making ends meet: mutations in genes and altered expression of proteins of the MRN complex and cancer. *Mutat Res* 2008; **659**: 262-273 [PMID: 18606567 DOI: 10.1016/j.mrrev.2008.05.005]

27 **Hsu HM**, Wang HC, Chen ST, Hsu GC, Shen CY, Yu JC. Breast cancer risk is associated with the genes encoding the DNA double-strand break repair Mre11/Rad50/Nbs1 com-

plex. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 2024-2032 [PMID: 17932350 DOI: 10.1158/1055-9965.EPI-07-0116]
28 **Angèle S**, Treilleux I, Brémond A, Tanière P, Hall J. Altered expression of DNA double-strand break detection and repair proteins in breast carcinomas. *Histopathology* 2003; **43**: 347-353 [PMID: 14511253]
29 **Helleday T**. The underlying mechanism for the PARP and BRCA synthetic lethality: clearing up the misunderstandings. *Mol Oncol* 2011; **5**: 387-393 [PMID: 21821475 DOI: 10.1016/j.molonc.2011.07.001]
30 **Calvert H**, Azzariti A. The clinical development of inhibitors of poly(ADP-ribose) polymerase. *Ann Oncol* 2011; **22** Suppl 1: i53-i59 [PMID: 21285153 DOI: 10.1093/annonc/mdq667]
31 **Irshad S**, Ashworth A, Tutt A. Therapeutic potential of PARP inhibitors for metastatic breast cancer. *Expert Rev Anticancer Ther* 2011; **11**: 1243-1251 [PMID: 21916578 DOI: 10.101586/era.11.52]
32 **Leung M**, Rosen D, Fields S, Cesano A, Budman DR. Poly(ADP-ribose) polymerase-1 inhibition: preclinical and clinical development of synthetic lethality. *Mol Med* 2011; **17**: 854-862 [PMID: 21424107 DOI: 10.2119/molmed.2010.00240]
33 **Ebi H**, Matsuo K, Sugito N, Suzuki M, Osada H, Tajima K, Ueda R, Takahashi T. Novel NBS1 heterozygous germ line mutation causing MRE11-binding domain loss predisposes to common types of cancer. *Cancer Res* 2007; **67**: 11158-11165 [PMID: 18056440 DOI: 10.1158/0008-5472.CAN-07-1749]
34 **Friedenson B**. BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. *MedGenMed* 2005; **7**: 60 [PMID: 16369438]
35 **Hu H**, Zhang Y, Zou M, Yang S, Liang XQ. Expression of TRF1, TRF2, TIN2, TERT, KU70, and BRCA1 proteins is associated with telomere shortening and may contribute to multistage carcinogenesis of gastric cancer. *J Cancer Res Clin Oncol* 2010; **136**: 1407-1414 [PMID: 20127252 DOI: 10.1007/s00432-010-0795-x]
36 **Toller IM**, Neelsen KJ, Steger M, Hartung ML, Hottiger MO, Stucki M, Kalali B, Gerhard M, Sartori AA, Lopes M, Müller A. Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells. *Proc Natl Acad Sci USA* 2011; **108**: 14944-14949 [PMID: 21896770 DOI: 10.1073/pnas.1100959108]
37 **Falchetti M**, Saieva C, Lupi R, Masala G, Rizzolo P, Zanna I, Ceccarelli K, Sera F, Mariani-Costantini R, Nesi G, Palli D, Ottini L. Gastric cancer with high-level microsatellite instability: target gene mutations, clinicopathologic features, and long-term survival. *Hum Pathol* 2008; **39**: 925-932 [PMID: 18440592 DOI: 10.1016/j.humpath.2007.10.024]
38 **Peshkin BN**, Alabek ML, Isaacs C. BRCA1/2 mutations and triple negative breast cancers. *Breast Dis* 2010; **32**: 25-33 [PMID: 21778580 DOI: 10.3233/BD-2010-0306]
39 **Galizia E**, Giorgetti G, Piccinini G, Santinelli A, Loretelli C, Bianchi F, Gagliardini D, Carbonari G, Pisa E, Belvederesi L, Bracci R, Ferretti C, Corradini F, Cellerino R. BRCA1 expression in triple negative sporadic breast cancers. *Anal Quant Cytol Histol* 2010; **32**: 24-29 [PMID: 20701084]
40 **Semba S**, Yokozaki H, Yasui W, Tahara E. Frequent microsatellite instability and loss of heterozygosity in the region including BRCA1 (17q21) in young patients with gastric cancer. *Int J Oncol* 1998; **12**: 1245-1251 [PMID: 9592181]
41 **Chen XR**, Zhang WZ, Lin XQ, Wang JW. Genetic instability of BRCA1 gene at locus D17S855 is related to clinicopathological behaviors of gastric cancer from Chinese population. *World J Gastroenterol* 2006; **12**: 4246-4249 [PMID: 16830385]
42 **Liu WB**, Ao L, Cui ZH, Zhou ZY, Zhou YH, Yuan XY, Xiang YL, Cao J, Liu JY. Molecular analysis of DNA repair gene methylation and protein expression during chemical-induced rat lung carcinogenesis. *Biochem Biophys Res Commun* 2011; **408**: 595-601 [PMID: 21530494 DOI: 10.1016/j.bbrc.2011.04.067]
43 **Kang B**, Guo RF, Tan XH, Zhao M, Tang ZB, Lu YY. Expression status of ataxia-telangiectasia-mutated gene correlated with prognosis in advanced gastric cancer. *Mutat Res* 2008; **638**: 17-25 [PMID: 17928013 DOI: 10.1016/j.mrfmmm.2007.08.013]
44 **Lewis KA**, Bakkum-Gamez J, Loewen R, French AJ, Thibodeau SN, Cliby WA. Mutations in the ataxia telangiectasia and rad3-related-checkpoint kinase 1 DNA damage response axis in colon cancers. *Genes Chromosomes Cancer* 2007; **46**: 1061-1068 [PMID: 17879369 DOI: 10.1002/gcc.20486]
45 **Ottini L**, Falchetti M, Saieva C, De Marco M, Masala G, Zanna I, Paglierani M, Giannini G, Gulino A, Nesi G, Mariani Costantini R, Palli D. MRE11 expression is impaired in gastric cancer with microsatellite instability. *Carcinogenesis* 2004; **25**: 2337-2343 [PMID: 15319296 DOI: 10.1093/carcin/bgh257]
46 **Patel AG**, De Lorenzo SB, Flatten KS, Poirier GG, Kaufmann SH. Failure of iniparib to inhibit poly(ADP-Ribose) polymerase in vitro. *Clin Cancer Res* 2012; **18**: 1655-1662 [PMID: 22291137 DOI: 10.1158/1078-0432.CCR-11-2890]
47 **Ledermann J**, Harter P, Gourley C, Friedlander M, Vergote I, Rustin G, Scott C, Meier W, Shapira-Frommer R, Safra T, Matei D, Macpherson E, Watkins C, Carmichael J, Matulonis U. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* 2012; **366**: 1382-1392 [PMID: 22452356 DOI: 10.1056/NEJMoa1105535]
48 **Gelmon KA**, Tischkowitz M, Mackay H, Swenerton K, Robidoux A, Tonkin K, Hirte H, Huntsman D, Clemons M, Gilks B, Yerushalmi R, Macpherson E, Carmichael J, Oza A. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *Lancet Oncol* 2011; **12**: 852-861 [PMID: 21862407 DOI: 10.1016/S1470-2045(11)70214-5]

P- Reviewer Joosse SA **S- Editor** Gou SX **L- Editor** Ma JY **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1975

World J Gastroenterol 2013 March 28; 19(12): 1975-1983
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Prognostic assessment of different metastatic lymph node staging methods for gastric cancer after D2 resection

Jia Xu, Yu-Hai Bian, Xin Jin, Hui Cao

Jia Xu, Yu-Hai Bian, Xin Jin, Hui Cao, Department of General Surgery, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200127, China
Author contributions: Xu J performed the majority of the research and wrote the first draft; Bian YH and Jin X contributed to the follow-up of patients and were also involved in editing the manuscript; Cao H was the guarantor and designed the study.
Correspondence to: Dr. Hui Cao, Department of General Surgery, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine, Dongfang Road No. 1630, Shanghai 200127, China. caohuishcn@hotmail.com
Telephone: +86-21-68383751 Fax: +86-21-58394262
Received: October 6, 2012 Revised: February 21, 2013
Accepted: February 28, 2013
Published online: March 28, 2013

Abstract

AIM: To compare the prognostic assessment of lymph node ratio and absolute number based staging system for gastric cancer after D2 resection.

METHODS: The clinical, pathologic, and long-term follow-up data of 427 patients with gastric cancer that underwent D2 curative gastrectomy were retrospectively analyzed. The relationships between the metastatic lymph node ratio (MLR), log odds of positive lymph nodes (LODDS), and positive lymph nodes (pN) staging methods and the long-term prognoses of the patients were compared. In addition, the survival curves, accuracy, and homogeneity were compared with stratification to evaluate the prognostic assessment of the 3 methods when the number of tested lymph nodes was insufficient (< 10 and 10-15).

RESULTS: MLR [hazard ratio (HR) = 1.401, P = 0.012], LODDS (HR = 1.012, P = 0.034), and pN (HR = 1.376, P = 0.005) were independent risk factors for gastric cancer patients. The receiver operating characteristic (ROC) curves showed that the prognostic accuracy of the 3 methods was comparable (P > 0.05). Spearman correlation analysis confirmed that MLR, LODDS, and pN were all positively correlated with the total number of tested lymph nodes. When the number of tested lymph node was < 10, the value of survival curves staged by MLR and LODDS was superior to those of pN staging. However, the difference in survival curves between adjacent stages was not significant. In addition, the survival rate of stage 4 patients using the MLR and LODDS staging methods was 26.7% and 27.3% with < 10 lymph node, respectively which were significantly higher than the survival rate of patients with > 15 tested lymph nodes (< 4%). The ROC curve showed that the accuracy of the prognostic assessment of MLR, LODDS, and pN staging methods was comparable (P > 0.05), and the area under the ROC curve of all 3 methods were increased progressively with the enhanced levels of examined lymph nodes. In addition, the homogeneity of the 3 methods in patients with ≤ 15 tested lymph nodes also showed no significant difference.

CONCLUSION: Neither MLR or LODDS could reduce the staging bias. A sufficient number of tested lymph nodes is key to ensure an accurate prognosis for patients underwent D2 radical gastrectomy.

Key words: Gastric cancer; Metastatic lymph node ratio; Lymph node metastasis; Prognosis

Xu J, Bian YH, Jin X, Cao H. Prognostic assessment of different metastatic lymph node staging methods for gastric cancer after D2 resection. *World J Gastroenterol* 2013; 19(12): 1975-1983 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1975.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1975

INTRODUCTION

Gastric carcinoma is one of the most common cancers in

many Asian countries including South Korea and Japan, and the second most common cause of cancer-related death worldwide[1]. There are nearly 470 000 newly diagnosed cases every year in China. Of these cases, approximately 75% of the patients will die, making gastric cancer the third leading cause of cancer deaths[2]. Because of its long-term efficacy, D2 radical gastrectomy has been accepted in most countries, including those in the Europe and the United States, as the standard surgery for gastric cancer[3-5]. The pathological staging of gastric cancer after D2 radical gastrectomy is not only closely related to the long-term survival of patients but is also the main basis to guide subsequent adjuvant therapy. In the currently accepted criteria of postoperative tumor-node-metastasis (TNM) staging of gastric cancer, the staging of regional lymph node metastasis (N) is of great significance. This staging is currently controversial and changes frequently. In both the latest 7th edition of the American Joint Cancer Committee (AJCC)[6] and the 14th edition of the Statute of Gastric Cancer Treatment in Japan[7] in 2010, the absolute number of positive lymph nodes (pN) in the perigastric region was used as the staging basis, and the staging criteria for each stage were unified. Meanwhile, many studies have supported the N staging by computing the metastatic lymph node ratio. Currently, there are 2 main methods in the staging of relative number of positive lymph nodes, the metastatic lymph node ratio (MLR)[8] and the log odds of positive lymph nodes (LODDS)[9]. The former calculates the ratio of the number of pN over the total number of the tested lymph nodes, while the latter calculates the log value, log[(pnod + 0.5)/(tnod - pnod + 0.5)], of the ratio between positive and negative lymph nodes. Previous studies have shown that, especially when the number of the tested lymph nodes was insufficient, the staging of MLR and LODDS could more accurately assess the prognosis of patients with gastric cancer than staging using the absolute value (pN)[9-13]. However, a unified standard of specific staging for relative number of positive lymph nodes is not currently available, and whether this ratio is superior to the pN staging is also unknown[14,15]. Therefore, the clinical data and long-term follow-up results of the gastric cancer patients that received D2 radical gastrectomy were retrospectively analyzed in this study, and the values of the above staging methods for regional lymph node metastasis in assessing patient prognosis were compared.

MATERIALS AND METHODS

Clinical data

The clinical data of 427 gastric cancer patients who were admitted and underwent standard D2 radical gastrectomy at Affiliated Renji Hospital, Shanghai Jiaotong University School of Medicine from June 2005 to December 2008 and had complete follow-up data were collected. All patients underwent either distal partial gastrectomy, proximal partial gastrectomy or total gastrectomy with regional lymph nodes dissection to D2 with curative intent by the same gastrointestinal professional operation team. However, due to the defects of pathological examination, the number of examined lymph nodes of most patients (65.1%) failed to reach the 7th edition of AJCC requirement, which recommended at least 16 lymph nodes should be retrieved for adequate staging. The clinical and pathological data are shown in Table 1. All surviving cases were followed for 39-81 mo with a median follow-up time of 55 mo. The last follow-up was on March 11, 2012. The overall survival rate was 52.5% for all patients. The survival rate was 38.9% for the patients with lymph node metastasis and 80.6% for the patients without lymph node metastasis. The overall median survival time was 44 mo.

Lymph node staging

Of the 427 patients, those without lymph node metastasis were staged as MLR 0. For the remaining patients, the ratio of the number of pN over the number of tested lymph nodes was calculated, and 20 layers were established from 0 to 1 in 5% intervals. The log-rank test was used to compare differences in the survival curves of 2 adjacent layers. The layers with no differences were merged. Finally, based on prognosis, all patients with lymph node metastases were staged MLR 1-4. Similarly, the patients were staged LODDS 0-4 by the log-rank survival test. The pN staging criteria was defined in accordance to the 2010 AJCC/UICC 7th edition TNM staging criteria. The staging criteria and the number of cases for each group are shown in Table 2.

Statistical analysis

The cumulative survival rate was obtained using a Kaplan-Meier curve, and the differences in cumulative survival rates were compared by the log-rank test. The multivariate prognostic analysis was conducted with the Cox proportional risk regression model. The correlation between MLR, LODDS, and pN, as well as the total number of the tested lymph nodes, was analyzed with the Spearman correlation analysis. The accuracy of the prognosis assessment of each staging method was compared using the receiver operating characteristic curve (ROC) and the area under the curve (AUC). The group in each pN stage was re-grouped in accordance with MLR and LODDS, and the overall survival differences within groups and between groups were analyzed using the log-rank survival test to compare the homogeneity of the 3 staging methods. All statistical analyses were completed with SPSS 17.0 software; $P < 0.05$ was considered significant.

RESULTS

Correlation between MLR, LODDS, and pN and the prognosis of patients with gastric cancer

The results of univariate analysis of the correlation between various prognostic factors related to lymph node status and the prognosis of gastric cancer patients after D2 radical gastrectomy showed that the total number of

Table 1 Clinical and histopathological characteristics of the patients

Factors	*n*
Gender (male/female)	281/146
Age (≤ 60 yr/> 60 yr)	200/227
Site (antrum/body/fundus/others)	234/163/22/8
Size (< 3 cm/3-6 cm/≥ 6 cm)	36/216/175
Histological grade (well/moderately/poorly)	11/313/103
Depth of invasion (T1/T2/≥ T3)	4/79/344
Lymphatic/venous invasion (absence/presence)	359/68
Perineural invasion (absence/presence)	396/31
Examined lymph nodes (< 10/10-15/> 15)	126/152/149

the tested lymph nodes, MLR, LODDS, and pN staging all had an impact on the patient prognosis (Table 3). When the above factors were individually fitted into the Cox proportional risk model, the results showed that MLR [hazard ratio (HR) = 1.401, P = 0.012], LODDS (HR = 1.012, P = 0.034), and pN (HR = 1.376, P = 0.005) were independent risk factors for the prognosis of patients with gastric cancer.

Comparison between MLR, LODDS, and pN staging methods in the prognostic assessment of gastric cancer patients

The 5-year survival of the 427 patients after surgery was used as the gold standard to draw the ROC curve to compare the accuracy of the 3 staging methods in the prognostic assessment of gastric cancer patients. In the groups with no staging, the corresponding area under the curve for MLR, LODDS, and pN was 0.784 ± 0.022, 0.790 ± 0.022, and 0.765 ± 0.023 respectively (Figure 1A), with no significant differences (P > 0.05). In the groups with staging, the corresponding areas under the curve for MLR, LODDS, and pN were 0.775 ± 0.023, 0.767 ± 0.023, and 0.765 ± 0.023, respectively (Figure 1B), with no significant differences.

Correlation between the MLR, LODDS, and pN staging methods and the total number of the tested lymph nodes

The results of Spearman correlation analysis showed that MLR, LODDS, and pN staging were all positively correlated with the total number of the tested lymph nodes, with a correlation coefficient of 0.177, 0.053, and 0.410, respectively, and all P values were < 0.01, which suggested that all of the 3 staging methods were more or less affected by the total mumber of tested lymph nodes. pN was positively correlated with MLR and LODDS with a correlation coefficient of 0.919 and 0.871, respectively, and the P values were both < 0.001.

Assessment value of the MLR, LODDS, and pN staging methods in patients with an insufficient number of tested lymph nodes

Some previous studies have suggested that, for the patients with an insufficient number of tested lymph nodes,

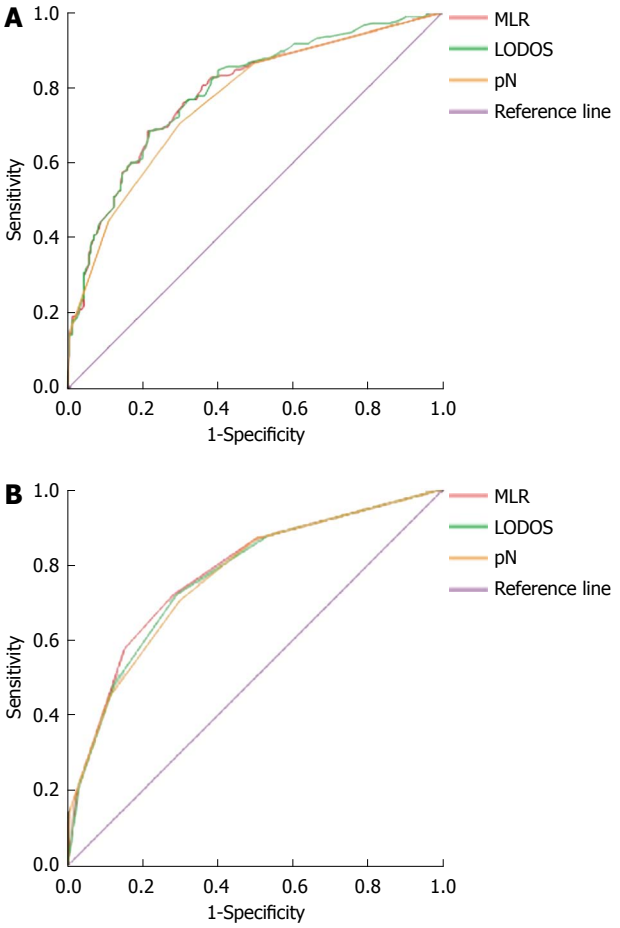


Figure 1 Comparison of receiver operating characteristic curves with metastatic lymph node ratio, log odds of positive lymph nodes, and positive lymph nodes staging methods. A: Receiver operating characteristic (ROC) curves with no staging; B: ROC curves with staging. MLR: Metastatic lymph node ratio; LODDS: Log odds of positive lymph nodes; pN: Positive lymph nodes.

the prognosis-assessment value of MLR staging was superior to that of the staging based on absolute number of positive lymph nodes[9-12]. Therefore, all patients were divided into 3 subgroups according to the total number of tested lymph nodes: the number of the tested lymph nodes was < 10 (n = 126), 10-15 (n = 152) or > 15 (n = 149). A comparison was performed to compare the differences in the postoperative survival curve, the prognostic accuracy, and the homogeneity of the 3 staging methods in the patients with < 15 tested lymph nodes.

Comparison of survival curves

For the patient group with < 10 tested lymph nodes, the 5-year survival rate of patients exhibited a downward trend with the enhanced levels of MLR and LODDS staging. A log-rank test was conducted to compare the difference between adjacent stages, and the results showed that only the difference in survival curves between stage MLR 0 and MLR 1, and stage LODDS 2 and LODDS 3 was significant, with the P values of 0.026 and 0.028 respectively; The difference of remaining survival curves between adjacent stages was not significant. The value of

Table 2 Staging criteria of positive lymph nodes, metastatic lymph node ratio and log odds of positive lymph nodes classifications *n* (%)

Grade	MLR		LODDS		pN	
0	Nr = 0	139 (32.6)	Nr < -1	129 (30.2)	0	139 (32.6)
1	0 < Nr ≤ 0.2	79 (18.5)	-1 ≤ Nr < -0.5	87 (20.4)	1-2	78 (18.3)
2	0.2 < Nr ≤ 0.4	58 (13.6)	-0.5 ≤ Nr < 0	85 (19.9)	3-6	94 (22.0)
3/3a	0.4 < Nr ≤ 0.7	104 (24.4)	0 ≤ Nr < 0.5	76 (17.8)	7-15	86 (20.1)
4/3b	0.7 < Nr ≤ 1	47 (11.0)	Nr ≥ 0.5	50 (11.7)	> 15	30 (7.0)

MLR: Metastatic lymph node ratio; LODDS: Log odds of positive lymph nodes; pN: Positive lymph nodes.

prognostic assessment based on pN staging system was not satisfactory, and the 5-year survival rate for each pN stage was 76.3% for pN 0, 44.4% for pN 1, 34.5% for pN 2, and 45.5% for pN 3a.

For the patient group with 10-15 tested lymph nodes, no significant difference in survival curves between any adjacent stages was found in the subgroup of MLR. Similar with MLR staging, the difference in survival curves also was not significant between any adjacent stages of LODDS.

For the group of patients with > 15 tested lymph nodes, the 5-year survival rates for each stage between MLR, LODDS and were comparable. However, the survival curves of pN staging appeared to better assess prognosis than the ratio-based staging methods, with significant difference in survival curves between any various stages ($P < 0.05$; Figure 2).

Comparison of the accuracy of prognostic assessment

The ROC curves showed that, regardless of staging, the corresponding areas under the curves of the MLR, LODDS, and pN staging methods were all increased progressively with the enhanced levels of examined lymph nodes. the AUC using the MLR, LODDS and pN staging methods increased from 0.716 ± 0.047, 0.718 ± 0.046 and 0.688 ± 0.048 with < 10 lymph node to 0.843 ± 0.031, 0.818 ± 0.034 and 0.836 ± 0.032 with > 15 tested lymph nodes, which were significantly larger than former groups. However, the AUC was not significantly different between the 3 methods within groups.

Comparison of the homogeneity of prognostic assessment

The various pN groups in which patients had < 10 or 10-15 tested lymph nodes were re-grouped according to MLR staging, and the results were shown in Table 4. When the numbers of retrieved lymph nodes were less than 10, only for patients in stage pN 1, the difference in the 5-year survival rate among different MLR stages was significant ($P < 0.05$). Furthermore, there was no significant difference in the 5-year survival rate among the different pN groups within the one MLR group. In 10-15 retrieved-node group, there was no significant difference of 5-year survival rates between the different MLR groups in one pN group. In addition, the difference of

Table 3 Univariate analysis of various prognostic factors correlated to retrieved lymph nodes *n* (%)

Variable		5-yr survival rate	Log rank χ^2 value	*P* value
Examined lymph nodes				
< 10	126 (29.5)	57.1%	4.256	0.039
10-15	152 (35.6)	55.9%		
> 15	149 (34.9)	45.0%		
pN				
0	139 (32.6)	80.6%	97.014	0.000
1-2	78 (18.3)	57.7%		
3-6	94 (22.0)	44.7%		
7-15	86 (20.1)	27.9%		
> 15	30 (7.0)	3.3%		
MLR				
Nr = 0	139 (32.6)	80.6%	103.984	0.000
0 < Nr ≤ 0.2	79 (18.5)	62.0%		
0.2 < Nr ≤ 0.4	58 (13.6)	50.0%		
0.4 < Nr ≤ 0.7	104 (24.4)	26.9%		
0.7 < Nr ≤ 1	47 (11.0)	12.8%		
LODDS				
Nr < -1	129 (30.2)	80.6%	96.214	0.000
-1 ≤ Nr < -0.5	87 (20.4)	63.2%		
-0.5 ≤ Nr < 0	85 (19.9)	43.5%		
0 ≤ Nr < 0.5	76 (17.8)	27.6%		
Nr ≥ 0.5	50 (11.7)	14.0%		

MLR: Metastatic lymph node ratio; LODDS: Log odds of positive lymph nodes; pN: Positive lymph nodes.

5-year survival rates between different pN groups in one MLR group was also not significant.

Because the staging of the patients in MLR 0 (no pN detected) was the same as in pN 0, some studies have stated that the prognostic assessment of LODDS staging was more accurate for these patients[9,16]. Therefore, when comparing the homogeneity of pN staging and LODDS staging, according to different numbers of the tested lymph nodes, the stage pN 0 was divided into two layers. The results showed that the pN0 patients with < 10 tested lymph nodes could be further staged LODDS 0-2, and the 5-year survival rate for the 3 stages was 81.8%, 70.0%, and 66.7%, respectively (Figure 3). However, the log-rank survival test showed that the differences between the 3 stages were not significant ($P = 0.476$). Furthermore, the pN 0 patients with 10-15 tested lymph nodes had the same LODDS stage. Generally, the difference in the 5-year survival rates between the different LODDS groups in one pN group was not significant. In addition, the difference in the 5-year survival rates between the different pN groups in one LODDS group was also not significant (Table 5).

DISCUSSION

Due to the decent long-term survival rate, surgical resection, represented by standard D2 radical gastrectomy, is currently the preferred treatment for gastric cancer. However, in recent years, with the rise of the concept of individualized treatment and the application of new adjunct treatment in clinical practice, an accurate prognostic assessment of patients with gastric cancer after surgery

Table 4 Five-year overall survival of patients with ≤ 15 tested lymph nodes based on positive lymph nodes and metastatic lymph node ratio staging system

		MLR 0		MLR 1		MLR 2		MLR 3		MLR 4		χ^2	*P* value
		n	5-YSR	*n*	5-YSR	*n*	5-YSR	*n*	5-YSR	*n*	5-YSR		
< 10 LN	pN 0	59	76.30%	-	-	-	-	-	-	-	-	-	-
	pN 1	-	-	8	50%	14	57.10%	2	0%	3	0%	22.293	0
	pN 2	-	-	-	-	4	50%	22	31.80%	3	33.30%	0.51	0.775
	pN 3a	-	-	-	-	-	-	3	66.70%	8	37.50%	0.78	0.377
	χ^2	-		-		0.258		9.278		1.658			
	P	-		-		0.611		0.098		0.437			
10-15 LN	pN 0	56	80.40%	-	-	-	-	-	-	-	-	-	-
	pN 1	-	-	24	62.50%	-	-	-	-	-	-	-	-
	pN 2	-	-	1	100%	20	50%	18	44.40%	-	-	0.755	0.686
	pN 3a	-	-	-	-	-	-	23	17.40%	10	20%	0.068	0.794
	χ^2	-		0.836		-		3.613		-			
	P	-		0.658		-		0.057		-			

MLR: Metastatic lymph node ratio; 5-YSR: 5-year survival rate; LN: Examined lymph nodes; pN: Positive lymph nodes.

Table 5 Five-year overall survival of patients with ≤ 15 tested lymph nodes based on positive lymph nodes and log odds of positive lymph nodes staging system

		LODDS 0		LODDS 1		LODDS 2		LODDS 3		LODDS 4		χ^2	*P* value
		n	5-YSR	*n*	5-YSR	*n*	5-YSR	*n*	5-YSR	*n*	5-YSR		
< 10 LN	pN 0	33	81.80%	20	70%	6	66.7.0%	-	-	-	-	1.486	0.476
	pN 1	-	-	10	50%	12	58.30%	4	0	1	0	22.349	0
	pN 2	-	-	-	-	14	42.90%	13	30.80%	2	0	4.202	0.122
	pN 3a	-	-	-	-	-	-	3	66.70%	8	37.5%	0.78	0.377
	χ^2	-		1.44		0.969		5.689		1.083			
	P	-		0.23		0.619		0.128		0.582			
10-15 LN	pN 0	56	80.40%	-	-	-	-	-	-	-	-	-	-
	pN 1	-	-	24	62.50%	-	-	-	-	-	-	-	-
	pN 2	-	-	4	75%	23	43.50%	12	50%	-	-	1.241	0.538
	pN 3a	-	-	-	-	2	0%	19	15.80%	12	25%	3.413	0.182
	χ^2	-		0.222		6.785		3.614		-			
	P	-		0.638		0		0.057		-			

LODDS: Log odds of positive lymph nodes; 5-YSR: 5-year survival rate; LN: Examined lymph nodes; pN: Positive lymph nodes.

is essential to the development of relevant follow-up treatment strategies[17,18]. Currently, the postoperative pathological TNM staging is accepted and widely applied as the prognostic evaluation indicator in clinical practice. With regard to the N portion of the TNM staging, there has been considerable controversy ranging from earlier staging based on anatomical sites of metastatic lymph nodes[19] to the specific staging criteria based on the number of regional metastatic lymph nodes[20,21]. The N staging criteria were not unified until the 7th edition of the AJCC[4] and the 14th edition of the Statute of Gastric Cancer Treatment in Japan[5] unified the criteria for the first time in 2010. However, many researchers still believe that when the staging is based on the absolute number of metastatic lymph nodes, the number of pN is easily influenced by the numbers of removed and tested lymph nodes. When the number of tested lymph nodes is insufficient, staging bias may occur, affecting the accuracy of the prognostic assessment[22,23]. The N staging based on MLR can overcome the above shortcomings[24,25]. Therefore, when comparing the prognostic assessment of different lymph node metastasis staging methods in gastric cancer patients after D2 radical gastrectomy, this study focused on the impact of the 3 staging methods on long-term survival rate when the number of pathologically tested lymph nodes after surgery was insufficient.

To date, neither MLR nor LODDS staging has accurate and widely accepted criteria; therefore, the log-rank survival test was first conducted to verify the staging criteria of MLR and LODDS (Table 2). The 5-year survival rates of various stages according to the above criteria were similar to those of the corresponding pN stages (TNM staging criteria in the 7th edition of AJCC/UICC). The correlation analysis of the 3 staging methods also showed that MLR and LODDS were significantly positively correlated with pN. The ROC curves also showed that the accuracy of prognosis assessment of the 3 staging methods in gastric cancer patients was not significantly different. The subsequent univariate and multivariate analyses both showed that the MLR, LODDS, and pN staging methods were all closely related to patient prognosis-they were all independent risk factors for the

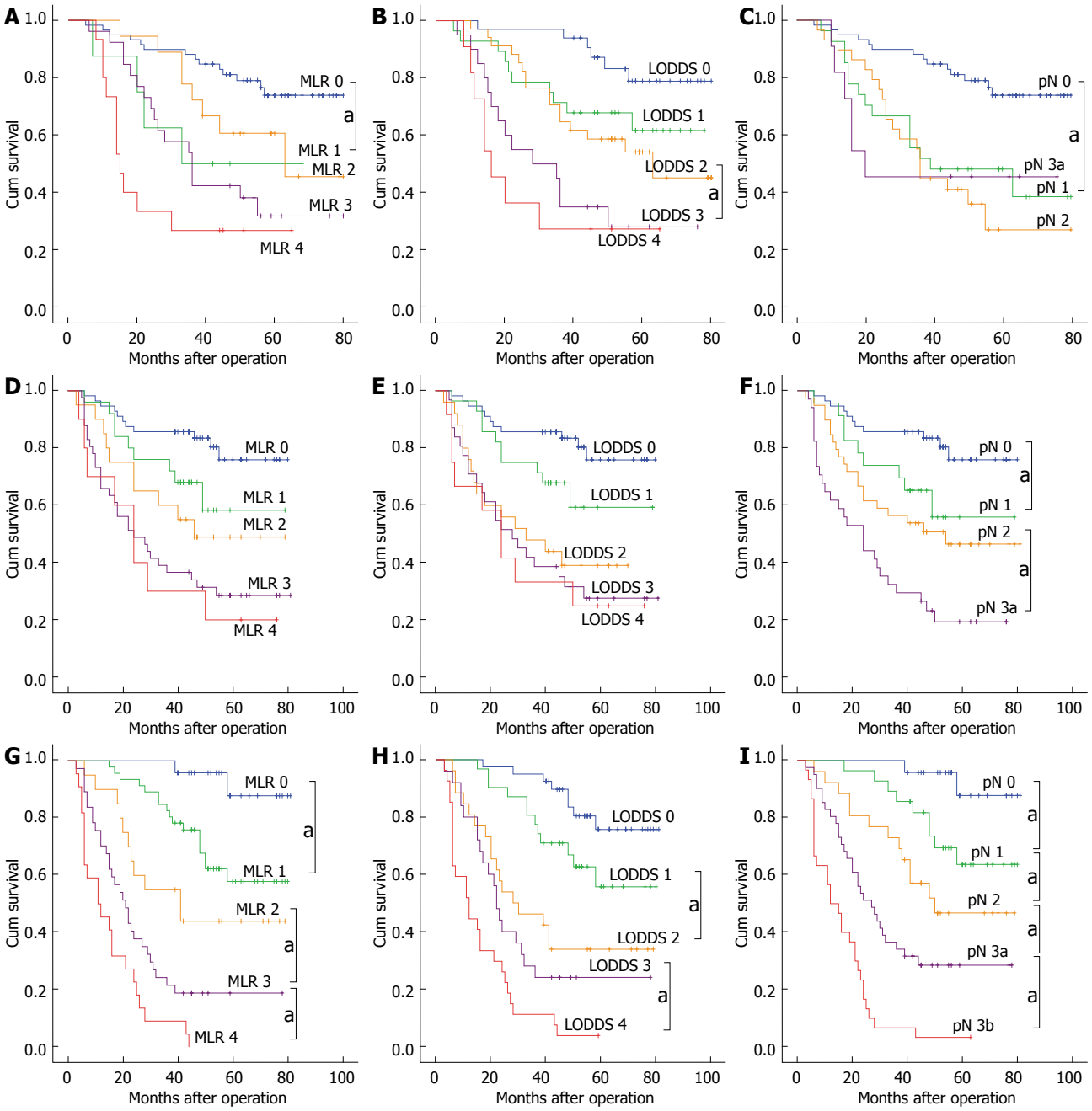


Figure 2 Comparison of survival curves in 3 staging system according to the number of lymph nodes retrieved. [a]*P* < 0.05 between adjacent stages. A: MLR, LN < 10; B: LODDS, LN < 10; C: pN, LN < 10; D: MLR, 10 ⩽ LN ⩽ 15; E: LODDS, 10 ⩽ LN ⩽ 15; F: pN, 10 ⩽ LN ⩽ 15; G: MLR, LN > 15; H: LODDS, LN > 15; I: pN, LN > 15. MLR: Metastatic lymph node ratio; LODDS: Log odds of positive lymph nodes; pN: Positive lymph nodes; LN: Examined lymph nodes.

prognoses of gastric cancer patients. The above results suggest that MLR, LODDS, and pN staging methods can all be used for the prognostic assessment of gastric cancer, and the assessment efficacies of the 3 methods were similar.

Although the total number of tested lymph nodes in the Cox proportional risk regression model was not a significant independent risk factor for patient prognosis, univariate analysis showed that as the number of tested lymph nodes increased, the 5-year survival rate of patients exhibited a downward trend ($P = 0.039$); moreover, a correlation analysis showed that MLR, LODDS, and pN were all positively correlated with the number of tested lymph nodes. When only the correlation coefficient of the number of tested lymph nodes was considered (pN > MLR > LODDS), the impact of the number of tested lymph nodes on the MLR and LODDS was smaller than that of the absolute number of pN, which suggests that compared with pN staging system, the MLR and LODDS were less affected by the total number of tested lymph nodes. The subsequent results of the survival curve of patients with insufficient tested lymph nodes also showed that, when the number of tested lymph node was < 10, the MLR and LODDS

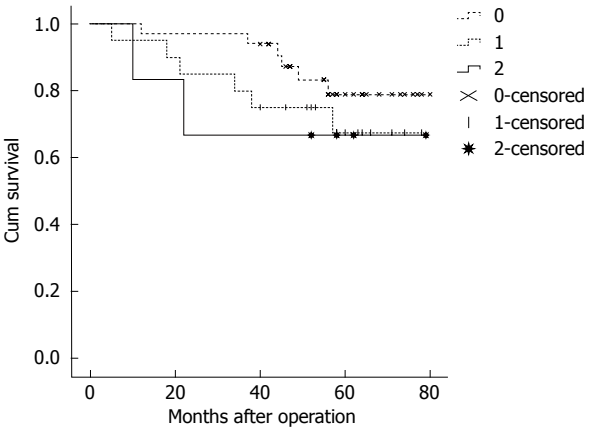


Figure 3 Survival curve of no positive lymph nodes patients with < 10 tested lymph nodes re-staged with the log odds of positive lymph nodes staging method.

staging methods appeared to better assess prognosis than pN staging. However, as shown in Figure 2, although the MLR and LODDS staging methods could more accurately assess the 5-year postoperative survival rate of gastric cancer patients at the early-middle stages (stage 0-2), the difference in the prognostic assessment of the patients at middle-late stages (stage 3 and 4) was not significant. The main reason was that, although the ratio could reduce the impact of sampling error compared with the absolute number, as the number of pN increased, the impact of the sampling error due to the insufficient number of tested lymph nodes also increased. Therefore, the difference in the prognoses of patients in the middle-late stages was not significant. In addition, for the patients in the middle-late stages of MLR and LODDS, especially those in stage 4, the 5-year survival rates were all $\geqslant$ 20%, which was significantly higher than that of the patients with > 15 (< 4%) tested lymph nodes. The reason for this result may be that when the total number of tested lymph nodes was insufficient, the sampling sites were too concentrated near the lesion; therefore, the ratio of pN was higher, resulting in an overestimation of the actual pathological staging of patients. Moreover, the comparison of the 5-year survival rate of the 3 methods in patients with $\leqslant$ 15 tested lymph nodes also confirmed that the accuracy and homogeneity of the staging methods based on the MLR, LODDS or the absolute number of pN were similar, with no significant difference. At the same time, the comparison of the survival curve and ROC curve of the 3 staging methods in the < 10, 10-15 and > 15 group showed that the difference in the 5-year survival rate between stages and the assessment accuracy of survival rate were all increased progressively with the enhanced levels of examined lymph nodes, and the 3 staging methods exhibited no significant difference. The above results all confirmed that, regardless of the staging method, a sufficient number of tested lymph nodes was the key factor. When the number of tested lymph nodes was $\leqslant$ 15, the staging based on MLR or LODDS could not compensate for the inadequacy of pN staging, and thus could not accurately assess patient prognosis.

Although the number of cases did not affect the results of the statistical analysis significantly, it could be observed from the survival curve that the LODDS staging method appeared to better assess prognosis for patients at MLR and pN stage 0 with an insufficient number of tested lymph nodes. However, the advantage of the LODDS staging method was only apparent when the number of tested lymph nodes was < 10. Additionally, because the 5-year survival rate for patients in stage pN0 was relatively high, the survival rates of patients in various LODDS stages were not significantly different after re-staging. Finally, the calculation method of LODDS was complicated. All of the above factors limited the practical application of LODDS staging method, and the practical value was low.

Some studies have found that the staging methods based on the MLR and LODDS could more accurately predict the prognosis of gastric cancer patients than the staging method based on the absolute number (pN), especially when the number of tested lymph nodes was insufficient[9-13]. The above conclusions in this study appeared to be inconsistent with those previous findings. Different surgical methods may be the main cause of the contradictory findings[26]. In a study recently published in Annals of Surgery in 2012[13], the postoperative clinical, pathologic and follow-up data of 18 043 gastric cancer patients retrieved from the Surveillance, Epidemiology, and End Results database of United States were retrospectively analyzed, and the results showed that when the number of tested lymph nodes was insufficient, the MLR staging method could more accurately assess the patient prognosis than the pN staging method. However, only 10% of the patients in this study underwent D2 radical gastrectomy, and the scope of lymph node removal in the remaining patients was D1 or below. The insufficient number of tested lymph nodes was mainly limited by the scope of lymph node removal. Some studies have confirmed that the average number of removed lymph nodes during D2 radical gastrectomy could reach 32[27]. The smaller the number of tested lymph nodes, the greater the sampling error. A sufficient number of tested lymph nodes is key to reducing sampling error. Therefore, to accurately assess the prognosis of patients after D2 radical gastrectomy, no staging method can replace a sufficient number of tested lymph nodes.

In summary, the MLR, LODDS and pN are all independent risk factors for the long-term postoperative survival of gastric cancer patients. The accuracy of the prognostic assessment of the MLR and LODDS staging methods is comparable to that of the pN staging method in gastric cancer patients. However, for the patients that undergo a D2 radical gastrectomy, when the number of tested lymph nodes is insufficient ($\leqslant$ 15), neither the staging method based on metastatic lymph node ratio nor the pN staging method can avoid staging bias. Therefore, as D2 radical gastrectomy is increasingly accepted, a sufficient number of tested lymph nodes is the only key to

ensure an accurate prediction of gastric cancer patient prognosis.

COMMENTS

Background

Gastric cancer is one of the leading fatal malignancies worldwide. D2 radical gastrectomy has been accepted in most countries as the standard surgery for gastric cancer. In the currently widely applied criteria of postoperative tumor-node-metastasis staging system, the staging of regional lymph node metastasis (N) is of great significance for accurate prognosis-assessment.

Research frontiers

Currently, the metastatic lymph node ratio has been considered as an alternative to the absolute number of positive lymph nodes. Although the 7th edition of the American Joint Cancer Committee and the 14th edition of the Statute of Gastric Cancer Treatment in Japan unified the pN staging criteria for the first time in 2010, many researchers still believe that the prognostic assessment of ratio staging was superior to that of the staging based on absolute number of positive lymph nodes, which demands the examination of at least 15 lymph nodes.

Innovations and breakthroughs

The clinical, pathologic, and long-term follow-up data of 427 patients underwent D2 radical gastrectomy were stratified and compared to evaluate the prognostic assessment of the 3 metastatic lymph node staging methods. The findings from this study suggested that neither metastatic lymph node ratio nor log odds of positive lymph nodes could avoid the staging bias due to the insufficient number of tested lymph nodes for patients underwent D2 radical gastrectomy.

Applications

This study compared three lymph node based N staging systems for gastric cancer patients with radical resection and D2 lymphadenectomy, and then demonstrated that a sufficient number of tested lymph nodes was key to ensure an accurate prognosis-assessment. The results are clinical significance in the prognostic assessment of patients with gastric cancer after surgery.

Peer review

This is a retrospective research of 427 gastric cancer patients undergoing radical resection plus D2 lymphadenectomy, with a median follow-up of 55 mo. The authors have analyzed patient outcomes in considerable depth, their data is well characterized. They provide an in depth analysis of factors contributing to survival and have utilized multivariate analysis in doing this. The information in the manuscript is highly relevant and useful.

REFERENCES

1 **Jemal A,** Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385 DOI: 10.3322/caac.20006]

2 **Peng J,** Wang Y. Epidemiology, pathology and clinical management of multiple gastric cancers: a mini-review. *Surg Oncol* 2010; **19**: e110-e114 [PMID: 20566282 DOI: 10.1016/j.suronc.2010.05.002]

3 **Songun I,** Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. *Lancet Oncol* 2010; **11**: 439-449 [PMID: 20409751 DOI: 10.1016/S1470-2045(10)70070-X]

4 **Sasako M,** Sano T, Yamamoto S, Kurokawa Y, Nashimoto A, Kurita A, Hiratsuka M, Tsujinaka T, Kinoshita T, Arai K, Yamamura Y, Okajima K. D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. *N Engl J Med* 2008; **359**: 453-462 [PMID: 18669424 DOI: 10.1056/NEJMoa0707035]

5 **Degiuli M,** Sasako M, Calgaro M, Garino M, Rebecchi F, Mineccia M, Scaglione D, Andreone D, Ponti A, Calvo F. Morbidity and mortality after D1 and D2 gastrectomy for cancer: interim analysis of the Italian Gastric Cancer Study Group (IGCSG) randomised surgical trial. *Eur J Surg Oncol* 2004; **30**: 303-308 [PMID: 15028313 DOI: 10.1016/j.ejso.2003.11.020]

6 **Sobin LH,** Gospodarowicz MK, Wittekind C. TNM classification of malignant tumors. 7th ed. Oxford: Wiley-Blackwell, 2009

7 **Japanese Gastric Cancer Association**. Japanese classification of gastric carcinoma. 14th ed. Tokyo: Kanehara and Co. Ltd., 2010

8 **Bando E,** Yonemura Y, Taniguchi K, Fushida S, Fujimura T, Miwa K. Outcome of ratio of lymph node metastasis in gastric carcinoma. *Ann Surg Oncol* 2002; **9**: 775-784 [PMID: 12374661 DOI: 10.1007/BF02574500]

9 **Sun Z,** Xu Y, Li de M, Wang ZN, Zhu GL, Huang BJ, Li K, Xu HM. Log odds of positive lymph nodes: a novel prognostic indicator superior to the number-based and the ratio-based N category for gastric cancer patients with R0 resection. *Cancer* 2010; **116**: 2571-2580 [PMID: 20336791 DOI: 10.1002/cncr.24989]

10 **Sun Z,** Zhu GL, Lu C, Guo PT, Huang BJ, Li K, Xu Y, Li DM, Wang ZN, Xu HM. The impact of N-ratio in minimizing stage migration phenomenon in gastric cancer patients with insufficient number or level of lymph node retrieved: results from a Chinese mono-institutional study in 2159 patients. *Ann Oncol* 2009; **20**: 897-905 [PMID: 19179553 DOI: 10.1093/annonc/mdn707]

11 **Inoue K,** Nakane Y, Iiyama H, Sato M, Kanbara T, Nakai K, Okumura S, Yamamichi K, Hioki K. The superiority of ratio-based lymph node staging in gastric carcinoma. *Ann Surg Oncol* 2002; **9**: 27-34 [PMID: 11829427 DOI: 10.1245/aso.2002.9.1.27]

12 **Lee SR,** Kim HO, Son BH, Shin JH, Yoo CH. Prognostic significance of the metastatic lymph node ratio in patients with gastric cancer. *World J Surg* 2012; **36**: 1096-1101 [PMID: 22382768 DOI: 10.1007/s00268-012-1520-5]

13 **Wang J,** Dang P, Raut CP, Pandalai PK, Maduekwe UN, Rattner DW, Lauwers GY, Yoon SS. Comparison of a lymph node ratio-based staging system with the 7th AJCC system for gastric cancer: analysis of 18,043 patients from the SEER database. *Ann Surg* 2012; **255**: 478-485 [PMID: 22330040 DOI: 10.1097/SLA.0b013e31824857e2]

14 **Espín F,** Bianchi A, Llorca S, Feliu J, Palomera E, García O, Remon J, Suñol X. Metastatic lymph node ratio versus number of metastatic lymph nodes as a prognostic factor in gastric cancer. *Eur J Surg Oncol* 2012; **38**: 497-502 [PMID: 22445061 DOI: 10.1016/j.ejso.2012.01.012]

15 **Xiao LB,** Yu JX, Wu WH, Xu FF, Yang SB. Superiority of metastatic lymph node ratio to the 7th edition UICC N staging in gastric cancer. *World J Gastroenterol* 2011; **17**: 5123-5130 [PMID: 22171148]

16 **Qiu MZ,** Qiu HJ, Wang ZQ, Ren C, Wang DS, Zhang DS, Luo HY, Li YH, Xu RH. The tumor-log odds of positive lymph nodes-metastasis staging system, a promising new staging system for gastric cancer after D2 resection in China. *PLoS One* 2012; **7**: e31736 [PMID: 22348125 DOI: 10.1371/journal.pone.0031736]

17 **Joensuu H,** Vehtari A, Riihimäki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Braconi C, Bordoni A, Magnusson MK, Linke Z, Sufliarsky J, Federico M, Jonasson JG, Dei Tos AP, Rutkowski P. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. *Lancet Oncol* 2012; **13**: 265-274 [PMID: 22153892 DOI: 10.1016/S1470-2045(11)70299-6]

18 **Ohtsu A.** Chemotherapy for metastatic gastric cancer: past, present, and future. *J Gastroenterol* 2008; **43**: 256-264 [PMID: 18458840 DOI: 10.1007/s00535-008-2177-6]

19 **Karpeh MS,** Leon L, Klimstra D, Brennan MF. Lymph node staging in gastric cancer: is location more important than Number? An analysis of 1,038 patients. *Ann Surg* 2000; **232**: 362-371 [PMID: 10973386 DOI: 10.1097/00000658-200009000-00008]

20 **Katai H,** Yoshimura K, Maruyama K, Sasako M, Sano T. Evaluation of the New International Union Against Can-

cer TNM staging for gastric carcinoma. *Cancer* 2000; 88: 1796-1800 [PMID: 10760754]
21 **Rausei S,** Dionigi G, Boni L, Rovera F, Dionigi R. How does the 7th TNM edition fit in gastric cancer management? *Ann Surg Oncol* 2011; **18**: 1219-1221 [PMID: 20878488 DOI: 10.1245/s10434-010-1346-z]
22 **Kulig J,** Sierzega M, Kolodziejczyk P, Popiela T. Ratio of metastatic to resected lymph nodes for prediction of survival in patients with inadequately staged gastric cancer. *Br J Surg* 2009; **96**: 910-918 [PMID: 19591164 DOI: 10.1002/bjs.6653]
23 **Lemmens VE,** Dassen AE, van der Wurff AA, Coebergh JW, Bosscha K. Lymph node examination among patients with gastric cancer: variation between departments of pathology and prognostic impact of lymph node ratio. *Eur J Surg Oncol* 2011; **37**: 488-496 [PMID: 21444177 DOI: 10.1016/j.ejso.2011.03.005]
24 **Kim CY,** Yang DH. Adjustment of N stages of gastric cancer by the ratio between the metastatic and examined lymph nodes. *Ann Surg Oncol* 2009; **16**: 1868-1874 [PMID: 19434459 DOI: 10.1245/s10434-009-0430-8]
25 **Celen O,** Yildirim E, Berberoglu U. Prognostic impact of positive lymph node ratio in gastric carcinoma. *J Surg Oncol* 2007; **96**: 95-101 [PMID: 17443727 DOI: 10.1002/jso.20797]
26 **Maduekwe UN,** Lauwers GY, Fernandez-Del-Castillo C, Berger DL, Ferguson CM, Rattner DW, Yoon SS. New metastatic lymph node ratio system reduces stage migration in patients undergoing D1 lymphadenectomy for gastric adenocarcinoma. *Ann Surg Oncol* 2010; **17**: 1267-1277 [PMID: 20099040 DOI: 10.1245/s10434-010-0914-6]
27 **Ahn HS,** Lee HJ, Hahn S, Kim WH, Lee KU, Sano T, Edge SB, Yang HK. Evaluation of the seventh American Joint Committee on Cancer/International Union Against Cancer Classification of gastric adenocarcinoma in comparison with the sixth classification. *Cancer* 2010; **116**: 5592-5598 [PMID: 20737569 DOI: 10.1002/cncr.25550]

P- Reviewer Szczepanik AM **S- Editor** Wen LL **L- Editor** A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1984

World J Gastroenterol 2013 March 28; 19(12): 1984-1991
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Effect evaluation of interleukin-1 receptor antagonist nanoparticles for mesenchymal stem cell transplantation

Xiao-Lei Shi, Wei Zhu, Jia-Jun Tan, Jiang-Qiang Xiao, Lin Zhang, Qian Xu, Zheng-Liang Ma, Yi-Tao Ding

Xiao-Lei Shi, Jia-Jun Tan, Jiang-Qiang Xiao, Yi-Tao Ding, Department of Hepatobiliary Surgery, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu Province, China
Xiao-Lei Shi, Yi-Tao Ding, Key Medical Center of Jiangsu Province for Hepatobiliary Diseases, Nanjing 210008, Jiangsu Province, China
Wei Zhu, Gulou Clinical Medical College of Combined TCM and Western Medicine, Nanjing University of Chinese Medicine, Nanjing 210008, Jiangsu Province, China
Wei Zhu, Zheng-Liang Ma, Department of Anesthesiology, the Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing 210008, Jiangsu Province, China
Lin Zhang, Qian Xu, School of Public Health, Southeast University, Nanjing 210009, Jiangsu Province, China
Author contributions: Shi XL and Zhu W contributed equally to this work; Shi XL, Zhu W, Ma ZL and Ding YT designed the research; Zhu W and Tan JJ wrote the paper; Xiao JQ, Zhang L and Xu Q performed the research; Tan JJ and Xu Q did data analysis.
Supported by National Natural Science Foundation of China, No. 30901431
Correspondence to: Yi-tao Ding, Professor, Department of Hepatobiliary Surgery, the Affiliated Drum Tower Hospital of Nanjing University Medical School, No. 321 Zhongshan Road, Nanjing 210008, China. yitaoding@hotmail.com
Telephone: +86-25-83105502 Fax: +86-25-83107080
Received: September 21, 2012 Revised: January 20, 2013
Accepted: February 5, 2013
Published online: March 28, 2013

Abstract

AIM: To study the efficacy of marrow mesenchymal stem cells (MSCs) transplantation combined with interleukin-1 receptor antagonist (IL-1Ra) for acute liver failure (ALF).

METHODS: Chinese experimental miniature swine were randomly divided into four groups (n = 7), and all animals were given D-galactosamine (D-gal) to induce ALF. Group A animals were then injected with 40 mL saline *via* the portal vein 24 h after D-gal induction; Group B animals were injected with 2 mg/kg IL-1Ra *via* the ear vein 18 h, 2 d and 4 d after D-gal induction; Group C received approximately 1×10^8 green fluorescence protein (GFP)-labeled MSCs (GFP-MSCs) suspended in 40 mL normal saline *via* the portal vein 24 h after D-gal induction; Group D animals were injected with 2 mg/kg IL-1Ra *via* the ear vein 18 h after D-gal induction, MSCs transplantation was then carried out at 24 h after D-gal induction, and finally 2 mg/kg IL-1Ra was injected *via* the ear vein 1 d and 3 d after surgery as before. Liver function, serum inflammatory parameters and pathological changes were measured and the fate of MSCs was determined.

RESULTS: The optimal efficiency of transfection (97%) was achieved at an multiplicity of infection of 80, as observed by fluorescence microscopy and flow cytometry (FCM). Over 90% of GFP-MSCs were identified as CD44+ CD90+ CD45- MSCs by FCM, which indicated that most GFP-MSCs retained MSCs characteristics. Biochemical assays, the levels of serum inflammatory parameters and histological results in Group D all showed a significant improvement in liver injury compared with the other groups ($P < 0.05$). The number of GFP-MSCs in Group D was also greater than that in Group B, and the long-term cell proliferation rate was also better in Group D than in the other groups.

CONCLUSION: MSCs transplantation is useful in ALF, IL-1Ra plays an important role in alleviating the inflammatory condition, and combination therapy with MSCs transplantation and IL-1Ra is a promising treatment for ALF.

Key words: Interleukin-1 receptor antagonist; Mesenchymal stem cells; Cell transplantation; Acute liver failure; Inflammatory environment

Shi XL, Zhu W, Tan JJ, Xiao JQ, Zhang L, Xu Q, Ma ZL, Ding YT. Effect evaluation of interleukin-1 receptor antagonist

nanoparticles for mesenchymal stem cell transplantation. *World J Gastroenterol* 2013; 19(12): 1984-1991 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1984.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1984

INTRODUCTION

Acute liver failure (ALF) is defined by the development of coagulopathy and encephalopathy within a short period of time in patients with no previous history of liver disease[1]. The essence of ALF is severe inflammation leading to cell necrosis in a large number of liver cells caused by paracetamol, idiosyncratic drug reactions, hepatitis B, or seronegative hepatitis[2,3]. The key to the treatment of ALF is the reduction of liver cell necrosis and the stimulation of liver cell regeneration.

Liver transplantation is the only efficient treatment for ALF; however, difficulties including severe donor shortage, numerous complications, immunological rejection and high medical costs limit its use[1,4]. Mesenchymal stem cells (MSCs) due to their sufficient source, low immunogenicity and the potential ability for differentiation into hepatocyte-like cells make MSCs transplantation a promising treatment for ALF[5-7]. In order to achieve better results, we transplanted MSCs into a pig model of acute liver failure in addition to interleukin-1 receptor antagonist (IL-1Ra) injection which is used to improve liver inflammation[8,9]. IL-1 is primarily a proinflammatory cytokine due to its ability to stimulate the expression of a number of inflammation-associated genes through the IL-1 signaling cascade[10-12]. IL-1Ra can bind to the IL-1 receptor and blocks IL-1 action through competitive inhibition, but will not initiate the IL-1 signaling cascade due to its IL-1-like structure which will not induce a signal at all[8]. In this study, we evaluated the efficiency of combination therapy with IL-1Ra and MSCs transplantation for the treatment of ALF in swine.

MATERIALS AND METHODS

Animals

Chinese experimental miniature swine (10 ± 3 kg, aged approximately 5 to 8 mo) were obtained from the Laboratory Animal Centre of the Affiliated Drum Tower Hospital of Nanjing University Medical School. All experiments were approved by the Institutional Animal Care and Use Committee.

In vitro experiment

MSCs were isolated by density gradient centrifugation from pig bone marrow and cultured in L-Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (all from Gibco BRL, Grand Island, NY, United States). The cells were then transfected with a lentiviral vector carrying the gene encoding green fluorescence protein (GFP), and the multiplicity of infection (MOI) of transfection was determined by fluorescent inverted phase contrast microscopy and flow cytometry (FCM). The surface markers (CD44, CD45, and CD90) of GFP-labeled MSCs (GFP-MSCs) were identified by FCM for their MSCs characteristics.

ALF model

The animals received a single intravenous injection of D-galactosamine (D-gal) 0.3 g/kg to induce experimental hepatic injury.

Experimental groups and treatments

Twenty-eight pigs were randomly divided into four groups, Group A (control, $n = 7$), Group B (IL-1Ra, $n = 7$), Group C (MSCs transplantation, $n = 7$), Group D (combined therapy, $n = 7$). Group A received 40 mL normal saline *via* the portal vein 24 h after D-gal induction; Group B received 2 mg/kg IL-1Ra (Institute of Process Engineering, Chinese Academy of Sciences, China) *via* the ear vein 18 h, 2 d and 4 d after D-gal induction; Group C received approximately 1×10^8 GFP-MSCs suspended in 40 mL normal saline *via* the portal vein 24 h after D-gal induction; Group D received 2 mg/kg IL-1Ra *via* the ear vein 18 h after D-gal induction, MSCs transplantation was carried out 24 h after D-gal induction, and finally 2 mg/kg IL-1Ra was injected *via* the ear vein 1 d and 3 d after surgery as before. Liver function, and inflammatory cytokines IL-1β, IL-2 and tumor necrosis factor α (TNF-α) were measured using enzyme-linked immuno sorbent assay Kits (Corbett Life Science, Australia) pre-operatively, intra-operatively, 1-6 d and 1-4 wk after surgery.

Histological analysis

Swine were humanely killed for histological examination at 3 d and every week after surgery. Liver tissues were immersion fixed, embedded in paraffin and sectioned at 5 μm, and the slices were submitted for hematoxylin and eosin (HE) and anti-Ki67 (Abcam Ltd., United Kingdom) staining. To determine liver cell proliferation, six high-powered fields of vision were obtained for each slice, and the average number of $Ki67^+$ cells was used for statistical analysis. To trace transplanted MSCs, cells expressing GFP were analyzed by fluorescent microscopy and conventional immunohistochemistry using anti-GFP (Abcam Ltd., United Kingdom).

Statistical analysis

All statistical analysis were performed using SPSS 16.0. The data are reported as mean ± SD. The statistical significance was analyzed by two-way analysis of variance. $P < 0.05$ was considered to denote statistical significance.

RESULTS

In vitro experiment

The optimal efficiency of transfection (97%) was achieved at an MOI of 80, as observed by fluorescence microscopy (Figure 1A and B) and FCM (Figure 1C). Over 90% of

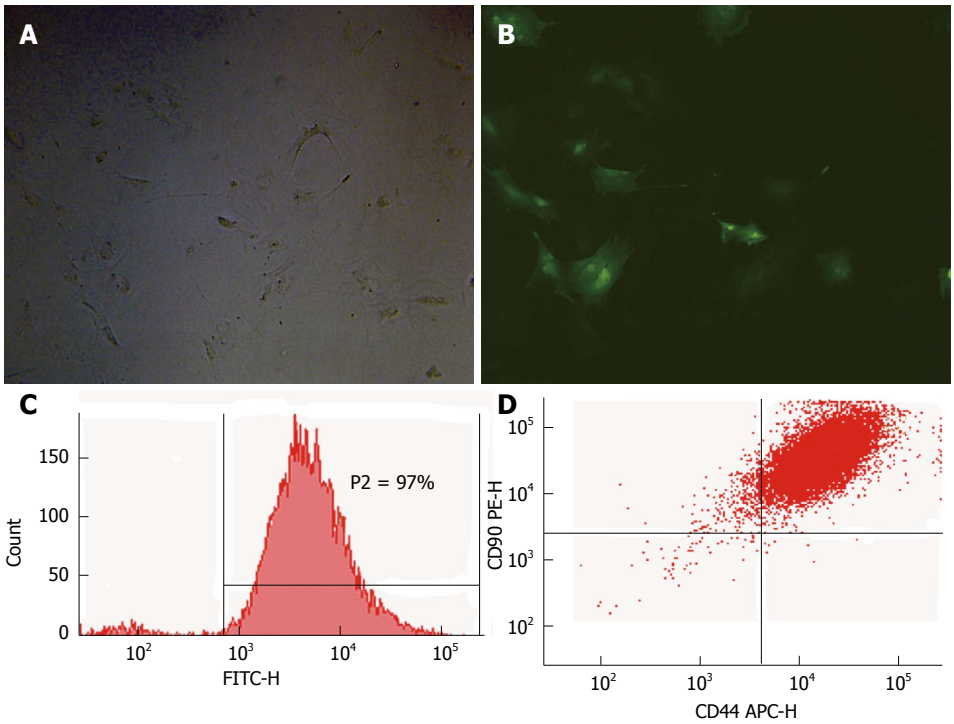


Figure 1 Mesenchymal stem cells transfected with a lentiviral vector carrying the gene encoding green fluorescence protein *in vitro*. A: GFP-MSCs cultivated for 3 d and observed by light microscopy (×400); B: GFP-MSCs with green fluorescence (×400); C: Over 97% of GFP-MSCs successfully expressed GFP after propagation; D: Most GFP-MSCs were identified as CD44+ CD90+. GFP: Green fluorescence protein; MSCs: Mesenchymal stem cells; GFP-MSCs: GFP-labeled MSCs.

GFP-MSCs were identified as CD44+ CD90+ CD45- MSCs (Figure 1D) by FCM, which indicated that most GFP-MSCs retained MSCs characteristics.

Liver function

Dramatic changes in alanine aminotransferase (ALT), aspertate aminotransferase (AST), total bilirubin (TB), ammonia and prothrombin time (PT) demonstrated that acute liver injury was successfully achieved by D-gal induction in all four groups. Highly significant increases in ALT, AST, TB, ammonia and PT were found in all groups within 48 h after D-gal injection, and then gradually declined. The most significant improvements were found in Group D following combination therapy compared with the other groups; ALT and ammonia in Group D were significantly lower than those in Group A ($P < 0.05$) within 1 to 4 d after combination therapy, and the low level of TB in Group D lasted longer than that in Group A ($P < 0.05$) and Group B ($P < 0.05$) from the first day to 1 wk, the improvement in ALB in Group D appeared the second day after treatment; Group B showed a significant reduction in ALT level within 1 to 3 d after IL-1Ra injection compared with Group A ($P < 0.05$); MSCs transplantation showed a slight effect on reducing TB and ammonia level in Group C (Table 1).

Serum inflammatory cytokines

Following D-gal induction, the levels of inflammatory cytokines, IL-1, IL-2 and TNF-α, in all groups increased significantly and reached a peak within 3 d, which lasted for several days before gradually declining. Group B and Group D had a faster improvement in IL-1 and TNF-α than the other groups, Group D had the lowest inflammatory level of all, with IL-1 and TNF-α levels significantly lower than Group A ($P < 0.05$) from the first injection of 2 mg/kg IL-1Ra; serum inflammatory cytokine levels were better in Group C than in Group A, however, no statistically significant difference between the groups was observed (Figure 2).

Histological analysis

D-gal-induced liver damage was observed by HE staining in all groups, however, less inflammatory cell infiltration and relatively complete lobular architecture were found in Group D, and liver damage was worst in Group A (Figure 3). The number of Ki-67$^+$ cells increased quickly after D-gal induction and reached a peak within 1 wk in all groups, and then a sharp decline was observed in Group A to a normal proliferation level at the end of 3 wk after operation, however, the Ki-67 positive cell index in Group D was maintained at a high level ($P < 0.05$); the performance of Group B and Group C were better than Group A, but there were no significant differences between the groups (Figure 4). Fluorescent microscopy revealed that there were more GFP-MSCs in Group D than in Group B at 1 wk after treatment, and most of these were distributed in the hepatic lobule along the central vein. Similar results were obtained by immunohistochemistry using anti-GFP (Figure 5).

DISCUSSION

ALF is a severe liver disease with large quantities of liver cell necrosis; liver transplantation is the only effective treatment, but has a number of difficulties. MSCs can be easily obtained from bone marrow and have multilineage potential. Petersen *et al*[13] and Schwartz *et al*[5] showed that MSCs possess the potential ability for hepatocyte differ-

Table 1 Biochemical parameters of Group A (control), Group B (interleukin-1 receptor antagonist), Group C (mesenchymal stem cells transplantation) and Group D (combination therapy) (mean ± SD)

	Group	Pre-operation	Intra-operation	Time after operation							
				D1	D2	D3	D4	D5	W1	W2	W4
ALT	A	54.7 ± 22.7	111.4 ± 44.1	167.25 ± 25.8	108.8 ± 25.2	89.3 ± 32.8	63.7 ± 24.6	54.1 ± 13.7	48.3 ± 17.1	38.7 ± 16.3	32.4 ± 4.2
(U/L)	B	48.9 ± 17.3	127.5 ± 20.8	108.5 ± 21.8[a]	77.6 ± 18.9[a]	78.5 ± 16.6[a]	68.7 ± 10.8	55.6 ± 12.9	51.6 ± 10.7	42.8 ± 11.6	45.6 ± 6.9
	C	52.7 ± 14.7	113.1 ± 23.5	157.8 ± 31.3	118.6 ± 16.7	83.6 ± 19.3	70.5 ± 11.2	58.8 ± 14.9	50.6 ± 7.3	51.7 ± 9.1	44.5 ± 6.9
	D	45.2 ± 7.7	112.6 ± 22.9	75.4 ± 10.1[a,e]	66.1 ± 16.7[a,e]	48.2 ± 11.9[a,e]	55.7 ± 9.3	51.9 ± 10.8	44.7 ± 6.1	35.9 ± 2.6	33.6 ± 4.2
TB	A	2.9 ± 1.3	24.0 ± 8.5	32.3 ± 7.4	26.2 ± 8.8	17.5 ± 5.1	12.9 ± 3.8	7.9 ± 1.9	5.8 ± 1.9	3.1 ± 1.2	1.9 ± 0.5
(μmol/L)	B	2.3 ± 1.5	20.8 ± 7.6	28.3 ± 7.2	19.4 ± 5.7	9.7 ± 2.8	9.2 ± 1.8	8.6 ± 2.2	6.5 ± 0.8	3.6 ± 0.9	2.1 ± 0.5
	C	1.3 ± 0.9	18.9 ± 5.7	27.5 ± 5.3	15.8 ± 4.1[a]	10.7 ± 2.6	7.3 ± 0.8	5.1 ± 0.8	1.9 ± 0.3[a]	1.7 ± 0.6	0.98 ± 0.1
	D	1.52 ± 0.73	24.2 ± 4.4	17.1 ± 2.7[a,c,e]	8.9 ± 3.51[a,c]	5.11 ± 3.3[a]	2.68 ± 2.03[a,c]	1.96 ± 1.51[a,c]	1.47 ± 0.75[a,c]	1.25 ± 0.7[a]	0.43 ± 0.05
NH3	A	33 ± 5.2	344.5 ± 102.1	267.5 ± 134.6	179 ± 33.6	159.2 ± 41.3	111.7 ± 32.6	88.5 ± 30.7	64.8 ± 7.3	99.3 ± 16.4	69 ± 24.2
(μmol/L)	B	39 ± 10.4	318 ± 67.8	206.5 ± 22.1	160.6 ± 18.2	128.8 ± 18.5	92.5 ± 11.3	114.2 ± 25.6	88.5 ± 19.7	73 ± 16	55.6 ± 11.8
	C	59 ± 12.4	335.8 ± 81.5	210.1 ± 21.7	163.5 ± 18.2	92.8 ± 23.3[a]	121.4 ± 24.5	110.8 ± 53.2	97.2 ± 26.8	48.9 ± 10.4[a]	57.2 ± 10.5
	D	38 ± 13.5	369.3 ± 104.2	148.7 ± 39.4[a,c,e]	106.1 ± 23.8[a,c]	81.3 ± 24.2[a,c]	84.6 ± 17.5[a]	87.7 ± 23.9	62 ± 17.8	42.7 ± 13.3[a,c]	41.7 ± 11.3
ALB	A	30.5 ± 4.3	27.7 ± 2.4	26.2 ± 3.0	25.1 ± 2.1	24.4 ± 1.6	25.2 ± 1.9	25.6 ± 1.4	26.3 ± 1.2	26.7 ± 1.7	28.5 ± 2.5
(g/L)	B	31.4 ± 3.3	28.7 ± 2.6	27.1 ± 1.8	25.7 ± 2.4	25.9 ± 2.7	25.6 ± 1.5	26.9 ± 0.9	26.5 ± 3.2	26.8 ± 3.7	28.8 ± 2.3
	C	28.8 ± 2.8	27.2 ± 2.2	26.6 ± 3.3	24.9 ± 1.8	25.1 ± 1.1	25.8 ± 1.6	26.3 ± 0.8	27.1 ± 4.2	27.9 ± 1.5	30.5 ± 2.1
	D	30.6 ± 3.5	26.7 ± 1.2	25.1 ± 2.2	26.2 ± 2.1	27.7 ± 1.5	30.1 ± 0.9[a,c,e]	30.7 ± 1.4[a,c,e]	31.7 ± 2.6	32.2 ± 2.1[a,c,e]	33.5 ± 2.5

[a]$P < 0.05$ *vs* Group A; [c]$P < 0.05$ *vs* Group B; [e]$P < 0.05$ *vs* Group C. ALT and ammonia in Group D were significantly lower than those in Group A ($P < 0.05$) within 1 to 4 d after combination therapy, and the low level of TB in Group D lasted longer than that in Group A ($P < 0.05$) and Group B ($P < 0.05$) from the 1 d to 1 wk. The improvement in ALB in Group D was seen on the second d after treatment; Group B showed a significant reduction in ALT level within 1 to 3 d after interleukin-1 receptor antagonist injection compared with Group A ($P < 0.05$); MSCs transplantation displayed a slight effect on reducing TB and ammonia level in Group C. MSCs: Mesenchymal stem cells; ALT: Alanine aminotransferase; ALB: Albumin; TB: Total bilirubin.

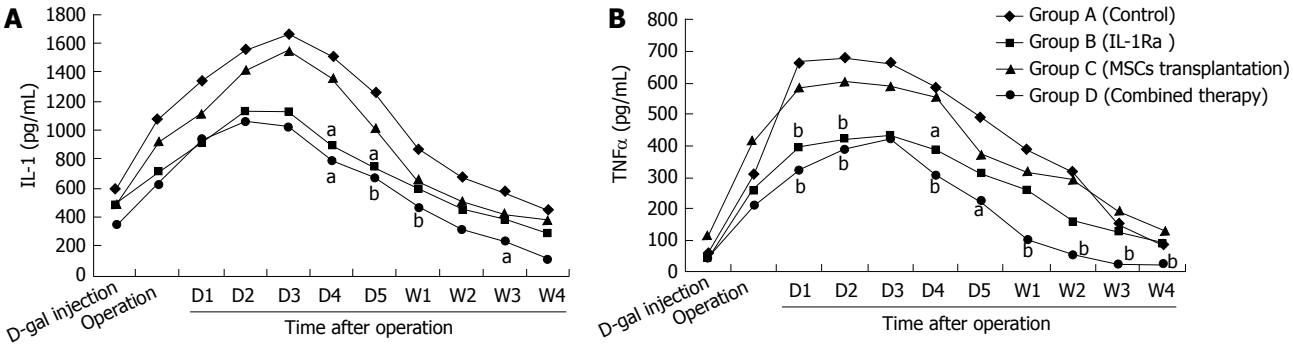


Figure 2 Changes in inflammatory cytokines (interleukin-1 and tumor necrosis factor α) levels. Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) levels in all groups increased after D-galactosamine (D-gal) injection, and then declined slowly. Group D showed a faster reduction in these cytokines following IL-1 receptor antagonist (IL-1Ra) injection than the other groups. [a]$P < 0.05$, [b]$P < 0.01$ *vs* control group. MSCs: Mesenchymal stem cells.

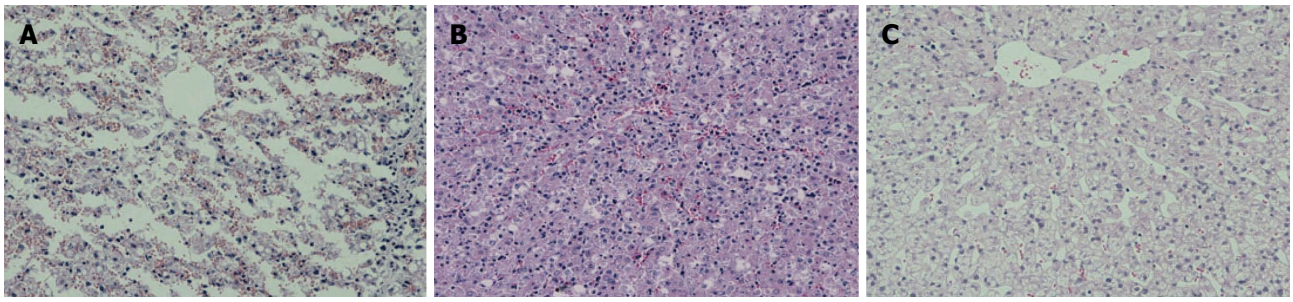


Figure 3 Hematoxylin and eosin staining of liver tissues 3 d after surgery. A: Extensive neutrophil infiltration and lobular architecture collapse was seen in Group A (control group); B: Lobular architecture can be seen in Group B (interleukin-1 receptor antagonist injection group), however, hepatic lobules were filled with cell necrosis and inflammatory cells; C: The lobular architecture in Group C (mesenchymal stem cells transplantation group) was destroyed but can still be recognized; D: Group D had clear hepatic lobular architecture and slight inflammatory cell infiltration. Magnification, ×200.

entiation *in vitro* and *in vivo*. Sakaida *et al*[14] confirmed that MSCs were involved in both liver repair and reconstruction. In their research, MSCs transplantation was used to reduce CCl4-induced liver fibrosis in mice, and their

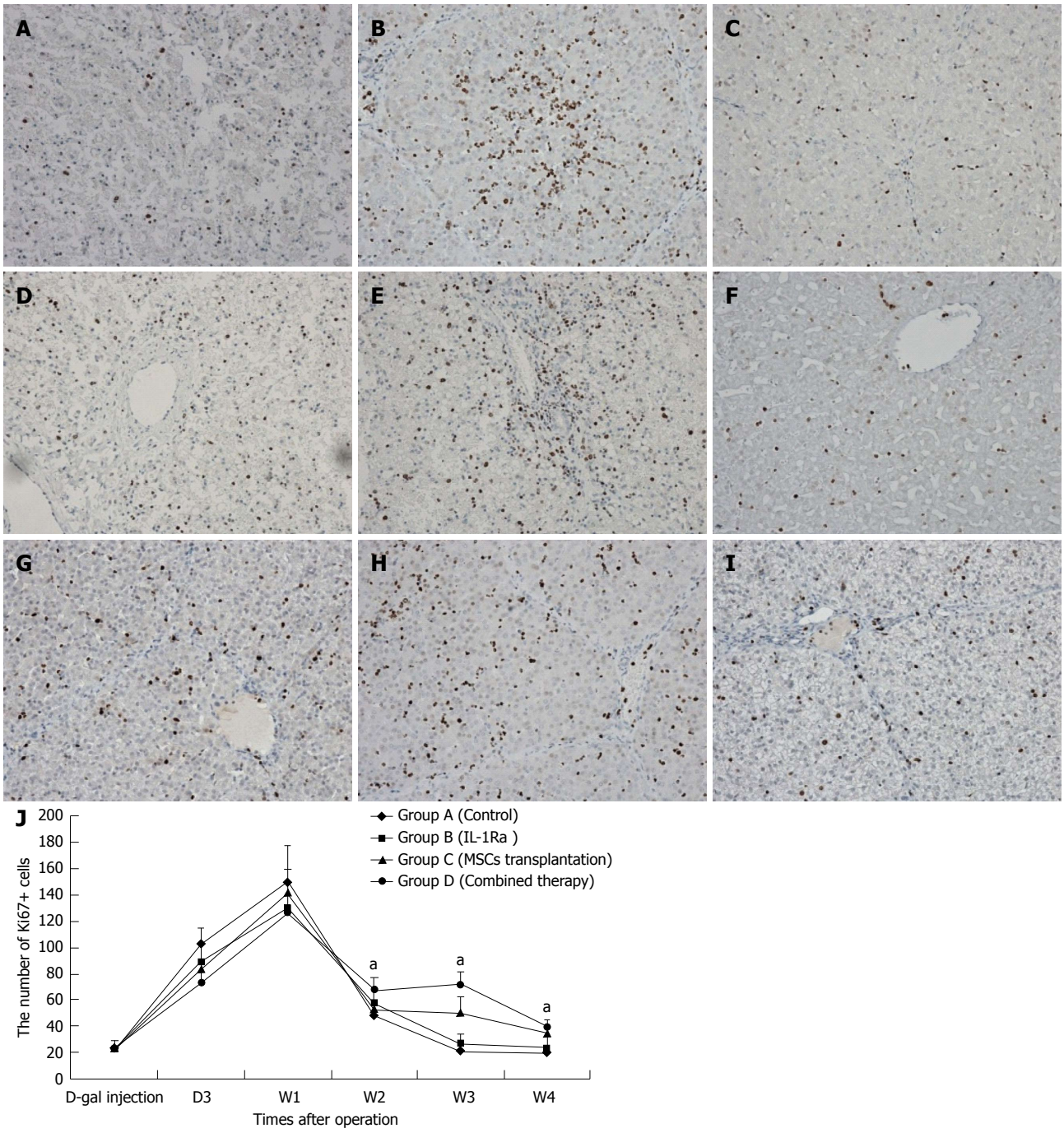


Figure 4 Changes in liver cell proliferation in all four groups. A, B and C showed the anti-Ki67 stain of Group A at the time point of D3, W1 and W4, respectively (×200); D, E, F and G, H, I showed anti-Ki67 stain of Group B and Group C, respectively, just as Group A did (×200); J: Changes in the number of Ki67+ cells demonstrated that Group D had better long-term proliferation. [a]$P < 0.05$ *vs* control group. IL-1Ra: Interleukin-1 receptor antagonist; MSCs: Mesenchymal stem cells; D-gal: D-galactosamine.

findings showed that MSCs transplantation was an ideal candidate treatment for liver disease. What will happen if MSCs transplantation is used for the treatment of ALF? di Bonzo *et al*[15] xenografted human MSCs into acute liver injured NOD/SCID mice and CCl4-induced liver injury in mice and demonstrated that the number of original human MSCs in acute liver injured mice was less than in chronically injured livers, and the number of hepatocytes undergoing differentiation was even less. In our study, there was no obvious improvement in liver function in the MSCs transplantation group (Group C), few GFP-MSCs were observed on fluorescent microscopy, and little differentiation was seen. Therefore, we concluded that MSCs transplantation for the treatment of ALF is largely limited by its low implantation and differentiation rate in acute liver injured patients.

In recent years, experimental studies have demonstrated that microcirculatory dysfunction and an inflammatory environment are determinants of ALF, and pro-inflammatory mediators such as IL-1, IL-2 and TNF-α are the key players[9,12,16-18]. Vodovotz also proved that the levels of these cytokines in ALF patients were significant-

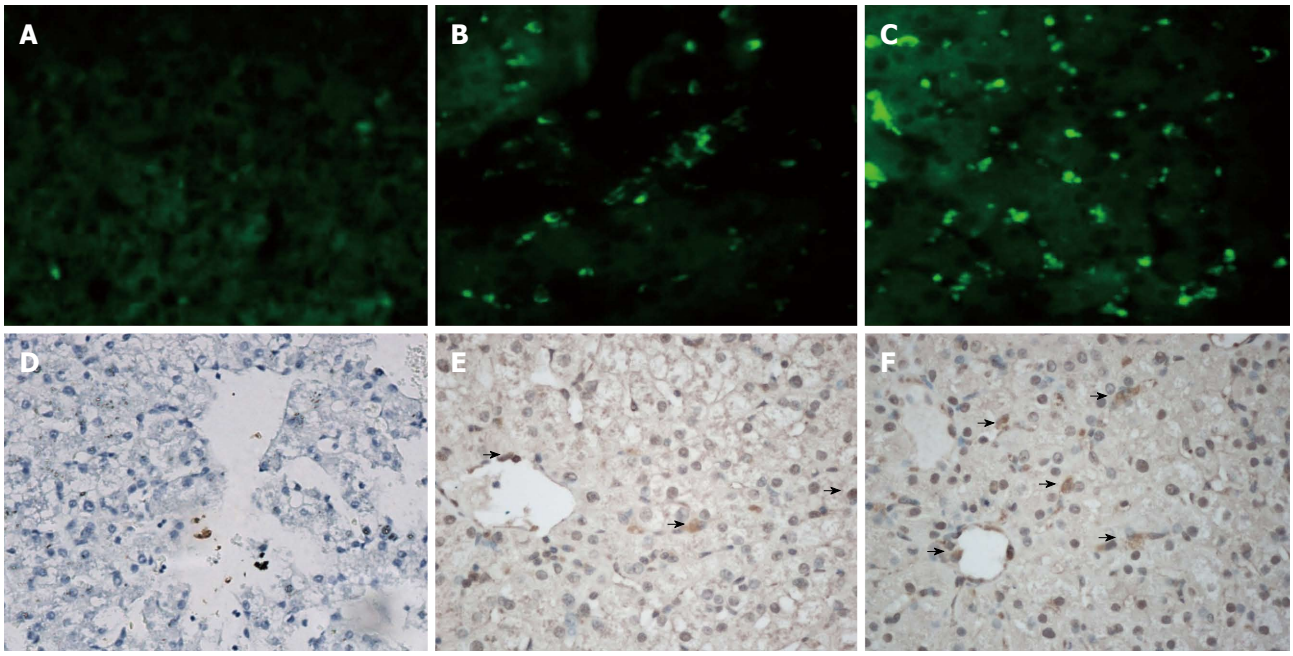


Figure 5 Fluorescence and anti-green fluorescence protein stained images of liver tissue 2 wk after surgery. A, B and C are fluorescence images of Group B, Group C and Group D, respectively; D, E and F are anti-green fluorescence protein immunohistochemical images of Group B, Group C and Group D, respectively. More cells expressing green fluorescence protein were seen in Group D (arrow). Magnification, ×400.

ly higher than in normal and chronic hepatitis patients; IL-1 may be a main driver of late inflammation which leads to further injury[3,9,19]. Given this, we presume that the inflammatory environment in ALF patients may be largely related to the low efficiency of MSCs transplantation, that is the inflammatory environment caused by proinflammatory mediators leads to the low survival and differentiation rate of transplanted MSCs. Our pre-experiment results proved this assumption: animals with lower levels of inflammatory cytokines had a higher MSCs implantation rate and differentiation rate. Therefore, we realized that reducing the inflammation level in the acutely injured liver may be a way of improving the efficacy of MSCs transplantation in ALF patients. IL-1Ra is a natural IL-1 antagonist, and can block the inflammatory process by competitively binding to the IL-1 receptor with an avidity equal to that of IL-1, but fails to stimulate downstream signals, thereby reducing the inflammation level[8,20]. An imbalance between IL-1 and IL-1Ra can be observed in a variety of inflammatory diseases including ALF[3,8,12,20]. IL-1Ra is significantly associated with the level of liver inflammation and is an independent marker unaffected by obesity, alcohol consumption, and insulin resistance[21], and can inhibit the process of hepatocellular apoptosis in mice with acetaminophen-induced ALF significantly improving their survival rate[22]. Therefore, will MSCs transplantation become an efficient treatment for ALF when it is combined with IL-1Ra used to relieve liver inflammation?

In this research, the combined therapy of IL-1Ra with MSCs transplantation was administered for acute liver injury, and the results obtained were very promising. Increased levels of proinflammatory cytokines such as IL-1, IL-2 and TNF-α were seen in all animals injected with D-gal, a slight improvement was observed when MSCs were transplanted in Group B animals, and better results were achieved in Group C and Group D animals following IL-1Ra injection. Thus, exogenous IL-1Ra had an enormous effect in reducing some proinflammatory mediators, and improving the inflammatory environment. This effect may last for at least a month as the animals in Group C and Group D showed a continuous reduction in these proinflammatory mediators compared with the other two groups in later experiments, which was thought to be mainly related to the damaged inflammatory cycle caused by IL-1Ra in the very early phase. Improved liver inflammation was then observed following MSCs transplantation. As shown in the results section, Group D treated with combination therapy had the highest GFP-MSCs implantation rate and the best liver function. In addition, the trend in proliferation level in the four groups was different, although the level in all groups peaked at a similar time point after surgery, Group D had a higher proliferation level than the other groups at the end of 2 wk of combination therapy and this difference was maintained for at least 2 wk; which was thought to be caused by both IL-1Ra and MSCs transplantation. IL-1Ra improved the liver inflammatory environment then increased liver cell proliferation rate and MSCs transplantation efficiency, and high MSCs transplantation efficiency may directly lead to a higher hepatocyte differentiation rate, proliferation level and better liver function.

Thus, IL-1Ra can improve liver inflammation and then enhance the effect of MSCs transplantation. Combination therapy with IL-1Ra and MSCs transplantation can promote the restoration and reconstruction of acute

liver injury in swine, and is a promising future treatment for patients with ALF.

COMMENTS

Background

Cell transplantation is an effective therapy for acute liver failure; however, the activity and function of transplanted cells are largely limited by the inflammatory environment of acute liver failure (ALF) liver. Interleukin-1 (IL-1) is primarily a proinflammatory cytokine and IL-1 receptor antagonist (IL-1Ra) is the most effective antagonist. The combination therapy with IL-1Ra and mesenchymal stem cell (MSC) transplantation for the treatment of ALF is an interesting way and responded well.

Research frontiers

The essence of ALF is severe inflammation leading to cell necrosis in a large number of liver cells. Microcirculatory dysfunction and an inflammatory environment are determinants of ALF, and proinflammatory mediators such as IL-1, IL-2 and tumor necrosis factor α are the primary players. The key to the treatment of ALF is the reduction of liver cell necrosis and the stimulation of liver cell regeneration.

Innovations and breakthroughs

Recent reports have highlighted the effect of IL-1Ra injection on ALF models. In this study, the authors investigated the effect of bone marrow MSCs transplantation combined with IL-1Ra injection on ALF swine. Based on the results of the study, the authors concluded that MSCs transplantation is somewhat useful for ALF swine and that the combined therapy of IL-1Ra with MSCs transplantation is a promising treatment for ALF.

Applications

According to this article, it may represent a future strategy for therapeutic intervention in the treatment of patients with ALF.

Terminology

IL-1Ra is the interleukin-1 receptor antagonist, which can bind to the IL-1 receptor and blocks IL-1 action through competitive inhibition, but will not initiate the IL-1 signaling cascade due to its IL-1-like structure which will not induce a signal at all.

Peer review

The authors evaluated the effect of bone marrow mesenchymal stem cell transplantationcombined with IL-1Ra injection on ALF swine. Group D (combine therapy of MSC transplantation + IL-1Ra) showed significantly improvement of biochemical assay, serum inflammation level and histological results compared with other groups ($P < 0.05$), labeled MSCs of Group D were more than Group B, and the long-term cell proliferation rate was the best in all groups too. They concluded that MSC transplantation is somewhat useful for ALF swine, IL-1Ra plays an important role in alleviating inflammatory condition, and the combined therapy is a promising treatment for ALF. These results are interesting and important in the therapy for ALF.

REFERENCES

1 **Foston TP**, Carpentar D. Acute liver failure. *Crit Care Nurs Clin North Am* 2010; **22**: 395-402 [PMID: 20691389 DOI: 10.1016/j.ccell.2010.05.001]

2 **Nguyen NT**, Vierling JM. Acute liver failure. *Curr Opin Organ Transplant* 2011; **16**: 289-296 [PMID: 21519261 DOI: 10.1097/MOT.0b013e328346c8ee]

3 **Vodovotz Y**, Constantine G, Faeder J, Mi Q, Rubin J, Bartels J, Sarkar J, Squires RH, Okonkwo DO, Gerlach J, Zamora R, Luckhart S, Ermentrout B, An G. Translational systems approaches to the biology of inflammation and healing. *Immunopharmacol Immunotoxicol* 2010; **32**: 181-195 [PMID: 20170421 DOI: 10.3109/08923970903369867]

4 **Laleman W**, Wilmer A, Evenepoel P, Verslype C, Fevery J, Nevens F. Review article: non-biological liver support in liver failure. *Aliment Pharmacol Ther* 2006; **23**: 351-363 [PMID: 16422994 DOI: 10.1111/j.1365-2036.2006.02765.x]

5 **Schwartz RE**, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, Lenvik T, Johnson S, Hu WS, Verfaillie CM. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; **109**: 1291-1302 [PMID: 12021244 DOI: 10.1172/JCI15182]

6 **Pournasr B**, Mohamadnejad M, Bagheri M, Aghdami N, Shahsavani M, Malekzadeh R, Baharvand H. In vitro differentiation of human bone marrow mesenchymal stem cells into hepatocyte-like cells. *Arch Iran Med* 2011; **14**: 244-249 [PMID: 21726099]

7 **Cho KA**, Lim GW, Joo SY, Woo SY, Seoh JY, Cho SJ, Han HS, Ryu KH. Transplantation of bone marrow cells reduces CCl4 -induced liver fibrosis in mice. *Liver Int* 2011; **31**: 932-939 [PMID: 21092070 DOI: 10.1111/j.1478-3231.2010.02364.x]

8 **Volarevic V**, Al-Qahtani A, Arsenijevic N, Pajovic S, Lukic ML. Interleukin-1 receptor antagonist (IL-1Ra) and IL-1Ra producing mesenchymal stem cells as modulators of diabetogenesis. *Autoimmunity* 2010; **43**: 255-263 [PMID: 19845478 DOI: 10.3109/08916930903305641]

9 **Vollmar B**, Menger MD. The hepatic microcirculation: mechanistic contributions and therapeutic targets in liver injury and repair. *Physiol Rev* 2009; **89**: 1269-1339 [PMID: 19789382 DOI: 10.1152/physrev.00027.2008]

10 **Jensen LE**, Muzio M, Mantovani A, Whitehead AS. IL-1 signaling cascade in liver cells and the involvement of a soluble form of the IL-1 receptor accessory protein. *J Immunol* 2000; **164**: 5277-5286 [PMID: 10799889]

11 **Orelio C**, Haak E, Peeters M, Dzierzak E. Interleukin-1-mediated hematopoietic cell regulation in the aorta-gonad-mesonephros region of the mouse embryo. *Blood* 2008; **112**: 4895-4904 [PMID: 18805969 DOI: 10.1182/blood-2007-12-123836]

12 **Sekiyama KD**, Yoshiba M, Thomson AW. Circulating proinflammatory cytokines (IL-1 beta, TNF-alpha, and IL-6) and IL-1 receptor antagonist (IL-1Ra) in fulminant hepatic failure and acute hepatitis. *Clin Exp Immunol* 1994; **98**: 71-77 [PMID: 7923888 DOI: 10.1111/j.1365-2249.1994.tb06609.x]

13 **Petersen BE**, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168-1170 [PMID: 10325227 DOI: 10.1126/science.284.5417.1168]

14 **Sakaida I**, Terai S, Yamamoto N, Aoyama K, Ishikawa T, Nishina H, Okita K. Transplantation of bone marrow cells reduces CCl4-induced liver fibrosis in mice. *Hepatology* 2004; **40**: 1304-1311 [PMID: 15565662 DOI: 10.1002/hep.20452]

15 **di Bonzo LV**, Ferrero I, Cravanzola C, Mareschi K, Rustichell D, Novo E, Sanavio F, Cannito S, Zamara E, Bertero M, Davit A, Francica S, Novelli F, Colombatto S, Fagioli F, Parola M. Human mesenchymal stem cells as a two-edged sword in hepatic regenerative medicine: engraftment and hepatocyte differentiation versus profibrogenic potential. *Gut* 2008; **57**: 223-231 [PMID: 17639088 DOI: 10.1136/gut.2006.111617]

16 **Bode JG**, Albrecht U, Häussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins--regulation by IL-6- and IL-1-type cytokines involving STAT3 and its crosstalk with NF-κB-dependent signaling. *Eur J Cell Biol* 2011; **91**: 496-505 [PMID: 22093287 DOI: 10.1016/j.ejcb.2011.09.008]

17 **Yazdi AS**, Drexler SK, Tschopp J. The role of the inflammasome in nonmyeloid cells. *J Clin Immunol* 2010; **30**: 623-627 [PMID: 20582456 DOI: 10.1007/s10875-010-9437-y]

18 **Kim YS**, Park HJ, Hong MH, Kang PM, Morgan JP, Jeong MH, Cho JG, Park JC, Ahn Y. TNF-alpha enhances engraftment of mesenchymal stem cells into infarcted myocardium. *Front Biosci* 2009; **14**: 2845-2856 [PMID: 19273239]

19 **Girard S**, Kadhim H, Larouche A, Roy M, Gobeil F, Sébire G. Pro-inflammatory disequilibrium of the IL-1 beta/IL-1ra ratio in an experimental model of perinatal brain damages induced by lipopolysaccharide and hypoxia-ischemia. *Cytokine* 2008; **43**: 54-62 [PMID: 18511291 DOI: 10.1016/

j.cyto.2008.04.007]
20 **Arend WP**. Cytokine imbalance in the pathogenesis of rheumatoid arthritis: the role of interleukin-1 receptor antagonist. *Semin Arthritis Rheum* 2001; **30**: 1-6 [PMID: 11357165 DOI: 10.1053/sarh.2001.23693]
21 **Pihlajamäki J**, Kuulasmaa T, Kaminska D, Simonen M, Kärjä V, Grönlund S, Käkelä P, Pääkkönen M, Kainulainen S, Punnonen K, Kuusisto J, Gylling H, Laakso M. Serum interleukin 1 receptor antagonist as an independent marker of non-alcoholic steatohepatitis in humans. *J Hepatol* 2012; **56**: 663-670 [PMID: 22027586 DOI: 10.1016/j.jhep.2011.10.005]
22 **Hu J**, Yan D, Gao J, Xu C, Yuan Y, Zhu R, Xiang D, Weng S, Han W, Zang G, Yu Y. rhIL-1Ra reduces hepatocellular apoptosis in mice with acetaminophen-induced acute liver failure. *Lab Invest* 2010; **90**: 1737-1746 [PMID: 20644519 DOI: 10.1038/labinvest.2010.127]

P- Reviewers Yamakawa M, Iizuka M, Sumi S, Sakata N
S- Editor Huang XZ **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1992

World J Gastroenterol 2013 March 28; 19(12): 1992-1996
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Usefulness of positron emission tomography in primary intestinal follicular lymphoma

Akira Tari, Hideki Asaoku, Masaki Kunihiro, Shinji Tanaka, Tadashi Yoshino

Akira Tari, Masaki Kunihiro, Division of Gastroenterology, Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima 734-8551, Japan
Hideki Asaoku, Department of Clinical Laboratory, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima 734-8551, Japan
Shinji Tanaka, Department of Endoscopy, Hiroshima University Hospital, Hiroshima 734-8551, Japan
Tadashi Yoshino, Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8530, Japan
Author contributions: Tari A performed the endoscopic examination, diagnosed and decided treatment policy, analyzed the data and wrote the paper; Asaoku H performed the hematological examination, diagnosed and decided treatment policy; Kunihiro M performed the endoscopic examination and diagnosis; Tanaka S performed the double-balloon enteroscopy and diagnosis; and Yoshino T performed the pathological diagnosis.
Correspondence to: Dr. Akira Tari, Division of Gastroenterology, Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, 1-9-6 Sendamachi, Naka-ku, Hiroshima 734-8551, Japan. stomach2@hiroshima-med.jrc.or.jp
Telephone: +81-82-2413111 Fax: +81-82-2460676
Received: November 20, 2012 Revised: December 25, 2012
Accepted: January 5, 2013
Published online: March 28, 2013

Abstract

Double-balloon enteroscopy (DBE) and video capsule endoscopy are useful for the diagnosis of lymphoma in the small intestine. However, DBE cannot be safely performed in cases with passage disturbance due to wall thickening and stenosis. Additionally, video capsule endoscopy cannot be performed in such cases because of the risk of retention. Here, we report 4 cases of primary follicular lymphoma of the gastrointestinal tract that could be detected using ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography (PET-CT). The endoscopic findings of these 4 cases included lesions with wall thickening, which comprised macroscopically clusters of nodules, dense clusters of whitish granules or small nodules, fold thickening and ulcers with irregular margins that occupied the whole lumen with edematous mucosa. All patients fulfilled the World Health Organization grade 1 criteria. ^{18}F-fluorodeoxyglucose PET-CT can help predict the risks that may result from certain endoscopic examinations, such as DBE and video capsule endoscopy.

Key words: Capsule endoscopy; Double-balloon enteroscopy; Follicular lymphoma; Positron-emission tomography; Computed tomography; Small intestine

Tari A, Asaoku H, Kunihiro M, Tanaka S, Yoshino T. Usefulness of positron emission tomography in primary intestinal follicular lymphoma. *World J Gastroenterol* 2013; 19(12): 1992-1996 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1992.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1992

INTRODUCTION

Primary follicular lymphoma of the gastrointestinal tract (FL-GI) is often diagnosed by initially detecting duodenal lesions using esophago-gastro-duodenoscopy (EGD)[1,2]. FL-GI lesions can exist in broad areas, ranging from the descending portion of the duodenum to the ileum, and may include lymph node involvement[1,2]. The correct diagnosis of the locations of lesions is vital to decisions regarding therapeutic plans[3]. Double-balloon enteroscopy (DBE) and video capsule endoscopy (VCE) are useful for the diagnosis of lesions in the small intestine[4].

However, DBE cannot be safely performed in cases with passage disturbance due to wall thickening and stenosis, and VCE cannot be performed in these cases because of the risk of retention[4]. Conversely, ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography combined with computed tomography (PET-CT) has been reported to be useful for detecting lymph node involvement in the diagnosis of the clinical stages of follicular lymphomas[5]. This study reports the cases of 4 patients for whom PET-CT was useful in the detection of FL-GI in the digestive tract, the location of which ranged from the duodenum to the ileocecal valve.

CASE REPORT

Twenty FL-GI patients (male/female 9/11, age 46-82 years, mean 58 years) consulted the Division of Gastroenterology, Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital from December 2004 to May 2010. In 2 of the 18 patients with duodenal lesions, FL-GI lesions were detected using PET-CT. One of the 14 patients with jejunal lesions and 2 of the 12 patients with ileal lesions had abnormal accumulations according to the PET-CT results.

Table 1 shows the 4 FL-GI patients with abnormal accumulations by PET-CT who had gastrointestinal lesions detected from the duodenum to the ileocecal valve. Case 1 had duodenal lesion, case 2 had jejunal lesions, case 3 had lesions from the terminal ileum to the ileocecal valve and case 4 had lesions in broad areas from the duodenum to the ileum. All lesions were diagnosed by endoscopic examination with biopsy.

The mandatory examinations included palpation of the superficial lymph nodes, blood tests, urinalysis, chest radiography, abdominal ultrasonography, contrast-enhanced computed tomography (CT) scan (General Electric, Fairfield, CT, United States) (CT/CE+) of the neck, chest, abdomen and pelvis, PET-CT (General Electric, Fairfield, Connecticut, United States), bone marrow aspiration, endoscopy with biopsies [EGD (Olympus, Tokyo, Japan), colonoscopy (Fujinon, Tokyo, Japan), DBE (Fujinon, Tokyo, Japan)] and VCE (Given Imaging, Yoqneam, Israel). Each patient was classified by the location of the lesions, clinical stage (Lugano International classification[6]), FL histological grade (World Health Organization grade)[7] and follicular lymphoma international prognosis index[8]. The macroscopic findings of FL-GI were classified by endoscopy into the following 6 types: whitish granules, multiple small nodules, fold swelling and thickening, mass forming, ulcers with irregular margins and rough mucosa[1].

Case 1 is a 52-year-old man who had no symptoms but was diagnosed to have abnormalities in the descending portion of the duodenum by EGD for gastric cancer screening (Table 1). This patient had wall thickening with abnormal accumulation at the descending portion of the duodenum and at the duodenojejunal flexure [maximum standardized uptake value (SUVmax) 6.6 and 5.5, respectively] (Figure 1A) by PET-CT. However, there were no abnormal findings in the small intestine by PET-CT. EGD showed lesions with dense clusters of whitish granules, making it difficult to see the folds in the descending and horizontal portions of duodenum (Figure 1B). The jejunum showed only rough clusters of whitish granules. The terminal ileum of this patient showed mucosa with normal lymphoid tissues.

Case 2 is a 62-year-old woman who presented with swelling of the mesenteric lymph nodes (max, 1.5 cm in diameter) by abdominal ultrasonography and a mild accumulation of FDG (SUVmax, 3.0) by PET-CT. A laparotomic lymph node biopsy led to the diagnosis of FL. Regarding the intestinal lesions, localized accumulation was demonstrated by PET-CT (SUVmax, 3.9) performed in the jejunum 25 mo later. The EGD showed swelling and thickening of the folds that occupied half of the lumen (Table 1).

Case 3 is a 66-year-old woman who underwent EGD because of a 1-wk history of postprandial epigastric discomfort and dyspepsia. There were abnormal findings in the papillae of vater (Table 1). This case showed intestinal wall thickening with abnormal accumulation from the terminal ileum to the cecum by PET-CT (SUVmax, 6.73) (Figure 2A and C). The endoscopic findings in the duodenum included eruptions with mild swelling and erosion at the papillae of Vater. The jejunum and the ileum had sparse clusters of small nodules. Portions from the terminal ileum (Figure 2B) to the ileocecal valve (Figure 2D) had dense clusters of granules and nodules.

Case 4 is a 61-year-old woman undergoing examinations for leukocytosis (white blood cells, 29 740) (Table 1). The PET-CT of this patient showed abnormal accumulations (SUVmax, 5.6) in broad areas from the descending portion of the duodenum through the jejunum to the ileum (Figure 3A). The color of the duodenal bulb was normal, and the macroscopic finding was rough mucosa. The mucosa of the descending and horizontal portions of the duodenum showed clusters of numerous whitish granules. The jejunum and the ileum had multiple ulcers with irregular margins that occupied the whole lumen, with edematous change in broad areas of the mucosa (Figure 3B).

DISCUSSION

FL-GI patients frequently have duodenal and small intestinal lesions, as has been already reported[2]. The macroscopic findings of patient endoscopies that were also detected using PET-CT were clusters of numerous whitish granules spreading over the mucosa, concealing the folds, obvious swelling and thickening of folds and dense clusters of granules and nodules (Cases 1-3). PET-CT could detect lesions of ulcers with irregular margins that occupied the whole lumen with edematous mucosa as well as lesions with clusters of numerous whitish granules

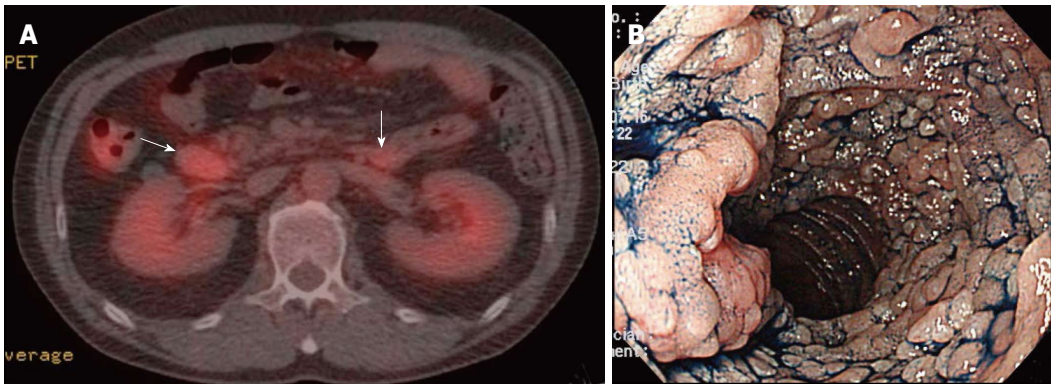


Figure 1 A 52-year-old man with follicular lymphoma of the gastrointestinal tract. A: ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography in transaxial images showed focal hypermetabolic activities (maximum standardized uptake value, 6.6 and 5.5, respectively) in the lesions of the descending portion of the duodenum and the duodenojejunal flexure (arrows), respectively; B: The esophago-gastro-duodenoscopic view with indigo carmine dye-spray of the descending portion of the duodenum. Numerous whitish granules densely clustered together.

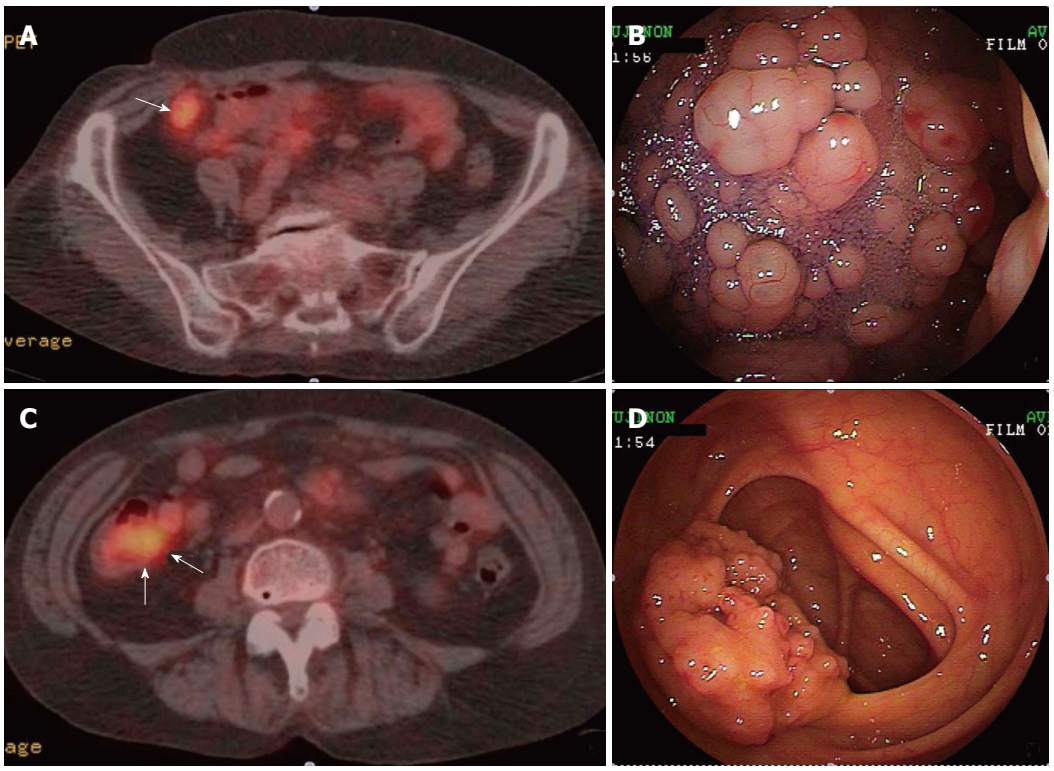


Figure 2 A 66-year-old woman with follicular lymphoma of the gastrointestinal tract. A, C: ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography in transaxial images showed focal hypermetabolic activities (maximum standardized uptake value, 6.73) in the lesion of the terminal ileum (A) (arrow) and the cecum (C) (arrows); B: The colonoscopic view with indigo carmine dye-spray of the terminal ileum. Numerous polypoid lesions of varying sizes (granules to small nodules) were densely clustered; D: The colonoscopic view of the ileocecal valve. Numerous polypoid lesions of varying sizes (granules to small nodules) were densely clustered.

(Case 4). Lesions with a scattered distribution of whitish granules and small nodules were not shown as abnormal accumulations by PET-CT (Cases 1 and 3). It is assumed that a greater wall thickness of a GI lesion results in a greater possibility of abnormal accumulation according to the PET-CT results.

There were 2 FL-GI patients with small intestinal tumors whose initial symptom was ileus (4.3% of FL-GI at our division in April 2012). PET-CT is a valuable tool for the detection of the lesions in these patients (PET-CT findings not shown). The detection rate of the GI lesions of FL by PET-CT is generally reported to be rather low[9,10]. However, among the gastrointestinal lesions that were detected by PET-CT were cases with wall thickening and macroscopic clusters of nodules, dense clusters of whitish granules or small nodules, fold thickening or ulcers that showed irregular margins and occupied the whole lumen with edematous mucosa. Therefore, PET-CT is useful in cases with lesions that are difficult to approach using DBE and in cases that have a risk of

Table 1 Patients detected to have primary follicular lymphoma of gastrointestinal tract by ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography

Case No.	Age (yr)	Sex	Diagnosis (WHO grade)	Clinical stage (Lugano)	FLIPI	Locations in GI tract	Endoscopic appearances	PET-CT
1	52	M	FL (grade 1)	I	Low	Duodenum descending portion-duodenojejunal flexure	Dense cluster of whitish granules	(+) descending portion and duodenojejunal flexure of duodenum
						Jejunum	Cluster of whitish granules	(-)
						Terminal ileum	Normal lymph follicles	(-)
2	62	F	FL (grade 1)	II 2	Intermediate	Jejunum	Swelling and thickening of folds	(+) jejunum
						Ileum	Normal lymph follicles	(-)
3	66	F	FL (grade 1)	II 2	Intermediate	Papilla vater	Mild swelling and erosion	(-)
						Jejunum	Sparse cluster of small nodules	(-)
						Ileum	Sparse cluster of small nodules	(-)
						Terminal ileum-ileocecal valve	Dense cluster of granules and nodules	(+) ileocecal valve
4	61	F	FL (grade 1)	IV	High	Duodenal bulb	Rough mucosa	(-)
						Duodenum descending portion-jejunum	Cluster of numerous whitish granules	(+) duodenum-jejunum
						Ileum	Multiple ulcers with irregular margin	(+) ileum

M: Male; F: Female; FL: Follicular lymphoma; WHO: World Health Organization; FLIPI: Follicular lymphoma international prognostic index; PET-CT: Positron emission tomography combined with computed tomography; GI: Gastrointestinal.

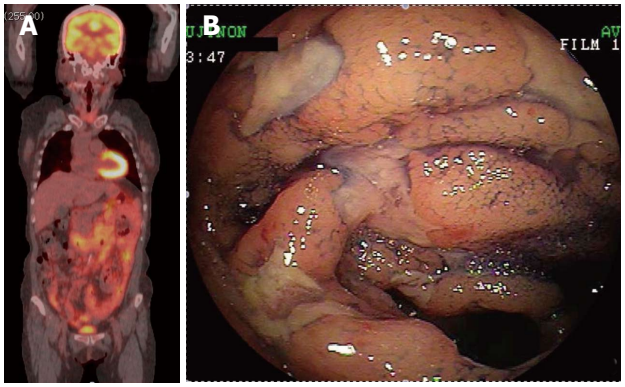


Figure 3 A 61-year-old woman with follicular lymphoma of the gastrointestinal tract. A: ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography in a projected image showed hypermetabolic foci in broad areas of the gastrointestinal tract from the 2nd portion of the duodenum to the terminal ileum (maximum standardized uptake value, 5.6); B: The colonoscopic view with indigo carmine dye-spray of the terminal ileum. Multiple ulcers with irregular margins were observed.

retention with VCE because of stenosis due to tumors or wall thickening of the deep portion of small intestine. PET-CT is a useful tool for detecting the involvement of lymph nodes and other organs in follicular lymphoma[3]. It is not only useful for deciding whether VCE should be performed, but also for deciding the method by which lesions in the small intestine are approached using DBE, which is necessary for the pathological diagnosis of the biopsy.

REFERENCES

1 **Tari A**, Asaoku H, Kunihiro M, Tanaka S, Fujihara M, Yoshino T. Clinical features of gastrointestinal follicular lymphoma: comparison with nodal follicular lymphoma and gastrointestinal MALT lymphoma. *Digestion* 2011; **83**: 191-197 [PMID: 21266815 DOI: 10.1159/000321814]

2 **Takata K**, Okada H, Ohmiya N, Nakamura S, Kitadai Y, Tari A, Akamatsu T, Kawai H, Tanaka S, Araki H, Yoshida T, Okumura H, Nishisaki H, Sagawa T, Watanabe N, Arima N, Takatsu N, Nakamura M, Yanai S, Kaya H, Morito T, Sato Y, Moriwaki H, Sakamoto C, Niwa Y, Goto H, Chiba T, Matsumoto T, Ennishi D, Kinoshita T, Yoshino T. Primary gastrointestinal follicular lymphoma involving the duodenal second portion is a distinct entity: a multicenter, retrospective analysis in Japan. *Cancer Sci* 2011; **102**: 1532-1536 [PMID: 21561531 DOI: 10.1111/j.1349-7006.2011.01980.x]

3 **Bendandi M**. Aiming at a curative strategy for follicular lymphoma. *CA Cancer J Clin* ; **58**: 305-317 [PMID: 18755938 DOI: 10.3322/CA.2008.0011]

4 **Akamatsu T**, Kaneko Y, Ota H, Miyabayashi H, Arakura N, Tanaka E. Usefulness of double balloon enteroscopy and video capsule endoscopy for the diagnosis and management of primary follicular lymphoma of the gastrointestinal tract in its early stages. *Dig Endosc* 2010; **22**: 33-38 [PMID: 20078662 DOI: 10.1111/j.1443-1661.2009.00915.x]

5 **Hofman MS**, Hicks RJ. Imaging in follicular NHL. *Best Pract Res Clin Haematol* 2011; **24**: 165-177 [PMID: 21658616 DOI: 10.1016/j.beha.2011.02.005]

6 **Rohatiner A**, d'Amore F, Coiffier B, Crowther D, Gospodarowicz M, Isaacson P, Lister TA, Norton A, Salem P, Shipp M. Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. *Ann Oncol* 1994; **5**: 397-400 [PMID: 8075046]

7 **Swerdlow SH,** Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classifications of tumors of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press, 2008

8 **Solal-Céligny P**, Roy P, Colombat P, White J, Armitage JO, Arranz-Saez R, Au WY, Bellei M, Brice P, Caballero D, Coiffier B, Conde-Garcia E, Doyen C, Federico M, Fisher RI, Garcia-Conde JF, Guglielmi C, Hagenbeek A, Haïoun C, LeBlanc M, Lister AT, Lopez-Guillermo A, McLaughlin P, Milpied N, Morel P, Mounier N, Proctor SJ, Rohatiner A, Smith P, Soubeyran P, Tilly H, Vitolo U, Zinzani PL, Zucca E, Montserrat E. Follicular lymphoma international prognostic index. *Blood* 2004; **104**: 1258-1265 [PMID: 15126323 DOI: 10.1182/blood-2003-12-4434]

9 **Hoffmann M**, Chott A, Püspök A, Jäger U, Kletter K, Raderer M. 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) does not visualize follicular lymphoma of the duodenum. *Ann Hematol* 2004; **83**: 276-278 [PMID: 15060746 DOI: 10.1007/s00277-003-0827-6]

10 **Higuchi N**, Sumida Y, Nakamura K, Itaba S, Yoshinaga S, Mizutani T, Honda K, Taki K, Murao H, Ogino H, Kanayama K, Akiho H, Goto A, Segawa Y, Yao T, Takayanagi R. Impact of double-balloon endoscopy on the diagnosis of jejunoileal involvement in primary intestinal follicular lymphomas: a case series. *Endoscopy* 2009; **41**: 175-178 [PMID: 19214900 DOI: 10.1055/s-0028-1119467]

P- Reviewers Bendandi M, Seto M
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.1997

World J Gastroenterol 2013 March 28; 19(12): 1997-1999
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Transmesocolic hernia with strangulation in a patient without surgical history: Case report

Peel Jung, Min Dae Kim, Tae Hyun Ryu, Sung Ho Choi, Han Se Kim, Kang Hun Lee, Jhong Hyun Park

Peel Jung, Tae Hyun Ryu, Sung Ho Choi, Han Se Kim, Kang Hun Lee, Department of Internal Medicine, Bongseng Memorial Hospital, Busan 601-723, South Korea
Min Dae Kim, Department of Gastroenterology, Bongseng Memorial Hospital, Busan 601-723, South Korea
Jhong Hyun Park, Department of General Surgery, Bongseng Memorial Hospital, Busan 601-723, South Korea
Author contributions: Jung P drafted and edited the manuscript; Ryu TH and Choi SH treated the patient; Kim HS and Lee KH contributed to the literature review; Park JH performed the operation; Kim MD contributed to the final approval.
Correspondence to: Min Dae Kim, MD, Department of Gastroenterology, Bongseng Memorial Hospital, 401 Jwacheon 1-dong, Dong-gu, Busan 601-723,
South Korea. mdmdk69@hanmail.net
Telephone: +82-51-6644000 Fax: +82-51-6644059
Received: November 25, 2012 Revised: December 10, 2012
Accepted: December 22, 2012
Published online: March 28, 2013

Abstract

Transmesenteric hernias have bimodal distribution and occur in both pediatric and adult patients. In the adult population, the cause is iatrogenic, traumatic, or inflammatory. We report a case of transmesocolic hernia in an elderly person without any preoperative history. An 84-year-old Korean female was admitted with mid-abdominal pain and distension for 1 d. On abdominal computed tomography, we diagnosed transmesocolic hernia with strangulated small bowel obstruction, and performed emergency surgery. The postoperative period was uneventful and she was discharged 11 d after surgery. Hence, it is important to consider the possibility of transmesocolic hernia in elderly patients with signs and symptoms of intestinal obstruction, even in cases with no previous surgery.

Key words: Transmesocolic hernia; Strangulation; Operation; Abdominal computed tomography; Small bowel obstruction; Internal hernia

Jung P, Kim MD, Ryu TH, Choi SH, Kim HS, Lee KH, Park JH. Transmesocolic hernia with strangulation in a patient without surgical history: Case report. *World J Gastroenterol* 2013; 19(12): 1997-1999 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/1997.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.1997

INTRODUCTION

The incidence of internal hernia is less than 1%[1], and transmesocolic hernia is a particularly rare type of internal hernia. The overall mortality is more than 50% in cases with strangulated small bowel obstruction[2]. In adults, transmesocolic hernias are most often caused by previous surgical procedures, abdominal trauma or intraperitoneal inflammation, and transmesocolic hernia in a person without a surgical history is extremely rare. We report such a case of transmesocolic hernia with strangulated intestinal obstruction.

CASE REPORT

An 84-year-old Korean female, with no past history of surgery, was admitted with mid-abdominal pain and distension for 1 d. Upon admission, her blood pressure was 120/80 mmHg, heart rate 72 beats/min, and body temperature 36.6 ℃. On physical examination of the area of concern, mid-abdominal tenderness was observed. On admission, laboratory assessments were as follows: white blood cell count 14 500/mm^3 (segmented neutrophil 91.4%), hemoglobin concentration 12.8 g/dL, platelet count 272 000/mm^3, sodium 135 mmol/L, potassium 4.5 mmol/L, blood urea nitrogen 21.5 mg/dL, creatinine 0.9 mg/dL, aspartate aminotransferase 18 IU/L, alanine aminotransferase 10 IU/L, alkaline phosphatase 256 IU/L,

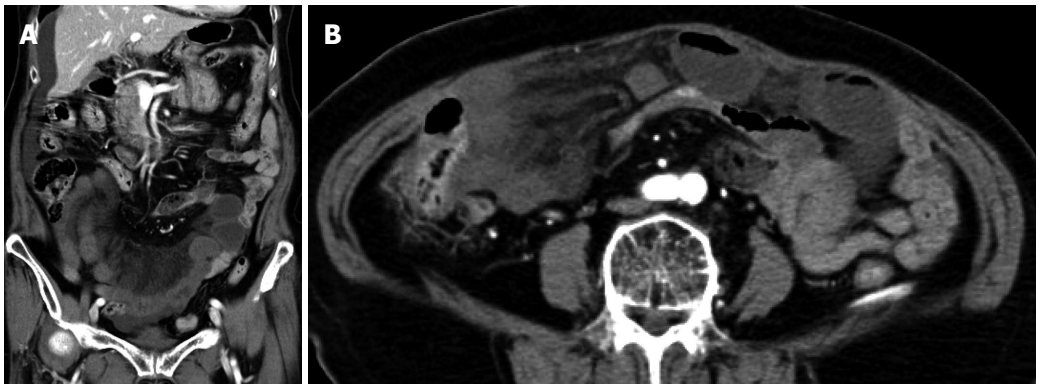


Figure 1 Abdominal computed tomography findings. Crowded small bowel loop (from distal jejunum to proximal ileum) with circumferential wall thickening and decreased enhancement in the middle and lower abdomen, stretched mesenteric vessels with mesenteric edema. A: Sagittal view; B: Transverse view.

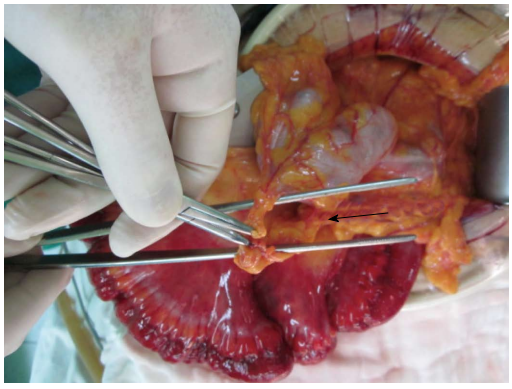


Figure 2 Intraoperative findings. Herniated small intestine with strangulation by perforated omentum (arrow) below the transverse colon.

lactate dehydrogenase 415 IU/L, γ-glutamyltransferase 17 IU/L, and C-reactive protein 1.04 mg/dL. A simple abdominal X-ray showed distended small bowel loops. Abdominal computed tomography (CT) revealed crowded small bowel loops (from distal jejunum to proximal ileum), with circumferential wall thickening and decreased enhancement in the middle and lower abdomen, and stretched mesenteric vessels with mesenteric edema (Figure 1). We diagnosed transmesocolic hernia with intestinal obstruction and performed emergency surgery. During the operation, a herniated small intestine with strangulation by perforated omentum was noted below the transverse colon (Figure 2). Strangulated herniation was seen 190 cm from the ligament of Treiz, for a length of 160 cm. The strangulated small intestine herniation was resected and the omentum defect was closed. The postoperative course was uneventful and the patient was discharged on postoperative day 11, with a favorable follow-up as an outpatient for 1 mo.

DISCUSSION

An internal hernia is the protrusion of an abdominal organ through a normal or abnormal mesenteric or peritoneal aperture[3]. An internal hernia can be acquired as a result of trauma or a surgical procedure, or may be constitutional and related to congenital peritoneal defects. In the broad category of internal hernias, there are several main types based on their location, as traditionally described by Meyers. These consist of paraduodenal (53%), pericecal (13%), foramen of Winslow-related (8%), transmesentric and transmesocolic (8%), intersigmoid (6%), and retroanastomotic (5%), with the overall incidence of internal hernia being 0.2%-0.9%[2]. The transmesenteric hernia has three main types: transmesocolic, through a small-bowel mesenteric defect, and Peterson's hernia[1]. Most transmesocolic hernias in children result from a congenital defect in the small bowel mesentery close to the ileocecal region. Congenital defects occur following the embryonic formation of an intestinal loop in thin avascular areas of the mesentery (*e.g.*, the mesenteries of the lower ileum, the sigmoid mesocolon, and the transverse mesocolon). As a consequence, there are multiple theories of congenital causes of such mesenteric defects[4]. It is likely that the congenital condition is associated with prenatal intestinal ischemic accidents due to the observed frequently in infants with atretic bowel segments. In adults, transmesocolic hernias are most often caused by previous surgical procedures, abdominal trauma or intraperitoneal inflammation. When the small bowel is herniated through a defect in the mesentery or omentum, the herniated bowel is compressed against the abdominal wall. In this case, the herniated bowel is clustered and lies outside the colon which is displaced centrally[5].

Transmesocolic hernias are more likely than other subtypes to develop volvulus and strangulation, or ischemia, the reported incidence of which are as high as 30% and 40%, respectively, with mortality rates of 50% for treated groups and 100% for non-treated subgroups[2,6]. Clinically, internal hernias can be asymptomatic, or can cause discomfort ranging from constant vague epigastric pain to intermittent colicky periumbilical pain, while additional symptoms include nausea and vomiting[1].

An internal hernia is difficult to diagnosis by physical examination, and the most important diagnostic method is abdominal CT. It has been suggested that the two findings of a peripherally located small bowel, and lack

of omental fat between the loops and the anterior abdominal wall, might be the most helpful CT signs, with an overall sensitivity of 85% and 92% for each respective finding[6,7]. Observation of the clustering of small bowel loops and an abnormality in the mesenteric vessels are helpful findings on abdominal CT.

In adults, a previous surgical procedure, as well as trauma or inflammation are the most common causes of transmesocolic hernia. Our case was a rare presentation in an elderly person without a history of trauma, and without previous surgery[8-12]. The patient had non-specific symptoms and signs on plain abdominal X-ray, but also non-specific abdominal distension upon physical examination. In the case of internal hernia, these defects may be idiopathic, but we can speculate that a small congenital defect existed without any hernia, and enlarged due to the aging. A transmesocolic hernia is difficult to diagnosis preoperatively despite the array of diagnostic techniques currently available. In patients suspected of having an internal hernia, early surgical intervention may be advisable due to the high morbidity and mortality rates. Therefore, it is important to consider the possibility of a transmesocolic hernia when patients have signs and symptoms of intestinal obstruction, even in cases of elderly patients with no previous surgical history.

REFERENCES

1 **Martin LC**, Merkle EM, Thompson WM. Review of internal hernias: radiographic and clinical findings. *AJR Am J Roentgenol* 2006; **186**: 703-717 [PMID: 16498098 DOI: 10.2214/AJR.05.0644]

2 **Meyers MA.** Dynamic radiology of the abdomen: normal and pathologic anatomy, 5th ed. New York, NY: Springer-Verlag, 2000: 711-748

3 **Mathieu D**, Luciani A. Internal abdominal herniations. *AJR Am J Roentgenol* 2004; **183**: 397-404 [PMID: 15269032]

4 **Treves F**. Lectures on the Anatomy of the Intestinal Canal and Peritoneum in Man. *Br Med J* 1885; **1**: 470-474 [PMID: 20751187 DOI: 10.1136/bmj.1.1262.470]

5 **Tauro LF**, Vijaya G, D'Souza CR, Ramesh HC, Shetty SR, Hegde BR, Deepak J. Mesocolic hernia: an unusual internal hernia. *Saudi J Gastroenterol* 2007; **13**: 141-143 [PMID: 19858633 DOI: 10.4103/1319-3767.33468]

6 **Blachar A**, Federle MP. Internal hernia: an increasingly common cause of small bowel obstruction. *Semin Ultrasound CT MR* 2002; **23**: 174-183 [PMID: 11996230]

7 **Takeyama N**, Gokan T, Ohgiya Y, Satoh S, Hashizume T, Hataya K, Kushiro H, Nakanishi M, Kusano M, Munechika H. CT of internal hernias. *Radiographics* 2005; **25**: 997-1015 [PMID: 16009820 DOI: 10.1148/rg.254045035]

8 **Park HS**, Kim JI, Kim MS, Kim SS, Cho SH, Park SH, Han JY, Kim JK. [A case of transmesocolic hernia in elderly person without a history of operation]. *Korean J Gastroenterol* 2006; **48**: 286-289 [PMID: 17060724]

9 **Ueda J**, Yoshida H, Makino H, Yokoyama T, Maruyama H, Hirakata A, Ueda H, Watanabe M, Uchida E, Uchida E. Transmesocolic hernia of the ascending colon with intestinal obstruction. *Case Rep Gastroenterol* 2012; **6**: 344-349 [PMID: 22740809 DOI: 10.1159/000339691]

10 **Sivit CJ**. CT scan of mesentery-omentum peritoneum. *Radiol Clin North Am* 1996; **34**: 863-884 [PMID: 8677314]

11 **Yoo E**, Kim JH, Kim MJ, Yu JS, Chung JJ, Yoo HS, Kim KW. Greater and lesser omenta: normal anatomy and pathologic processes. *Radiographics* 2007; **27**: 707-720 [PMID: 17495288 DOI: 10.1148/rg.273065085]

12 **Chatterjee S**, Chatterjee S, Kumar S, Gupta S. Acute intestinal obstruction: a rare aetiology. *Case Rep Surg* 2012; **2012**: 501209 [PMID: 22792504 DOI: 10.1155/2012/501209]

P- Reviewer Mizrahi S **S- Editor** Gou SX
L- Editor Cant MR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.2000

World J Gastroenterol 2013 March 28; 19(12): 2000-2004
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Rare case of *Helicobacter pylori*-related gastric ulcer: Malignancy or pseudomorphism?

Ting-Ting Li, Feng Qiu, Zhi-Qiang Wang, Lu Sun, Jun Wan

Ting-Ting Li, Jun Wan, Department of Geriatric Gastroenterology, Chinese PLA General Hospital, Beijing 100853, China
Feng Qiu, Department of Neurology, Chinese Navy General Hospital, Beijing 100037, China
Zhi-Qiang Wang, Department of Geriatric Gastrointestinal Endoscopy, Chinese PLA General Hospital, Beijing 100853, China
Lu Sun, Department of Pathology, Chinese PLA General Hospital, Beijing 100853, China
Author contributions: Li TT and Qiu F substantially contributed to conception and design, acquisition of data, analysis and interpretation of data; Wang ZQ and Sun L drafted the article and revised it critically for important intellectual content; Wan J approved the final version to be published.
Correspondence to: Dr. Jun Wan, Department of Geriatric Gastroenterology, Chinese PLA General Hospital, Beijing 100853, China. wanjun301@126.com
Telephone: +86-10-66876266 Fax: +86-10-66876266
Received: December 7, 2012 Revised: January 5, 2013
Accepted: January 18, 2013
Published online: March 28, 2013

Abstract

Helicobacter pylori (*H. pylori*) is a pathogen and the most frequent cause of gastric ulcers. There is also a close correlation between the prevalence of *H. pylori* infection and the incidence of gastric cancer. We present the case of a 38-year-old woman referred by her primary care physician for screening positron emission tomography-computed tomography (PET-CT), which showed a nodular strong accumulation point with standardized uptake value 5.6 in the gastric fundus. Gastroscopy was then performed, and a single arched ulcer, 12 mm in size, was found in the gastric fundus. Histopathological examination of the lesion revealed chronic mucosal inflammation with acute inflammation and *H. pylori* infection. There was an obvious mitotic phase with widespread lymphoma. Formal anti-*H. pylori* treatment was carried out. One month later, a gastroscopy showed a single arched ulcer, measuring 10 mm in size in the gastric fundus. Histopathological examination revealed chronic mucosal inflammation with acute inflammation and a very small amount of *H. pylori* infection. The mitotic phase was 4/10 high power field, with some heterotypes and an obvious nucleolus. Follow-up gastroscopy 2 mo later showed the gastric ulcer in stage S2. The mucosal swelling had markedly improved. The patient remained asymptomatic, and a follow-up PET-CT was performed 6 mo later. The nodular strong accumulation point had disappeared. Follow-up gastroscopy showed no evidence of malignant cancer. *H. pylori*-associated severe inflammation can lead to neoplastic changes in histiocytes. This underscores the importance of eradicating *H. pylori*, especially in those with mucosal lesions, and ensuring proper follow-up to prevent or even reverse early gastric cancer.

Key words: *Helicobacter pylori*; Gastric ulcer; Gastric cancer; Positron emission tomography-computed tomography; Gastroscopy

Li TT, Qiu F, Wang ZQ, Sun L, Wan J. Rare case of *Helicobacter pylori*-related gastric ulcer: Malignancy or pseudomorphism? *World J Gastroenterol* 2013; 19(12): 2000-2004 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/2000.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.2000

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic bacterium that colonizes the stomach of approximately two-thirds of the human population and is involved in the pathogenesis of various gastroenterological diseases including gastric ulcer and gastric cancer. *H. pylori*'s interaction with the host has an impact on the severity of these diseases and their clinical outcome[1,2].

The mechanisms of *H. pylori*-related colonization are not fully understood. However, different types of *H. py-*

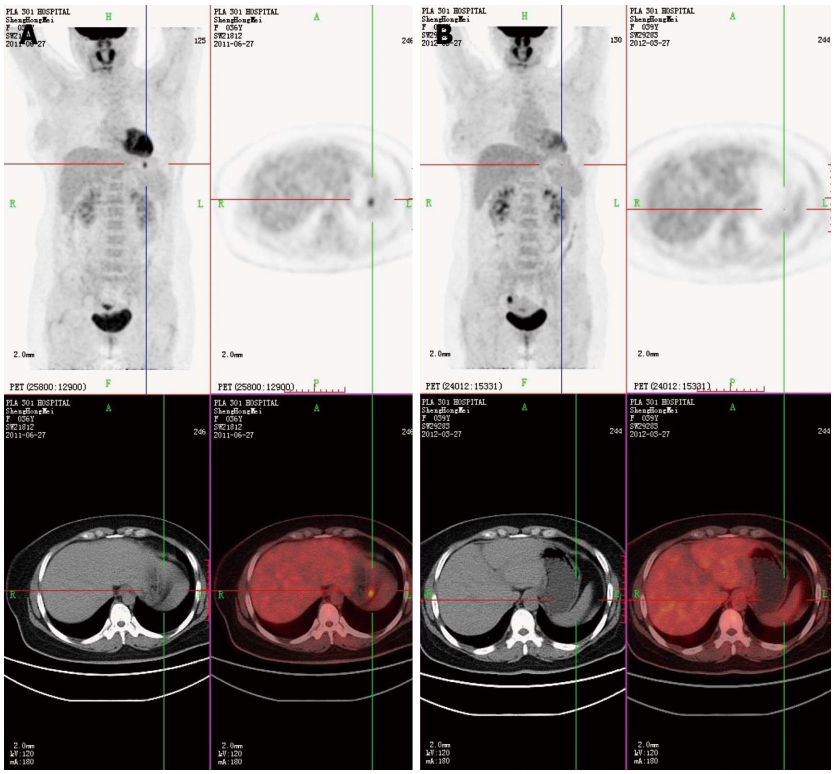


Figure 1 Positron emission tomography-computed tomography. A: Positron emission tomography-computed tomography showed a nodular strong accumulation point with standardized uptake value 5.6 in the gastric fundus; B: After treatment, the nodular strong accumulation point in the gastric fundus had disappeared.

lori virulence factors, especially cytotoxin-associated gene A (CagA), vacuolating cytotoxin A (VacA), outer inflammation protein A and so on are reported to be correlated with *H. pylori*-related diseases. One of the major bacterial virulence factors, the VacA, seems to be involved in the physiologic mechanism. The VacA protein encoded by the polymorphic *H. pylori VacA* gene, is produced and secreted by all bacterium strains and induces the formation of intracellular vacuoles in epithelial cell lines *in vitro*. Environmental and demographic data also interfere with the pathophysiology of *H. pylori*-associated gastric diseases[3].

CASE REPORT

We present a case of a 38-year-old woman with a history of thyroid cancer who was referred by her primary care physician for a screening positron emission tomography-computed tomography (PET-CT). She was essentially asymptomatic and did not report any abdominal pain, dysphagia, nausea, or vomiting. Findings of a physical examination were unremarkable.

PET-CT showed a nodular strong accumulation point with standardized uptake value (SUV) 5.6 in the gastric fundus (Figure 1A). Gastroscopy was then performed, and demonstrated a single arched ulcer, measuring 12 mm in size, in the gastric fundus (Figure 2A and B). Histopathological examination revealed that the lesion had chronic mucosal inflammation with acute inflammation and *H. pylori* infection. There was an obvious mitotic phase with widespread lymphoma immunohistochemical staining was positive for CD4 (T cell), CD3 (T cell), CD20, Ki-67 (+25%), CD79a (+++), PAX-5, CD45RO and negative for CD56, TIA-1, TIF-1, Bcl-6, CD10, CD30, CD34, CD117, CK, MUM-1, MPO (Figure 3A).

The patient was given *H. pylori* eradication therapy, based on proton pump inhibitor-clarithromycin-amoxicillin-mucosal protective agent treatment, the so-called quadruple 14 d therapy. One month later, gastroscopy was performed and showed a single arched ulcer, measuring 10 mm in size in the gastric fundus (Figure 2C). Histopathological examination revealed that the lesion had chronic mucosal inflammation with acute inflammation and a small amount of *H. pylori* infection (Figure 3B). The mitotic phase was 4/10 high power field with some heterotypes and an obvious nucleolus. Immunohistochemical staining showed tissue cell-like cells positive for S-100, vimentin, CD68, Ki-67 (30%) and negative for CD1a, CD21,Bcl-2, CD3, CD20, CD30, CD45RO, CD117 and PAX-5 (Figure 3C). For further examination, immunohistochemical staining was repeated by the Beijing Cancer Hospital and showed that staining for CD1a was positive for focal lesions (Figure 3D). The shape and immunophenotype indicated Langerhans histiocytosis. Because of the active growth of cancer cells, the patient was referred for medical oncology evaluation for this unusual pathologic finding with malignant potential.

Follow-up gastroscopy 2 mo later showed that the gastric ulcer was in stage S2 (Figure 2D). The mucosal swelling was markedly reduced. Endoscopic ultrasonography showed that the local echo was normal and each layer was clearly divided (Figure 2E). Histopathological examination showed chronic mucosal inflammation with lymphoid tissue hyperplasia in the lamina propria (Figure 3E).

The patient remained asymptomatic, and a follow-up PET-CT was performed 6 mo later. The nodular strong accumulation point with SUV 5.6 in the gastric fundus

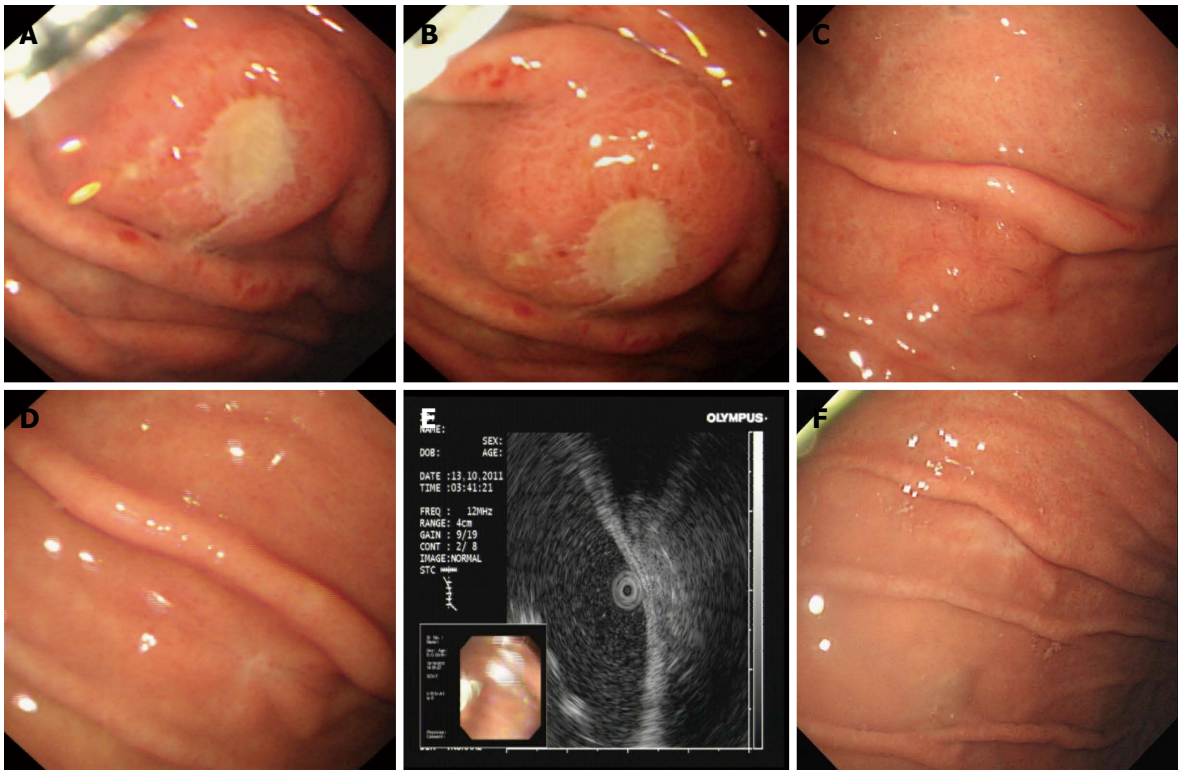


Figure 2 Gastroscopy showed a single arched ulcer and changes after treatment. A, B: A single arched ulcer, measuring 12 mm in size, was found in the gastric fundus (July 1, 2011); C: A single arched ulcer, measuring 10 mm in size, was found in the gastric fundus (August 16, 2011); D: The gastric ulcer was in stage S2 (October 13, 2011); E: Endoscopic ultrasonography showed that the local echo was normal and each layer was clearly divided (October 13, 2011); F: The gastric ulcer was in stage S2 (April 25, 2012).

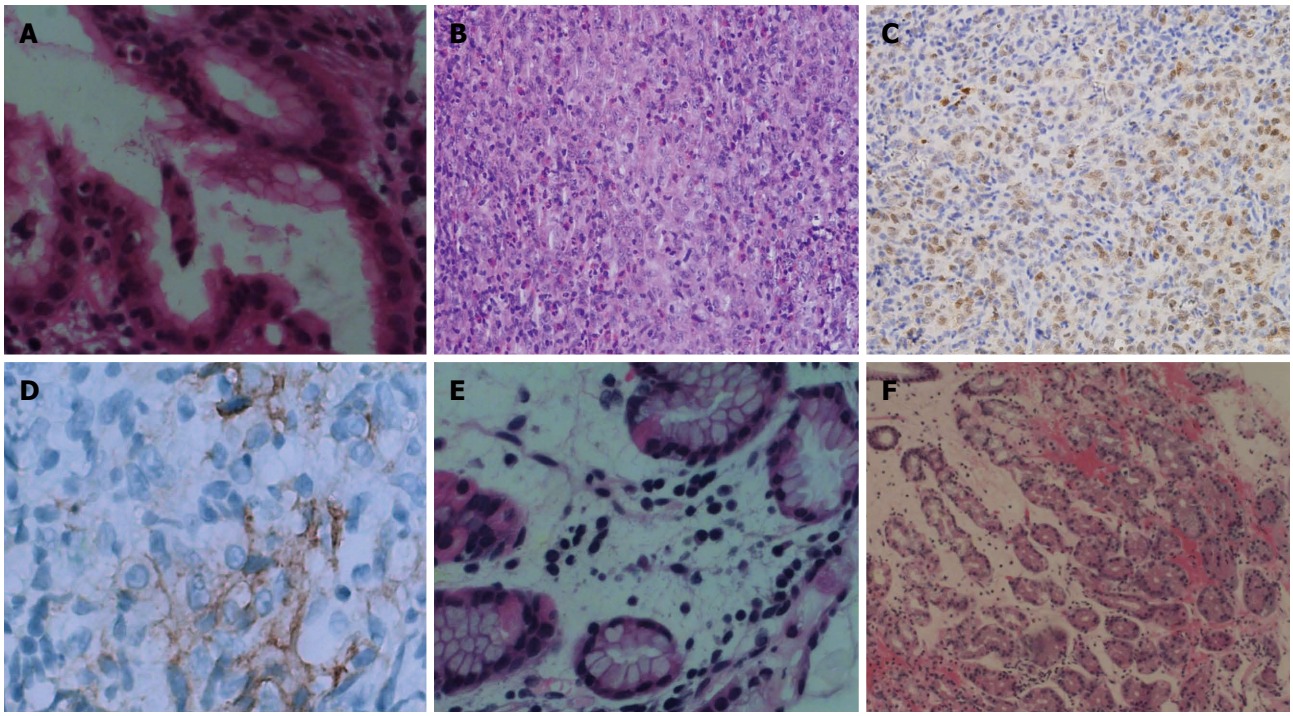


Figure 3 Histopathology changes after treatment. A: Histopathological examination revealed that the lesion had chronic mucosal inflammation with acute inflammation and a small amount of *Helicobacter pylori* (*H. pylori*) infection. The mitotic phase was obvious and lymphoma was widespread [hematoxylin and eosin (HE), × 400, July 2, 2011]; B: Histopathological examination revealed chronic mucosal inflammation with acute inflammation and a only a small amount of *H. pylori* infection. The mitotic phase was 4/10 high power field (HE, ×400, August 17, 2011); C: Immunohistochemical staining showed tissue cell-like cells with S-100 (×400, August 17, 2011); D: Immunohistochemical staining was repeated by the Beijing Cancer Hospital and showed that staining for CD1a was positive for focal lesions (×400, August 25, 2011); E: Histopathological examination showed chronic mucosal inflammation with lymphoid tissue hyperplasia in the lamina propria (HE, ×400, October 14, 2011); F: Histopathological examination revealed that chronic mucosal inflammation with acute inflammation (HE, ×10 , May 8, 2012).

had disappeared (Figure 1B). Follow-up gastroscopy at the same time showed that the gastric ulcer was in stage S2 (Figure 2F). Histopathological examination revealed that the lesion had chronic mucosal inflammation with acute inflammation (Figure 3F). Therefore, we found no evidence of malignant cancer.

DISCUSSION

H. pylori infection is a worldwide disease, with about half of the world's population harboring this bacterium in their stomach. The infection is asymptomatic in most individuals. However, it is the leading cause of non-ulcer dyspepsia, peptic ulcers and gastric tumors[4].

H. pylori is able to survive in the gastric acidic environment because of its ability to synthesize urease, an enzyme which can neutralize the stomach acidic pH. It seems to play a role in the mechanisms which lead to gastric cancer by inducing methylation in different genes, interfering with apoptotic pathways and by causing inflammatory events leading to gastritis, then to atrophic gastritis and possibly to gastric cancer[5]. It may affect the acid secretion of the parietal cells by causing mucosal inflammation. Gastric acid secretion depends on the localization and the degree of the inflammation. Acute infection with *H. pylori* results in hypochlorhydria, whereas chronic infection can cause either hypo- or hyper-chlorhydria, depending on the distribution of the infection and the degree of corpus gastritis[6]. *H. pylori* is a powerful carcinogen, since it is able to induce genetic changes, such as hypermethylation events, contributing to cell transformation[5].

H. pylori is well recognized as a class I carcinogen because long-term colonization by this organism can provoke chronic inflammation and atrophy, which can further lead to malignant transformation[7]. Chronic inflammation plays important roles in the development of various cancers, particularly in digestive organs, including *H. pylori*-associated gastric cancer[8]. During chronic inflammation, *H. pylori* can induce genetic and epigenetic changes, including point mutations, deletions, duplications, recombinations, and methylation of various tumor-related genes through various mechanisms, which act in concert to alter important pathways involved in normal cellular function, and hence accelerate inflammation-associated cancer development[9]. Alfizah *et al*[10] reported that variant of *H. pylori* CagA proteins induce different magnitudes of morphological changes in gastric epithelial cells. In his study, the CagA protein was injected into gastric epithelial cells and supposedly induced morphological changes termed the "hummingbird phenotype", which is associated with scattering and increased cell motility. The molecular mechanisms leading to the CagA-dependent morphological changes are only partially known[11,12]. The activity of different CagA variants in the induction of the hummingbird phenotype in gastric epithelial cells depends at least in part on EPIYA motif variability. The difference in CagA genotypes might influence the potential of individual CagAs to cause morphological changes in host cells. Depending on the relative exposure of cells to CagA genotypes, this may contribute to the various disease outcomes caused by *H. pylori* infection in different individuals[10].

Epidemiologic studies have demonstrated that *H. pylori* infection is associated with increased risk of the development of gastric cancer[13-15]. Animal studies have also shown that *H. pylori* infection leads to gastric carcinogenesis, especially intestinal phenotypes. Yu *et al*[16] carried out an *in vitro* study of cell transformation induced by *H. pylori* and showed that *H. pylori* induced morphologic changes in GES-1 cells and significantly increased the proliferation of GES-1 cells. Because the transition from inflamed mucosa to atrophic change is a common route to carcinogenesis, the effect of *H. pylori* eradication on the incidence of this early precursor lesion is of interest[17]. Since *H. pylori* infection is associated with gastric carcinoma, therapy is warranted for its eradication[5,18,19].

This was a rare case of a *H. pylori*-related gastric ulcer that resembled gastric cancer. PET-CT SUV was high, and gastroscopy showed a large ulcer with malignant-like histopathological features. However, after *H. pylori* eradication treatment, the lesion recovered quickly and follow-up examination showed no evidence of malignant cancer.

REFERENCES

1 **Peterson WL**. Helicobacter pylori and peptic ulcer disease. *N Engl J Med* 1991; **324**: 1043-1048 [PMID: 2005942]
2 **Egan BJ**, Holmes K, O'Connor HJ, O'Morain CA. Helicobacter pylori gastritis, the unifying concept for gastric diseases. *Helicobacter* 2007; **12** Suppl 2: 39-44 [PMID: 17991175]
3 **Suzuki RB**, Cola RF, Cola LT, Ferrari CG, Ellinger F, Therezo AL, Silva LC, Eterovic A, Sperança MA. Different risk factors influence peptic ulcer disease development in a Brazilian population. *World J Gastroenterol* 2012; **18**: 5404-5411 [PMID: 23082057 DOI: 10.3748/wjg.v18.i38.5404]
4 **De Francesco V**, Ierardi E, Hassan C, Zullo A. Helicobacter pylori therapy: Present and future. *World J Gastrointest Pharmacol Ther* 2012; **3**: 68-73 [PMID: 22966485 DOI: 10.4292/wjgpt.v3.i4.68]
5 **Giordano A**, Cito L. Advances in gastric cancer prevention. *World J Clin Oncol* 2012; **3**: 128-136 [PMID: 23061031 DOI: 10.5306/wjco.v3.i9.128]
6 **Schubert ML**. Gastric secretion. *Curr Opin Gastroenterol* 2007; **23**: 595-601 [PMID: 17906434]
7 **Lee YC**, Chen TH, Chiu HM, Shun CT, Chiang H, Liu TY, Wu MS, Lin JT. The benefit of mass eradication of Helicobacter pylori infection: a community-based study of gastric cancer prevention. *Gut* 2012 Jun 14; Epub ahead of print [PMID: 22698649]
8 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789 [PMID: 11556297]
9 **Chiba T**, Marusawa H, Ushijima T. Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. *Gastroenterology* 2012; **143**: 550-563 [PMID: 22796521 DOI: 10.1053/j.gastro.2012.07.009]
10 **Alfizah H**, Ramelah M. Variant of Helicobacter pylori CagA proteins induce different magnitude of morphological changes in gastric epithelial cells. *Malays J Pathol* 2012; **34**: 29-34 [PMID: 22870595]

11 **Lin LL**, Huang HC, Ogihara S, Wang JT, Wu MC, McNeil PL, Chen CN, Juan HF. Helicobacter pylori Disrupts Host Cell Membranes, Initiating a Repair Response and Cell Proliferation. *Int J Mol Sci* 2012; **13**: 10176-10192 [PMID: 22949854 DOI: 10.3390/ijms130810176]

12 **Matsuhisa T**, Aftab H. Observation of gastric mucosa in Bangladesh, the country with the lowest incidence of gastric cancer, and Japan, the country with the highest incidence. *Helicobacter* 2012; **17**: 396-401 [PMID: 22967124 DOI: 10.1111/j.1523-5378.2012.00967.x]

13 **Liu YE**, Gong YH, Sun LP, Xu Q, Yuan Y. The relationship between H. pylori virulence genotypes and gastric diseases. *Pol J Microbiol* 2012; **61**: 147-150 [PMID: 23163215]

14 **Muhammad JS**, Zaidi SF, Sugiyama T. Epidemiological ins and outs of helicobacter pylori: a review. *J Pak Med Assoc* 2012; **62**: 955-959 [PMID: 23139983]

15 **Bhandari A**, Crowe SE. Helicobacter pylori in gastric malignancies. *Curr Gastroenterol Rep* 2012; **14**: 489-496 [PMID: 23054813 DOI: 10.1007/s11894-012-0296-y]

16 **Yu XW**, Xu Y, Gong YH, Qian X, Yuan Y. Helicobacter pylori induces malignant transformation of gastric epithelial cells in vitro. *APMIS* 2011; **119**: 187-197 [PMID: 21284736 DOI: 10.1111/j.1600-0463.2010.02709.x]

17 **Kato M**, Asaka M. Recent development of gastric cancer prevention. *Jpn J Clin Oncol* 2012; **42**: 987-994 [PMID: 23018579 DOI: 10.1093/jjco/hys151]

18 **Malfertheiner P**, Selgrad M, Bornschein J. Helicobacter pylori: clinical management. *Curr Opin Gastroenterol* 2012; **28**: 608-614 [PMID: 23010682 DOI: 10.1097/MOG.0b013e32835918a7]

19 **Derakhshan MH**, Lee YY. Gastric cancer prevention through eradication of helicobacter pylori infection: feasibility and pitfalls. *Arch Iran Med* 2012; **15**: 662-663 [PMID: 23102240]

P- Reviewers Mimeault M, Guo JM **S- Editor** Jiang L
L- Editor Cant MR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.2005

World J Gastroenterol 2013 March 28; 19(12): 2005-2008
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Coexistence of gastrointestinal stromal tumor, esophageal and gastric cardia carcinomas

Yong Zhou, Xu-Dong Wu, Quan Shi, Jing Jia

Yong Zhou, Department of General Surgery, Yancheng City No.1 People's Hospital, Yancheng 224005, Jiangsu Province, China
Xu-Dong Wu, Department of Gastroenterology, Yancheng City No.1 People's Hospital, Yancheng 224005, Jiangsu Province, China
Quan Shi, Department of Radiology, Yancheng City No.1 People's Hospital, Yancheng 224005, Jiangsu Province, China
Jing Jia, Department of Nephrology, Yancheng City No.1 People's Hospital, Yancheng 224005, Jiangsu Province, China
Author contributions: Zhou Y, Wu XD, Shi Q and Jia J contributed to the manuscript writing and revision.
Correspondence to: Xu-Dong Wu, PhD, Department of Gastroenterology, Yancheng City No.1 People's Hospital, 16 Yuehe Road, Yancheng 224005, Jiangsu Province, China. hnjsycwxd@163.com
Telephone: + 86-510-88508910 Fax: +86-510-88508910
Received: December 6, 2012 Revised: December 27, 2012
Accepted: January 11, 2013
Published online: March 28, 2013

Abstract

Gastric gastrointestinal stromal tumor (GIST), esophageal squamous cell carcinoma and gastric cardia adenocarcinoma are distinct neoplasms originating from different cell layers; therefore, simultaneous development of such carcinomas is relatively rare. Auxiliary examinations revealed coexistence of esophageal and gastric cardia carcinoma with lymph node metastasis in a 77-year-old man. Intraoperatively, an extraluminal tumor (about 6.0 cm × 5.0 cm × 6.0 cm) at the posterior wall of the gastric body, a tumor (about 2.5 cm × 2.0 cm) in the lower esophagus, and an infiltrative and stenosing tumor (about 1.0 cm × 2.0 cm) in the gastric cardia were detected. Wedge resection for extraluminal gastric tumor, radical esophagectomy for lower esophageal tumor, and cardiac resection with gastroesophageal (supra-aortic arch anastomoses) were performed. Postoperative histological examination showed synchronous occurrence of gastric GIST, esophageal squamous cell carcinoma, and gastric cardia adenocarcinoma. Furthermore, immunohistochemistry indicated strong staining for c-Kit/CD117, Dog-1, Ki-67 and smooth muscle, while expression of S-100 and CD34 was negative.

Key words: Gastrointestinal stromal tumor; Esophageal squamous cell carcinoma; Gastric cardia adenocarcinoma

Zhou Y, Wu XD, Shi Q, Jia J. Coexistence of gastrointestinal stromal tumor, esophageal and gastric cardia carcinomas. *World J Gastroenterol* 2013; 19(12): 2005-2008 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/2005.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.2005

INTRODUCTION

Recently, cases of synchronous development of a gastrointestinal stromal tumor (GIST) and another neoplasm with different incidence, etiology, evolution and prognosis have been reported more frequently[1-3]. Although squamous cell carcinoma and adenocarcinoma constitute the most common type of esophageal and gastric cardia tumor, respectively, simultaneous development of a GIST is relatively rare. Here, we report a case of synchronous occurrence of gastric GIST, esophageal squamous cell carcinoma, and gastric cardia adenocarcinoma.

CASE REPORT

A 77-year-old man presented with dysphagia for 2 mo. Upper gastrointestinal endoscopy was performed, which showed a tumor arising from the lower esophagus and extending into the lumen, and an ulcerated tumor located in the cardia, just below the gastroesophageal junction.

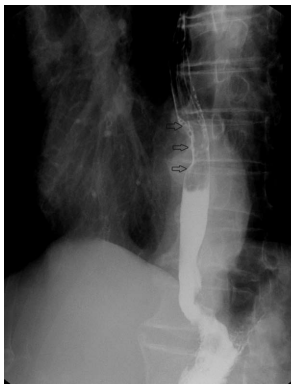


Figure 1 Esophagography showed a filling defect in the anterior wall of the lower esophagus.

He had no relevant past history or family history. Clinical examination did not find any palpable abdominal mass. Laboratory examination was normal. Esophagography showed a filling defect in the anterior wall of the lower esophagus (Figure 1). Computed tomography (CT) showed circumferential thickening of the lower esophageal wall with loss of the lumen. Scanning at a lower level displayed focal thickening of the gastric cardia wall with marked enhancement. Furthermore, scans obtained at lower levels displayed a large, heterogeneous, round mass close to the greater curvature of the stomach. The patient was diagnosed presumptively with synchronous esophageal and gastric cardia carcinoma with lymph node metastasis (Figure 2).

Intraoperatively, an extraluminal tumor (about 6.0 cm × 5.0 cm × 6.0 cm) at the posterior wall of the gastric body, a tumor (about 2.5 cm × 2.0 cm) in the lower esophagus, and an infiltrative and stenosing tumor (about 1.0 cm × 2.0 cm) in the gastric cardia was detected. Wedge resection for extraluminal gastric tumor, radical esophagectomy for lower esophageal tumor, and cardiac resection with gastroesophageal (supra-aortic arch anastomoses) were performed.

On histopathological examination, the gastric cardia tumor was a mid-differentiated gastric adenocarcinoma ($pT_{1b}N_0M_0$), and the lower esophageal tumor was a low-mid-differentiated squamous cell carcinoma ($pT_3N_0M_0$) (Figure 3). There was no vascular invasion and no lymph node metastasis.

Further histopathological examination of the extraluminal gastric tumor revealed GIST of the high-risk category, which showed a high mitotic index (> 10 mitoses/50 high-power fields). Immunohistochemistry indicated strong staining for c-Kit/CD117, Dog-1, Ki-67 and smooth muscle, while expression of S-100 and CD34 was negative (Figure 3). The patient was diagnosed with high-grade gastric GIST due to large tumor size (> 5 cm) and unfavorable histopathological features (high mitotic index and strong positivity for Ki-67).

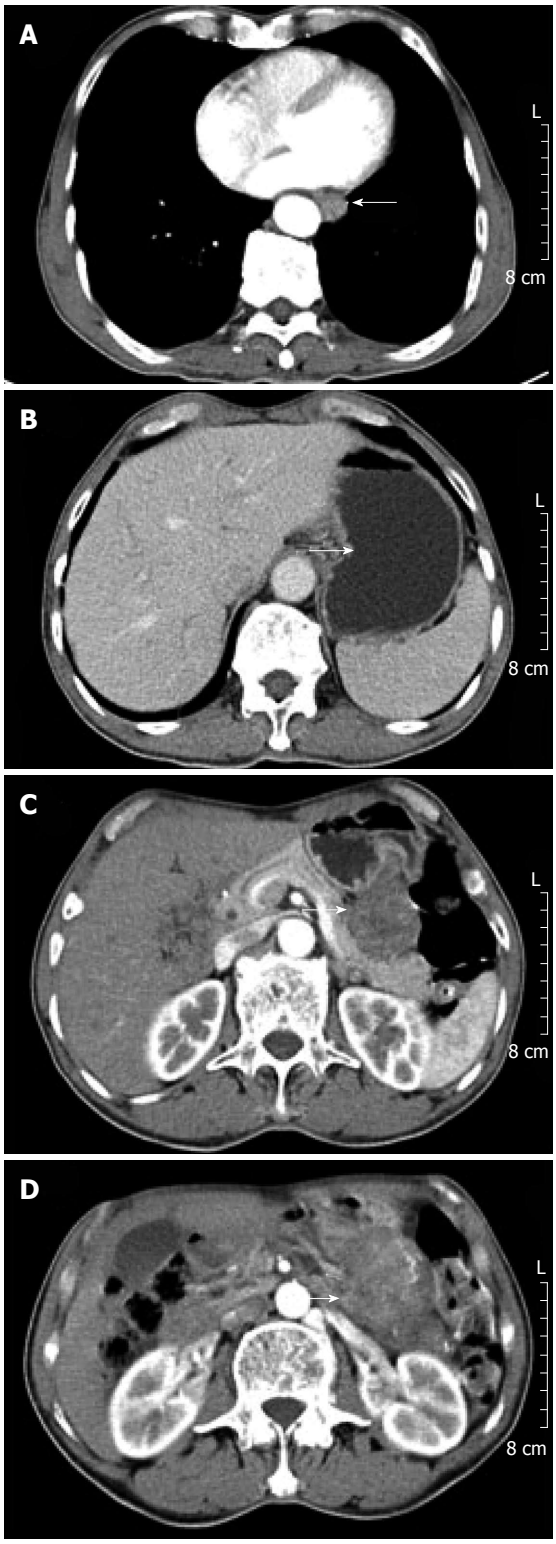


Figure 2 Computed tomography scan. A: Circumferential thickening (arrow) of the lower esophageal wall with loss of lumen; B: Lower level displayed focal thickening (arrow) of the gastric wall with marked enhancement; C and D: Lower levels displayed a large, heterogeneous, round mass close to the greater curvature of the stomach (arrows).

DISCUSSION

GISTs are rare, accounting for only 0.1%-3% of all gastrointestinal malignancies. Primary GISTs arise most commonly in the stomach (50%-70%), followed by the small intestine (25%-35%), colon and rectum (5%-10%) and esophagus (< 5%)[4]. These tumors are considered

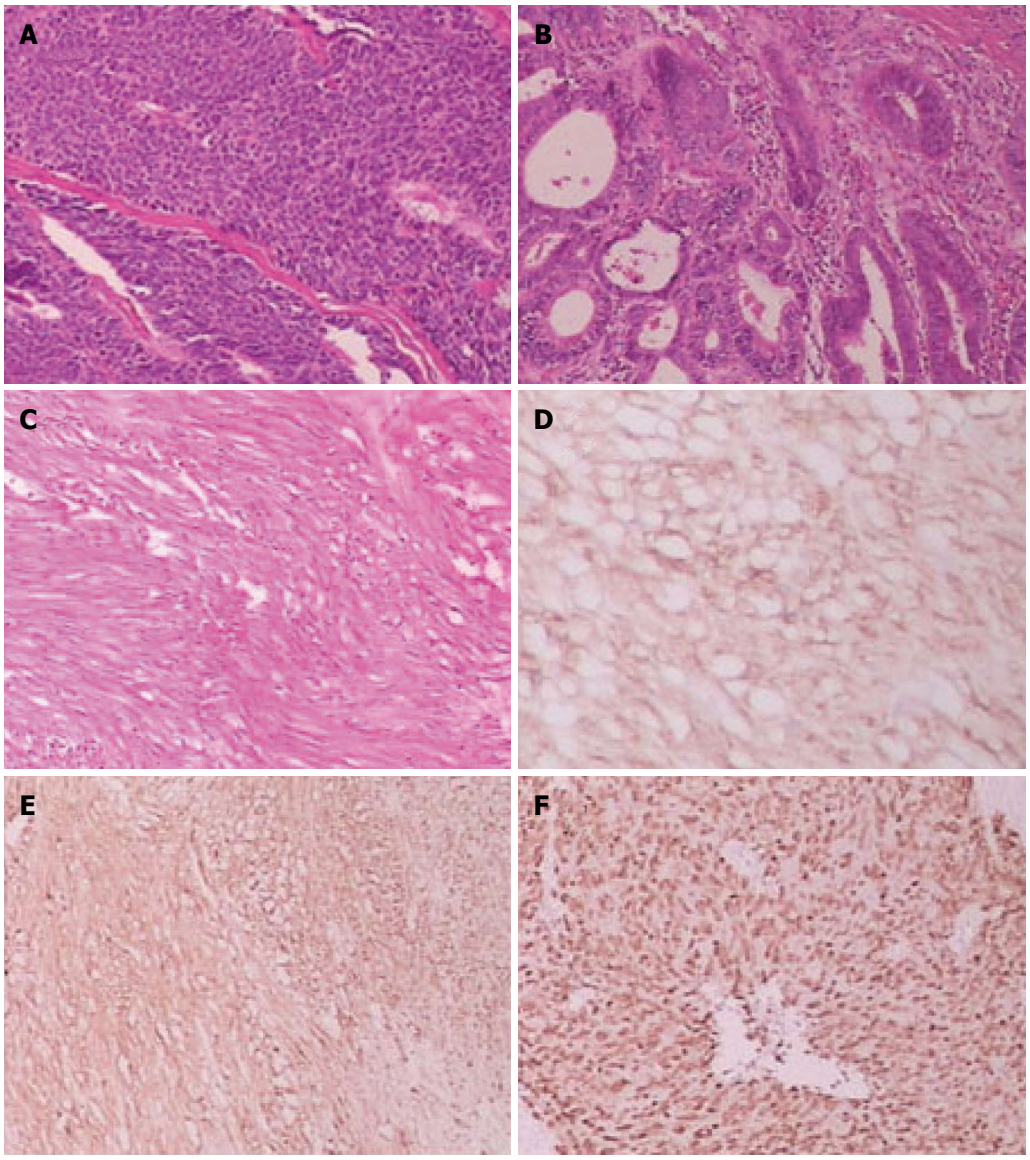


Figure 3 Microscopic images. A: Esophageal squamous cell carcinoma (×10); B: Gastric cardia adenocarcinoma (×10); C: Gastric gastrointestinal stromal tumor (× 10); D-F: Immunohistochemistry indicated strong staining for Dog-1 (D, × 40), c-Kit/CD117 (E, ×10), Ki-67 (F, ×10).

to originate from interstitial cells of Cajal or their precursors, because both strongly express the c-Kit protein (CD117; a type Ⅲ tyrosine kinase receptor encoded by the c-Kit proto-oncogene)[5].

Radical surgery is the main treatment in primary resectable GISTs. Recurrence, metastatic disease or unresectable tumors could be treated with imatinib (a small-molecule tyrosine-kinase inhibitor)[6].

Adenocarcinoma of the stomach ranks as the second most common cancer worldwide, which comprises 80% of all stomach cancers. Squamous cell carcinoma mainly occurs in the mid to lower esophagus, and is not commonly accompanied by other cancerous lesions. Suzuki *et al*[7] have reported that most lesions are in the stomach (59.6%), followed by the colon and rectum (12.3%). Various hypotheses, such as gene mutation, expression of metallothioneins, neighboring tissues being influenced by the same carcinogens, have been proposed regarding the simultaneous development of GIST and other cancers[8,9]. However, at present, no data are available to support such hypotheses. Furthermore, simultaneous occurrence of gastric GIST, esophageal squamous cell carcinoma, and gastric adenocarcinoma has not often been reported in the literature. Simple coincidence could be the most reasonable explanation.

For patients with primary GIST, surgical resection is the only chance for cure. Resection can usually be accomplished with only wedge resection of the stomach or segmental resection of the small bowel for small GISTs, whereas extensive surgery is occasionally required for larger or poorly positioned GISTs[6].

The only curative treatment for esophageal or gastric cardia cancer is surgical resection. After esophagectomy, digestive tract reconstruction can be accomplished using the remaining stomach, depending on the location of the gastric tumor. The colon or jejunum is the frequent choice for esophageal substitution. In our opinion, digestive tract reconstruction with the remaining stomach

should be a reasonable choice for old people (age > 75 years). Although the GIST is large, only wedge resection of the stomach was performed to keep enough stomach for digestive tract reconstruction.

In our case, we considered gastric adenocarcinomas to be an early stage gastric cancer and esophageal squamous cell carcinoma to be a middle stage esophageal cancer. Meantime, the patient was diagnosed with high-grade gastric GIST due to large tumor size (> 5 cm) and unfavorable histopathological features (high mitotic index and strong positivity for Ki-67). Therefore, we suggested that the patient should undergo chemoradiation therapy and adjuvant imatinib treatment. However, the patient refused. A postoperative CT scan performed 3 mo later showed no evidence of tumor recurrence. The patient needs a long follow-up period.

REFERENCES

1 **Theodosopoulos T**, Dellaportas D, Psychogiou V, Gennatas K, Kondi-Pafiti A, Gkiokas G, Papaconstantinou I, Polymeneas G. Synchronous gastric adenocarcinoma and gastrointestinal stromal tumor (GIST) of the stomach: a case report. *World J Surg Oncol* 2011; **9**: 60 [PMID: 21615935 DOI: 10.1186/1477-7819-9-60]

2 **Yamamoto D**, Hamada Y, Tsubota Y, Kawakami K, Yamamoto C, Yamamoto M. Simultaneous development of adenocarcinoma and gastrointestinal stromal tumor (GIST) in the stomach: case report. *World J Surg Oncol* 2012; **10**: 6 [PMID: 22230934 DOI: 10.1186/1477-7819-10-6]

3 **Firat Ö**, Çalişkan C, Karaca C, Sezak M, Özütemız Ö, Ersın S, Güler A. Coexistence of gastric cancer and gastrointestinal stromal tumor: report of two cases. *Turk J Gastroenterol* 2010; **21**: 302-304 [PMID: 20931437]

4 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001; **438**: 1-12 [PMID: 11213830 DOI: 10.1007/s004280000338]

5 **Feng F**, Liu XH, Xie Q, Liu WQ, Bai CG, Ma DL. Expression and mutation of c-kit gene in gastrointestinal stromal tumors. *World J Gastroenterol* 2003; **9**: 2548-2551 [PMID: 14606094]

6 **Chaudhry UI**, DeMatteo RP. Advances in the surgical management of gastrointestinal stromal tumor. *Adv Surg* 2011; **45**: 197-209 [PMID: 21954688]

7 **Suzuki S**, Nishimaki T, Suzuki T, Kanda T, Nakagawa S, Hatakeyama K. Outcomes of simultaneous resection of synchronous esophageal and extraesophageal carcinomas. *J Am Coll Surg* 2002; **195**: 23-29 [PMID: 12113541]

8 **Rauf F**, Ahmad Z, Muzzafar S, Hussaini AS. Synchronous occurrence of gastrointestinal stromal tumor and gastric adenocarcinoma: a case report. *J Pak Med Assoc* 2006; **56**: 184-186 [PMID: 16711342]

9 **Nemes C**, Rogojan L, Surdea-Blaga T, Seicean A, Dumitrascu DL, Ciuce C. Gastrointestinal stromal tumor (GIST) associated with synchronous colon adenocarcinoma - a case report. *J Gastrointestin Liver Dis* 2012; **21**: 101-103 [PMID: 22457867]

P- Reviewer Peparini N **S- Editor** Gou SX
L- Editor Kerr C **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i12.2009

World J Gastroenterol 2013 March 28; 19(12): 2009-2010
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Tumor rupture during surgery for gastrointestinal stromal tumors: Pay attention!

Nadia Peparini, Piero Chirletti

Nadia Peparini, Azienda Sanitaria Locale Roma H, 00043 Ciampino, Italy
Piero Chirletti, Department of Surgical Sciences, Sapienza University of Rome, 00161 Rome, Italy
Author contributions: Peparini N conceived and drafted the manuscript, critically revised the manuscript and gave the final approval; Chirletti P critically revised the manuscript and gave its final approval.
Correspondence to: Nadia Peparini, MD, PhD, Azienda Sanitaria Locale Roma H, Via Mario Calò, 5-00043 Ciampino, Italy. nadiapeparini@yahoo.it
Telephone: +39-339-2203940 Fax: +39-76-488423
Received: December 24, 2012 Revised: February 18, 2013
Accepted: March 6, 2013
Published online: March 28, 2013

Abstract

In a recently published letter to the editor, we debated the proposal by Coccolini *et al* to treat gastrointestinal stromal tumors (GISTs) of the esophagogastric junction with enucleation and, if indicated, adjuvant therapy. We highlighted that, because the prognostic impact of a T1 high-mitotic rate esophageal GIST is worse than that of a T1 high-mitotic rate gastric GIST, enucleation may not be adequate surgery for esophagogastric GISTs with a high mitotic rate. In rebuttal, Coccolini *et al* pointed out the possible bias in assessment of the mitotic rates due to the lack of standardized methods and underlined that the site and features of the tumor need to be carefully considered in evaluation of the risk-benefit balance. Here we confirm that, apart from the problematic issue of mitotic counting, enucleation should not be indicated for GISTs at any site to reduce the risk of tumor rupture, which has been recently considered to be an unfavorable prognostic factor, and to avoid microscopic residual tumor.

Key words: Gastrointestinal stromal tumor; Esophagogastric junction; Surgery; Resection; Enucleation

Peparini N, Chirletti P. Tumor rupture during surgery for gastrointestinal stromal tumors: Pay attention! *World J Gastroenterol* 2013; 19(12): 2009-2010 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i12/2009.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i12.2009

TO THE EDITOR

In a recent issue of *World Journal of Gastroenterology*, we debated[1] the proposal by Coccolini *et al*[2] to treat gastrointestinal stromal tumors (GISTs) of the esophagogastric junction with enucleation and, if indicated, adjuvant therapy. We highlighted that, because the prognostic impact of a T1 high-mitotic rate esophageal GIST is worse than that of a T1 high-mitotic rate gastric GIST, enucleation may not be adequate surgery for esophagogastric GISTs with a high mitotic rate. In rebuttal, Coccolini *et al*[3] pointed out the possible bias in the assessment of the mitotic rate due to the lack of standardized methods and underlined that the site and features of the tumor need to be carefully considered in the evaluation of the risk-benefit balance.

Apart from the prognostic differences related to the anatomic localization of the gastric GISTs (gastroesophageal junction-body-distal antrum), problematic mitotic counting is a significant issue in the staging and therapy of GISTs. Controversies exist regarding how large the 50 high-power field areas should be[4], varying from 5 mm^2 to 10 mm^2. The area recommended by the European Guideline represents half of the area recommended by TNM Classification of Malignant Tumors[5,6].

However, tumor rupture is a highly unfavourable prognostic factor, which should be considered rather than the mitotic rate, tumor site and tumor size in planning an effective treatment for GISTs. According to the modified risk stratification proposed by Joensuu *et al*[7] and Rutkowski *et al*[8], patients with tumor rupture are in-

cluded in high-risk category GISTs.

On the other hand, according to updated National Comprehensive Cancer Network Guidelines[9], Coccolini *et al*[2] pointed out the value of complete resection, leaving a negative margin and an intact pseudocapsule. GISTs may be soft and fragile because of intratumoral hemorrhage and/or necrosis; anyway they are surrounded by a pseudocapsule that should not be torn during surgery to avoid intra-abdominal seeding. From technical point of view, enucleation of GIST implies that the plane of dissection is conducted along the pseudocapsule with no distance margin on the entire surface of the tumor - *i.e.*, at best microscopic residual tumor (R1) surgery - or rather, enucleation maximizes the risks of R1 and tumor rupture.

We think that complete resection should remain the standard surgical treatment for localized GISTs at any site through wedge resection for small size favorably positioned GISTs and variably extended segmental organ resection depending on the size and site for large and/or unfavourably positioned GISTs. To reduce the risk of tumor rupture with consequent risk of tumor relapse and avoid microscopic residual tumor enucleation should not be indicated for any GISTs. For the risk of tumor rupture , laparoscopic surgery should be avoided with large GISTs[5].

REFERENCES

1 **Peparini N**, Carbotta G, Chirletti P. Enucleation for gastrointestinal stromal tumors at the esophagogastric junction: is this an adequate solution? *World J Gastroenterol* 2011; **17**: 2159-2160 [PMID: 21547139 DOI: 10.3748/wjg.v17.i16.2159]

2 **Coccolini F**, Catena F, Ansaloni L, Lazzareschi D, Pinna AD. Esophagogastric junction gastrointestinal stromal tumor: resection vs enucleation. *World J Gastroenterol* 2010; **16**: 4374-4376 [PMID: 20845503 DOI: 10.3748/wjg.v16.i35.4374]

3 **Coccolini F**, Catena F, Ansaloni L, Pinna AD. Gastrointestinal stromal tumor and mitosis, pay attention. *World J Gastroenterol* 2012; **18**: 587-588 [PMID: 22363128 DOI: 10.3748/wjg.v18.i6.587]

4 **Agaimy A**. Gastrointestinal stromal tumors (GIST) from risk stratification systems to the new TNM proposal: more questions than answers? A review emphasizing the need for a standardized GIST reporting. *Int J Clin Exp Pathol* 2010; **3**: 461-471 [PMID: 20606727]

5 **ESMO/European Sarcoma Network Working Group.** Gastrointestinal stromal tumors: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2012; **23** Suppl 7: vii49-vii55 [PMID: 22997454 DOI: 10.1093/annonc/mds252]

6 **Sobin LH**, Gospodarowicz MK, Wittekind Ch. TNM Classification of Malignant Tumors. 7th ed. Wiley-Blackwell, 2010

7 **Joensuu H**. Risk stratification of patients diagnosed with gastrointestinal stromal tumor. *Hum Pathol* 2008; **39**: 1411-1419 [PMID: 18774375 DOI: 10.1016/j.humpath.2008.06.025]

8 **Rutkowski P**, Bylina E, Wozniak A, Nowecki ZI, Osuch C, Matlok M, Switaj T, Michej W, Wroński M, Głuszek S, Kroc J, Nasierowska-Guttmejer A, Joensuu H. Validation of the Joensuu risk criteria for primary resectable gastrointestinal stromal tumour - the impact of tumour rupture on patient outcomes. *Eur J Surg Oncol* 2011; **37**: 890-896 [PMID: 21737227 DOI: 10.1016/j.ejso.2011.06.005]

9 **Demetri GD**, von Mehren M, Antonescu CR, DeMatteo RP, Ganjoo KN, Maki RG, Pisters PW, Raut CP, Riedel RF, Schuetze S, Sundar HM, Trent JC, Wayne JD. NCCN Task Force report: update on the management of patients with gastrointestinal stromal tumors. *J Natl Compr Canc Netw* 2010; **8** Suppl 2: S1-41; quiz S42-44 [PMID: 20457867]

P- Reviewers Chen P, Perakath B **S- Editor** Zhai HH
L- Editor Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2013 March 28; 19(12): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access (OA) journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

Aims and scope

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

WJG is published by Baishideng Publishing Group (BPG) in both electronic and online forms. All *WJG* articles are published in *WJG* website and PubMed Central. The major advantages of OA journals are faster release and delivery, no page or graph restrictions, and increased visibility, usage and impact. Full-text PDF articles and electronic/online versions are freely available to global readers. After the paper is published, the author(s) can obtain high-quality PDF files, which contain the journal cover, a list of editorial board members, table of contents, text, and back cover of the journal. BPG has a strong professional editorial team composed of editorial board members, editors-in-chief, science editors, language editors, and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJG* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in gastroenterology and hepatology; (12) Brief

Articles: To briefly report the novel and innovative findings in gastroenterology and hepatology; (13) Meta-Analysis: To evaluate the clinical effectiveness in gastroenterology and hepatology by using data from two or more randomised control trials; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Launch date
October 1, 1995

Frequency
Weekly

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
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

Publisher
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Production center
Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

Representative office
USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

Indexed and abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Thomson Reuters, 2011 Impact Factor: 2.471 (32/74 Gastroenterology and Hepatology).

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they

might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece.

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc*. It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list,

e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nihgov/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.00000 35706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15.

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315222607.htm

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

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.

Publication fee

WJG is an international, peer-reviewed, open access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 1365 USD per article. All invited articles are published free of charge.

百世登
Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

ISSN 1007-9327
9 771007 932045 12>