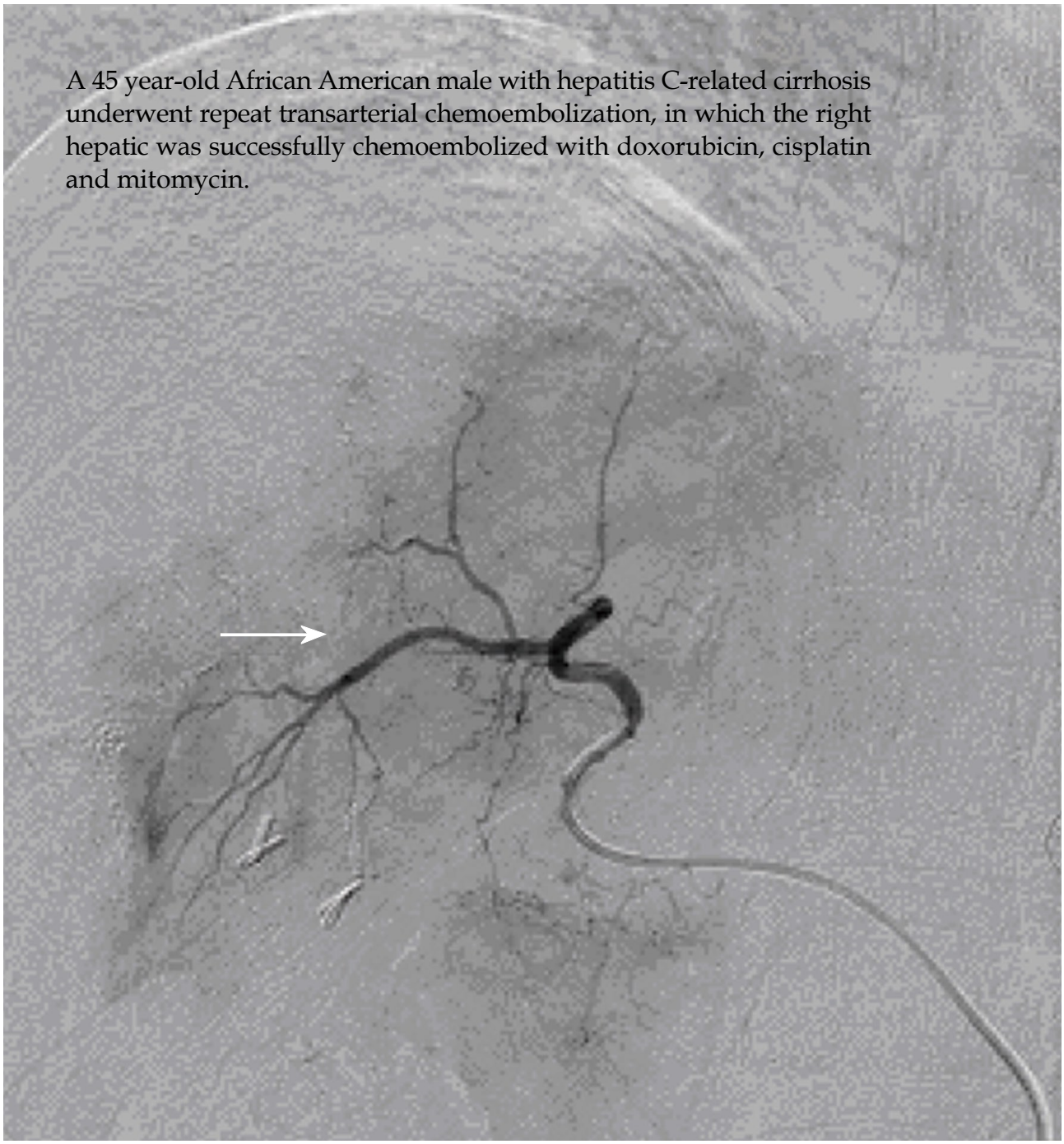
ISSN 1948-5182 (online)

World Journal of Hepatology

World J Hepatol 2010 July 27; 2(7): 251-294

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



WJH World Journal of Hepatology

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

Editorial Board

2009-2013

The *World Journal of Hepatology* Editorial Board consists of 572 members, representing a team of worldwide experts in hepatology. They are from 45 countries, including Argentina (4), Australia (7), Austria (2), Bangladesh (1), Belgium (3), Botswana (2), Brazil (8), Brunei Darussalam (1), Bulgaria (1), Canada (10), Chile (1), China (89), Egypt (3), Finland (1), France (15), Gambia (1), Germany (28), Greece (8), Hungary (3), India (20), Ireland (1), Israel (7), Italy (65), Japan (45), Malaysia (1), Mexico (4), Netherlands (4), Pakistan (2), Poland (1), Portugal (1), Philippines (1), Romania (1), Saudi Arabia (1), Singapore (4), South Korea (17), Spain (22), Sri Lanka (1), Sudan (1), Switzerland (2), Thailand (6), Tunisia (2), Turkey (13), United Kingdom (17), United States (144), and Venezuela (1).

Egypt

Finland

France

Gambia

Germany

Greece

Hungary

India

Ireland

Israel

Italy

Luca Aasaloni, *Bologna*
Giovanni Addolorato, *Rome*
Luigi E Adinolfi, *Naples*
Pietro Andreone, *Bologna*
M Appetecchia, *Rome*
Antonio Ascione, *Napoli*
Ferruccio Bonino, *Milano*
Bruno D Bruno, *Benevento*
Savino Bruno, *Milano*
Melchiorre Cervello, *Palermo*
Claudio Chiesa, *Rome*
Stefano Colagrande, *Firenze*
Massimo G Colombo, *Milan*
Samuele De Minicis, *Montegranaro*
Alessandro Vitale, *alessandro*
Fabio Farinati, *Padova*
Paolo Feltracco, *Padova*
Domenico Ferri, *Bari*
Amalia Gastaldelli, *Pisa*
Domenico Girelli, *Verona*
Fernando Goglia, *Benevento*
Alessandro Grasso, *Savona*
Ignazio Grattagliano, *Bari*
Pietro Invernizzi, *Milan*
Francesco Izzo, *Naples*
Amedeo Lonardo, *Modena*
Malaguarnera Lucia, *Trecastagni*
Massimo Di Maio, *Rossano*
Melania Manco, *Rome*
Andrea Mancuso, *Palermo*
F Marotta, *Milano*
Fabio Marra, *Florence*
Roberto Mazzanti, *Florence*
Giulia Morsica, *Milan*
Antonio Moschetta, *Bari*
Massimo Negrini, *Ferrara*
Andrea Nicolini, *Pisa*
Giuseppe R Nigri, *Rome*
Valerio Nobili, *Rome*
Valentina Pallottini, *Rome*
Adriano M Pellicelli, *Rome*
Marcello Persico, *Naples*
Massimo Pinzani, *Firenze*
Giovanni Polimeni, *Messina*
Camillo Porta, *Pavia*
Piero Portincasa, *Bari*
Emilio Quaia, *Trieste*
Giuseppe Remuzzi, *Bergamo*
Domenico Ribatti, *Bari*
Massimo Roncalli, *Rozzano*
Carlo Sabbà, *Bari*
Orazio Schillaci, *Rome*
Gaetano Serviddio, *Foggia*
Aurelio Sonzogni, *Bergamo*
Paolo Sorrentino, *Salerno*
Enea Spada, *Roma*
Giovanni Tarantino, *Naples*
Luciano Tarantino, *Naples*
Claudio Tiribelli, *Trieste*
Pierluigi Toniutto, *Udine*
Pietro Vajro, *Naples*
Luca Vigano, *Torino*

Japan

Yuichiro Eguchi, *Saga*
Munechika Enjoji, *Fukuoka*
Jiro Fujimoto, *Osaka*
Atsushi Hosui, *Osaka*
Kazuo Ikeda, *Nagoya*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Satoru Kakizaki, *Gunma*
Naoya Kato, *Tokyo*
Takumi Kawaguchi, *Kurume*
Kiminori Kimura, *Tokyo*
Tsuneo Kitamura, *Chiba*
Keiichi Kubota, *Tochigi*
Sabina Mahmood, *Okayama*
Hitoshi Maruyama, *Chiba*
Sachiko Matsuhashi, *Saga*
Toshihiro Mitaka, *Sapporo*
Eiji Miyoshi, *Yamada-oka Suita*
Zenichi Morise, *Toyoake Aichi*
Ryuichi Morisihita, *Osaka*
Yoshiki Murakami, *Kyoto*
Satoru Murata, *Tokyo*
Atsushi Nakajima, *Kanagawa*
Yasuni Nakanuma, *Kanazawa*
Waka Ohishi, *Hiroshima*
Morikazu Onji, *Matsuyama*
Toshiji Saibara, *Nankoku*
Hiroaki Shiba, *Tokyo*
Ikuo Shoji, *Hyogo*
Ryo Sudo, *Yokohama*
Yoshio Sumida, *Nara*
Shinji Tanaka, *Tokyo*
Takuji Tanaka, *Gifu*
Akihiko Tsuchida, *Tokyo*
Takato Ueno, *Kurume*
Shinichi Ueno, *Kagoshima*
Kiyohito Yagi, *Osaka*
Yo-ichi Yamashita, *Hiroshima*
Teruyoshi Yanagita, *Saga*
Shuang-Qin Yi, *Kanazawa*
Hiroshi Yoshida, *Tokyo*
Hitoshi Yoshiji, *Nara*

Malaysia

Kamsiah Jaarin, *Kuala Lumpur*

Mexico

Norberto C Chavez-Tapia, *Tlalpan*
Javier Lizardi Cervera, *Tlalpan CP*
Saúl Villa-Treviño, *México DF*
Florencia V Vorackova, *México DF*

Netherlands

Robert Jacobus de Knegt, *Rotterdam*
TU Hoogenraad, *Heidelberglaan*
Maarten E Tushuizen, *MB Amsterdam*
Robert C Verdonk, *RB Groningen*

Pakistan

Syed Hamid Ali, *Karachi*
Huma IQ TI, *Islamabad*

Poland

Maria ES Lotowska, *Bialystok*

Portugal

Felix Dias Carvalho, *Porto*

Philippines

Janus P Ong, *Manila*

Romania

Eugen Georgescu, *Craiova*

Saudi Arabia

Ahmed Helmy, *Riyadh*

Singapore

Wei Ning Chen, *Singapore*
Si-Shen Feng, *Singapore*
Lang Zhuo, *Singapore*
Chun-Tao Wai, *Singapore*

South Korea

Sang Hoon Ahn, *Seoul*
Sun Pyo Hong, *Yongin*
Byung Ihn Choi, *Seoul*
Seok Joo Han, *Seoul*
Kyung Lib Jang, *Busan*
Bum-Joon Kim, *Seoul*
Dong Goo Kim, *Seoul*
Kyung Sik Kim, *Seoul*
Meehyein Kim, *Yongin*
Young Chul Kim, *Seoul*
Mi-Kyung Lee, *Jeonnam*
Young-Ik Lee, *Taejon*
Kwan-Kyu Park, *Daegu*
Hyunchul Rhim, *Seoul*
In Kyoung Lim, *Gyunggi-do*
Dae-Yeul Yu, *Daejeon*
Jong Won Yun, *Kyungbuk*

Spain

Jose AG Agundez, *Badajoz*
Maria Angeles, *Madrid*
Agustin Castiella, *Mendaro*
Ruben Ciria, *Cordoba*
Joan Clari, *Barcelona*
Maria Buti Ferret, *Barcelona*

Puri Fortes, *Pamplona*
Joan Genescà, *Barcelona*
María J Gómez-Lechón, *Valencia*
Arias Jaime, *Madrid*
Ángeles Pajares María, *Madrid*
Jordi Muntane, *Cordoba*
Jose JG Marin, *Salamanca*
Julia P Onsurbe, *Barcelona*
Albert Parés, *Barcelona*
Sonia Ramos, *Madrid*
Cristina Ripoll, *Madrid*
Isabel F Romero, *Barcelona*
Marta R Romero, *Salamanca*
Juan Macias Sanchez, *Sevilla*
Juan Sastre, *Valencia*
Manuel Vázquez-Carrera, *Barcelona*

Sri Lanka

EGD Shaman Rajindrajith, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Switzerland

Beat Mullhaupt, *Zurich*
Maurer A Christoph, *Liestal*

Thailand

Nattiya Hirankarn, *Bangkok*
Somchai Pinlaor, *Khon Kaen*
Yong Poovorawan, *Bangkok*
Abhasnee Sobhonslidsuk, *Bangkok*
Chanitra Thuwajit, *Bangkok*
Sopit Wongkham, *Khon Kaen*

Tunisia

Olfa Bahri, *Tunis-Belvedere*
Chadli Dziri, *Tunis*

Turkey

Inci Alican, *Istanbul*
Ahmet Atessahin, *Elazig*
Yasemin Hatice Balaban, *Ankara*
Hayrullah Derici, MD, *Izmir*
Cigdem Ulukaya Durakbasa, *Istanbul*
Muhsin MM Harputluoglu, *Malatya*
Abdurrahman Kadayifci, *Gaziantep*
Adnan Kadayifci, *Antalya*
Ali Sazci, *Kocaeli*
Ilker Tasci, *Ankara*
Mehmet Yalniz, *Elazig*
Serkan Yener, *Izmir*
Yusuf Yilmaz, *Istanbul*

United Kingdom

Alastair David Burt, *Newcastle*
David O Cosgrove, *London*
Anil Dhawan, *London*
Indra Neil Guha, *Nottingham*
Phillip M Harrison, *London*
Hübscher SG Hübscher, *Birmingham*
Long R Jiao, *London*
AT Koulaouzidis, *Edinburgh*
Patricia Lalor, *Birmingham*
David A Lomas, *Cambridge*
Rajeshwar P Mookerjee, *London*
Gareth J Morris-Stiff, *Wales*
Kathryn L Nash, *Southampton*
Derek Anthony O'Reilly,
Christian P Selinge, *Bolton*
Konstantinos Tziomalos, *London*
Feng Wu, *Oxford*

United States

Gary A Abrams, *Montgomery*
Hassan H A-Kader, *Tucson*
Hans-Olov Adami, *Massachusetts*
Joseph Ahn, *Maywood*
Shannon Marie Bailey, *Alabama*
Numan Cem Balci, *St Louis MO*
Edmund J Bini, *New York*
Victor E Buckwold, *Frederick*
Roniel Cabrera, *Gainesville*
Guoqing Cao, *Indiana*
Disaya Chavalitdhamrong, *New York*
Chien-Shing Chen, *Loma Linda*
Fei Chen, *Morgantown*
Su Chen, *San Antonio*
Youhai H Chen, *Philadelphia*
Anne M Covey, *New York*
Mark J Czaja, *New York*
Srikanta Dash, *New Orleans*
Anthony JB Demetris, *Pittsburgh*
Sridevi Devaraj, *California*
Lisa Ross Dixon, *Gainesville*
Terrence M Donohue, *Omaha*
Q Ping Dou, *Detroit*
Murray N Ehrinpreis, *Detroit*
Marwan Ghazi Fakih, *Buffalo*
Shengyun Fang, *Maryland*
Claus J Fimmel, *Illinois*
Robert Anthony Fisher, *Virginia*
Samuel W French, *Torrance*
Phillip A Furman, *Princeton*
M Eric Gershwin, *California*
Jalal K Ghali, *Michigan*
Grace Liejun Guo, *Kansas City*
Dieter Haemmerich, *Charleston*
Young S Hahn, *Charlottesville*
Stephen A Harrison, *Texas*
Dee Harrison-Findik, *Nebraska*
Sidhartha Hazari, *Louisiana*
Thomas S Helling, *Jackson*
Alan W Hemming, *Florida*
Iryna S Hepburn, *Evans*
Ai-Xuan L Holterman, *Chicago*
Ke-Qin Hu, *California*
Guangcun Huang, *Ohio*
Wendong Huang, *California*
Rachel M Hudacko, *New Brunswick*
Michael John Jacobs, *Michigan*
Hartmut W Jaeschke, *Kansas City*
Ravi Jhaveri, *North Carolina*
Lynt B Johnson, *Washington*
Neil Louis Julie, *Bethesda*
Sanjay Kakar, *San Francisco*
Sanjeeva P Kalva, *Boston*
Jing X Kang, *Massachusetts*
Hetal Karsan, *Georgia*
Emmet B Keeffe, *California*
Nancy Ellen Kemeny, *New York*
Andrew Scott Kennedy, *Cary*
Kusum K Kharbanda, *Omaha*
David H Kirn, *California*
Hyam Lerner Leffert, *La Jolla*
Stacee Marie Lerret, *Milwaukee*
Fengzhi Li, *New York*
Wei Li, *Houston*
Shuang Liu, *Indiana*
Su Hao Lo, *Davis*
Daniel G Maluf, *Richmond*
Jose E Manautou, *Storrs*
Richard S Mangus, *Indiana*
Mary Ko Manibusan, *Virginia*
Paul Martin, *Miami,*
Jochen Mattner, *Ohio*
James A McCubrey, *North Carolina*
Valentina Medici, *Sacramento*
George Michalopoulos, *Pittsburgh*
Smruti R Mohanty, *Illinois*
John T Moore, *GlaxoSmithKline*
Ravi Murthy, *Texas*
Laura E Nagy, *Cleveland*
Sagar U Nigwekar, *Rochester*
Eileen M O'Reilly, *New York*
Kevin FS O'Carroll, *Hershey*
Melissa Kay Osborn, *Atlanta*
Helieh Saatara Oz, *Kentucky*
Igor P Pogribny, *Arkansas*
Nicholas C Popescu, *Bethesda Maryland*
Daniel S Pratt, *Boston*
Ratna B Ray, *Louis*
Nancy Reau, *Chicago*
Janardan K Reddy, *Chicago*
Martin J Ronis, *Little Rock*
Phillip Ruiz, *Florida*
Tanios B Saab, *Columbus*
Adnan Said, *Madison*
Neeraj Saxena, *Georgia*
Raymund R Saxena, *Minnesota*
Ann Scheimann, *Baltimore*
Timothy M Schmitt, *Charlottesville*
Bernd Schnabl, *La Jolla*
Kunwar Shailubhai, *Pennsylvania*
Muhammad Y Sheikh, *California*
Perry Shen, *Winston-Salem*
Viji Shridhar, *Rochester*
Shivendra D Shukla, *Missouri*
Ashwani K Singal, *Galveston*
Keshav K Singh, *New York*
Omar Skalli, *Shreveport*
Byoung-Joon Song, *Maryland*
Branko Stefanovic, *Tallahassee*
Stephen Strom, *Pennsylvania*
Xiao Su, *San Francisco*
Wing-Kin Syn, *North Carolina*
Gyongyi Szabo, *Massachusetts*
Shinako Takada, *Houston*
Yueming Tang, *Chicago*
John M Taylor, *Philadelphia*
Swee H The, *Springfield*

WJH World Journal of Hepatology

Contents

Monthly Volume 2 Number 7 July 27, 2010

World Journal of Hepatology
Volume 2 Number 7 July 27, 2010

Contents

NAME OF JOURNAL
World Journal of Hepatology

LAUNCH DATE
October 31, 2009

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Hepatology*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

ONLINE SUBSCRIPTION
One-Year Price 216.00 USD

PUBLICATION DATE
July 27, 2010

CSSN
ISSN 1948-5182 (online)

EDITORIAL OFFICE
Hai-Ning Zhang, Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0038
Fax: +86-10-8538-1893
E-mail: wjh@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm. If you do not have web access please wcontact the editorial office.

ONLINE SUBMISSION
http://www.wjgnet.com/1948-5182office

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.251

World J Hepatol 2010 July 27; 2(7): 251-255
ISSN 1948-5182 (online)

EDITORIAL

Non-invasive methods for liver fibrosis prediction in hemochromatosis: One step beyond

Agustin Castiella, Eva Zapata, José M Alústiza

Agustin Castiella, Eva Zapata, Gastroenterology Service, Mendaro Hospital, Mendaro 20850, Spain
José M Alústiza, Radiology Service, Osatek Donostia, San Sebastián 20014, Spain
Author contributions: Castiella A, Zapata E, and Alústiza JM contributed equally to the article in accordance with the standard proposed by the International Committee of Medical Journal Editors.
Correspondence to: Agustin Castiella, MD, Gastroenterology Service, Mendaro Hospital, Barrio Mendarozabal, Mendaro 20850, Spain. agustincastiella@yahoo.es
Telephone: +34-94-3032800 Fax: +34-94-3032836
Received: April 10, 2010 Revised: July 7, 2010
Accepted: July 14, 2010
Published online: July 27, 2010

Abstract

Advances in recent years in the understanding of, and the genetic diagnosis of hereditary hemochromatosis (HH) have changed the approach to iron overload hereditary diseases. The ability to use a radiologic tool (MRI) that accurately provides liver iron concentration determination, and the presence of non-invasive serologic markers for fibrosis prediction (serum ferritin, platelet count, transaminases, *etc*), have diminished the need for liver biopsy for diagnosis and prognosis of this disease. Consequently, the role of liver biopsy in iron metabolism disorders is changing. Furthermore, the irruption of transient elastography to assess liver stiffness, and, more recently, the ability to determine liver fibrosis by means of MRI elastography will change this role even more, with a potential drastic decline in hepatic biopsies in years to come. This review will provide a brief summary of the different non-invasive methods available nowadays for diagnosis and prognosis in HH, and point out potential new techniques that could come about in the next years for fibrosis prediction, thus avoiding the need for liver biopsy in a greater number of patients. It is possible that liver biopsy will remain useful for the diagnosis of associated diseases, where other non-invasive means are not possible, or for those rare cases displaying discrepancies between radiological and biochemical markers.

Key words: Hemochromatosis; Iron overload; Liver fibrosis; Non-invasive; Magnetic resonance imaging; Ultrasound elastography

Peer reviewers: Waka Ohishi, MD, PhD, Senior Scientist, Chief, Division of Clinical Laboratories, Department of Clinical Studies, Radiation Effects Research Foundation, Hiroshima 732-0815, Japan; Regina Coeli dos Santos Goldenberg, Professor, Department of Carlos Chagas filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

Castiella A, Zapata E, Alústiza JM. Non-invasive methods for liver fibrosis prediction in hemochromatosis: One step beyond. *World J Hepatol* 2010; 2(7): 251-255 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/251.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.251

INTRODUCTION

Liver biopsy with histological study and liver iron concentration quantification has long been the gold standard for the diagnosis and prognosis of hemochromatosis[1]. The great developments that have occurred in the field of iron overload diseases in the last 15 years - identification of the HFE gene and the mutations responsible of hemochromatosis, liver iron concentration (LIC) determination by Magnetic resonance imaging (MRI) and non-invasive liver fibrosis prediction with laboratory tests[1-3] - have all diminished the role of hepatic biopsy in hemochromatosis study. Consequently, it is usually only used for prognosis purposes[4]. The development of liver cirrhosis is crucial for hemochromatosis patients, as it changes both the prognosis and the management of the disease[5].

Nevertheless, liver biopsy has associated risks inherent

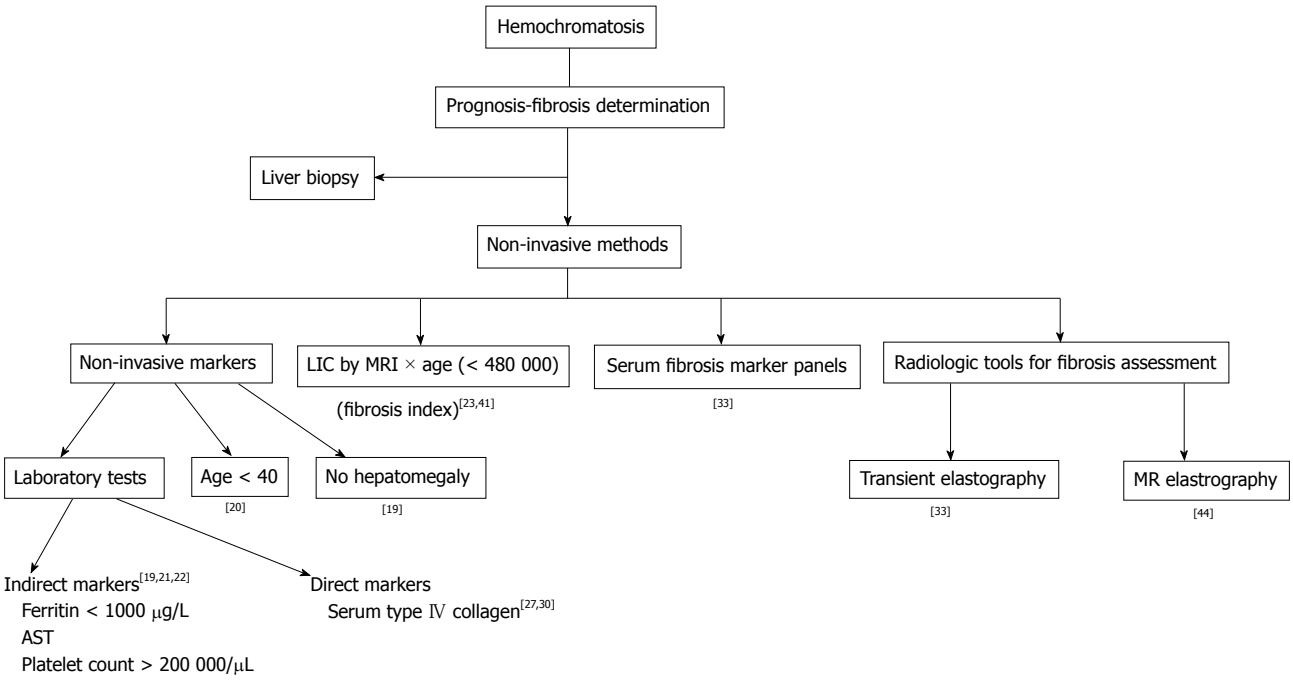


Figure 1 Non-invasive assessment of liver fibrosis in hereditary hemochromatosis in 2010. MRI: magnetic resonance imaging; LIC: liver iron concentration; AST: aspartate aminotransferase.

to the technique, with a related mortality around 1/1 000-1/10 000[6,7], as well as wide variations in the results. Sampling error studies have shown that a single biopsy will miss cirrhosis in 10%-30% of patients and incorrectly classify fibrosis by at least one stage in 20%-30%[5,8]. In addition, both patients and hemochromatosis associations have their objections to the procedure[9]. Furthermore, liver fibrosis, and, in some cases, cirrhosis, may regress after treatment with phlebotomy[10]. Concerns about complications and sampling errors have resulted in a search for non-invasive tests for cirrhosis[5].

ROLE OF LIVER BIOPSY IN GENETIC HEMOCHROMATOSIS

The liver is the most easily accessible tissue in which it is possible to determine the presence of iron overload. This is done by LIC measurement in hepatic biopsy[11], but there is a wide variation in measures of hepatic iron load, especially in patients with liver cirrhosis[12]. Liver biopsy no longer has a primary role in the diagnosis of hereditary hemochromatosis (HH) (Figure 1). In patients with HH, liver biopsy is nowadays performed for three main reasons[13]: (1) To determine the prognosis of the disease, calculating the fibrosis grade in the liver sample; a Serum Ferritin > 1 000 μg/L, raised aspartate aminotransferase (AST) and platelet count < 200 000/μL are considered markers of advanced liver disease, and liver biopsy has been recommended for these patients[1]; (2) To diagnose the presence of other diseases which produce iron overload, such as alcoholic liver disease and non-alcoholic speatohepatitis (NASH), and to determine their severity. When there is a coexistent pathology, liver biopsy may help to clarify the main cause of liver disease; and (3) To identify preneoplastic lesions, including iron-free foci[14] and dysplastic nodules. All of these three main reasons have been able to be assessed by non-invasive tools for quite some time[15-17].

NON-INVASIVE FIBROSIS MARKERS

Laboratory tests

Serological markers for non-invasive liver fibrosis determination are classified in two groups: (1) indirect markers, such as those which do not directly reflect the extracellular matrix metabolism, including transaminases, serum ferritin, and platelet count; and (2) direct markers, such as products of the extracellular matrix degradation or synthesis, e.g. procollagen-Ⅲ N-terminal peptide (PⅢNP), serum type Ⅳ collagen, laminin, hyaluronic acid, and tissue inhibitors of metalloproteinases (TIMP), *etc*[18].

Indirect markers: In the last decade, reports of non-invasive approaches for fibrosis prediction in HH have been published[19-23]. The most obvious of these are serum transaminases, but they are found to have normal levels in up to 50% of patients with cirrhosis[20].

Guyader *et al*[19] revised the clinical and laboratory variables of 197 HH (C282Y homozygote) patients in France. Their findings were validated in a group of 113 patients from Canada. No patient with high-degree fibrosis was found without hepatomegaly, raised AST, or serum ferritin > 1000 μg/L.

A study of 66 HH (C282Y homozygote) patients in the USA[20] revealed that age was an important feature, and that no high-degree fibrosis in patients younger than

40 was found. It has been previously suggested that patients over 45 run a greater risk of developing significant fibrosis or cirrhosis in HH[23,24].

More recently, Beaton *et al*[21] studied the non-invasive variables for cirrhosis prediction in 193 HH patients (C282Y homozygotes) in Canada, and the study was validated in a group of 162 patients from France. The combination of ferritin > 1 000 μg/L, platelets < 200 000/μL and raised AST correctly diagnosed the presence of cirrhosis in 77% of Canadian patients and in 90% of French patients. Morrison *et al*[22] proved in a USA multicenter study of phenotypic hemochromatosis patients that a ferritin value < 1 000 μg/L makes the presence of cirrhosis unlikely, regardless of patient age or transaminase values, thus avoiding the need for liver biopsy for a prognosis in these patients. Our group in the Basque Country[25,26], recently reported the utility of various non-invasive methods for fibrosis prediction in hemochromatosis, with 32 patients being included in the study of which nine presented with F3 or F4 fibrosis (four patients had cirrhosis). In our study, the combination of raised AST and a platelet count < 200 000/μL revealed a negative predictive value of 100% for high-degree fibrosis. Platelets alone had a 94% negative predictive value for high degree fibrosis; the four patients with cirrhosis had a platelet count < 200 000/μL, and three of them had a serum ferritin value < 1 000 μg/L. This is particularly unusual[27], revealing potential differences in different populations[26], but cirrhosis with a serum ferritin value < 1000 μg/L has also been reported by other groups in Canada and Australia[28,29]. Crawford *et al*[27] have evaluated the utility of current diagnostic algorithms for detecting cirrhosis (serum ferritin values, platelet count, and AST levels), in combination with serum markers of fibrosis (collagen type Ⅳ, hyaluronic acid), in predicting cirrhosis in HH patients. No patient with a serum ferritin < 1 000 μg/L were cirrhotic. A combination of a platelet count < 200 000/μL, ferritin > 1 000 μg/L, and raised AST failed to detect 30% (3/10) of the patients with cirrhosis (they did not have all the predicting factors), but none of the patients with cirrhosis had a platelet count > 200 000/μL and normal AST. We think that the combination of a platelet count < 200 000/μL and raised AST is very useful indication for cirrhosis prediction in HH[26].

Direct markers: Type Ⅳ collagen is an important component of the normal extracellular matrix, and serum components of type Ⅳ collagen are thought to primarily reflect matrix degradation. A serum type Ⅳ collagen level higher than 115 ng/mL has been found to be 100% sensitive in the prediction of underlying cirrhosis in HH[30]. Recently, the same group has reported similar 100% sensitivity with values > 113, but with only 56% specificity for cirrhosis[27]. Other fibrosis markers, like serum laminin and tissue inhibitor of metalloproteinase (TIMP- Ⅰ) levels (or concentrations), seem to be of little value for fibrosis prediction in iron overloaded livers[30].

Jensen *et al*[31] showed that the serum procollagen Ⅲ N-propeptide, previously studied by Colombo *et al*[32], and serum laminin, seem to be of little value in iron-loaded disorders.

Crawford *et al*[27] recently reported that serum hyaluronic acid with serum ferritin can accurately predict cirrhosis and thus reduce the need for liver biopsy in C282Y hemochromatosis. In their study, serum hyaluronic acid concentration > 46.5 ng/mL was 100% sensitive and 100% specific in identifying C282Y hemochromatosis patients with cirrhosis. In HH patients with serum ferritin values >1 000 μg/L, the measurement of hyaluronic acid is a noninvasive, accurate, and cost-effective method for the diagnosis of cirrhosis, and can assist in the clinical assessment of the eventual need, or not, for liver biopsy.

Serum fibrosis marker panels: In 2008, Adhoute *et al*[33] published a study about the diagnosis of liver fibrosis using FibroScan and other non-invasive methods in patients with hemochromatosis. They studied Fibro Test, Hepascore, APRI, FIB-4, Forns, Lok, and GUCI scores in 57 patients with HH, and 46 controls. No statistical difference was observed between the two groups in any of the non-invasive tests. In both groups, a significant correlation was found between FibroScan and Fibro Test values, Forns score, Hepascore and GUCI score. No correlation was found between Fibro Scan values and Lok score, FIB-4 score or APRI score. When comparing patients with a recent HH diagnosis (n = 10) and those with iron-depletion (n = 47), no significant differences were observed between the two groups for non-invasive methods for liver fibrosis evaluation, with the exception of the APRI and GUCI scores. A slight correlation was found between serum ferritin values and FibroScan. They concluded that biochemical markers and FibroScan may constitute reliable non-invasive means for liver fibrosis determination.

IRON CONCENTRATION DETERMINATION AND FIBROSIS

The risk of significant fibrosis or cirrhosis has been associated with the level of LIC[23]. Bassett *et al*[34] introduced the concept of a threshold for LIC above which cirrhosis was more likely, and Sallie *et al*[24] reported that, in addition to LIC, an age greater than 45 years may be a risk factor for significant fibrosis or cirrhosis. In 2005 Olynyk *et al*[23] showed that the duration of iron exposure by the liver increases the risk of significant fibrosis in HH, and considered patient's age as a significant factor for fibrosis prediction. The product of age and LIC (fibrosis-index) obtained by liver biopsy or by MRI, with a 480 000 cut-off resulted in a 100% sensitivity and 86% specificity for the diagnosis of high degree- fibrosis (F3-F4)[23]. MRI can now be used for assessing iron load[35-38]; consequently, liver biopsy is no longer required for the evaluation of iron load[39,40], and the presence of iron in the reticuloendothelial system can be assessed by MRI of the spleen[40], thus discarding secondary hemochromatosis cases (Figure 2). This fibrosis index has been validated

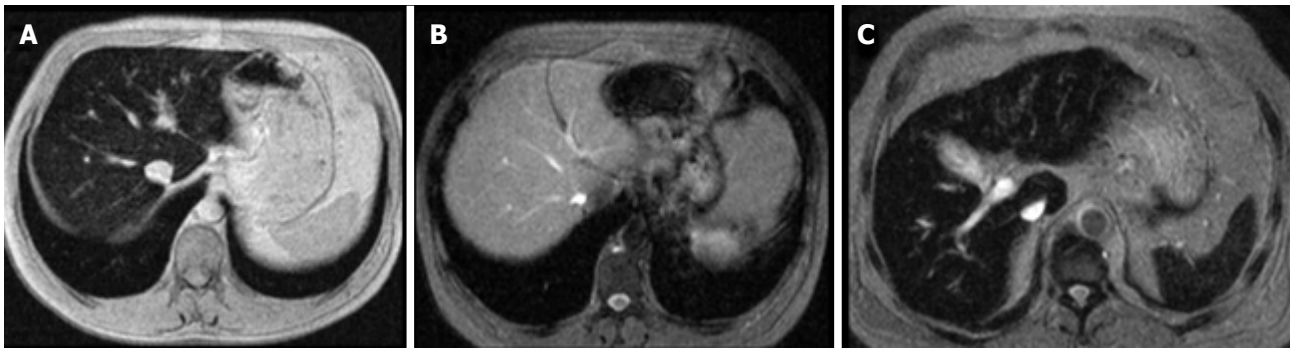


Figure 2 Quantification of liver iron concentration using magnetic resonance imaging. A: Hereditary hemochromatosis. Liver iron overload: Important reduction in signal intensity from the liver; B: Prolonged treatment with phlebotomies. Liver signal intensity is normal; C: Secondary hemochromatosis. Reduction in signal intensity in the liver and the spleen.

externally by our group[41]. The results we obtained were close to those in the original paper, but we think that this index must be taken into account in conjunction with other predictive parameters.

RADIOLOGIC TOOLS FOR FIBROSIS ASSESSMENT

Transient elastography

Transient elastography (FibroScan) is a new non-invasive, rapid, reproducible method, allowing assessment of liver fibrosis by measuring liver rigidity[42]. Adhoute *et al*[33] have studied the utility of FibroScan and other non-invasive methods in patients with hemochromatosis. They included 57 cases with 46 controls, obtaining a strong correlation between FibroScan and many biochemical markers, although ferritin levels did not correlate with FibroScan values. The prevalence of patients with FibroScan values higher than 7.1 kPa (cut-off level for significant fibrosis), was 22.8% in patients with hemochromatosis and 0% in the controls ($P < 0.0001$). However, the technique must be improved, because liver stiffness measurements are uninterpretable in nearly one in five cases of a large prospective series[43], mainly due to obesity, particularly increased waist circumference, and limited operator experience.

Magnetic resonance elastography

Recently, another non-invasive radiologic tool has been developed for liver fibrosis study: MR Elastography[44]. Large Az values for elasticity (> 0.990 for scores ⩾ F2, ⩾ F3, and F4) show that MR elastography was accurate in liver fibrosis staging and that it was superior to biochemical testing with APRIs. It seems that it will provide a higher technical success rate and a better diagnostic accuracy than ultrasound elastography and APRI for staging liver fibrosis[44]. To the best of our knowledge, this promising new non-invasive method has not yet been utilised for the study of hemochromatosis patients.

CONCLUSION

Based on the advances during the last few years, biochemical markers, LIC determination by MRI (Fibrosis index) and FibroScan and, probably, MR Elastography, all constitute reliable non-invasive means for detecting liver fibrosis. The role of liver biopsy in the study of hemochromatosis is decreasing. In future, it seems that lLiver biopsy will only be performed for diagnosis of associated diseases, or in patients where discrepancies between radiologic and biochemical markers exist. We think it is time to take a step forward and to reduce our "faith" in liver biopsy in favour of non-invasive methods for liver fibrosis prediction.

ACKNOWLEDGEMENTS

The authors thank the translation department of the "Instituto Vasco de Investigaciones Sanitarias" (BIOEF) for their translation help.

REFERENCES

1 **Pietrangelo A**. Hereditary hemochromatosis--a new look at an old disease. *N Engl J Med* 2004; **350**: 2383-3297
2 **Feder JN**, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR, Wolff RK. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996; **13**: 399-408
3 **Gandon Y**, Olivié D, Guyader D, Aubé C, Oberti F, Sebille V, Deugnier Y. Non-invasive assessment of hepatic iron stores by MRI. *Lancet* 2004; **363**: 357-362
4 **Castiella A**, Alustiza JM, Artetxe J. Hereditary hemochromatosis. *N Engl J Med* 2004; **351**: 1263-1264; author reply 1263-1264
5 **Wood MJ**, Skoien R, Powell LW. The global burden of iron overload. *Hepatol Int* 2009; **3**: 434-444
6 **Froehlich F**, Lamy O, Fried M, Gonvers JJ. Practice and complications of liver biopsy. Results of a nationwide survey in Switzerland. *Dig Dis Sci* 1993; **38**: 1480-1484
7 **Thampanitchawong P**, Piratvisuth T. Liver biopsy:complications and risk factors. *World J Gastroenterol* 1999; **5**: 301-304
8 **Bedossa P**, Dargère D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. *Hepatology* 2003; **38**: 1449-1457
9 **de Sterke**. Should we continue to perform liver biopsies in hereditary hemochromatosis (HH)? Open letter to all

doctors involved in hereditary hemochromatosis. Update on January 5th 2010. Available from: URL: http://www.hemochromatosis.co.uk/liverbiopy/index.htm
10 **Falize L**, Guillygomarc'h A, Perrin M, Lainé F, Guyader D, Brissot P, Turlin B, Deugnier Y. Reversibility of hepatic fibrosis in treated genetic hemochromatosis: a study of 36 cases. *Hepatology* 2006; **44**: 472-477
11 **Clark P**, Britton LJ, Powell LW. The diagnosis and management of hereditary haemochromatosis. *Clin Biochem Rev* 2010; **31**: 3-8
12 **Villeneuve JP**, Bilodeau M, Lepage R, Côté J, Lefebvre M. Variability in hepatic iron concentration measurement from needle-biopsy specimens. *J Hepatol* 1996; 25: 172-177
13 **Hübschner SG**. Role of liver biopsy in disorders of iron metabolism. *Diagnostic Histopathology* 2008; **14**: 577-585
14 **Deugnier YM**, Charalambous P, Le Quilleuc D, Turlin B, Searle J, Brissot P, Powell LW, Halliday JW. Preneoplastic significance of hepatic iron-free foci in genetic hemochromatosis: a study of 185 patients. *Hepatology* 1993; **18:** 1363-1369
15 **Guyader D**, Gandon Y, Sapey T, Turlin B, Mendler MH, Brissot P, Deugnier Y. Magnetic resonance iron-free nodules in genetic hemochromatosis. *Am J Gastroenterol* 1999; **94**: 1083-1086
16 **Alústiza JM**, Castiella A. Liver fat and iron at in-phase and opposed-phase MR imaging. Radiology 2008; 246: 641
17 **Springer F**, Machann J, Claussen CD, Schick F, Schwenzer NF. Liver fat content determined by magnetic resonance imaging and spectroscopy. *World J Gastroenterol* 2010; **16**: 1560-1566
18 **Marín Gabriel JC**, Solís Herruzo JA. [Predicting liver fibrosis with non-invasive tests--a hope for the future]. *Rev Esp Enferm Dig* 2008; **100**: 605-610
19 **Guyader D**, Jacquelinet C, Moirand R, Turlin B, Mendler MH, Chaperon J. Noninvasive prediction of fibrosis in C282Y homozygous hemochromatosis. *Gastroenterology* 1998; **115**: 929-936
20 **Bacon BR**, Olynyk JK, Brunt EM, Britton RS, Wolff RK. HFE genotype in patients with hemochromatosis and other liver diseases. *Ann Intern Med* 1999; **130**: 953-962
21 **Beaton M**, Guyader D, Deugnier Y, Moirand R, Chakrabarti S, Adams P. Noninvasive prediction of cirrhosis in C282Y-linked hemochromatosis. *Hepatology* 2002; **36**: 673-678
22 **Morrison ED**, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El-Serag HB, Gordon SC, Galan MV, Tung BY, Ioannou GN, Kowdley KV. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. *Ann Intern Med* 2003; **138**: 627-633
23 **Olynyk JK**, St Pierre TG, Britton RS, Brunt EM, Bacon BR. Duration of hepatic iron exposure increases the risk of significant fibrosis in hereditary hemochromatosis: a new role for magnetic resonance imaging. *Am J Gastroenterol* 2005; **100**: 837-841
24 **Sallie RW**, Reed WD, Shilkin KB. Confirmation of the efficacy of hepatic tissue iron index in differentiating genetic haemochromatosis from alcoholic liver disease complicated by alcoholic haemosiderosis. *Gut* 1991; **32**: 207-210
25 **Castiella A**, Zapata E, Otazua P, Fernández J, Alustiza JM, Ugarte M, Legasa L, Galardi A, Ugalde A, Barredo I, Arriola JA. [Utility of various non-invasive methods for fibrosis prediction among Basque Country patients with phenotypic hemochromatosis]. *Rev Esp Enferm Dig* 2008; **100**: 611-614
26 **Castiella A**, Zapata E, Otazua P. Hemochromatosis: platelets and aspartate aminotransferase are useful high-degree fibrosis marker. *Hepatology* 2009; **49**: 1781; author reply 1781-1782
27 **Crawford DH**, Murphy TL, Ramm LE, Fletcher LM, Clouston AD, Anderson GJ, Subramaniam VN, Powell LW, Ramm GA. Serum hyaluronic acid with serum ferritin accurately predicts cirrhosis and reduces the need for liver biopsy in C282Y hemochromatosis. *Hepatology* 2009; **49**: 418-425
28 **Beaton M**, Adams PC. Assessment of silent liver fibrosis in hemochromatosis C282Y homozygotes with normal transaminase levels. *Clin Gastroenterol Hepatol* 2008; **6**: 713-714
29 **Olynyk JK**, Hagan SE, Cullen DJ, Beilby J, Whittall DE. Evolution of untreated hereditary hemochromatosis in the Busselton population: a 17-year study. *Mayo Clin Proc* 2004; **79**: 309-313
30 **George DK**, Ramm GA, Walker NI, Powell LW, Crawford DH. Elevated serum type IV collagen: a sensitive indicator of the presence of cirrhosis in haemochromatosis. *J Hepatol* 1999; **31**: 47-52
31 **Jensen PD**, Heickandorff L, Helweg-Larsen HM, Jensen FT, Christensen T, Ellegaard J. Serum procollagen III peptide concentration in iron overload. *Eur J Haematol* 1996; **57**: 157-164
32 **Colombo M**, Annoni G, Donato MF, Conte D, Martines D, Zaramella MG, Bianchi PA, Piperno A, Tiribelli C. Serum type III procollagen peptide in alcoholic liver disease and idiopathic hemochromatosis: its relationship to hepatic fibrosis, activity of the disease and iron overload. *Hepatology* 1985; **5**: 475-479
33 **Adhoute X**, Foucher J, Laharie D, Terrebonne E, Vergniol J, Castéra L, Lovato B, Chanteloup E, Merrouche W, Couzigou P, de Lédinghen V. Diagnosis of liver fibrosis using FibroScan and other noninvasive methods in patients with hemochromatosis: a prospective study. *Gastroenterol Clin Biol* 2008; **32**: 180-187
34 **Bassett ML**, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. *Hepatology* 1986; **6**: 24-29
35 **Queiroz-Andrade M**, Blasbalg R, Ortega CD, Rodstein MA, Baroni RH, Rocha MS, Cerri GG. MR imaging findings of iron overload. *Radiographics* 2009; **29**: 1575-1589
36 **Alústiza JM**, Castiella A, De Juan MD, Emparanza JI, Artetxe J, Uranga M. Iron overload in the liver diagnostic and quantification. *Eur J Radiol* 2007; **61**: 499-506
37 **Wood JC**. Magnetic resonance imaging measurement of iron overload. *Curr Opin Hematol* 2007; **14**: 183-190
38 **Tziomalos K**, Perifanis V. Liver iron content determination by magnetic resonance imaging. *World J Gastroenterol* 2010; **16**: 1587-1597
39 **Pietrangelo A**. Non-invasive assessment of hepatic iron overload: are we finally there? *J Hepatol* 2005; **42**: 153-154
40 **Pietrangelo A**, Corradini E, Ferrara F, Vegetti A, De Jong G, Luca Abbati G, Paolo Arcuri P, Martinelli S, Cerofolini E. Magnetic resonance imaging to identify classic and nonclassic forms of ferroportin disease. *Blood Cells Mol Dis* 2006; **37**: 192-196
41 **Castiella A**, Emparanza JI. External validation for fibrosis predicting index in hereditary hemochromatosis. *Am J Gastroenterol* 2005; **100**: 2366-2367
42 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847
43 **Castéra L**, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828-835
44 **Huwart L**, Sempoux C, Vicaut E, Salameh N, Annet L, Danse E, Peeters F, ter Beek LC, Rahier J, Sinkus R, Horsmans Y, Van Beers BE. Magnetic resonance elastography for the noninvasive staging of liver fibrosis. *Gastroenterology* 2008; **135**: 32-40

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.256

World J Hepatol 2010 July 27; 2(7): 256-260
ISSN 1948-5182 (online)

GUIDELINES FOR CLINICAL PRACTICE

Alpha-fetoprotein specific CD4 and CD8 T cell responses in patients with hepatocellular carcinoma

Shahriar Behboudi, Stephen P Pereira

Shahriar Behboudi, Stephen P Pereira, UCL Institute of Hepatology, University College London, Royal Free Hospitals, London NW3 2QG, United Kingdom
Author contributions: Behboudi S and Pereira SP contributed equally to the paper.
Supported by a project grant from Association for International Cancer Research
Correspondence to: Shahriar Behboudi, PhD, UCL Institute of Hepatology, University College London, 69-75 Chenies Mews, London WC1E 6HX, United Kingdom. s.behboudi@ucl.ac.uk
Telephone: +44-20-76796515 Fax: +44-20-73800405
Received: June 1, 2009 Revised: June 17, 2010
Accepted: June 24, 2010
Published online: July 27, 2010

Abstract

The presence of CD8 T cell responses to tumor associated antigens have been reported in patients with different malignancies. However, there is very little information on a comparable CD8 and CD4 T cell response to a tumor antigen in liver cancer patients. Here, we re-examine the kinetic and the pattern of T helper 1 and cytotoxic T lymphocyte responses to alpha-fetoprotein (AFP), a tumor rejection antigen in hepatocellular carcinoma (HCC). Then, we discuss the possibility of using AFP-based immunotherapy in combination with necrotizing treatments in HCC patients.

Key words: Hepatocellular carcinoma; Alpha-fetoprotein; Cell-mediated immunity; Immunotherapy

Peer reviewers: Nabil Mohie Abdel-Hamid, Professor, Chairman of Biochemistry Department, Pharmacy College, Minia University, Al Minya 61519, Egypt; Nattiya Hirankarn, MD, Associated Professor, Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Rama 4 road, Bangkok 10330, Thailand

Behboudi S, Pereira SP. Alpha-fetoprotein specific CD4 and CD8 T cell responses in patients with hepatocellular carcinoma. *World J Hepatol* 2010; 2(7): 256-260 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/256.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.256

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and a major health problem in parts of Asia and Africa. The majority of cases of HCC arise in cirrhotic livers with hepatitis B being the main cause of nodules developing in the cirrhotic liver with malignant transformation resulting in HCC. Survival of untreated patients is poor and surgical resection provides the only chance of cure. However, surgery is not suitable for majority of patients in whom the tumor is metastasized and/or liver function is seriously undermined. For the majority of the HCC patients, non-surgical treatments such as transarterial chemoembolization and radiofrequency ablation are the only option[1]. These nonsurgical treatments have been shown to reduce tumor burden and improve survival rate but tumor relapse is common and thus more effective treatments are required to control tumor growth[2]. Early diagnosis and the development of novel systemic therapies such as immunotherapeutic strategies may be very important. It has been demonstrated that the immune system is able to induce responses against tumors and these responses can be enhanced using a number of strategies. T cells or T lymphocytes are a group of immune system cells that play a central role in cell-mediated immunity. Cytotoxic T cells (CD8 T cells) and helper T cells (CD4 T cells) recognize tumor associated antigens presented on MHC class Ⅰ or Ⅱ of antigen presenting cells via their T cell receptors. The activated T cells develop into effector and memory T

cells that recognize and lyse tumor cells. Molecular and immunological approaches have been applied to identify HCC associated antigens. HCC over-express several tumor associated antigens[3]. Some of these antigens such as MAGE, Glypican-3 and NY-ESO-1 are also expressed by many other types of cancer cells[4,5]. Among these antigens, alpha-fetoprotein (AFP) is shown to be specific to HCC and testicular carcinoma. AFP is a glycoprotein with a molecular weight of around 70 kDa which is produced in the endodermal cells of the yolk sac and fetal liver. The synthesis of AFP decreases dramatically after birth and only trace amounts are expressed in the adult liver. However, expression of the AFP gene is reactivated in adults during liver regeneration and hepatocarcinogenesis[6] with the majority of HCC patients showing an increase of AFP in serum. The measurement of serum AFP plays an important role in the diagnosis of HCC and monitoring responses to the treatment[6]. In some cases, over-expression of AFP can be detected in HCC cells even when serum AFP levels are normal[7].

The induction of anti-AFP cell-mediated immune responses has been demonstrated to control tumor growth in animal models. In one study, anti-AFP cytotoxic T lymphocyte (CTL) response was induced and a significant survival benefit was observed in mice immunized with mAFP expression vector DNA while no hepatocyte damage was detectable despite low-level endogenous hepatic mAFP expression, showing that AFP is a tumor rejection antigen[8]. In humans, it has been shown that B and T cells can recognize peptide epitopes within the AFP sequence and develop into effector and/or regulatory lymphocytes[9-17]. Here, we discuss naturally occurring CD8 and CD4 T cell responses to AFP in different groups of HCC patients and discuss the possibility of combining trans-arterial chemoembolization (TACE)/trans-arterial embolisation (TAE) treatment with AFP-based immunotherapy.

AFP-SPECIFIC CD8 T CELL RESPONSES

CD8 T cells recognize AFP derived peptide epitopes in the context of MHC class I molecules and develop into cytotoxic T cells with the ability to recognize and kill tumor cells. An existing immunological paradigm is that high concentrations of soluble protein contribute to the maintenance of peripheral tolerance/ignorance to self protein. However, that is not the case as many AFP-specific CD8 T cell clones are not deleted during ontogeny and AFP derived epitopes are recognized by both murine and human T cells. AFP-derived peptides with high probability to bind MHC class I (HLA-A2) were synthesized and tested *in vitro*. T cell clones recognizing several AFP-derived peptides were identified. Initially, four peptides were identified and termed "immunodominant" based on their binding efficiency to MHC class I (HLA-A2) and the ability to stimulate IFN-γ production by CD8 T cells from healthy donors. These peptide epitopes were also found to be immunogenic and immuno-dominant in HLA-A2 transgenic mice[18]. Later, five other AFP-derived peptides containing HLA-A2402 binding motifs were also identified. CD8 T cells recognized these epitopes presented on HLA-A2402 positive hepatoma cells and were developed into cytotoxic T cells with the ability to lyse tumor cells and produce IFN-γ[13]. Although several HLA-A2 and HLA-24 restricted CD8 T cell epitopes have been identified and some are classified as immuno-dominant and some as sub-dominant[11,13], it has been recently suggested that a high frequency of AFP-specific IFN-γ producing CD8 T cells (CTL) are directed against different epitopes spreading over the entire AFP sequence with no single immuno-dominant epitope[19]. Our recent data support these findings and demonstrate that there is no immunodominant CD8 T cell epitope within the AFP sequence[20]. Another important finding is that AFP-specific CD8 T cells can be detected in patients with HCC, patients with non-HCC liver diseases as well as healthy donors[19,21,22]. This is in contrast with the results demonstrating the absence of anti-AFP CD4 T cell responses in healthy donors or patients with non-HCC liver diseases[14,16,20]. The presence of CD8 T cells recognizing AFP, Glypican-3, NY-ESO-1 and MAGE-1 have been confirmed in healthy donors[10,19,23,24]. Although AFP-CD8 T cell responses are detected in non-HCC patients as well as HCC patients, it has been suggested that this response is stronger in HCC patients[19]. We were unable to confirm these findings and no significant differences were observed in the frequencies of anti-AFP CD8 T cell responses in healthy donors and patients with HCC and the same percentages of responders were observed in HCC patients and non-HCC patients[20]. The results from several studies including our recent report support the fact that the detection of AFP-specific CD8 T cells does not correlate with elevation of serum AFP, vascular invasion, liver function and the type of viral infection[19-21,25]. Similarly, no association was found between the levels of serum AFP and Okuda stage in HCC patients and the presence of CD8 T cell responses to non-AFP HCC associated antigens such as NY-ESO-1[26]. However, for the first time we have demonstrated that there is an association between the stage of liver cirrhosis and the presence of anti-AFP CD8 T cell responses. Anti AFP-CD8 T cell responses were observed in 17% of HCC patients with Child-Pugh A score while this response was detected in 46% of HCC patients with Child-Pugh B or C[20], demonstrating that anti-AFP CD8 T cell responses are expanded as liver cirrhosis progresses. Another interesting finding is that the frequency of AFP-specific CD8 T cells in the liver of HCC patients is no higher than that in peripheral blood[19]. This is an unusual finding, as tumor specific CD8 T cells are generally enriched in the liver[24]. It is possible that liver infiltrating AFP-specific CD8 T cells undergo apoptosis or stop responding to peptide stimulation due to exhaustion or expression of inhibitory molecules such as PD-1. This negative regulation by PD-1 on NY-ESO-1 specific CD8 T cell responses has been demonstrated in patients

with ovarian cancer[27] and further studies are required to establish its effects in patients with HCC. More sensitive assays for the detection of antigen specific CD8 T cells such as tetramer staining of AFP-specific CD8 T cells in liver and peripheral blood are required to confirm these findings. In a study performed in HLA-A24+ HCC patients, it was demonstrated that HCC treatments such as radiofrequency ablation (RF) or TACE can augment the frequency of circulating AFP-specific (*ex vivo*) but not viral-specific, CD8 T cells in some HCC patients[13]. This may suggest that tumor burden may suppress the expansion of anti-AFP CD8 T cell responses in HCC patients or that tumor necrosis stimulates the expansion of anti-tumor immune responses.

AFP-SPECIFIC CD4 T CELL RESPONSES

It has been shown that CD4 T cells play a crucial role in the control of tumor growth in both animal models and cancer patients. It is believed that CD4 T cells provide help required for the induction of CD8 T cell responses with the ability to lyse tumor cells in a MHC class Ⅰ, Fas ligand and perforin dependent manner. T helper (Th) 1 cells can also directly eradicate tumor cells without any significant involvement of CD8 T cells[28]. This is also confirmed in experiments involving adaptive transfer of IFN-γ producing CD4 T cells recognizing tumor antigens. The adaptive transfer of Th1 cells but not CTLs provided protection against various transplanted or endogenous tumors in animal models[29,30], suggesting that Th1 cells play a more important role in tumor immunity than was initially postulated. This is also shown in a cancer patient where adaptive transfer of a Th1 clone recognizing NY-ESO-1 antigen provided sustained clinical remission[31]. Therefore, it is crucial to explore the concept of targeting HCC-specific Th1 cells in anti-HCC immunotherapy.

We have extensively studied the magnitude and characteristics of circulating AFP-specific CD4+ T cell responses in HCC patients[14-16,20]. In these studies, several HLA-DR restricted CD4 T cell epitopes within the AFP sequence have been identified. In contrast to CD8 T cell responses, an immuno-dominant epitope is established for CD4 T cells and it is shown that more than 20% of HCC patients have a detectable response to this immuno-dominant epitope[14]. Th1 responses to the immunodominant epitopes were only observed in HCC patients and no response was detected in patients with non-HCC liver disease or healthy controls[14,16,20]. However, this does not exclude the possibility that Th1 responses to other yet unknown epitopes could be detected in patients with non-HCC liver disease or healthy controls. The presence of anti-AFP IgG in the serum of patients with non-HCC liver disease[17] suggests that AFP-specific CD4 T cell responses may be present in this group of patients. This notion is also supported by results showing that CD4 T cells from healthy donors respond to protein (AFP)-pulsed dendritic cells by producing IFN-γ[21].

Anti-AFP CD4 T cell response are more likely to be present in patients with early stage disease (Okuda stage Ⅰ) and low or moderately elevated serum AFP[14]. It is still unclear why AFP-specific CD8 T cell responses are detected in all groups of HCC patients (early and late stage cancer) and there is no association with the levels of serum AFP levels. We have recently performed a parallel analysis of AFP-specific CD4 and CD8 T cell responses in the same group of HCC patients and healthy donors. The results confirm this trend and demonstrated that anti-AFP Th1 response is detectable in 58% of HCC patients with Okuda stage Ⅰ tumors and 15.8% of patients with Okuda stage Ⅱ or Ⅲ tumors[20]. When the patients were classified based on their liver function, anti-AFP Th1 response was observed in 44% of HCC patients with a Child-Pugh A score (early stage of cirrhosis) whereas this response was detected in only 15% with a B or C score (late-stage cirrhosis). These results suggest that anti-AFP Th1 responses are more likely to be present in patients who are in an early stage of disease (for both tumor stage and liver cirrhosis). This indicates that there is a difference in the activation of anti-AFP CD4 *vs* CD8 T cells in HCC patients, with a CD4 T-cell response expanding in early stages of disease usually associated with low concentrations of serum AFP and with exhaustion of this response in later stages of disease in which there is a high concentration of serum AFP. This is in accordance with our earlier reports showing that high concentrations of AFP suppress immune cell function *in vitro*[32] and CD4 T cells isolated from HCC patients with high concentrations of serum AFP are impaired[14]. It seems that anti-AFP CD4 T cell response is impaired or exhausted in late stage HCC patients and any effective immunotherapy should be combined with treatment strategies that restore the function of these cells. It is possible that CD4 T cells are more susceptible to immuno-regulatory effects of tumor cells than that by CD8 T cells. In this case, the removal of tumor cells, the immuno-regulatory molecules or cells induced by tumor cells should improve the function of anti-tumor Th1 cells. In fact, we have shown that tumor necrotizing treatments such as TACE/TAE that reduce tumor burden improve the function of AFP-specific CD4 T cells[16]. Different immune-regulatory mechanisms have been reported in patients with HCC[15,32-34]. For example, an expansion of $CD4^+CD25^+$ regulatory T cells in the peripheral blood and tumors tissues of patients with HCC has been reported[33,35,36] and this expansion has an inverse correlation with the recurrence-free survival[37]. A number of preclinical murine studies suggest that the depletion of regulatory T cells augments the effects of immune-based therapies such as anti-tumor vaccines[38]. The lack of a specific marker for the detection of $CD4^+$ regulatory T cells makes their *in vivo* depletion in patients difficult. However, low-dose cyclophosphamide treatment has been shown to deplete $CD4^+CD25^+$ regulatory T cells in a murine tumor model[39]. In a recent report, the effect of a low-dose cyclophosphamide treatment on the frequency and function of regulatory T cells in patients with advanced HCC were analyzed. A systemic treatment of HCC patients with low-dose cyclophosphamide decreased

the frequency and suppressor function of circulating $CD4^+CD25^+Foxp^+$ regulatory T cells in peripheral blood and unmasked anti-AFP T cell responses[40]. The results suggest that this procedure could be used in combination with immunotherapeutic approaches in HCC.

In a clinical trial, when both CD4 and CD8 T cells were targeted by administration of tumor lysate-pulsed dendritic cells in HCC patients, a partial clinical response was observed in one out of 35 HCC patients[41]. In this clinical trial[41], the induction or expansion of a pre-existing AFP-specific CD4 T cell response was not analyzed. However, we believe that this vaccination strategy can induce or activate both AFP-specific T helper and cytotoxic T cell responses required for the generation of potent anti-tumor immune responses. It is possible but not proven that tumor lysate-pulsed dendritic cells can also activate the expansion of regulatory T cells and this can diminish the effectiveness of this immunotherapy strategy[42]. In fact, it has been suggested that transforming growth factor-beta1 producing CD4 T cells could be induced by the AFP-derived peptide epitope[15] and this may hamper anti-tumor immunity.

REFERENCES

1 **Schafer DF**, Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999; **353**: 1253-1257
2 **Ebied OM**, Federle MP, Carr BI, Pealer KM, Li W, Amesur N, Zajko A. Evaluation of responses to chemoembolization in patients with unresectable hepatocellular carcinoma. *Cancer* 2003; **97**: 1042-1050
3 **Kittaka N**, Takemasa I, Takeda Y, Marubashi S, Nagano H, Umeshita K, Dono K, Matsubara K, Matsuura N, Monden M. Molecular mapping of human hepatocellular carcinoma provides deeper biological insight from genomic data. *Eur J Cancer* 2008; **44**: 885-897
4 **Capurro MI**, Shi W, Sandal S, Filmus J. Processing by convertases is not required for glypican-3-induced stimulation of hepatocellular carcinoma growth. *J Biol Chem* 2005; **280**: 41201-41206
5 **Gnjatic S**, Nishikawa H, Jungbluth AA, Gure AO, Ritter G, Jager E, Knuth A, Chen YT, Old LJ. NY-ESO-1: review of an immunogenic tumor antigen. *Adv Cancer Res* 2006; **95**: 1-30
6 **Mizejewski Gj**. Physiology of alpha-fetoprotein as a biomarker for perinatal distress: relevance to adverse pregnancy outcome. *Exp Biol Med (Maywood)* 2007; **232**: 993-1004
7 **Sato K**, Tanaka M, Kusaba T, Fukuda H, Tanikawa K. Immunohistochemical demonstration of alpha-fetoprotein in small hepatocellular carcinoma. *Oncol Rep* 1998; **5**: 355-358
8 **Grimm CF**, Ortmann D, Mohr L, Michalak S, Krohne TU, Meckel S, Eisele S, Encke J, Blum HE, Geissler M. Mouse alpha-fetoprotein-specific DNA-based immunotherapy of hepatocellular carcinoma leads to tumor regression in mice. *Gastroenterology* 2000; **119**: 1104-1112
9 **Butterfield LH**, Koh A, Meng W, Vollmer CM, Ribas A, Dissette V, Lee E, Glaspy JA, Mcbride WH, Economou JS. Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from alpha-fetoprotein. *Cancer Res* 1999; **59**: 3134-3142
10 **Butterfield LH**, Meng WS, Koh A, Vollmer CM, Ribas A, Dissette VB, Faull K, Glaspy JA, Mcbride WH, Economou JS. T cell responses to HLA-A*0201-restricted peptides derived from human alpha fetoprotein. *J Immunol* 2001; **166**: 5300-5308
11 **Liu Y**, Daley S, Evdokimova VN, Zdobinski DD, Potter DM, Butterfield LH: Hierarchy of alpha fetoprotein (AFP)-specific T cell responses in subjects with AFP-positive hepatocellular cancer. *J Immunol* 2006; **177**: 712-721
12 **Meng WS**, Butterfield LH, Ribas A, Heller JB, Dissette VB, Glaspy JA, Mcbride WH, Economou JS. Fine specificity analysis of an HLA-A2.1-restricted immunodominant T cell epitope derived from human alpha-fetoprotein. *Mol Immunol* 2000; **37**: 943-950
13 **Mizukoshi E**, Nakamoto Y, Tsuji H, Yamashita T, Kaneko S. Identification of alpha-fetoprotein-derived peptides recognized by cytotoxic T lymphocytes in HLA-A24+ patients with hepatocellular carcinoma. *Int J Cancer* 2006; **118**: 1194–1204
14 **Alisa A**, Ives A, Pathan AA, Navarrete CV, Williams R, Bertoletti A, Behboudi S. Analysis of CD4+ T-Cell Responses to a Novel {alpha}-Fetoprotein-Derived Epitope in Hepatocellular Carcinoma Patients. *Clin Cancer Res* 2005; **11**: 6686-6694
15 **Alisa A**, Boswell S, Pathan AA, Ayaru L, Williams R, Behboudi S. Human CD4(+) T cells recognize an epitope within alpha-fetoprotein sequence and develop into TGF-beta-producing CD4(+) T cells. *J Immunol* 2008; **180**: 5109-5117
16 **Ayaru L**, Pereira SP, Alisa A, Pathan AA, Williams R, Davidson B, Burroughs AK, Meyer T, Behboudi S. Unmasking of {alpha}-Fetoprotein-Specific CD4+ T Cell Responses in Hepatocellular Carcinoma Patients Undergoing Embolization. *J Immunol* 2007; **178**: 1914-1922
17 **Bei R**, Budillon A, Reale MG, Capuano G, Pomponi D, Budillon G, Frati L, Muraro R. Cryptic epitopes on alpha-fetoprotein induce spontaneous immune responses in hepatocellular carcinoma, liver cirrhosis, and chronic hepatitis patients. *Cancer Res* 1999; **59**: 5471-5474
18 **Butterfield LH**. Immunotherapeutic strategies for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S232-S241
19 **Thimme R**, Neagu M, Boettler T, Neumann-Haefelin C, Kersting N, Geissler M, Makowiec F, Obermaier R, Hopt UT, Blum HE, Spangenberg HC. Comprehensive analysis of the alpha-fetoprotein-specific CD8+ T cell responses in patients with hepatocellular carcinoma. *Hepatology* 2008; **48**: 1821-1833
20 **Behboudi S**, Alisa A, Boswell S, Anastassiou J, Pathan AA, Williams R. Expansion of anti-AFP Th1 and Tc1 responses in hepatocellular carcinoma occur in different stages of disease. *Br J Cancer* 2010; **102**: 748-753
21 **Ritter M**, Ali MY, Grimm CF, Weth R, Mohr L, Bocher WO, Endrulat K, Wedemeyer H, Blum HE, Geissler M. Immunoregulation of dendritic and T cells by alpha-fetoprotein in patients with hepatocellular carcinoma. *J Hepatol* 2004; **41**: 999-1007
22 **Hanke P**, Rabe C, Serwe M, Böhm S, Pagenstecher C, Sauerbruch T, Caselmann WH. Cirrhotic patients with or without hepatocellular carcinoma harbour AFP-specific T-lymphocytes that can be activated in vitro by human alpha-fetoprotein. *Scand J Gastroenterol* 2002; **37**: 949-955
23 **Hiroishi K**, Eguchi J, Baba T, Shimazaki T, Ishii S, Hiraide A, Sakaki M, Doi H, Uozumi S, Omori R, Matsumura T, Yanagawa T, Ito T, Imawari M. Strong CD8 (+) T-cell responses against tumor-associated antigens prolong the recurrence-free interval after tumor treatment in patients with hepatocellular carcinoma. *J Gastroenterol* 2010; **45**: 451-458
24 **Bricard G**, Bouzourene H, Martinet O, Rimoldi D, Halkic N, Gillet M, Chaubert P, Macdonald HR, Romero P, Cerottini JC, Speiser DE. Naturally acquired MAGE-A10- and SSX-2-specific CD8+ T cell responses in patients with hepatocellular carcinoma. *J Immunol* 2005; **174**: 1709-1716
25 **Butterfield LH**, Ribas A, Potter DM, Economou JS. Spontaneous and vaccine induced AFP-specific T cell phenotypes in subjects with AFP-positive hepatocellular cancer. *Cancer Immunol Immunother* 2007; **56**: 1931-1943
26 **Gehring AJ**, Ho ZZ, Tan AT, OO Aung M, Lee KH, Tan KC, Lim SG, Bertoletti A. Profile of tumor antigen-specific CD8 T cells in patients with hepatitis B virus-related hepatocellular carcinoma. *Gastroenterology* 2009; **137**: 682-690
27 **Matsuzaki J**, Gnjatic S, Mhawech-Fauceglia P, Beck A,

Miller A, Tsuji T, Eppolito C, Qian F, Lele S, Shrikant P, Old LJ, Odunsi K. Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer. *Proc Natl Acad Sci USA* 2010; **107**: 7875-7880

28 **Schurmans LR**, Diehl L, Den Boer AT, Sutmuller RP, Boonman ZF, Medema JP, Van Der Voort EI, Laman J, Melief CJ, Jager MJ, Toes RE. Rejection of intraocular tumors by CD4(+) T cells without induction of phthisis. *J Immunol* 2001; **167**: 5832-5837

29 **Egeter O**, Mocikat R, Ghoreschi K, Dieckmann A, Rocken M. Eradication of disseminated lymphomas with CpG-DNA activated T helper type 1 cells from nontransgenic mice. *Cancer Res* 2000; **60**: 1515-1520

30 **Muller-Hermelink N**, Braumuller H, Pichler B, Wieder T, Mailhammer R, Schaak K, Ghoreschi K, Yazdi A, Haubner R, Sander CA, Mocikat R, Schwaiger M, Forster I, Huss R, Weber WA, Kneilling M, Rocken M. TNFR1 signaling and IFN-gamma signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. *Cancer Cell* 2008; **13**: 507-518

31 **Hunder NN**, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, Jungbluth A, Gnjatic S, Thompson JA, Yee C. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N Engl J Med* 2008; **358**: 2698-2703

32 **Um S**, Mulhall C, Alisa A, Ives AR, Karani J, Williams R, Bertoletti A, Behboudi S. Alpha-fetoprotein impairs APC function and induces their apoptosis. *J Immunol* 2004; **173**: 1772-1778

33 **Yang XH**, Yamagiwa S, Ichida T, Matsuda Y, Sugahara S, Watanabe H, Sato Y, Abo T, Horwitz DA, Aoyagi Y. Increase of CD4+ CD25+ regulatory T-cells in the liver of patients with hepatocellular carcinoma. *J Hepatol* 2006; **45**: 254-262

34 **Hoechst B**, Ormandy LA, Ballmaier M, Lehner F, Krüger C, Manns MP, Greten TF, Korangy F. A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells. *Gastroenterology* 2008; **135**: 234-243

35 **Ormandy LA**, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 2005; **65**: 2457-2464

36 **Fu J**, Xu D, Liu Z, Shi M, Zhao P, Fu B, Zhang Z, Yang H, Zhang H, Zhou C, Yao J, Jin L, Wang H, Yang Y, Fu YX, Wang FS. Increased regulatory T cells correlate with CD8 T-cell impairment and poor survival in hepatocellular carcinoma patients. *Gastroenterology* 2007; **132**: 2328-2339

37 **Gao Q**, Qiu SJ, Fan J, Zhou J, Wang XY, Xiao YS, Xu Y, Li YW, Tang ZY. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007; **25**: 2586-2593

38 **Beyer M**, Schultze JL. Regulatory T cells in cancer. *Blood* 2006; **108**: 804-811

39 **Ghiringhelli F**, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C, Chauffert B, Solary E, Bonnotte B, Martin F. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol* 2004; **34**: 336-344

40 **Greten TF**, Ormandy LA, Fikuart A, Höchst B, Henschen S, Hörning M, Manns MP, Korangy F. Low-dose cyclophosphamide treatment impairs regulatory T cells and unmasks AFP-specific CD4+ T-cell responses in patients with advanced HCC. *J Immunother* 2010; **33**: 211-218

41 **Palmer DH**, Midgley RS, Mirza N, Torr EE, Ahmed F, Steele JC, Steven NM, Kerr DJ, Young LS, Adams DH. A phase II study of adoptive immunotherapy using dendritic cells pulsed with tumor lysate in patients with hepatocellular carcinoma. *Hepatology* 2009; **49**: 124-132

42 **Grauer OM**, Sutmuller RP, van Maren W, Jacobs JF, Bennink E, Toonen LW, Nierkens S, Adema GJ. Elimination of regulatory T cells is essential for an effective vaccination with tumor lysate-pulsed dendritic cells in a murine glioma model. *Int J Cancer* 2008; **122**: 1794-1802

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.261

World J Hepatol 2010 July 27; 2(7): 261-274
ISSN 1948-5182 (online)

GUIDELINES FOR CLINICAL PRACTICE

Acute esophageal variceal bleeding: Current strategies and new perspectives

Salvador Augustin, Antonio González, Joan Genescà

Salvador Augustin, Antonio González, Joan Genescà, Liver Unit, Department of Internal Medicine, Hospital Universitari Vall d'Hebron, Institut de Recerca, Universitat Autònoma de Barcelona, Barcelona 08035, Spain

Author contributions: Augustin S, González A, and Genescàs A contributed equally to the design, writing and revision of this paper.

Supported partially by a Grant from Ministerio de Ciencia e Innovación SAF2009-08354; Salvador Augustin is a recipient of a 'Río Hortega' Fellowship Grant from the Instituto de Salud Carlos III and is enrolled in the postgraduate programme of the Universitat Autònoma de Barcelona

Correspondence to: Salvador Augustin, MD, Liver Unit, Department of Internal Medicine, Hospital Universitari Vall d'Hebron, Institut de Recerca, Passeig Vall d´Hebron 119-129, Barcelona 08035, Spain. saugustin@vhebron.net
Telephone: +34-93-2746140 Fax: +34-93-2746068

Received: February 18, 2010 Revised: June 14, 2010
Accepted: June 21, 2010
Published online: July 27, 2010

Abstract

Management of acute variceal bleeding has greatly improved over recent years. Available data indicates that general management of the bleeding cirrhotic patient by an experienced multidisciplinary team plays a major role in the final outcome of this complication. It is currently recommended to combine pharmacological and endoscopic therapies for the initial treatment of the acute bleeding. Vasoactive drugs (preferable somatostatin or terlipressin) should be started as soon as a variceal bleeding is suspected (ideally during transfer to hospital) and maintained afterwards for 2-5 d. After stabilizing the patient with cautious fluid and blood support, an emergency diagnostic endoscopy should be done and, as soon as a skilled endoscopist is available, an endoscopic variceal treatment (ligation as first choice, sclerotherapy if endoscopic variceal ligation not feasible) should be performed. Antibiotic prophylaxis must be regarded as an integral part of the treatment of acute variceal bleeding and should be started at admission and maintained for at least 7 d. In case of failure to control the acute bleeding, rescue therapies should be immediately started. Shunt therapies (especially transjugular intrahepatic portosystemic shunt) are very effective at controlling treatment failures after an acute variceal bleeding. Therapeutic developments and increasing knowledge in the prognosis of this complication may allow optimization of the management strategy by adapting the different treatments to the expected risk of complications for each patient in the near future. Theoretically, this approach would allow the initiation of early aggressive treatments in high-risk patients and spare low-risk individuals unnecessary procedures. Current research efforts will hopefully clarify this hypothesis and help to further improve the outcomes of the severe complication of cirrhosis.

Key words: Portal hypertension; Variceal bleeding; Complications of cirrhosis

Peer reviewer: Hitoshi Maruyama, MD, Department of Medicine and Clinical Oncology, Chiba University Graduate School of Medicine, 1-8-1, Inohana, Chuou-ku, Chiba 260-8670, Japan

Augustin S, González A, Genescà J. Acute esophageal variceal bleeding: Current strategies and new perspectives. *World J Hepatol* 2010; 2(7): 261-274 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/261.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.261

INTRODUCTION

Variceal bleeding is a major complication of portal hypertension and represents a leading cause of death in patients with cirrhosis[1,2]. Diagnostic and therapeutic developments have led to a significant improvement in the prognosis of this complication over the past two decades. However, early mortality after an episode of acute variceal

bleeding (AVB) remains high (15%-24%)[1-5]. This paper reviews the current knowledge, most recent advancements and research prospects in the management of patients with cirrhosis presenting with AVB of esophageal origin. The prognostic and therapeutic approach to patients bleeding from gastric varices is clearly different and is not considered in the present review.

NATURAL HISTORY OF ACUTE VARICEAL BLEEDING

Ruptured esophageal varices cause approximately 70% of all upper gastrointestinal (GI) hemorrhages in cirrhosis[6]. Therefore, a variceal origin should be suspected in any cirrhotic patient presenting with a GI bleeding until a diagnostic endoscopy is performed.

It is known from placebo-controlled trials that approximately 40%-50% of variceal hemorrhages stop spontaneously[7]. Current available therapies further increase control of bleeding in about 80% of patients. However, despite the application of the most effective treatments available, one out of four patients will still show either a failure to control the bleeding or an early recurrence of the hemorrhage in the first 6 wk after the initial bleeding[4-6]. This risk peaks during the first 5 d, the period in which 40% of all rebleedings occur. Afterwards, it decreases slowly, equaling at 6 wk the risk previous to the bleeding episode[1,4-6].

A similar improvement in the early mortality of AVB has been recognized in the past 30 years from the 42% of the seminal study by Graham and Smith[1] to 15%-24% with current therapies[1,4-6]. A recent population-based study from the USA showed a crude in-hospital mortality decrease from 18% to 11.5% between 1988 and 2004[8]. However, this early mortality rate is still very high and probably underestimates the true risk since pre-hospital mortality data are scarce, the only available estimation being 3%-4%[9]. The risk of death after an AVB episode shows a similar evolution to that of rebleeding, peaking during the first 5-10 d and slowly returning to the base line after 6 wk[1,5].

Due to the difficulties in recognizing a single cause of death after a variceal hemorrhage, the general consensus is that any death occurring within 6 wk of admission from the index bleeding should be considered as a bleeding-related death[10]. However, it is currently estimated that 20%-40% of deaths after AVB are secondary to uncontrolled bleeding and exsanguination while the majority of remaining cases are due to liver failure, infections and hepatorenal syndrome[6,10]. Therefore, management of these patients should require a global approach including hemostatic therapies but also prophylactic strategies to avoid the above mentioned complications.

PROGNOSTIC STUDIES IN ACUTE VARICEAL BLEEDING

The value of different clinical and hemodynamic variables in predicting the outcome after an AVB has been the subject of a number of studies in the past years[5,6,11-16] (Table 1).

Prognostic indicators of rebleeding have been assessed in most studies together with initial failure to control the acute bleeding and 5 d mortality as a composite endpoint referred to as "5 d failure". Severity of liver disease, quantified as Child-Pugh and Model for End-Stage Liver Disease (MELD) scores or its individual components, has been widely recognized as a robust independent predictor of 5 d failure. Active bleeding at initial endoscopy has also been identified as an important risk factor for 5 d failure in several studies[6,13,14]. The prognostic value of other reported factors (platelet count, etiology of cirrhosis, hematocrit, transfusion needs, shock, portal vein thrombosis) seem to be less reproducible between studies.

Regarding early mortality, severity of liver disease (mainly Child-Pugh class C) is also the main and most constant prognostic indicator. The presence of hepatocellular carcinoma or occurrence of early rebleeding when included in multivariate analysis have been also recognized as important independent risk factors for 6 wk mortality. Recognition of the prognostic relevance of potentially modifiable factors such as bacterial infection or renal failure is increasing since these complications could be regarded as targets of specific therapies aiming to improve global outcomes after AVB. Other prognostic clinical variables reported in different studies are shown in Table 1.

The hepatic venous pressure gradient (HVPG) has proven an excellent prognostic value for both treatment failure and survival after AVB[17]. A HVPG value $\geqslant$ 20 mmHg in the first 48 h after admission has been associated with higher treatment failure and mortality in several studies. However, the discriminating ability of HVPG after an AVB does not seem to be superior to the combined use of clinical variables[11].

In summary, available prognostic studies suggest that the combined use of clinical variables (mainly Child and/or MELD scores, active bleeding, hepatocellular carcinoma, bacterial infection and renal failure) along with HVPG measurements when available are likely to accurately predict prognosis after AVB. Although it is worth remarking that no available prognostic models based on these variables are suited for individual prognostication, it is nevertheless important to highlight the potential practical value of these predictive tools. A precise early classification of patients into different risk strata would make it possible to adapt the therapeutic approach to the expected outcomes of each stratum. The use of new statistical approaches based on techniques such as Classification and Regression Tree analysis (CART) may facilitate the recognition of prognostic subgroups as targets for specific interventions[5,18]. This method is especially adept at detecting relevant interactions between variables and provides intuitive decision trees allowing the identification of subgroups of patients that share a specific combination of clinical characteristics and a similar prognosis, as illustrated in Figure 1. The

Table 1 Most relevant prognostic studies in acute variceal bleeding published between 1999-2009

Authors (year)(Ref.)	*n*	Universal antibiotic prophylaxis	Hemostatic-treatment	Early rebleeding/ treatment failure	Prognostic factors for rebleeding/treatment failure	Early mortality	Prognostic factors for early mortality	Statistical technique	Validation of prognostic models
Ben Ari *et al* (1999)[13]	529	No	EST or VAD (+ EST if failure)	224 (42%)	Active bleeding at endoscopy, platelet count, time to admission, alcohol, heart rate, encephalopathy	92 (17%)	Encephalopathy, bilirubin, ascites, plasma urea, heart rate, 5 d failure	Cox regression	Yes (bootstrapping)
Moitinho *et al* (1999)[14]	65	No	EST or VAD	23 (35%)	HVPG ⩾ 20 mmHg	6 (9%)	Not reported	Logistic regression	No
D'Amico *et al* (2003)[6]	297	No	VAD, Endoscopy or combination	49 (15%)	Child, portal vein thrombosis, AST, active bleeding, transfusion volume	70 (21%)	Encephalopathy, bilirubin, HCC, albumin	Logistic regression	Yes (split sample)
Thomopoulous *et al* (2003)[15]	121	No	VAD + EVL	15 (10%)	Not reported	26 (18%)	Child, shock	Logistic regression	No
Lecleire *et al* (2005)[16]	275	No	Endoscopy	Not reported	Not reported	107 (23%)	Prothrombin time, digestive cancer, hematemesis, corticoids, age, in-patients	Logistic regression	No
Abraldes *et al* (2008)[14]	117	Yes	VAD + Endoscopy	18 (15%)	HVPG ⩾ 20 mmHg, shock, Child, non-alcoholic cirrhosis	7 (6%)	Not reported	Logistic regression	Yes (bootstrapping)
Bambha *et al* (2008)[12]	256	Yes	EVL + (VAD or placebo)	37 (15%)	MELD ⩾ 18, clot on varix	35 (14%)	MELD ⩾ 18, transfusion volume, active bleeding at endoscopy, early rebleeding	Bivariate cox analysis	No
Augustin *et al*. (2009)[5]	267	Yes	VAD + Endoscopy	55 (21%)	Not reported	63 (24%)	Child, infection, plasma creatinine, HCC	Logistic regression + CART analysis	Yes (split sample)

n: total number of patients (only those with variceal bleeding considered); EST: endoscopic sclerotherapy; EVL: endoscopic variceal ligation; VAD: vasoactive drug; HVPG: hepatic venous pressure gradient; AST: aspartate aminotransferase; HCC: hepatocellular carcinoma; CART: classification and regression tree; Ref: references.

efficacy of this "a la carte" strategy and the value of the different stratification approaches should be evaluated in future randomized controlled trials (RCTs). Finally, it seems important to remark that the relative weight of the different variables in the proposed prognostic models may be substantially affected by the treatments applied in the cohort. Therefore, new models drawn from cohorts receiving the current standard of care (i.e. combined vasoactive drug plus endoscopic ligation plus antibiotics) will be needed. A recent study by our group showed that when this standard of care is applied, renal dysfunction is the main modifiable indicator of bad prognosis in AVB[18].

GENERAL MANAGEMENT

There is evidence that current treatment strategies for AVB have improved survival in different countries[8,19,20]. However, early rebleeding and mortality rates remain high (15%-24%) in this scenario. For this reason, AVB is considered a medical emergency and therefore current guidelines state that it should be managed by a multidisciplinary team of experienced staff including nurses, hepatologists, endoscopists, interventional radiologists and surgeons, preferably in an intensive care unit (ICU). Diagnostic and therapeutic decisions should be driven by a written protocol developed to optimize the resources of each center. These recommendations have so far been based mainly on experts' opinion since objective data on the issue has been scarce. Nevertheless, over the last years, a number of studies published evaluating different aspects of the quality of general management of these patients may help to optimize current strategies.

On one hand it is clear that the management of patients with AVB and organ dysfunction can be extremely challenging. Admission of these patients to high-dependency or intensive care units is highly advisable. However, it is worth noting that the outcome of cirrhotic patients admitted to ICU correlates directly with the number of organs failing. Sepsis and multiorgan failure, especially if requiring renal replacement therapy, confer a dismal prognosis with over 90% mortality[21]. Therefore, consideration should be given to the futility of ICU admission and escalating organ support measures in this subset of patients, especially if they are not suitable for liver trans-

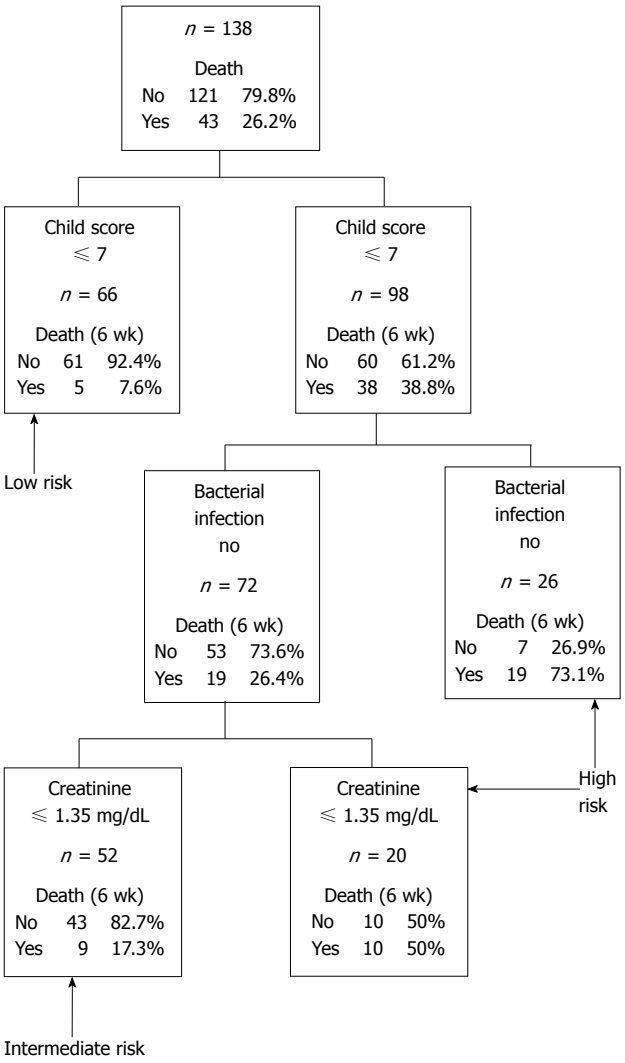


Figure 1 Prognostic model for 6 wk mortality based on an inductive tree generated by classification and regression tree analysiss in a cohort of 164 consecutive patients after an episode of acute esophageal variceal bleeding.

plantation. Besides, recent studies have evaluated the influence of hospital volume and time of admission (weekdays or weekends) as indirect measures of quality of care. Regarding the importance of hospital volume, a recent retrospective study was unable to show a direct relationship between hospital volume and better outcomes[22]. However, it still seems advisable that lack of the above mentioned facilities should require immediate referral to an experienced center until more data is available. Finally, a large cross-sectional study the USA identified a possible "weekend" effect leading to increased mortality for patients with non-variceal upper GI bleeding which did not reach statistical significance for variceal hemorrhages. This weekend effect may be attributable to many considerations including patients presenting later in the course of the disease or system issues (such as the availability and quality of supportive care with disparities in staff patterns on weekends).

The therapeutic approach to the cirrhotic patient with AVB should include initial resuscitation and specific hemostatic measures aimed at correcting the hemorrhagic shock as well as early prevention of severe and frequent complications that worsen the prognosis of these patients (mainly bacterial infection and renal dysfunction).

Initial resuscitation

Initial resuscitation in AVB follows the general ABC (Airway, Breathing, Circulation) scheme and it is aimed at maintaining an appropriate delivery of oxygen to the tissues.

Extreme care of the airway should be maintained as the patient is at high risk of bronchial aspiration of gastric contents and blood. This risk is especially high in encephalopathic patients and is further exacerbated by endoscopic procedures. Endotracheal intubation is thus mandatory if there is any concern about the safety of the airway. Pulse oxymetry and oxygen administration are essential to maintain adequate blood oxygen saturation. Variceal bleeding is often massive; therefore, it is essential to obtain adequate peripheral venous access in order to administer fluids and blood products if required.

Optimal volume replacement remains controversial. Blood volume restitution should be undertaken as soon as possible with the goal of maintaining systolic blood pressure around 100 mmHg. Avoidance of hypovolemia and prolonged hypotension is particularly important in order to prevent renal failure and infection which are associated with increased risk of rebleeding and death[6,7,23]. Nevertheless, blood volume replacement and transfusion should be cautious and conservative since over-transfusion has been associated with rebound increases in portal pressure and more rebleeding and mortality, as suggested by experimental studies[24,25] and in a recent RCT published in abstract form[26]. Blood transfusion should be aimed at maintaining hemoglobin at 7-8 g/L[10,26] except in patients with rapid ongoing bleeding or ischemic heart or cerebral disease in which case this threshold should be raised. The use of vasoactive drugs has been shown to blunt the increase in portal pressure induced by volume expansion[27,28]. It has been suggested that volume replacement should be done with human albumin fraction or gelatin-based colloid which have been associated with less effect on clotting compared to dextran, although clinical data is lacking. Similarly, vigorous resuscitation with saline solution should be avoided because it can worsen or precipitate a recurrent variceal hemorrhage and accumulation of ascites and fluid at other extravascular sites.

Patients with cirrhosis often present with abnormalities in coagulation tests and platelet counts. The derangement of hemostasis in these patients has long been thought to play an important role in variceal hemorrhage. However, these abnormalities seem to be poorly correlated with bleeding[29]. Recent advancements in the pathophysiology of hemostasis in cirrhosis have led several authors to challenge these concepts and give new insights on potential new therapeutic approaches. The thrombocytopenia that is usually encountered in these

patients is now considered to impair not only primary hemostasis but also thrombin generation[30]. Transfusion of fresh frozen plasma and platelets can be considered in these patients although the exact role of these measures has not been evaluated appropriately. Another possibility still to be investigated in clinical trials is treatment with thrombopoietin[31]. Additionally, several drugs that act on coagulation and fibrinolytic pathways have been tested. Desmopressin (DDAVP), a drug that significantly decreases bleeding time in cirrhosis, has shown no clinical benefits in the setting of variceal bleeding[31,32]. The potential benefit of therapy with anti-fibrinolytic agents, useful in liver transplantation, has not been evaluated in clinical trials[33]. The use of recombinant activated factor Ⅶ which corrects prothrombin time in cirrhotics[34] has been assessed in two RCTs[35,36]. These studies failed to show a beneficial effect of this factor over standard therapy on preventing treatment failure and so this expensive therapy cannot be currently recommended.

Diagnostic endoscopy

The gold standard for the diagnosis of variceal hemorrhage is endoscopy. A diagnosis of bleeding varices is accepted if certain pre-specified criteria are met[37]. Current guidelines recommend performing an emergency endoscopy as soon as safely possible after admission[10,19,20] in order to confirm a variceal origin of the hemorrhage which represents the leading cause of upper GI bleeding in cirrhotics. However, these recommendations are based on experts' opinions and not on objective evidence drawn from adequately designed studies. On one hand, a certain amount of indirect data suggests that early performance of endoscopy may indeed be preferable. First, documentation of a non-variceal origin associates a much better prognosis and directly influences management. Second, endoscopic therapy clearly improves outcomes in AVB so the presumption is that early application of endotherapy may lower treatment failures and mortality. In fact, a recently published retrospective study[38] identified delayed endoscopy (defined as performed 15 h after admission) as a risk factor for in-hospital mortality although important methodological drawbacks hamper the external validity of these results. Finally, early endoscopy has proven to lower costs when performed in patients with non-variceal upper GI bleeding[39] so the assumption is that it may well be the same case for variceal bleeding. Unfortunately, these hypotheses remain unproven in RCTs so far.

On the other hand, several authors suggest that endoscopy-related complications (such as aspiration pneumonia) may compromise the potential benefits of early endotherapy[40]. Moreover, based on available evidence, it has been argued that early administration of vasoactive drugs might justify the delay of endoscopy and that endotherapy could be added only in case of failure of drugs to control bleeding[41]. Finally, since performing endoscopic therapy at the time of diagnostic endoscopy would spare the patient a second procedure, it is advisable that a skilled endoscopist is available. A recent retrospective study from Korea compared the outcomes of patients presenting with after-hours AVB according to the timing of initial endoscopy[42]. In the early endoscopy group (< 12 h after admission), the rate of finding the bleeding source was lower and 30 d mortality was higher than in the delayed endoscopy (12-24 h after admission) cohort. Another recent study with similar design showed that a shorter time to endoscopy was not associated with better outcomes[43]. Again, the retrospective nature of these studies limits the validity of these observations. In conclusion, although consensus seems to exist that an emergency endoscopy should be performed as soon as safely possible after admission, more studies are needed to adequately address the potential benefits and drawbacks of this strategy.

PREVENTION OF COMPLICATIONS AND DETERIORATION IN LIVER FUNCTION

Prophylaxis and treatment of infection

Up to 20% of cirrhotic patients who are hospitalized due to GI bleeding present with bacterial infections and an additional 50% will develop an infection while hospitalized[44]. This risk is especially high in those patients with poor liver function (i.e. Child B and C)[45,46]. The most frequent infections are spontaneous bacterial peritonitis and spontaneous bacteriemia (50%), followed by urinary tract infections (25%) and pneumonia (25%)[47]. Presence of these infections should be systematically ruled out in a bleeding cirrhotic patient (performing chest x-ray, urinary analysis and diagnostic paracentesis).

Infection is one of the strongest prognostic indicators in AVB and is associated with early rebleeding and greater mortality[5,48,49]. It has been proven that antibiotic prophylaxis significantly reduces the percentage of patients who develop infection and rebleeding[50] and that it increases survival[51]. Therefore, all cirrhotic patients (with or without ascites) with upper GI bleeding must receive prophylactic antibiotic therapy at admission. The current recommended antibiotic schedule is oral norfloxacin at dose of 400 mg BID for 7 d although ciprofloxacin could also be used[10,19,20]. When the oral route is not possible, quinolones can be administered intravenously. A recent RCT suggests that intravenous (Ⅳ) ceftriaxone (1 g/d) might be more effective than oral norfloxacin in preventing bacterial infections in Child B and C patients[52]. It seems advisable that the final choice of antibiotic should be nevertheless adjusted to the prevalence of quinolone-resistant microorganisms at each center. The potential beneficial effect of prophylactic schemes that cover the high risk 6 wk period after the bleeding remains unexplored.

Ascites and renal function

Tense ascites should be treated with paracentesis along with albumin replacement when indicated. This has been shown to decrease portal and variceal pressure[53].

The development of renal failure in cirrhotic patients after an AVB which occur in approximately 11% of cases is associated with a dismal prognosis[23]. Moreover, serum

creatinine level at admission of AVB has also proven to be a robust marker of severity in this setting, regardless of the evolution of renal function. Although current consensus set the creatinine level to define renal failure at 1.5 mg/dL, a lower cut-off (1.35 mg/dL) may allow an early identification of a high-risk population among variceal bleeders[5]. The need for aggressive management of renal dysfunction in cirrhotic patients is widely encouraged[10]. Renal function should be supported by adequate fluid and electrolyte replacement (saline solutions should be avoided), and should be closely monitored. Urine output should be maintained at a minimum of 40 mL/h; an output below 20 mL/h indicates poor renal function and impending renal failure[10]. In this case, active search and prompt treatment of potential (even non-apparent) precipitating factors (rebleeding, infection) is mandatory. Nephrotoxic drugs should be avoided, particularly amynoglycosides and non-steroidal anti-inflammatory drugs. The potential beneficial effect of specific strategies (e.g. short-term albumin infusion) aimed at preventing and/or treating renal dysfunction after AVB require evaluation in future studies.

Nutrition

Malnutrition is frequent in cirrhosis[54] and may contribute to an increased susceptibility to infections and renal dysfunction. Therefore, feeding should be resumed as soon as a 24 h interval free of rebleeding has been achieved. Enteral nutrition is always preferable due to lower cost and complications when compared to parenteral nutrition. There is currently no empirical evidence to continue recommending low protein diets which could further impair the nutritional status of these patients[55].

Encephalopathy

Variceal bleeding can precipitate hepatic encephalopathy. There is insufficient data to support the prophylactic use of lactulose or lactitol[10] although they can be given to patients who already present encephalopathy. It is important to be forewarned about the possibility of alcohol withdrawal. Judicious use of benzodiazepines or clomethiazole may be necessary to control an acute deprivation/withdrawal syndrome. Thiamine administration should also be considered to prevent Wernicke syndrome in alcoholic and/or malnourished patients.

HEMOSTATIC THERAPIES

The treatment of the AVB is aimed at controlling the acute hemorrhage, preventing early rebleeding and, ultimately, reducing mortality. Current recommended initial management is based on the combination of pharmacological and endoscopic therapy[10,19,20]. Rescue therapies such as local tamponade or portal-systemic shunts may be necessary in case of treatment failures.

Pharmacological therapy

Vasoactive drugs exert their action by reducing portal pressure. The rationale of the use of vasoactive drugs is the assumption that this reduction of portal pressure leads to a reduction in variceal pressure and a better control of hemorrhage[28,56]. Indeed, treatment with vasoactive drugs alone controls bleeding in up to 83% of patients[41].

Whenever a variceal bleeding is suspected, vasoactive drugs should be started as soon as possible, even before diagnostic confirmation, and ideally during transfer to the hospital since a quarter of deaths occur very early after bleeding onset[9]. Furthermore, a number of trials[57-59] have shown that early administration of these drugs reduces the rate of active bleeding during endoscopy thus facilitating endoscopic procedures. This might lead to a reduction of side effects, treatment failures and bleeding related mortality. The optimal duration of therapy with vasoactive drugs is not well established. Current guidelines recommend maintaining vasoactive treatment for 2-5 d since this is the time period in which rebleeding is more frequent[10,19,20].

Several drugs are available to treat AVB. Published data does not permit firm conclusions about the superiority of any of them over the rest and the choice should be based according to local resources[10,19,20,60].

Terlipressin: Terlipressin is a synthetic analogue of vasopressin with longer activity and fewer side effects. It reduces portal pressure and its effects are still significant 4 hours after administration[61-63]. The overall efficacy of terlipressin in controlling variceal bleeding is 75%-80% at 48 h[64] and 67% at 5 d[65]. Terlipressin has been shown to significantly improve control of bleeding and survival when compared to placebo[64,66-68] and is the only drug that has shown to improve survival. However, terlipressin can provoke ischemic complications and severe dysrhythmias. Therefore, it should be used with extreme caution or even avoided in those patients with a history of ischemic heart or cerebral disease, limb or gut vascular disease or heart rhythm disorders.

Terlipressin is given as a 2 g bolus dose every 4 hours during the first 2 d. The dose is halved after bleeding is controlled and can be maintained for up to 5 d. Administration of terlipressin at low doses in continuous perfusion has been tested in cirrhotic patients with septic shock with promising results[69,70] but its use in AVB has not been explored yet and cannot be recommended.

Somatostatin: Natural somatostatin also causes splanchnic vasoconstriction at therapeutical doses and has proven to reduce portal pressure and HVPG during active bleeding[28,71-73]. Additionally, somatostatin blocks the postprandial increase in portal blood flow and portal pressure.

Randomized trials and meta-analyses[58,73,74] have demonstrated that somatostatin significantly improves control of bleeding when compared to placebo (63% *vs* 46%) but not survival[75]. On the other hand, its beneficial effect on control of bleeding, early rebleeding and mortality is similar to that of terlipressin with a better safety profile. Major side effects with somatostatin are extremely rare. Minor

side effects such as vomiting and hyperglycemia occur in up to 21% of patients and are usually easy to manage.

Somatostatin is usually given at a continuous perfusion dose of 250 mcg/h after an initial 250 mcg bolus (which can be repeated up to 3 times during the first hour). The infusion should be maintained for 5 d[76] or until a 24 h period free of rebleeding has been achieved. The use of 500 mcg/h doses has been associated with greater decreases in HVPG[71] and may be more effective in patients with more severe bleedings[77].

Octreotide and other somatostatin analogues: Octreotide is a synthetic analogue of natural somatostatin with similar mechanism of action and longer half life. However, this does not result in longer hemodynamic effects[78,79], probably due to the development of tachyphylaxis or rapid desensitization[80]. The effect of octreotide as single therapy in AVB is controversial. The only RCT addressing the issue did not show any benefit of octreotide over placebo in prevention of rebleeding or mortality[81]. On the other hand, octreotide appeared to be equivalent to terlipressin in two other trials which were nevertheless underpowered and not double-blinded[75]. Overall, the result of a recent meta-analysis suggests that the beneficial effect of octreotide as single therapy in AVB is negligible[82]. No placebo-controlled trials have been published using octreotide before endoscopy, the setting in which it is frequently used in clinical practice. However, results of another meta-analysis suggest that, when used on top of endoscopic sclerosis, octreotide is indeed effective in preventing early rebleeding with no apparent effect on mortality[83]. It has been speculated that this beneficial effect of octreotide may be related to its capacity of blunting postprandial increases in portal pressure[84]. The safety profile of octreotide is similar to that of somatostatin. The drug is usually given in continuous infusion of 25-50 mcg/h with an optional initial iv or subcutaneous bolus of 50 mcg. As for somatostatin, it can be given for up to 5 d to prevent early rebleeding. In summary, octreotide may be beneficial when used along with endoscopic therapy but has uncertain effects when used alone and therefore should be considered a second choice when terlipressin or somatostatin is available.

Vapreotide and lanreotide are two other synthetic an alogues of somatostatin with comparable affinity for somatostatin receptors[84]. They both have been shown to reduce portal pressure in animals but their clinical hemodynamic effect in humans is controversial[85]. One study showed that, when used before endotherapy, vapreotide was more effective than placebo in controlling variceal bleeding[86]. Lanreotide did not improve the efficacy of endotherapy in a recent cooperative RCT that remains unpublished[12].

Vasopressin: Vasopressin is the most potent splanchnic vasoconstrictor. It reduces blood flow to all splanchnic organs, leading to a secondary decrease in portal venous inflow and portal pressure. However, these same potent vasoconstrictive properties limit the clinical usefulness of vasopressin. Its use is associated with multiple side effects, including cardiac and peripheral ischemia, dysrhythmia and hypertension, with an overall withdrawal rate of up to 25%[87]. Although the association with nitrates improves the efficacy and reduces complications of vasopressin, side effects are still significantly higher than those of terlipressin or somatostatin and its analogues[75]. Therefore, it remains the last choice among pharmacological therapy. It should not be used at maximal doses beyond the first 24 h after the bleeding. Vasopressin is given at continuous IV perfusion of 0.2-0.4 U/min that can be increased to a maximal dose of 0.8 U/min. It should always be associated to IV nitroglycerine at a 40-400 mcg/min dose, adjusted to maintain blood pressure above 90 mmHg.

In summary, vasoactive drugs are effective and safe and should be used as first line treatment of AVB as soon as variceal bleeding is suspected. Available data do not permit firm conclusions regarding the superiority of one drug over the others, although the efficacy and safety profile of either terlipressin or somatostatin seems to be the most adequate, rendering these two drugs as first choice. Octreotide and vapreotide could also be used if combined with endoscopy.

Endoscopic therapy

Endoscopic therapy versus placebo or non-active treatment: Endoscopic sclerotherapy (EST) alone controls active bleeding in at least 62% of patients[88]. A meta-analysis of the 5 available studies comparing EST with either sham or non-active treatment demonstrated a significant reduction in control of acute bleeding, early rebleeding and mortality[89]. There is no available data comparing endoscopic variceal ligation (EVL) with placebo.

Endotherapy versus drugs: A number of studies have compared EST with active drug treatment for AVB. A meta-analysis of these 13 studies (8 versus octreotide and 5 versus somatostatin) was not able to find significant differences between the two therapies regarding bleeding control or mortality[41]. However, differences of serious adverse events significantly favored somatostatin. No head-to-head comparisons with drugs have been conducted using EVL as endoscopic modality.

Combined therapy *vs* drugs or endotherapy alone: Available individual RCTs and meta-analysis have shown that combined endoscopic and pharmacological therapy improves initial control of bleeding and decreases treatment failure when compared with either one of them alone. A systematic review[90] comparing EST alone *vs* combined therapy showed a significant reduction in initial and 5 d hemostasis for combined therapy, with no significant effect on 5 d mortality (Risk ratio, *RR*: 0.73; 95% Confidence Interval, 95CI: 0.45-1.18). The rate of serious adverse events appeared to be similar for both therapeutic regimens. The only study comparing both strategies using exclusively EVL as endoscopic modality

Table 2 Analysis of pooled data of the only two studies in acute variceal bleeding comparing combined therapy *vs* endoscopy alone in which endoscopic variceal ligation was used as endoscopic modality

	VAD + EVL *n/N* (%)	EVL *n/N* (%)
Calés *et al*[92]	14/98 (14)	21/98 (21)
Sung *et al*[91]	5/47 (11)	11/47 (23)
Total	19/145 (13)	32/145 (22)

Absolute risk difference for combined therapy: -9.0%, CI95 1.7%-17.7%; RR: 0.59, CI95 0.35-0.99, $P = 0.045$. In the study by Calés *et al*, EVL was not performed in all patients (the exact percentage of patients receiving EVL or EST is not provided). VAD: vasoactive drug; EVL: endoscopic variceal ligation; EST: endoscopic sclerotherapy; *n/N*: number of events/number of patients in each treatment arm; CI95: 95% confidence interval.

showed a significant reduction of early rebleeding (EVL alone 38% *vs* octreotide + EVL 9%, $P = 0.0007$) and a remarkable reduction in 30 d mortality (23% *vs* 11%, RR 0.45, 95CI 0.17-1.20), which nevertheless failed to reach statistical significance due to a lack of statistical power (total N: 94; β error for 50% risk reduction: 0.33; total N needed to make the observed RR 0.45 significant: 300 patients)[91]. When data from this study is pooled with data from the other available study in which EVL was used[92] (either EST or EVL where indistinctly performed in this study), combined therapy is significantly superior to endotherapy alone in reducing early mortality (Table 2).

Only two trials have been published comparing combined therapy (using EST) with vasoactive treatment alone: one with somatostatin[93], the other with octreotide[94] and the latter only as an abstract. Pooled results of these studies showed that combined therapy, despite causing more adverse effects, improved control of bleeding without apparent statistical influence on mortality (14% *vs* 21%, relative risk reduction 30%, RR 0.7 95CI 0.29-1.7, $P = 0.4$)[89]. Again, the study available as peer-reviewed article[93] was clearly underpowered to detect otherwise clinically relevant differences in mortality (total $n = 100$; total n needed to render this notable 0.7 RR statistically significant: 600).

Finally, the only trial comparing the current recommended combined therapy (using exclusively EVL) with drugs alone has been recently published [95]. In this study, combination of banding ligation and terlipressin infusion for 2 days was superior to only infusion of terlipressin for 5 days in the reduction of very early rebleeding (0% *vs* 15%, $P = 0.006$) and treatment failure (2% *vs* 24%, $P = 0.002$) in patients with inactive variceal bleeding at endoscopy.

In summary, combined endoscopic and vasoactive treatment is clearly more effective in controlling active bleeding and rebleeding than any of them alone but probably with the cost of more adverse effects. The net benefit on mortality might likely favor the combination but all available studies are clearly underpowered to address effect on mortality. More data are needed to draw firm conclusions on this key issue.

Sclerotherapy *vs* Ligation: Both EST and EVL (alone or combined with drugs) have proven to be effective to control AVB as explained above. Only two RCTs have specifically compared the efficacy of both endotherapies when used without vasoactive drugs. One of the studies, published only as an abstract[96], suggested that EST might be more effective, while the other study[96,97] showed that EVL was superior in terms of efficacy and safety. The only study comparing EST *vs* EVL as adjuvant therapy to drugs (somatostatin) has been recently published[96-98]. The study showed that the combination EVL-somatostatin was superior to EST-somatostatin in terms of bleeding control (treatment failure 10% *vs* 24%, RR 2.4, CI95 1.1-4.9, $P = 0.02$) and safety (overall side effects 14% *vs* 28%, RR 1.9, CI95 1.1-3.5, $P = 0.03$). Again, the beneficial effect of EVL on 6 wk mortality (13% *vs* 21%, RR 1.6, CI95 0.8-3.1, $P = 0.17$) did not reach statistical significance due to the small sample size (total $n = 179$; β error for 1.6 risk reduction: 0.48; total n needed to make the observed RR significant: 690 patients).

Additionally, a recent meta-analysis pooled data of 2 of these trials along with 8 other trials in which EST and EVL were compared both in acute bleeding and prevention of rebleeding[47]. The overall results of this review showed that EVL is better than EST in terms of controlling the initial bleeding and survival and is associated with less adverse events. Moreover, one of these studies also showed that EST but not EVL may induce sustained increases in HVPG which may affect control of bleeding and favor an early recurrence[99]. Finally, it has been claimed that emergency EVL may be more difficult to perform in the presence of massive bleedings due to a more reduced field of view compared to EST[100]. Nevertheless, the use of multi-shot ligation devices[48] as well as the reduction in the rate of active bleeding with early drug therapy have helped to overcome these difficulties[58,86,101]. In summary, all these data support the current consensus that EVL is the endotherapy of choice in AVB although some authors still consider EST acceptable if ligation is not available or technically not feasible.

Rescue therapies

Despite a careful observation of current recommended strategies, 10%-20% of patients will still experience treatment failure or early rebleeding[19,20]. Mortality of these patients is high (30%-50%)[102]. This section reviews the more recent advancements regarding rescue therapies for AVB.

Second endoscopy: Current guidelines[10] recommend that failure of the initial combined treatment can be managed with a second attempt at endoscopic therapy. However, this recommendation is based on experts' opinion since the exact role of a second attempt with endoscopy for uncontrolled bleedings has not yet been systematically evaluated.

Balloon tamponade and esophageal stents: Balloon tamponade is a very effective measure in controlling the

acute bleeding. The use of Sengstaken-Blakemore tube when a massive variceal bleeding is suspected allows initial control of bleeding in up to 80% of patients[103]. Nevertheless, its use is associated with potentially lethal complications such as aspiration, asphyxia due to balloon ligation and esophagus perforation which are associated with a high mortality. Besides, bleeding recurs after deflation in over 50% of cases. Therefore, its use should be restricted to patients with uncontrollable bleeding for a short period of time (< 24 h) as a bridge to a more definitive therapy[19,20]. Airway protection should be considered when balloon tamponade is used.

Recently, esophageal stents have been proposed as an alternative to balloon tamponade in the initial control of massive variceal hemorrhages. These removable self-expanding devices were able to control initially refractory bleedings in 70%-100% patients in 3 small non-controlled pilot studies[104-106]. Theoretically, they will have the advantage over tamponade of less severe complications and additional protection against early rebleeding since they can be left in place for up to 14 d. However, concerns do exist regarding the possibility of downstream migration (especially in patients with concomitant hiatus hernia). An ongoing multicentric RCT comparing balloon tamponade and self-expandable stents will hopefully provide useful information.

Shunting procedures: Both transjugular intrahepatic portosystemic shunts (TIPS) and surgical derivative procedures are extremely effective controlling variceal bleeding in patients who fail to respond to initial pharmacological and endoscopic therapies. However, the incidence of encephalopathy (which affects over 50% and worsens quality of life) and mortality (30% in the first month)[107] remain very high for shunt therapies[108,109], especially for patients with poor liver function (Child B or C).

Two studies by the same surgical group have shown almost universal control of bleeding and high long term survival after early (< 8 h from onset of bleeding) porto-caval shunt. The first study was an uncontrolled report from a large cohort of non-selected cirrhotic patients over a 30-year period[110]. The second study[111], a RCT comparing emergency porto-caval shunt with EST (n = 211), yielded similar results, with universal control of bleeding in the surgical arm and clear superiority of shunt over EST in terms of survival and adverse effects for all Child-Pugh classes. Unfortunately, these impressive results have not been yet equaled by other groups. Although it has been suggested that surgical shunts may remain an option in Child A patients[10], its use as first choice rescue therapy is not currently supported.

TIPS have been proven to be extremely effective in controlling treatment failures in AVB[112,113]. Final hemostasis with TIPS is achieved in 90%-95% of patients with uncontrolled bleeding[19,20]. However, mortality remains high in these patients, mostly due to worsening of liver function (and frequently multiorganic failure), as a consequence of multiple transfusions, repeated endoscopic procedures, infections and deterioration of renal function. In patients with Child-Pugh score > 13, early mortality after TIPS is almost inevitable. Moreover, quality of life of patients surviving salvage TIPS is hampered by the high incidence of encephalopathy which affects half of the patients.

According to the most recent guidelines, the current place of TIPS in AVB is as second line treatment, applicable only for those patients in whom the combined pharmacological and endoscopic therapy has failed. However, technical advances and new studies have stimulated the interest on readdressing the role of TIPS in AVB. On one hand, the development of extended polytetra-fluoroethylene- covered stents have shown to significantly improve the stent long term patency and reduce the incidence of encephalopathy when compared with bare stents[114]. This may contribute to improve overall outcomes of patients receiving TIPS.

Besides, two other recent RCTs have reconsidered the place of TIPS in the management of AVB[115,116]. Both studies are based on the hypothesis that the benefits of TIPS may be enhanced if placed early before the patient deteriorates too much. To this aim, patients at higher risk of complication should be rapidly identified. The first RCT used hemodynamic criteria (HVPG ⩾ 20 mmHg), uncovered TIPS as intervention arm and EST as control therapy[115]. The second study, still available only as abstract, used clinical criteria (Child-Pugh class B with active bleeding or Child C), covered TIPS and combined pharmacological and endoscopic therapy (either EST or EVL) for comparison[116]. In both studies, TIPS significantly reduced rebleeding and mortality without increasing the incidence of encephalopathy. Nevertheless, it should be noted that patients in the control arms of both studies presented mortality rates that were much higher than what would be expected if the current standard of care of AVB (i.e. drug + EVL + antibiotic treatment) had been universally applied so the actual relative benefits of TIPS could be overestimated.

CURRENT RECOMMENDATIONS FOR THE TREATMENT OF ACUTE ESOPHAGEAL VARICEAL BLEEDING (FIGURE 2)

Available data indicates that general management of the bleeding cirrhotic patient plays a major role in the final outcome of this complication. Advancements in this field are difficult due to inherent methodological issues (a variety of procedures performed by a multidisciplinary group influencing a single outcome). However, this growing body of evidence obtained from both RCTs and real-life data sources should help convince clinicians and decision makers alike that adequate resources need to be provided to allow for competent resuscitation, risk stratification, early endoscopy, the availability of timely skilled endoscopic intervention, as well as appropriate more specific therapy - all of which should be coordinated through a collaborative multidisciplinary group.

It can be currently recommended to combine phar-

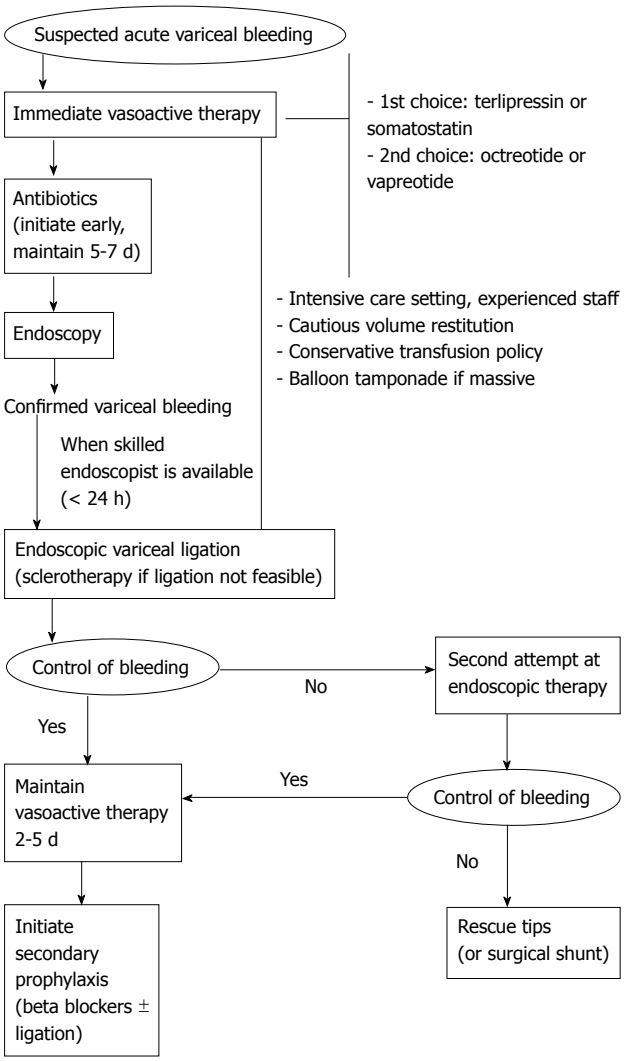


Figure 2 Current recommended management of patients with acute variceal bleeding.

macological and endoscopic therapies for the initial treatment of AVB. Vasoactive drugs (preferable somatostatin or terlipressin) should be started as soon as a variceal bleeding is suspected (ideally during transfer to hospital) and maintained afterwards for 2-5 d. After stabilizing the patient with cautious fluid and blood support, an emergency diagnostic endoscopy should be done and, as soon as a skilled endoscopist is available, an endoscopic variceal treatment (ligation as first choice, sclerotherapy if EVL not feasible) should be performed. Antibiotic prophylaxis must be regarded as integral part of the treatment of AVB and should be started at admission and maintained for at least 7 d. In case of failure to control the acute bleeding, rescue therapies should be immediately started. Shunt therapies (especially TIPS) are very effective at controlling treatment failures after AVB. In the near future, early identification of high-risk patients and use of covered TIPS may contribute to lower the high mortality of these patients. More studies are warranted to clarify which is the most rational management of patients presenting with a high risk of treatment failure.

CONCLUSION

Management of AVB has greatly improved over the past recent years. However, treatment failures and mortality remain high, especially in patients with poor liver function, even if the current standard of care is carefully applied. Therapeutic developments and increasing knowledge in the prognosis of this complication may allow optimization of the management strategy of AVB in the near future, adapting the different treatments to the expected risk of complications for each patient. Theoretically, this approach would allow the initiation of early aggressive treatments in high-risk patients and spare low-risk individuals unnecessary procedures. Current research efforts will hopefully clarify this hypothesis and help to further improve the outcomes of this severe complication of cirrhosis.

REFERENCES

1 **Graham DY**, Smith JL. The course of patients after variceal hemorrhage. *Gastroenterology* 1981; **80**: 800-809
2 **Gines P**, Quintero E, Arroyo V, Teres J, Bruguera M, Rimola A. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128
3 **El-Serag HB**, Everhart JE. Improved survival after variceal hemorrhage over an 11-year period in the Department of Veterans Affairs. *Am J Gastroenterol* 2000; **95**: 3566-3573
4 **McCormick PA**, O'Keefe C. Improving prognosis following a first variceal haemorrhage over four decades. *Gut* 2001; **49**: 682-685
5 **Augustin S**, Muntaner L, Altamirano JT, González A, Saperas E, Dot J, Abu-Suboh M, Armengol JR, Malagelada JR, Esteban R, Guardia J, Genescà J. Predicting early mortality after acute variceal hemorrhage based on classification and regression tree analysis. *Clin Gastroenterol Hepatol* 2009; **7**: 1347-1354
6 **D'Amico G**, De Franchis R. Upper digestive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. *Hepatology* 2003; **38**: 599-612
7 **D'Amico G**, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. Semin *Liver Dis* 1999; **19**: 475-505
8 **Jamal MM**, Samarasena JB, Hashemzadeh M, Vega KJ. Declining hospitalization rate of esophageal variceal bleeding in the United States. *Clin Gastroenterol Hepatol* 2008; **6**: 689-695; quiz 605
9 **Nidegger D**, Ragot S, Berthelémy P, Masliah C, Pilette C, Martin T, Bianchi A, Paupard T, Silvain C, Beauchant M. Cirrhosis and bleeding: the need for very early management. *J Hepatol* 2003; **39**: 509-514
10 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176
11 **Abraldes JG**, Villanueva C, Bañares R, Aracil C, Catalina MV, Garci A-Pagán JC, Bosch J. Hepatic venous pressure gradient and prognosis in patients with acute variceal bleeding treated with pharmacologic and endoscopic therapy. *J Hepatol* 2008; **48**: 229-236
12 **Bambha K**, Kim WR, Pedersen R, Bida JP, Kremers WK, Kamath PS. Predictors of early re-bleeding and mortality after acute variceal haemorrhage in patients with cirrhosis. *Gut* 2008; **57**: 814-820
13 **Ben Ari Z**, Cardin F, McCormick AP, Wannamethee G, Burroughs AK. A predictive model for failure to control bleeding during acute variceal haemorrhage. *J Hepatol* 1999; **31**: 443-450

14 **Moitinho E**, Escorsell A, Bandi JC, Salmerón JM, García-Pagán JC, Rodés J, Bosch J. Prognostic value of early measurements of portal pressure in acute variceal bleeding. *Gastroenterology* 1999; **117**: 626-631
15 **Thomopoulos KC**, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Iconomou G, Nikolopoulou VN. Non-invasive predictors of the presence of large oesophageal varices in patients with cirrhosis. *Dig Liver Dis* 2003; **35**: 473-478
16 **Lecleire S**, Di Fiore F, Merle V, Hervé S, Duhamel C, Rudelli A, Nousbaum JB, Amouretti M, Dupas JL, Gouerou H, Czernichow P, Lerebours E. Acute upper gastrointestinal bleeding in patients with liver cirrhosis and in noncirrhotic patients: epidemiology and predictive factors of mortality in a prospective multicenter population-based study. *J Clin Gastroenterol* 2005; **39**: 321-327
17 **Ripoll C**, Bañares R, Rincón D, Catalina MV, Lo Iacono O, Salcedo M, Clemente G, Núñez O, Matilla A, Molinero LM. Influence of hepatic venous pressure gradient on the prediction of survival of patients with cirrhosis in the MELD Era. *Hepatology* 2005; **42**: 793-801
18 **Augustin S**, Muntaner L, Gonzalez A, Esteban R, Guardia J, Genesca J. Renal dysfunction is the main modifiable determinant of early survival in cirrhotic patients with acute variceal bleeding treated with standard of care therapy. *Hepatology* 2009; **50**: 436A
19 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938
20 **Sarin SK**, Kumar A, Angus PW, Baijal SS, Chawla YK, Dhiman RK, Janaka de Silva H, Hamid S, Hirota S, Hou MC, Jafri W, Khan M, Lesmana LA, Lui HF, Malhotra V, Maruyama H, Mazumder DG, Omata M, Poddar U, Puri AS, Sharma P, Qureshi H, Raza RM, Sahni P, Sakhuja P, Salih M, Santra A, Sharma BC, Shah HA, Shiha G, Sollano J. Primary prophylaxis of gastroesophageal variceal bleeding: consensus recommendations of the Asian Pacific Association for the Study of the Liver. *Hepatol Int* 2008; **2**: 429-439
21 **Austin MJ**, Shawcross DL. Outcome of patients with cirrhosis admitted to intensive care. *Curr Opin Crit Care* 2008; **14**: 202-207
22 **Myers RP**, Papay KD, Shaheen AA, Kaplan GG. Relationship between hospital volume and outcomes of esophageal variceal bleeding in the United States. *Clin Gastroenterol Hepatol* 2008; **6**: 789-798
23 **Cárdenas A**, Ginès P, Uriz J, Bessa X, Salmerón JM, Mas A, Ortega R, Calahorra B, De Las Heras D, Bosch J, Arroyo V, Rodés J. Renal failure after upper gastrointestinal bleeding in cirrhosis: incidence, clinical course, predictive factors, and short-term prognosis. *Hepatology* 2001; **34**: 671-676
24 **Castañeda B**, Morales J, Lionetti R, Moitinho E, Andreu V, Pérez-Del-Pulgar S, Pizcueta P, Rodés J, Bosch J. Effects of blood volume restitution following a portal hypertensive-related bleeding in anesthetized cirrhotic rats. *Hepatology* 2001; **33**: 821-825
25 **Kravetz D**, Sikuler E, Groszmann RJ. Splanchnic and systemic hemodynamics in portal hypertensive rats during hemorrhage and blood volume restitution. *Gastroenterology* 1986; **90**: 1232-1240
26 **Colomo A**, Hernandez-Gea V, Muniz-Diaz E, Villanueva C. Transfusion strategy in patients with cirrhosis and acute gastrointestinal bleeding. *Hepatology* 2008; **48**: 413A
27 **Morales J**, Moitinho E, Abraldes JG, Fernández M, Bosch J. Effects of the V1a vasopressin agonist F-180 on portal hypertension-related bleeding in portal hypertensive rats. *Hepatology* 2003; **38**: 1378-1383
28 **Villanueva C**, Ortiz J, Miñana J, Soriano G, Sàbat M, Boadas J, Balanzó J. Somatostatin treatment and risk stratification by continuous portal pressure monitoring during acute variceal bleeding. *Gastroenterology* 2001; **121**: 110-117
29 **Tripodi A**, Mannucci PM. Abnormalities of hemostasis in chronic liver disease: reappraisal of their clinical significance and need for clinical and laboratory research. *J Hepatol* 2007; **46**: 727-733
30 **Tripodi A**, Primignani M, Chantarangkul V, Clerici M, Dell'Era A, Fabris F, Salerno F, Mannucci PM. Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology* 2006; **44**: 440-445
31 **Peck-Radosavljevic M**, Wichlas M, Zacherl J, Stiegler G, Stohlawetz P, Fuchsjäger M, Kreil A, Metz-Schimmerl S, Panzer S, Steininger R, Mühlbacher F, Ferenci P, Pidlich J, Gangl A. Thrombopoietin induces rapid resolution of thrombocytopenia after orthotopic liver transplantation through increased platelet production. *Blood* 2000; **95**: 795-801
32 **de Franchis R**, Arcidiacono PG, Carpinelli L, Andreoni B, Cestari L, Brunati S, Zambelli A, Battaglia G, Mannucci PM. Randomized controlled trial of desmopressin plus terlipressin vs. terlipressin alone for the treatment of acute variceal hemorrhage in cirrhotic patients: a multicenter, double-blind study. New Italian Endoscopic Club. *Hepatology* 1993; **18**: 1102-1107
33 **Molenaar IQ**, Warnaar N, Groen H, Tenvergert EM, Slooff MJ, Porte RJ. Efficacy and safety of antifibrinolytic drugs in liver transplantation: a systematic review and meta-analysis. *Am J Transplant* 2007; **7**: 185-194
34 **Ejlersen E**, Melsen T, Ingerslev J, Andreasen RB, Vilstrup H. Recombinant activated factor VII (rFVIIa) acutely normalizes prothrombin time in patients with cirrhosis during bleeding from oesophageal varices. *Scand J Gastroenterol* 2001; **36**: 1081-1085
35 **Bosch J**, Thabut D, Albillos A, Carbonell N, Spicak J, Massard J, D'Amico G, Lebrec D, de Franchis R, Fabricius S, Cai Y, Bendtsen F. Recombinant factor VIIa for variceal bleeding in patients with advanced cirrhosis: A randomized, controlled trial. *Hepatology* 2008; **47**: 1604-1614
36 **Bosch J**, Thabut D, Bendtsen F, D'Amico G, Albillos A, González Abraldes J, Fabricius S, Erhardtsen E, de Franchis R. Recombinant factor VIIa for upper gastrointestinal bleeding in patients with cirrhosis: a randomized, double-blind trial. *Gastroenterology* 2004; **127**: 1123-1130
37 **de Franchis R**, Pascal JP, Ancona E, Burroughs AK, Henderson M, Fleig W, Groszmann R, Bosch J, Sauerbruch T, Soederlund C. Definitions, methodology and therapeutic strategies in portal hypertension. A Consensus Development Workshop, Baveno, Lake Maggiore, Italy, April 5 and 6, 1990. *J Hepatol* 1992; **15**: 256-261
38 **Hsu YC**, Chung CS, Tseng CH, Lin TL, Liou JM, Wu MS, Hu FC, Wang HP. Delayed endoscopy as a risk factor for in-hospital mortality in cirrhotic patients with acute variceal hemorrhage. *J Gastroenterol Hepatol* 2009; **24**: 1294-1299
39 **Spiegel BM**, Vakil NB, Ofman JJ. Endoscopy for acute nonvariceal upper gastrointestinal tract hemorrhage: is sooner better? A systematic review. *Arch Intern Med* 2001; **161**: 1393-1404
40 **Yan BM**, Lee SS. Emergency management of bleeding esophageal varices: drugs, bands or sleep? *Can J Gastroenterol* 2006; **20**: 165-170
41 **D'Amico G**, Pietrosi G, Tarantino I, Pagliaro L. Emergency sclerotherapy versus vasoactive drugs for variceal bleeding in cirrhosis: a Cochrane meta-analysis. *Gastroenterology* 2003; **124**: 1277-1291
42 **Kim Y**, Kim SG, Kang HY, Kang HW, Kim JS, Jung HC, Song IS. [Effect of after-hours emergency endoscopy on the outcome of acute upper gastrointestinal bleeding]. *Korean J Gastroenterol* 2009; **53**: 228-234
43 **Sarin N**, Monga N, Adams PC. Time to endoscopy and outcomes in upper gastrointestinal bleeding. *Can J Gastroenterol* 2009; **23**: 489-493
44 **Soares-Weiser K**, Brezis M, Tur-Kaspa R, Leibovici L. Antibiotic prophylaxis for cirrhotic patients with gastrointestinal bleeding. *Cochrane Database Syst Rev* 2002; CD002907

45 **Blaise M**, Pateron D, Trinchet JC, Levacher S, Beaugrand M, Pourriat JL. Systemic antibiotic therapy prevents bacterial infection in cirrhotic patients with gastrointestinal hemorrhage. *Hepatology* 1994; **20**: 34-38
46 **Pauwels A**, Mostefa-Kara N, Debenes B, Degoutte E, Lévy VG. Systemic antibiotic prophylaxis after gastrointestinal hemorrhage in cirrhotic patients with a high risk of infection. *Hepatology* 1996; **24**: 802-806
47 **Abraldes JG**, Bosch J. The treatment of acute variceal bleeding. *J Clin Gastroenterol* 2007; **41** Suppl 3: S312-S317
48 **Goulis J**, Armonis A, Patch D, Sabin C, Greenslade L, Burroughs AK. Bacterial infection is independently associated with failure to control bleeding in cirrhotic patients with gastrointestinal hemorrhage. *Hepatology* 1998; **27**: 1207-1212
49 **Bernard B**, Cadranel JF, Valla D, Escolano S, Jarlier V, Opolon P. Prognostic significance of bacterial infection in bleeding cirrhotic patients: a prospective study. *Gastroenterology* 1995; **108**: 1828-1834
50 **Hou MC**, Lin HC, Liu TT, Kuo BI, Lee FY, Chang FY, Lee SD. Antibiotic prophylaxis after endoscopic therapy prevents rebleeding in acute variceal hemorrhage: a randomized trial. *Hepatology* 2004; **39**: 746-753
51 **Bernard B**, Grangé JD, Khac EN, Amiot X, Opolon P, Poynard T. Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology* 1999; **29**: 1655-1661
52 **Fernández J**, Ruiz del Arbol L, Gómez C, Durandez R, Serradilla R, Guarner C, Planas R, Arroyo V, Navasa M. Norfloxacin vs ceftriaxone in the prophylaxis of infections in patients with advanced cirrhosis and hemorrhage. *Gastroenterology* 2006; **131**: 1049-1056; quiz 1285
53 **Kravetz D**, Romero G, Argonz J, Guevara M, Suarez A, Abecasis R, Bildozola M, Valero J, Terg R. Total volume paracentesis decreases variceal pressure, size, and variceal wall tension in cirrhotic patients. *Hepatology* 1997; **25**: 59-62
54 **Loguercio C**, Sava E, Marmo R, del Vecchio Blanco C, Coltorti M. Malnutrition in cirrhotic patients: anthropometric measurements as a method of assessing nutritional status. *Br J Clin Pract* 1990; **44**: 98-101
55 **Córdoba J**, López-Hellín J, Planas M, Sabín P, Sanpedro F, Castro F, Esteban R, Guardia J. Normal protein diet for episodic hepatic encephalopathy: results of a randomized study. *J Hepatol* 2004; **41**: 38-43
56 **Garcia-Pagan JC**, Escorsell A, Moitinho E, Bosch J. Influence of pharmacological agents on portal hemodynamics: basis for its use in the treatment of portal hypertension. *Semin Liver Dis* 1999; **19**: 427-438
57 **Levacher S**, Letoumelin P, Pateron D, Blaise M, Lapandry C, Pourriat JL. Early administration of terlipressin plus glyceryl trinitrate to control active upper gastrointestinal bleeding in cirrhotic patients. *Lancet* 1995; **346**: 865-868
58 **Avgerinos A**, Nevens F, Raptis S, Fevery J. Early administration of somatostatin and efficacy of sclerotherapy in acute oesophageal variceal bleeds: the European Acute Bleeding Oesophageal Variceal Episodes (ABOVE) randomised trial. *Lancet* 1997; **350**: 1495-1499
59 **Calès P**, Masliah C, Bernard B, Garnier PP, Silvain C, Szostak-Talbodec N, Bronowicki JP, Ribard D, Botta-Fridlund D, Hillon P, Besseghir K, Lebrec D. Early administration of vapreotide for variceal bleeding in patients with cirrhosis. *N Engl J Med* 2001; **344**: 23-28
60 **Dell'Era A**, de Franchis R, Iannuzzi F. Acute variceal bleeding: pharmacological treatment and primary/secondary prophylaxis. *Best Pract Res Clin Gastroenterol* 2008; **22**: 279-294
61 **Nevens F**. Non-invasive variceal pressure measurements: validation and clinical implications. *Verh K Acad Geneeskd Belg* 1996; **58**: 413-437
62 **Moreau R**, Soubrane O, Hadengue A, Sogni P, Gaudin C, Kleber G, Lebrec D. [Hemodynamic effects of the administration of terlipressin alone or combined with nitroglycerin in patients with cirrhosis]. *Gastroenterol Clin Biol* 1992; **16**: 680-686
63 **Escorsell A**, Bandi JC, Moitinho E, Feu F, Garcia-Pagan JC, Bosch J. Time profile of the haemodynamic effects of terlipressin in portal hypertension. *J Hepatol* 1997; **26**: 621-627
64 **Ioannou GN**, Doust J, Rockey DC. Systematic review: terlipressin in acute oesophageal variceal haemorrhage. *Aliment Pharmacol Ther* 2003; **17**: 53-64
65 **Escorsell A**, Ruiz del Arbol L, Planas R, Albillos A, Bañares R, Calès P, Pateron D, Bernard B, Vinel JP, Bosch J. Multicenter randomized controlled trial of terlipressin versus sclerotherapy in the treatment of acute variceal bleeding: the TEST study. *Hepatology* 2000; **32**: 471-476
66 **Walker S**, Stiehl A, Raedsch R, Kommerell B. Terlipressin in bleeding esophageal varices: a placebo-controlled, double-blind study. *Hepatology* 1986; **6**: 112-115
67 **Freeman JG**, Cobden I, Record CO. Placebo-controlled trial of terlipressin (glypressin) in the management of acute variceal bleeding. *J Clin Gastroenterol* 1989; **11**: 58-60
68 **Soderlund C**, Magnusson I, Torngren S, Lundell L. Terlipressin (triglycyl-lysine vasopressin) controls acute bleeding oesophageal varices. A double-blind, randomized, placebo-controlled trial. *Scand J Gastroenterol* 1990; **25**: 622-630
69 **Morelli A**, Ertmer C, Lange M, Westphal M. Continuous terlipressin infusion in patients with septic shock: less may be best, and the earlier the better? *Intensive Care Med* 2007; **33**: 1669-1670
70 **Umgelter A**, Reindl W, Schmid RM, Huber W. Continuous terlipressin infusion in patients with persistent septic shock and cirrhosis of the liver. *Intensive Care Med* 2008; **34**: 390-391
71 **Cirera I**, Feu F, Luca A, García-Pagán JC, Fernández M, Escorsell A, Bosch J, Rodés J. Effects of bolus injections and continuous infusions of somatostatin and placebo in patients with cirrhosis: a double-blind hemodynamic investigation. *Hepatology* 1995; **22**: 106-111
72 **Nevens F**, Sprengers D, Fevery J. The effect of different doses of a bolus injection of somatostatin combined with a slow infusion on transmural oesophageal variceal pressure in patients with cirrhosis. *J Hepatol* 1994; **20**: 27-31
73 **Burroughs AK**, McCormick PA, Hughes MD, Sprengers D, D'Heygere F, McIntyre N. Randomized, double-blind, placebo-controlled trial of somatostatin for variceal bleeding. Emergency control and prevention of early variceal rebleeding. *Gastroenterology* 1990; **99**: 1388-1395
74 **Gøtzsche PC**, Gjørup I, Bonnén H, Brahe NE, Becker U, Burcharth F. Somatostatin v placebo in bleeding oesophageal varices: randomised trial and meta-analysis. *BMJ* 1995; **310**: 1495-1498
75 **D'Amico G**, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. *Semin Liver Dis* 1999; **19**: 475-505
76 **Escorsell A**, Bordas JM, Del Arbol LR, Jaramillo JL, Planas R, Banares R. Randomized controlled trial of sclerotherapy versus somatostatin infusion in the prevention of early rebleeding following acute variceal hemorrhage in patients with cirrhosis. Variceal Bleeding Study Group. *J Hepatol* 1998; **29**: 779-788
77 **Moitinho E**, Planas R, Bañares R, Albillos A, Ruiz-del-Arbol L, Gálvez C, Bosch J. Multicenter randomized controlled trial comparing different schedules of somatostatin in the treatment of acute variceal bleeding. *J Hepatol* 2001; **35**: 712-718
78 **Jenkins SA**, Nott DM, Baxter JN. Pharmacokinetics of octreotide in patients with cirrhosis and portal hypertension; relationship between the plasma levels of the analogue and the magnitude and duration of the reduction in corrected wedged hepatic venous pressure. *HPB Surg* 1998; **11**: 13-21
79 **Møller S**, Brinch K, Henriksen JH, Becker U. Effect of octreotide on systemic, central, and splanchnic haemodynamics in cirrhosis. *J Hepatol* 1997; **26**: 1026-1033
80 **Escorsell A**, Bandi JC, Andreu V, Moitinho E, García-Pa-

gán JC, Bosch J, Rodés J. Desensitization to the effects of intravenous octreotide in cirrhotic patients with portal hypertension. *Gastroenterology* 2001; **120**: 161-169
81 **Burroughs AK**. Double blind RCT of 5 day octreotide versus placebo, associated with sclerotherapy for trial/failures. *Hepatology* 1996; **24**: A352
82 **Gøtzsche PC**, Hróbjartsson A. Somatostatin analogues for acute bleeding oesophageal varices. *Cochrane Database Syst Rev* 2005; CD000193
83 **Corley DA**, Cello JP, Adkisson W, Ko WF, Kerlikowske K. Octreotide for acute esophageal variceal bleeding: a meta-analysis. *Gastroenterology* 2001; **120**: 946-954
84 **Abraldes JG**, Bosch J. Somatostatin and analogues in portal hypertension. *Hepatology* 2002; **35**: 1305-1312
85 **Bosch J**, Kravetz D, Mastai R, Navasa M, Silva G, Chesta J, Rodes J. Effects of somatostatin in patients with portal hypertension. *Horm Res* 1988; **29**: 99-102
86 **Calès P**, Masliah C, Bernard B, Garnier PP, Silvain C, Szostak-Talbodec N, Bronowicki JP, Ribard D, Botta-Fridlund D, Hillon P, Besseghir K, Lebrec D. Early administration of vapreotide for variceal bleeding in patients with cirrhosis. *N Engl J Med* 2001; **344**: 23-28
87 **Bosch J**, Garcia-Pagan JC. Complications of cirrhosis. I. Portal hypertension. *J Hepatol* 2000; **32**: 141-156
88 **de Franchis R**, Primignani M. Endoscopic treatments for portal hypertension. *Semin Liver Dis* 1999; **19**: 439-455
89 **Laine L**, Planas R, Nevens F, Bañares R, Patch D, Bosch J. Treatment of the acute bleeding episode. In: de Franchis R, editor. Portal Hipertension IV. Proceedings of the fourth Baveno International Consensus Workshop on methodology of diagnosis and treatment. Oxford: Blackwell Publishing, 2006: 217-242
90 **Bañares R**, Albillos A, Rincón D, Alonso S, González M, Ruiz-del-Arbol L, Salcedo M, Molinero LM. Endoscopic treatment versus endoscopic plus pharmacologic treatment for acute variceal bleeding: a meta-analysis. *Hepatology* 2002; **35**: 609-615
91 **Sung JJ**, Chung SC, Yung MY, Lai CW, Lau JY, Lee YT, Leung VK, Li MK, Li AK. Prospective randomised study of effect of octreotide on rebleeding from oesophageal varices after endoscopic ligation. *Lancet* 1995; **346**: 1666-1669
92 **Calès P**, Masliah C, Bernard B, Garnier PP, Silvain C, Szostak-Talbodec N, Bronowicki JP, Ribard D, Botta-Fridlund D, Hillon P, Besseghir K, Lebrec D. Early administration of vapreotide for variceal bleeding in patients with cirrhosis. *N Engl J Med* 2001; **344**: 23-28
93 **Villanueva C**, Ortiz J, Sàbat M, Gallego A, Torras X, Soriano G, Sáinz S, Boadas J, Cussó X, Guarner C, Balanzó J. Somatostatin alone or combined with emergency sclerotherapy in the treatment of acute esophageal variceal bleeding: a prospective randomized trial. *Hepatology* 1999; **30**: 384-389
94 **Novella MT**, Villanueva C, Ortiz J. Octreotide vs. sclerotherapy and octreotide for acute variceal bleeding. A pilot study. *Hepatology* 1996; **24**: A207
95 **Lo GH**, Chen WC, Wang HM, Lin CK, Chan HH, Tsai WL, Cheng LC, Yu HC, Tsay FW. Low-dose terlipressin plus banding ligation versus low-dose terlipressin alone in the prevention of very early rebleeding of oesophageal varices. *Gut* 2009; **58**: 1275-1280
96 **Jensen DM**, Kovacs TOG, Randall G. Emergency sclerotherapy vs rubber band ligation for actively bleeding esophageal varices in a randomized prospective study [abstract]. *Gastrointest Endosc* 1994; **40**: 241
97 **Lo GH**, Lai KH, Cheng JS, Lin CK, Huang JS, Hsu PI, Chiang HT. Emergency banding ligation versus sclerotherapy for the control of active bleeding from esophageal varices. *Hepatology* 1997; **25**: 1101-1104
98 **Villanueva C**, Piqueras M, Aracil C, Gómez C, López-Balaguer JM, Gonzalez B, Gallego A, Torras X, Soriano G, Sáinz S, Benito S, Balanzó J. A randomized controlled trial comparing ligation and sclerotherapy as emergency endoscopic treatment added to somatostatin in acute variceal bleeding. *J Hepatol* 2006; **45**: 560-567
99 **Avgerinos A**, Armonis A, Stefanidis G, Mathou N, Vlachogiannakos J, Kougioumtzian A, Triantos C, Papaxoinis C, Manolakopoulos S, Panani A, Raptis SA. Sustained rise of portal pressure after sclerotherapy, but not band ligation, in acute variceal bleeding in cirrhosis. *Hepatology* 2004; **39**: 1623-1630
100 **Burroughs AK**, Patch DW. Management of variceal haemorrhage in cirrhotic patients. *Gut* 2001; **48**: 738-740
101 **Laine L**. Ligation: endoscopic treatment of choice for patients with bleeding esophageal varices? *Hepatology* 1995; **22**: 663-665
102 **D'Amico M**, Berzigotti A, Garcia-Pagan JC. Risk stratification and treatment of acute variceal hemorrhage. In: Arroyo V, editor. Treatment of Liver Diseases. Barcelona: Ars Medica, 2009: 161-167
103 **Avgerinos A**, Armonis A. Balloon tamponade technique and efficacy in variceal haemorrhage. *Scand J Gastroenterol* 1994; **207**: 11-16
104 **Hubmann R**, Bodlaj G, Czompo M, Benkö L, Pichler P, Al-Kathib S, Kiblböck P, Shamyieh A, Biesenbach G. The use of self-expanding metal stents to treat acute esophageal variceal bleeding. *Endoscopy* 2006; **38**: 896-901
105 **Zehetner J**, Shamiyeh A, Wayand W, Hubmann R. Results of a new method to stop acute bleeding from esophageal varices: implantation of a self-expanding stent. *Surg Endosc* 2008; **22**: 2149-2152
106 **Wright G**, Lewis H, Hogan B, Burroughs A, Patch D, O'Beirne J. A self-expanding metal stent for complicated variceal hemorrhage: experience at a single center. *Gastrointest Endosc* 2010; **71**: 71-78
107 **Riggio O**, Angeloni S, Salvatori FM, De Santis A, Cerini F, Farcomeni A, Attili AF, Merli M. Incidence, natural history, and risk factors of hepatic encephalopathy after transjugular intrahepatic portosystemic shunt with polytetrafluoroethylene-covered stent grafts. *Am J Gastroenterol* 2008; **103**: 2738-2746
108 **D'Amico G**, Pagliaro L, Bosch J. The treatment of portal hypertension: a meta-analytic review. *Hepatology* 1995; **22**: 332-354
109 **Bosch J**. Salvage transjugular intrahepatic portosystemic shunt: is it really life-saving? *J Hepatol* 2001; **35**: 658-660
110 **Orloff MJ**, Orloff MS, Orloff SL, Rambotti M, Girard B. Three decades of experience with emergency portacaval shunt for acutely bleeding esophageal varices in 400 unselected patients with cirrhosis of the liver. *J Am Coll Surg* 1995; **180**: 257-272
111 **Orloff MJ**, Isenberg JI, Wheeler HO, Haynes KS, Jinich-Brook H, Rapier R, Vaida F, Hye RJ. Randomized trial of emergency endoscopic sclerotherapy versus emergency portacaval shunt for acutely bleeding esophageal varices in cirrhosis. *J Am Coll Surg* 2009; **209**: 25-40
112 **Escorsell A**, Bañares R, García-Pagán JC, Gilabert R, Moitinho E, Piqueras B, Bru C, Echenagusia A, Granados A, Bosch J. TIPS versus drug therapy in preventing variceal rebleeding in advanced cirrhosis: a randomized controlled trial. *Hepatology* 2002; **35**: 385-392
113 **Khan S**, Tudur Smith C, Williamson P, Sutton R. Portosystemic shunts versus endoscopic therapy for variceal rebleeding in patients with cirrhosis. *Cochrane Database Syst Rev* 2006; CD000553
114 **Bureau C**, Garcia-Pagan JC, Otal P, Pomier-Layrargues G, Chabbert V, Cortez C, Perreault P, Péron JM, Abraldes JG, Bouchard L, Bilbao JI, Bosch J, Rousseau H, Vinel JP. Improved clinical outcome using polytetrafluoroethylene-coated stents for TIPS: results of a randomized study. *Gastroenterology* 2004; **126**: 469-475
115 **Monescillo A**, Martínez-Lagares F, Ruiz-del-Arbol L, Sierra A, Guevara C, Jiménez E, Marrero JM, Buceta E, Sánchez J, Castellot A, Peñate M, Cruz A, Peña E. Influence of portal

hypertension and its early decompression by TIPS placement on the outcome of variceal bleeding. *Hepatology* 2004; **40**: 793-801

116 **Garcia-Pagan JC**, Caca K, Bureau C, Laleman W, Appenrodt B, Luca A . An early decision for PTFE-TIPS improves survival in high risk cirrhotic patients admitted with an acute variceal bleeding. A multicenter RCT. *Hepatology* 2008; **48**: A373

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.275

World J Hepatol 2010 July 27; 2(7): 275-288
ISSN 1948-5182 (online)

REVIEW

Pediatric nonalcoholic fatty liver disease: A clinical and laboratory challenge

Lucia Pacifico, Eleonora Poggiogalle, Vito Cantisani, Guendalina Menichini, Paolo Ricci, Flavia Ferraro, Claudio Chiesa

Lucia Pacifico, Eleonora Poggiogalle, Flavia Ferraro, Claudio Chiesa, Departments of 1 Pediatrics, Sapienza University of Rome, Rome 00161, Italy
Vito Cantisani, Guendalina Menichini, Paolo Ricci, Departments of Radiological Sciences, Sapienza University of Rome, Rome 00161, Italy
Lucia Pacifico, Claudio Chiesa, National Research Council, Institute of Neurobiology and Molecular Medicine, Rome 00133, Italy
Author contributions: Pacifico L, Poggiogalle E, and Chiesa C designed the study; Menichini G and Ferraro F were responsible for the review of the literature; Cantisani V and Ricci P analyzed the data and were responsible for the initial preparation of the paper;and Pacifico L and Chiesa C prepared the final version of the manuscript.
Correspondence to: Claudio Chiesa, MD, Institute of Neurobiology and Molecular Medicine, National Research Council, Via del Fosso del Cavaliere, Rome 100 00133, Italy. claudio.chiesa@artov.inmm.cnr.it
Telephone: +39-6-49979215 Fax: +39-6-49979216
Received: December 23, 2009 Revised: July 6, 2010
Accepted: July 13, 2010
Published online: July 27, 2010

Abstract

The true prevalence of pediatric nonalcoholic fatty liver disease (NAFLD) is unknown. Challenges in determining the population prevalence of NAFLD include the type of test (and the reference intervals used to define normal and abnormal), the type of population (general population, hospital series), the demographic characteristics of the population sampled, and the nature of the study design. The natural history of pediatric NAFLD remains uncertain. The issue of when to perform a liver biopsy in children with suspected NAFLD remains controversial. Children with NAFLD but normal alanine aminotransferase are rarely investigated. However, evidence of alterations in glucose metabolism parameters should prompt a better understanding of the natural history of pediatric NAFLD not only in terms of the progression of liver disease but also regarding its potential relationship with other health outcomes such as type 2 diabetes mellitus and cardiovascular disease. This evidence could make liver biopsy mandatory in the majority of cases at risk of progressive and severe hepatic and extrahepatic disease. This conclusion, however, raises the question of the feasibility of liver biopsy assessment in an extremely large at risk population, and of the cost/effectiveness of this policy. There is a considerable, continuous interest in reliable, noninvasive alternatives that will allow the prognosis of pediatric NAFLD to be followed in large community or population-based studies.

Key words: Nonalcoholic fatty liver disease; Children; Insulin resistance; Ultrasound; Magnetic resonance imaging

Peer reviewers: Papandreou Dimitrios, PhD, MD, RD, Assistant Professor of Nutrition, Department of Health Science, University of Nicosia, Cyprus; Head of Pediatric Obesity Unit, Aristotle University of Thessaloniki, School of Medicine, Ahepa General Hospital, P. Mela 22 GR 54622, Greece; Henning Gronbaek, MD, PhD, Department of Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, Aarhus C8000, Denmark

Pacifico L, Poggiogalle E, Cantisani V, Menichini G, Ricci P, Ferraro F, Chiesa C. Pediatric nonalcoholic fatty liver disease: A clinical and laboratory challenge. *World J Hepatol* 2010; 2(7): 275-288 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/275.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.275

INTRODUCTION

Over the last 2 decades, the rise in the prevalence rates

of overweight and obesity probably explains the emergence of nonalcoholic fatty liver disease (NAFLD) as the leading cause of liver disease in the pediatric population worldwide[1]. NAFLD is a clinicopathologic condition characterized by abnormal lipid deposition in hepatocytes (steatosis) in the absence of excess alcohol intake and represents a spectrum of liver disease ranging from bland (simple) steatosis to nonalcoholic steatohepatitis (NASH) that may lead to fibrosis and cirrhosis. It is a likely common cause of cryptogenic cirrhosis[2]. Once cirrhosis is present, hepatocellular carcinoma may also develop[3]. At present, the most commonly used noninvasive tests to detect NAFLD include measurement of serum aminotransferases and liver ultrasound. Although these tools are useful for the diagnosis of NAFLD, they lack the sensitivity and specificity to distinguish NASH from simple steatosis and determine the presence of fibrosis. Thus, currently, a liver biopsy remains the only reliable way to identify NASH[1]. Despite several advances, there are limited data on the epidemiology and natural history of the disease in children. This review will outline the current knowledge, recent advances, and challenges regarding the prevalence, pathogenesis, natural history, and diagnosis of pediatric NAFLD.

PREVALENCE OF PEDIATRIC NAFLD

The true prevalence of pediatric NAFLD is unknown (Table 1)[4-21]. There are inherent challenges in determining the population prevalence of NAFLD. The first challenge is the kind of test (and the cutoff-points used to define normal and abnormal) used in making diagnosis of NAFLD[21]. Diagnosis requires liver biopsy, which is not feasible in a population-based study. Therefore, most studies use serum aminotransferase elevations as a surrogate marker for fatty liver disease (in conjunction with negative markers for other types of liver disease)[22]. Especially germane to this issue is the common use of serum levels of aminotransferases during routine health care examinations to detect unsuspected liver disease[23]. However, the spectrum of fatty liver appears to encompass a wider range of subjects than identified by elevated serum liver chemistries. Franzese *et al*[11] demonstrated a lack of agreement between ultrasonography and serum aminotransferase levels in cases of fatty liver. Of the 53% of obese children with fatty liver identified by ultrasound, only 32% had abnormalities in serum aminotransferases. In subjects with more severe steatosis, a higher proportion of abnormalities in serum aminotransferases (56%) existed. These findings suggest that heavy infiltration is required for abnormalities in serum aminotransferases to occur. Previous studies have also demonstrated the insensitivity of serum alanine aminotransferase (ALT) in detecting hepatic fat accumulation in obese children as indicated by fast-gradient echo magnetic resonance imaging (fast-MRI) pulse sequences. Using fast-MRI Fishbein *et al*[24] first showed the insensitivity of serum aminotransferases to detect low levels (< 18%) of hepatic fat fraction (HFF) in obese children. Burger *et al*[25] then showed that only 48% of obese children with intrahepatic fat accumulation (as evidenced by HFF $\geqslant$ 5.5% using fast-MRI) had abnormal ALT levels. Finally, we recently showed that obese children with elevated ALT had a much higher mean level of MRI HFF than obese children with normal serum ALT[26].

On the other hand, the way in which clinical laboratories determine their own upper limits of normal values for aminotransferases is critically important in determining the absence as well as the presence of fatty liver disease[23]. Validated standards are not used to establish upper normal limits for ALT and aspartate aminotransferase (AST)[27]. Instead, laboratories use locally defined reference populations to establish their own reference intervals for these tests. Recently, Newschwander-Tetri and colleagues showed that the primary factor contributing to the widely divergent values of upper limits of normal ALT is related to the characteristics of the cohorts used by individual laboratories to establish their own reference intervals[23]. Among factors contributing to the variability in reference cohorts used to establish upper limits of normal values, obesity could be a major factor. As obesity increases in the general population, such reference populations could increasingly include individuals with unsuspected NAFLD, which would skew the upper reference limit to inappropriately high levels. Thus, laboratories should consider identifying healthy adults as well as children without risk factors for insulin resistance and fatty liver disease when establishing reference groups for testing serum ALT and AST levels[23]. Also, anthropomorphic, clinical, or demographic differences other than obesity could be responsible for the variation between laboratories. Care providers often use multiples of reported upper limits of normal values as criteria for further evaluation of the abnormality by imaging, and even liver biopsy. However, multiplying inaccurate upper limits of normal values only multiplies the errant value created by using the local reference population[23]. As expected, the sensitivity and specificity of aminotransferase measurements in the diagnosis of pediatric NAFLD is not established. Alternative approaches include imaging modalities such as liver ultrasound, although the diagnostic accuracy of this approach has its limitations (see section "Laboratory assessment of NAFLD").

A further challenge in assessing population prevalence is the selection of a representative population with minimal bias[22]. However, most prevalence studies have been conducted in cohorts of children selected for overweight or obesity, many of whom were referred for medical evaluation of obesity. The prevalence of elevated ALT in obese youth has been reported as 10%-14% in the United States adolescents[5,28], 24% in Hispanic youth[16], 25% in Italian youth[11], 24% in Japanese youth[29], and 48% in youth with type 2 diabetes[30]. There are also studies using ultrasound to assess the prevalence of suspected fatty liver in obese children. The prevalence of echogenic liver in obese youth has been reported as 77% in Chinese youth[13], 28% in German youth[18], 42%-53% in Italian

Table 1 Studies providing an estimate of nonalcoholic fatty liver disease in the pediatric population

Authors/year/country	Sample size (*n*)	Age range (yr)	Clinical characteristics	Criteria	Prevalence (%)
Tominaga *et al*/1995/Japan[4]	810	4-12	Population-based (Japan)	Ultrasound echogenicity	2.60
Strauss *et al*/2000/USA[5]	2450	12-18	Population-based (NHANES Ⅲ)	Elevated ALT (> 30 U/L)	3.00
Park *et al*/2005/Korea[6]	1594	10-19	Population-based (Korean NHANES)	Elevated ALT (> 40 U/L)	3.20
Schwimmer *et al*/2006/USA[7]	742	2-19	Population-based (Autopsy data, San Diego County)	Liver histology with ⩾ 5% hepatocytes containing fat	9.60
Tominaga *et al*/2009/Japan[8]	846	6-15	Population-based (Japan)	Ultrasound echogenicity	4.40
Alavian *et al*/2009/Iran[9]	966	7-18	Population-based (Iran)	Ultrasound echogenicity	7.10
Tazawa *et al*/1997/Japan[10]	310	6-11	Obese cohort (% IBW/height over 120)	Elevated ALT (> 30 U/L)	24.00
Franzese *et al*/1997/Italy[11]	72	4-15	Obese cohort (% IBW/height over 120)	Ultrasound echogenicty	53.00
Guzzaloni *et al*/2000/Italy[12]	375	8-16	Obese cohort (BMI > 2 SD for chronologic age)	Ultrasound echogenicity	42.00
Chan *et al*/2004/China[13]	84	7-18	Obese cohort (BMI > 95th percentile)	Ultrasound echogenicty	77.00
Flores-Calderon *et al*/2005/Mexico[14]	80	8-10	Overweight (> 85th) and obese (> 95th percentile) cohort	Elevated ALT (> 40 U/L)	42.00
Louthan *et al*/2005/USA[15]	181	4-17	Obese cohort (BMI > 95th percentile)	Elevated ALT (> 40 U/L)	8.00
Quiros-Tejeira *et al*/2007/USA[16]	517	4-19	Obese cohort (BMI > 95th percentile)	Elevated ALT (> 97.5th percentile for age-and sex-specific reference values)	24.00
Rocha *et al*/2009/Brazil[17]	175	12-15	Obese cohort (WC > 75th percentile)	Ultrasound echogenicity	1.70
Denzer *et al*/2009/Germany[18]	532	8-19	Obese cohort (BMI > 90th percentile)	Ultrasound echogenicity	28.00
Papandreou *et al*/2009/Greece[19]	43	9-13	Obese cohort	Ultrasound echogenicity	42.00

IBW: ideal body weight; BMI: body mass index; NHANES: national health and nutrition examination survey; ALT: alanine aminotransferase.

youth[11,12], 66% in Japanese youth[10], and 74% the United States adolescents[28].

Further challenges in assessing population prevalence are inherent variables based on the age, sex, race, and ethnicity of the population sampled[21]. Keeping this in mind, there are few population-based prevalence studies of pediatric NAFLD. The National Health and Nutrition Examination Survey (NHANES), cycle Ⅲ, examined a nationally representative sample of children and adults between 1988 and 1994[5]. The sample included 2450 adolescents, ages 12 through 18 yr. Abnormal serum ALT levels were found in 75 (3%) of adolescents[5]. Other factors associated with elevated ALT levels included increasing age. Data obtained from 1594 subjects aged 10-19 yr from the Korean National Health and Nutrition Examination Survey 1998, found that the prevalence of elevated ALT was 3.6% in boys and 2.8% in girls[6]. Population-based prevalence of pediatric NAFLD has also been estimated using imaging techniques. A study of 810 Japanese students (ages 4 to 12 yr) attending a public kindergarten and elementary school found a 2.6% prevalence of echogenic liver at ultrasound examination[4]. Fatty liver was found in children as young as 6 yr of age. Fatty liver prevalence was higher for boys than for girls.

Although liver biopsy cannot be used as a screening tool in (pediatric) population studies, autopsy series is another way of estimating prevalence based on the histological diagnosis within select populations[22]. Schwimmer *et al* conducted a retrospective analysis of 742 children between the ages of 2 and 19 yr who had an autopsy performed in San Diego County by a county medical examiner from 1993 to 2003. Fatty liver was defined as ⩾ 5% of hepatocytes containing macrovesicular fat[7]. After standardization for age, gender, race, and ethnicity, the estimated prevalence of fatty liver was 9.6%, which represented ~70 000 children ages 2 to 19 yr in the county of San Diego[7]. Fatty liver prevalence increased with age and differed significantly by race and ethnicity. The highest rate of fatty liver was seen in obese children. The results of this study confirm that fatty liver is the most common liver abnormality in children ages 2 to 19 yr.

PATHOLOGIC ASSESSMENT OF NAFLD

Liver biopsy remains an important tool in the diagnostic process in patients with NAFLD. The distinction between simple hepatic steatosis and potentially progressive NASH can only be made by liver biopsy, which can assess the presence and extent of necroinflammation and fibrosis. Nonetheless, even liver biopsy has important limitations that need to be considered. The basic assumption that the small fragment collected through percutaneous liver biopsy is representative of overall hepatic involvement has been seriously challenged. A needle biopsy sample usually represents around 1/50 000 of the total mass of the liver[31]. In addition, many studies have been published showing considerable sampling variability for

most histological features[32,33]. Ratziu *et al*[34] compared histological findings in 51 patients with NAFLD, each of whom had two samples collected through percutaneous liver biopsy. None of the features examined displayed high levels of agreement. Substantial agreement was only seen for steatosis grade; moderate agreement was seen for hepatocyte ballooning and perisinusoidal fibrosis; and lobular inflammation displayed only slight agreement. Six of 17 patients with bridging fibrosis (35%) in one sample had only mild or no fibrosis in the other and, therefore, could have been under-staged by a single biopsy. Ratziu *et al*[34] concluded that histological lesions of NASH were unevenly distributed throughout the liver parenchyma and that sampling error in liver biopsy can, therefore, result in substantial misdiagnosis and staging inaccuracies. Merriman *et al*, through a careful comparison of paired lobar biopsies in subjects at high risk of NAFLD, have also shown significant sampling variability in NAFLD. In their study, agreement for steatosis was excellent, moderate for fibrosis and only fair for most components of necroinflammation[35]. This variability can have an important impact on the diagnostic performance of liver biopsy specimens, as well as in the staging or grading of hepatic disease. Furthermore, liver biopsy is an invasive procedure, and not suitable for repeated evaluations.

It has been demonstrated that the higher the ALT levels, the higher the risk of NASH[36,37]. Not surprisingly, children are usually selected for liver biopsy to confirm NAFLD where it appears to be the only explanation for the either persistently or intermittently elevated serum aminotransferases, associated with diffusely hyperechogenic liver tissue at ultrasound examination[22,38-43]. At present, children with NAFLD but normal ALT are rarely investigated or indicated for liver biopsy[38], and the value of performing a biopsy in this situation is still debated[42,44-47]. All patients, including children, within the spectrum of NAFLD should be considered potentially affected, not only by hepatic but also by a multisystemic disease. This suspicion would be even stronger in the presence of elevated insulin resistance, which is a sensitive predictor of both progressive liver disease and severe extrahepatic disease. Diabetes and insulin resistance have been reported as the factors most closely associated with severe liver disease in adults as well as in children with biopsy-confirmed NAFLD but in the absence of ALT abnormalities[45,46]. Hypertension, one of the main features of the metabolic syndrome, has been reported to be more prevalent in adult patients with NAFLD and normal ALT than in those with increased liver enzymes[45]. The clinician should, therefore, be aware that the metabolic alterations related to steatosis and to adipose tissue-related endocrine dysfunction occur independently of overt liver damage and that even fatty liver *per se* is frequently associated with extrahepatic manifestations of insulin resistance syndrome[45,48]. Furthermore, studies using *a priori* selection based on the exclusion of children with normal ALT levels from liver biopsy will not reflect the true extent of NASH-related liver damage in the general pediatric population. Indeed, it is known that liver enzymes may be within the reference intervals in up to 70% of patients with diagnosed NAFLD and that normal liver enzymes cannot be reliably used as a criterion to argue against the usefulness of performing liver biopsy in at risk patients[49,50]. This evidence could make liver biopsy mandatory in the majority of cases at risk of progressive and severe hepatic disease, as well as of extrahepatic manifestations, unless accurate, noninvasive tests, which are currently unavailable, prove their efficacy. This conclusion, however, raises the question of the feasibility of liver biopsy assessment in an extremely large at risk population, and of the cost/effectiveness of this policy[45].

The histological features of NAFLD in children include a wide spectrum of alterations including simple steatosis (macrovesicular steatosis, characterized by a single or a few large droplets of fat and displacement of the nucleus, in hepatocytes without inflammation), NASH (macrovesicular steatosis in hepatocytes associated with inflammation and fibrosis), and cirrhosis. The distinction between simple hepatic steatosis and potentially progressive NASH can only be made by liver biopsy, which can assess the presence and extent of necroinflammation and fibrosis. The minimum criteria for NASH are: steatosis, with macrovesicular fat greater than microvescicular fat; mixed, mild lobular inflammation with scattered polymorphonuclear leukocytes and mononuclear cells; and ballooning degeneration of hepatocytes that are most apparent near steatotic liver cells[51]. A growing body of evidence suggests that children with NASH frequently show histopathological features that differ from those of adults[38,43]. A unique histological pattern, type 2 NASH, is reported in pediatrics[51]. In this pattern, inflammation and fibrosis are accentuated in the portal areas[38,43], in contrast to the perisinusoidal-pericellular injury typically observed in adults with NASH (type 1 NASH)[38]. The single-center, retrospective study by Schwimmer *et al*[38] performed on 100 pediatric patients with biopsy-proven NAFLD, identified type 1 NASH (characterized by steatosis, ballooning degeneration, and perisinusoidal fibrosis) in 17% of subjects, type 2 NASH (characterized by steatosis, portal inflammation, and portal fibrosis) in 51%, and an overlap in 16% of patients. Boys were significantly more likely to have type 2 NASH and less likely to have type 1 NASH than girls. The NASH type differed significantly by race and ethnicity. Type 1 NASH was more common in white children, whereas type 2 NASH was more common in children of Asian, Native American, and Hispanic ethnicity. In cases of advanced fibrosis, the pattern was that of type 2 NASH. The single-center, prospective study by Nobili *et al*[39] performed on 84 children (with unknown history of race and ethnicity) with biopsy-proven NAFLD, identified type 1 NASH in 2.4% of subjects, type 2 NASH in 28.6%, and an overlap in 52.4% of patients. Recently, in the multicenter, retrospective study by Carter-Kent *et al*[43] performed on 130 children with NAFLD (52% of patients, Caucasian; 1%, African American; 18%, Asian; and 30%, Hispanic),

overlapping features of both type 1 and type 2 NASH were found in 82% of patients. Thus, it is likely that a spectrum of disease patterns exists in pediatric NASH[51].

PATHOGENESIS OF NAFLD

A crucial question is that of the underlying difference between people who deposit fat in their liver and those who do not[52]. Similarly, it remains to be determined why some of those who deposit fat go on to develop NAFLD, cirrhosis, and liver failure[52]. A "two-hit" theory for the development of NASH has been proposed[53]. Hepatic steatosis resulting from obesity and hyperinsulinemia is then followed by a second hit of oxidative stress and lipid peroxidation. However, the linkage between inflammatory changes signified by elevation of ALT and further oxidation stress is still unknown[13].

A genetic predisposition is indisputably present in NAFLD, and one possibility is that genetics influence the observed heterogeneity in the development of these traits. Clinical case series have shown familial clustering of NAFLD[54,55]. Recent research on heritability of NAFLD has shown how family members of children with NAFLD should be considered at high risk for NAFLD even in the absence of obesity or increased serum aminotransferase levels[56]. Furthermore, there are racial and ethnic differences in the prevalence of NAFLD[7,57].

Genetic studies

A number of genes regulate a wide spectrum of mechanisms involved in NAFLD pathogenesis, including lipid accumulation into the liver, oxidative stress, inflammation, and fibrogenesis. Their expression relates not only to fat accumulation but also to the different mechanisms implicated in disease progression[58]. Several polymorphisms capable of increasing the severity of disease have been identified[59]. For example, a number of studies have analysed genes implicated in liver fat accumulation, adipokine/cytokine networks, oxidative stress, and fibrogenesis. The microsomal triglyceride transfer protein (MTP) is a key factor for the transfer of triglycerides to nascent apolipoprotein B, producing very low-density lipoprotein (VLDL) and removing lipid from the hepatocyte. The functional polymorphism 493 G/T in the MTP gene has been linked to the severity of liver disease in NAFLD: GG homozygosity, or carrying a lower MTP activity (which would lead to less triglyceride excretion as VLDL, and greater accumulation of lipid inside the hepatocytes) than the other genotypes, predicted more severe liver histology[60], independently of adipokine levels and insulin resistance[61]. Along the same lines, a recent study showed that adiponectin single-nucleotide polymorphisms 45GT and 276GT were more prevalent in NAFLD patients than in the general population, and independently predicted the severity of liver disease in NASH[62]. Similarly, a recent study in NASH patients and healthy volunteers evaluated the distribution of the 1183 T/C polymorphism in the mitochondrial targeting sequence of manganese superoxide dismutase (MnSOD), a potent scavenger localized to mitochondria with a key role in scavenging excessive oxidative stress to hepatocytes in NASH patients. This showed that the T/T genotype frequency was significantly higher in NASH patients in comparison with that in the controls. This results in a decrease of MnSOD capacity to detoxify superoxide anions produced in mitochondria, and, therefore, favours excessive oxidative damage inside hepatocytes and NASH progression[60]. Miele *et al* studied Kruppel-like factor 6, previously identified as a ubiquitous transcription factor and immediate early gene expressed in activated hepatic stellate cells after liver injury[63,64], and therefore possibly involved in the process of liver fibrogenesis. They found that the wild type gene was associated with the severity of fibrosis in NAFLD livers, independently of age, sex, body mass index (BMI), and blood glucose level[65]. They also showed preferential transmission of the wild type Kruppel-like factor 6 to children with fibrotic NAFLD[65]. Finally, in a population comprising Hispanic, African American, and European American individuals, Romeo *et al*[66] demonstrated that an amino acid sequence variant [rs 738409 (G), encoding 1148M] in patatin-like phospholipase A3 (PNPLA3), a protein of unknown function, was strongly associated with increased hepatic fat levels [evidenced by proton magnetic resonance spectroscopy (MRS)] and with hepatic inflammation (as shown by release of liver enzymes into the circulation). The allele was most common in Hispanics, the group most susceptible to NAFLD; and hepatic fat content was more than twofold higher in PNPLA3 homozygotes than in noncarriers. Resequencing revealed another allele of PNPLA3 [rs6006460 (T), encoding S4531] that was associated with reduced hepatic fat content in African Americans[66], the group at lowest risk of NAFLD. Thus, variation in PNPLA3 contributes to inter-individual differences in hepatic fat content and susceptibility to NAFLD.

NATURAL HISTORY OF NAFLD

Current studies suggest that the rate of progression of NAFLD relates to histological severity[67]. There is significant debate about the clinical significance and prognosis of simple or "bland"steatosis. This condition is thought to be readily reversible. Once significant fibrosis is present, however, it is unclear if this can be reversed[68]. Changes in fibrosis stage have been specifically evaluated in independent series[69-72]. Overall, fibrosis progresses over time, but it may remain stable for some years and may improve spontaneously in some cases[69-73]. Increased risk of fibrosis appears to be associated with central obesity, insulin resistance states including diabetes as well as features of the metabolic syndrome, in particular high triglyceride and low HDL levels[67]. More advanced stages of NAFLD appear to be associated with older age, higher BMI, diabetes, hypertension, high triglycerides, and/or insulin resistance[67]. An AST/ALT ratio > 1 may also indicate more severe disease[68,74]. The findings

from different studies are not completely consistent as to which factors are independently associated, and this may depend on the population studied (patients with elevated liver enzymes vs morbidly obese patients vs subjects in the general population)[68]. As fibrosis progresses over time, other features of NAFLD, including steatosis, inflammation and ballooning of hepatocytes, significantly improve or disappear[71]. In addition, aminotransferases, when elevated, improve or normalize spontaneously over time despite fibrosis progression[71]. Thus, fibrosis severity may be the only biopsy feature useful to predict the long-term prognosis in patients with NAFLD. Furthermore, it is possible that the long-term complications of NAFLD might have been underrecognized and underreported, as the characteristic features of macrovesicular steatosis may disappear in the late stages of the disease, leading to a picture of "bland"cirrhosis, which is frequently described as "cryptogenic", rather than NAFLD-related cirrhosis[67].

Most studies evaluating the long-term prognosis of patients with NAFLD originate from specialized care centers at which adult patients had been selected to undergo liver biopsy[75-79]. The retrospective analysis by Matteoni *et al*[75] comparing clinical characteristics and outcomes of 98 adult patients with different types of NAFLD over an average (SD) follow-up of 8.3 (5.4) years, demonstrated that the outcome of cirrhosis and liver-related death was not uniform across the spectrum of NAFLD. Poor outcomes were more common in types 3 and 4 NAFLD (currently designed as NASH)[75]. This study suggested that histological findings may have prognostic value in patients with NAFLD. Other hospital-based studies of the histological subgroup of NAFLD patients with NASH have documented progression to cirrhosis and hepatocellular carcinoma, but have been limited by the small numbers of adult patients and/or average follow-up of less than 5 years[76-78]. More recently, the study by Rafiq *et al*, with a median follow-up period of 18.5 years, has shown that liver-related mortality of the NASH cohort [mean (SD) age, 68.9 (10.8) yr] increased to 17.5% in comparison with only 2.7% in the non-NASH NAFLD cohort [mean (SD) age, 71.7 (11.3) yr][80]. These findings confirm that with longer follow-up periods, more NASH patients die as a result of liver-related disease. It also confirms that most patients with non-NASH (simple steatosis or steatosis with nonspecific inflammation) are not subject to liver-related death. This relatively nonprogressive course of non-NASH NAFLD has been reported by others[79-81].

Accurate data are also needed on the extent to which NAFLD causes morbidity and mortality in the general population. There are few population-based studies to determine the long-term prognosis of NAFLD. Using the resources of the Rochester Epidemiology, Project, Adams *et al*[82] conducted a population-based cohort study to examine the natural history of patients [mean (SD) age, 49 (15) yr] diagnosed with NAFLD on the basis of imaging studies or liver biopsy. Mean (SD) follow-up was 7.6 (4.0) years culminating in 3192 persons/years follow-up. Liver-related death was the third most common cause of death in those with NAFLD while it was the thirteenth most common cause of death in the general population. Utilizing data from the NHANES III and the NHANES III Linked Mortality File, Ong *et al*[83] conducted a study to determine the overall and liver-related mortality of NAFLD in the general population including 12822 persons. Liver disease was the third cause of death among persons with NAFLD after cardiovascular disease and malignancy[83]. This risk was independent of obesity or the presence of diabetes mellitus. On the other hand, liver disease was only the eleventh most common cause of death in persons without liver disease[83].

Although NAFLD is very common in the pediatric population, data on the prognosis of NAFLD in children remain scant. Given the large number of children affected, it is imperative that we establish a better understanding of the natural history of pediatric fatty liver in terms of the progression of liver disease[7]. Although some series have reported documented cases of cirrhotic stage disease in children[7,84] or cases of children with NAFLD who developed cirrhosis in young adulthood[85,86], cirrhosis is not considered to be a common component of pediatric NASH. Some Authors have speculated that the delay in presentation of cirrhosis until adulthood may be due to the short duration of the process or to introduction of a cofactor after childhood[87]. Notably, cirrhosis is reported, though rarely, in patients with NAFLD associated with pituitary dysfunction[88-90]. This liver disease, which is more likely to present with or include hepatopulmonary syndrome[88,91], will add to the morbidity and mortality of this patient population. Despite careful monitoring and treatment of endocrine abnormalities, recurrence of NASH after liver transplantation has been documented in two children who had a history of hypothalamic/pituitary dysfunction[88,89] and developed decompensated liver disease from NAFLD[91].

In contrast, hepatic fibrosis is frequently observed in pediatric NASH. Kinugasa *et al*[84] found, by routine laboratory examination, elevated levels of serum transaminases in 36 (12%) of the 299 obese children studied. Liver biopsies carried out in 11 of the 36 children showed fibrous changes in five patients. One patient, a 15-year-old girl with a long history of obesity, had cirrhosis along with maturity-onset diabetes mellitus and hyperlipidemia. Baldridge *et al*[87] reported on a series of 14 obese children with idiopathic hepatic steatosis, identified by retrospective review of all liver biopsies performed in a tertiary-care pediatric hospital. Thirteen of the 14 children had portal fibrosis, and many also had central sclerosis, central portal bridging, and portal-portal bridging. Rashid and Roberts reported on a series of 24 children who were predominantly obese and who underwent percutaneous liver biopsy; all showed large-droplet steatosis, and many had fibrosis (17% or 71%) of varying severity[92]. Fibrosis was moderately severe in seven patients. One additional patient had cirrhosis at diagnosis. In a retrospective study, defining the liver biopsy findings in 100 predominantly

obese children with clinical features consistent with NAFLD, Schwimmer *et al*[38] found that simple steatosis was present in 16% of subjects, and advanced fibrosis in 8%. In the study by Nobili *et al*[39], involving 84 obese/overweight children with elevated aminotransferases and diagnosis of NAFLD confirmed via liver biopsy, increased fibrosis was noted in 49 (58%) patients but was mostly of mild (stage 1) severity, with only 4 (4.7%) patients showing septal fibrosis (stage 3). None of the patients showed cirrhosis-stage disease on liver biopsy.

Feldstein *et al*[91] recently reported the first longitudinal study describing the long-term survival of children with NAFLD who underwent a follow-up for up to 20 years. That study demonstrated that NAFLD in children is a disease of progressive potential. Some children presented with cirrhosis, others progressed to advanced fibrosis or cirrhosis during follow-up, and some developed end-stage liver disease with the consequent need for liver transplantation. Feldstein *et al*[91] also showed that NAFLD in children is associated with significantly shorter long-term survival as compared to the expected survival in the general population of the same age and sex. Children with NAFLD had a 13.8-fold higher risk of dying or requiring liver transplantation than the general population of the same age and sex[91].

On the basis of the above information, an important goal must be to identify those children with advanced fibrosis, as well as the ones most likely to progress to end-stage liver disease. It is also imperative that noninvasive means be developed to identify children at greatest risk for progressive disease[40]. Age, sex, race, ethnicity, and severity of obesity have been reported to be associated with the steatohepatitis pattern types[38]. Older age has been found to be independently associated with increased liver fibrosis in some series[39]. However, in other series children with advanced stages of fibrosis tended to be younger than those with lesser degrees of fibrosis[40], suggesting that yet unidentified susceptibility genes predispose to the more aggressive course in these children[40]. Most published reports of pediatric NAFLD have reported males to be affected more commonly[4,12,41,87,92-94]. Sex-related differences have been also shown in an animal model of NASH, with male sex associated with more severe and diffuse injury[95]. In the study by Schwimmer *et al*[38], who tested potential associations of distinct patterns of NASH in children, girls with type 2 NASH were more likely to be prepubertal and, therefore, have a hormone profile more similar to young boys with type 2 NASH, whereas girls with type 1 NASH were more likely to be postmenarcheal and thus have higher estrogen levels. Thus, sex hormones are attractive candidates for mediators of the development of and/or protection from NASH. Beside changes in sex steroid hormones, another potential mediator of disease expression and progression is pubertal development[40]. During puberty, plasma insulin levels increase, and insulin sensitivity decreases along with multiple other physical and hormonal changes[96]. This decrease in sensitivity occurs early in puberty, between Tanner stages Ⅰ and Ⅱ, with a nadir at Tanner stage Ⅲ and recovery by stage Ⅴ[96,97]. A longitudinal study examining 60 children at Tanner stage Ⅰ (ages 9.2 ± 1.4 yr) as well as after 2.0 ± 0.6 yr, showed that, at follow-up, 29 children remained at Tanner stage Ⅰ while 31 had progressed to Tanner stage Ⅲ or Ⅳ[98]. In children remaining at Tanner stage Ⅰ, there was a slight increase in insulin sensitivity with no significant change in acute insulin response or fasting glucose and insulin. Pubertal transition from Tanner stage Ⅰ to Tanner stage Ⅲ was associated with a 32% reduction in insulin sensitivity, and increase in fasting glucose, insulin, and acute insulin response[98]. These changes were similar across sex, ethnicity, and obesity. Thus, sex steroid hormones and insulin resistance associated with pubertal development may account for the significance of developmental stage in the onset of fatty liver. In that context, the recent study of Patton *et al*[40] may be of significant interest. These authors found that lower Tanner stage was predictive of higher fibrosis scores, suggesting that hormonal changes associated with pubertal development may influence disease severity[40]. However, as pointed out by Patton *et al*[40], whether children with borderline zone 1 pattern may evolve into definite NASH and/or children with definite NASH regress to borderline zone 3 or simple steatosis upon further developmental maturation is unknown and will require longitudinal data to determine.

In some recent series, the presence and severity of fibrosis was associated with a higher BMI[39,41,42]. However, data obtained prospectively from children enrolled in the NASH Clinical Research Network reported no association of BMI with fibrosis severity[40], although the percentage of body fat was lower among subjects without fibrosis[40]. It may be that body fat distribution (or adiposity with a central distribution[99]) is a more important determinant of fibrosis than BMI. Body fat distribution affects insulin sensitivity and varies by race and ethnicity[41]. Ellis *et al*[100] showed that, after adjustment for body size, Hispanic children have significantly higher body fat and percentage fat than white or black children. In a study evaluating clinical correlation of histopathology in pediatric NAFLD, Hispanic ethnicity was predictive of fibrosis severity when comparing those with mild and moderate degrees of fibrosis[40]. In another study aiming to define key differences between the NASH subtypes, the majority of biopsies from children of the white race had type 1 NASH, while type 2 NASH was the major form seen in children of Asian and Native American race and Hispanic ethnicity[38]. In the same report, biopsies from children of the black race mostly showed simple steatosis[38].

Previous studies in adults with NAFLD have shown that components of metabolic syndrome may contribute to severe liver steatosis, NASH activity, fibrosis or isolated portal fibrosis[42]. It is, therefore, also important that we establish a better understanding of the natural history of pediatric NAFLD in terms of its potential relationship with other health outcomes including type 2 diabetes mellitus and cardiovascular disease[7]. Studies in children have demonstrated the relationship between

fasting hyperinsulinemia and dyslipidemia[101-103], hypertension[101,104-106],and impaired glucose tolerance[107]. Schwimmer *et al*[41] first extended these data to include NAFLD in children as being related to fasting hyperinsulinemia and insulin resistance. They showed that, even after subjects with diabetes were excluded, almost all children with biopsy-proven NAFLD had insulin resistance. Portal inflammation was predicted by the combination of ALT and fasting insulin, while portal fibrosis was indicated by the combination of right upper quadrant pain and homeostasis model assessment of insulin resis tance[41]. In another series of pediatric NAFLD, higher insulin levels also were predictive of moderate versus mild fibrosis[40]. Recently, Manco *et al*[42] showed that fasting insulin secretion trended to be increased in children with fibrosis compared to those without fibrosis. More recently, Patton *et al*[108] showed that severity of insulin resistance was the component most consistently associated with histological features of NAFLD, showing significant associations with severity of steatosis, fibrosis, hepatoce llular ballooning, and NAFLD pattern. Although in clinical practice insulin resistance is unlikely to be of use in distinguishing fibrosis stage, the above findings support insulin resistance as key variable in disease progression[109-111]. Evidence of alterations in glucose metabolism parameters should prompt a careful follow-up of pediatric patients to prevent major complications[44].

Higher levels of serum AST have been found asso ciated with fibrosis in some series of pediatric NAFLD[40,41,43]. However, neither AST levels alone nor the combination of routinely available laboratory data have shown sufficient specificity or sensitivity to predict the presence of severe fibrosis in children with NAFLD[43]. Recent data from adult as well as pediatric patients underscore that NAFLD has to be considered a potentially progressive disease even in the presence of normal ALT levels[42,45,46]. In that vein, it is remarkable that in one series of pediatric NAFLD 23% of patients had normal values of ALT at the time of biopsy even though fibrosis was observed in 60% of them[42]. Not surprisingly, the issue of when to perform a liver biopsy in children with suspected NAFLD remains controversial[38,42,47], and there is no clear standard[47]. Unfortunately, none of the clinical and laboratory predictors of histology appear sufficiently powerful to replace liver biopsy as an accurate noninvasive means of identifying the progression of disease[40]. Nonetheless, there is a considerable, continuous interest in reliable, noninvasive alternatives that will allow the prognosis of pediatric NAFLD to be followed in large community or population-based studies. Recently, transient elastography (TE), using the Fibroscan apparatus, has received increasing attention as a noninvasive means to measure liver stiffness and thus progression in chronic liver disease patients[112]. Accordingly, the study by Nobili *et al*[113] indicated that TE is an accurate and reproducible methodology to identify, in children and adolescents affected by NASH, those without any degree of fibrosis, or with advanced fibrosis. However, in that study an overlap was observed among patients with lower degr ees of liver fibrosis (stages 0 and 1 and stages 1 and 2). Further limitations of that study are related to the acquisition of a highly selected cohort typical of a specialized tertiary care referral center[113]. Thus the conclusions of the study cannot be applied to pediatric populations seen in primary care settings. Nonetheless, the recent results reported in a cohort of pediatric NAFLD indicate that measurement of specific circulating markers of fibrinogenesis or fibrosis through the enhanced liver fibrosis (ELF) test appears to be a promising alternative for discriminating between different stages of fibrosis[114]. Again, further characterization of this test's performance in larger and less selected cohorts of patients is needed before proposing the use of the ELF panel in clinical practice. Studies in this area are likely to continue.

CLINICAL PRESENTATION OF NAFLD

Most children with NAFLD are asymptomatic[47,51], and elevated levels of aminotransferases are frequently found incidentally or after screening for obesity-related comorbidites[51]. Children may also complain of vague right upper quadrant or epigastric pain, fatigue or malaise.

The typical child with NAFLD is an 11-13 year-old, usually male, usually overweight or obese[47]. Some children with NAFLD are tall with large bones and proportionally heavy body weight[92], consistent with being overnourished. A thorough history often reveals comorbid conditions related to metabolic syndrome, including hypertension, type 2 diabetes mellitus, dysplipidemia, obstructive sleep apnea, and polycystic ovarian syndrome[51]. In 36%-49% of children with NAFLD acanthosis nigricans, a brown to black pigmentation of skin folds and axillae, has been found[41,92]. Acanthosis nigricans may be subtle and can be missed without careful examination[92]. Although acanthosis nigricans may occur in simple childhood obesity, it has been shown to be a cutaneous marker of hyperinsuli nemia[115,116]. Keratinocytes have receptors for insulin, and insulin-like growth factors. In hyperinsulinemia, circulating insulin, because of its structural similarity to insulin-like growth factors, binds to these receptors and stimulates cell division, leading to acanthosis[92].

More than 90% of children with NAFLD are obese, with central adiposity[41]. On abdominal examination hepatomegaly, with or without splenomegaly, is evident in 33%-51% of patients[41,92]. Central adiposity can make organomegaly difficult to identify.

DIAGNOSIS OF NAFLD

Laboratory assessment

The NASH Clinical Research Network recently failed to identify routine laboratory tests with an adequate discriminating power to replace liver biopsy in evaluating NAFLD pattern and fibrosis severity in children and adolescents[40]. Serum aminotransferases are usually slightly to moderately elevated (less than 1.5 times the upper limit of normal) in NAFLD, but may be higher[114]. The AST: ALT ratio is usually less than one, but this

ratio increases as fibrosis advances[73]. However, aminotransferase levels may remain normal, even with biopsy-proven NASH[117]. In one study evaluating clinical correlation of histopathology in pediatric NAFLD[40], Patton *et al* showed that AST was superior to ALT in distinguishing NAFLD patterns and that the addition of ALT to AST did not improve performance. Although these results did not support the use of AST in place of liver biopsy, the strong association found in that study between AST and meaningful histological features in pediatric NAFLD supports current recommendations to use serum aminotransferase levels in screening overweight children[118]. Total and direct bilirubin are typically normal. One laboratory value that may be useful is gamma glutamyl transferase (GGT). In one series of pediatric NAFLD[91], GGT was elevated in 88% of patients. However, most of them (83.3%) presented with at least one feature of the metabolic syndrome whereas overt metabolic syndrome (i.e. > 3 features) was present in 28.8% children. Recently, elevated serum levels of GGT have been associated with several cardiovascular disease risk factors[119-120]. GGT may also act as a marker of the metabolic syndrome[121]. It mediates the uptake of glutathione, an important component of intracellular antioxidant defenses. Although the relationship between the metabolic syndrome and NAFLD in children has not been characterized, GGT may be used as a clinical marker in pediatric NAFLD because its expression is enhanced by oxidative stress. and it may be released by conditions inducing cellular stress and insulin resistance[91], which are key components in the development of NAFLD[122]. Prothrombin time and serum albumin levels are normal, until the development of cirrhosis and liver failure. Thirty per cent of adults with NAFLD have high serum ferritin and 6%-14% have elevated transferrin saturation[109]. These iron indices, however, are not routinely measured in youth, as hemochromatosis is rare in children[109]. Decreased serum adiponectin predicts severity of liver disease in NAFLD, even in the absence of diabetes and obesity, although it remains a research tool and not a diagnostic criterion[47]. Nonspecific autoantibodies, usually against smooth muscle determinants, may be detected in relatively low titers in up to 3% of adults with NAFLD. However, the prevalence in children is unknown[123].

In diagnosis of NAFLD, a through evaluation and systematic exclusion of other causes of liver disease is necessary, including Wilson's disease, viral hepatitis, autoimmune hepatitis, alpha-1-antitrypsin deficiency, fatty acid oxidation defects, lipodystrophy, and total parenteral nutrition. Furthermore,drug-induced liver injury (i.e. valproate, methotrexate, tetracycline, amiodarone, prednisone, and synthetic estrogens) should be considered and excluded. Alcohol use, especially in adolescents, must also be excluded.

Radiological assessment

Noninvasive imaging techniques, including ultrasound, computed tomography (CT), MRI, and MRS may detect fatty infiltration of the liver but, unlike liver biopsy, they are limited in their ability to detect coexisting inflammation and fibrosis[124]. It has been suggested that unenhanced CT might be useful in the noninvasive quantification of the degree of hepatic steatosis in experimental and *in vivo* human studies[125,126]. However, a recent study by Pak *et al*[127] concluded that diagnostic performance of unenhanced CT for quantitative assessment of macrovesicular steatosis is not clinically acceptable. Unenhanced CT does not provide high performance in qualitative diagnosis of macrovesicular steatosis of less than 30%[127]. In addition, CT scanning has the drawback of exposing subjects to ionizing radiation. These two factors limit its potential use in pediatric longitudinal studies[124]. The diagnosis of fatty liver on contrast-enhanced helical CT may also be accurate but is protocol-specific[128].

Ultrasound

Hepatic ultrasound is a relatively inexpensive, noninvasive technique, which is easy to perform and is, therefore, widely used in clinical practice to detect fatty infiltration of the liver. However, sonography is not typically quantitative and a hepatocyte fat content of ⩾ 15% to 30% is required to detect ultrasonographic changes[129]. In adults, ultrasound sensitivity has been shown to range from 60% to 94% and specificity from 84% to 95%, respectively[130-133]. In the presence of hepatic fat content of 10% to 19%, ultrasound has a sensitivity of 55%, which rises to 80% in the presence of > 30% fatty infiltration[134]. Furthermore, ultrasound may be technically challenging to perform in patients with significant central obesity. In the presence of morbid obesity[135], the sensitivity and specificity of ultrasound fall to 49% and 75%, respectively, possibly due to technical problems in performing ultrasound in such patients. Furthermore, ultrasound is operator-dependent[124,136], and the sonographic evaluation of the liver is based mainly on the subjective visual assessment of hepatic echogenicity and posterior attenuation of the ultrasound beam, with consequent substantial observer variability. In the report by Strauss *et al*[136], the mean interobserver and intraobserver agreement rates for the presence of fatty liver were 72% and 76%, respectively. In the same report by Strauss *et al*[136], on severity of fatty liver, the initial reading for pairs of observers had 47%-59% interobserver agreement, while the interobserver agreement for the second reading was 59%-64%. The mean agreement rates for pairs of observers were 53% and 62% on the first and second readings. Intraobserver agreement for severity of fatty liver ranged from 55% to 68%. Grading of hepatic fat content using ultrasound has been reported, but it is somewhat subjective and only broad categories of involvement have been reported[130,133,137,138].

Magnetic resonance imaging

MRI, though more costly, is more sensitive than ultrasound in detecting fat and allows for more definitive

hepatic fat quantification when performed using the modified Dixon technique[24,138-140]. Like ultrasound, hepatic MRI involving fast gradient echo does not require conscious sedation in (compliant) children. In fact, the sequence of scan parameters allows simultaneous acquisition of both in-phase and out-of-phase images during the multibreath-hold interval required to cover the entire liver. However, MRI is more appealing than ultrasound to detect minor changes in hepatic fat content associated with steatogenic disorders[138]. While hepatic fibrosis can limit the ability of ultrasound to grade hepatic steatosis, hepatic MRI, based upon chemical shift imaging, is not influenced by the presence of fibrosis in the accurate quantification of the hepatic fat content[138]. In a previous study evaluating hepatic steatosis severity in a series of obese children through both MRI and ultrasound, we found evidence of limitations in ultrasound with regard to grading of steatosis, in comparison with quantitative assessment of HFF by MRI[26]. Ultrasound scores varied across MRI hepatic fat contents. In children in whom ultrasound revealed moderate to severe steatosis, MRI delineated a wide range of hepatic fat content within both categories of ultrasound steatosis severity[26]. This suggests that the utility of ultrasound would appear to be limited by its incapacity to identify fat regression or progression in subjects with NAFLD[138]. The progression of NAFLD in children can be prevented by early weight reduction, which can lessen the degree of fatty infiltration and elicit reversion of the biochemical abnormalities. Thus, a child with NAFLD undergoing a reduction of MRI HFF from 40% to 20% through successful intervention would be unlikely to have a corresponding alteration in ultrasound appearance[141]. However, in our series of children with mild steatosis severity as determined by ultrasound, the pattern of a slight increase in liver echogenicity conflicted with the finding of normal, minimal levels of MRI HFF[26]. Previous investigations have found the slight alterations or accumulation of hepatic fat content as indicated by ultrasound to be equivocal[12]. In contrast, HFF, which is derived from the signal differences between fat and water, gives unequivocal data for the entire spectrum of fatty liver and unlike sonography, is not subject to interpretation or interobserver variation[24]. The clinical efficacy of this technique has been previously demonstrated. Burgert *et al* showed that obese children with a high HFF were significantly more insulin resistant, compared with those with a low HFF, and had higher triglycerides and lower adiponectin, even after adjustment for BMI-z scores, race/ethnicity, gender, and age[25]. Furthermore, obese children with a high HFF had a significantly greater prevalence of the metabolic syndrome, after controlling for the above confounders[25]. We also previously showed that the increasing severity of MRI fat accumulation were strongly related to fasting hyperinsulinemia and insulin resistance after correction for confounding variables such as SD score-BMI, sex, age and pubertal status[26].

MRS is currently considered being the most accurate for determination of HFF (as a measure of liver triglyceride concentration[21]), especially in patients with less than 10% of fat in the liver[22]. However, MRS demonstrates some limitations in that it is time-consuming, restricted in spatial coverage, and requires off-scan analysis by an expert[21]. Because of these limitations, MRS is not appropriate for widespread use. Furthermore, the main limitation of MRS is that it provides information from small regions of interest in the liver. By contrast, MRI (using the modified Dixon technique) allows evaluation of the presence of fat in the entire liver. To date, however, none of the above imaging modalities allow differentiation of benign steatosis from NASH or have the ability to grade the severity of inflammation[51].

CONCLUSION

Over the last decade, pediatric NAFLD has become the most common form of liver disease in the preadolescent and adolescent age groups. Liver biopsy remains the gold standard and is currently the only way to diagnose NASH. However, the issue of when to perform a liver biopsy in children with suspected NAFLD remains controversial, and there is no clear standard. Thus, it is imperative that reliable, noninvasive means be developed to identify children at greatest risk for progressive disease. Large population-based epidemiologic studies in children are needed to understand the true impact of pediatric NASH on long-term morbidity and mortality.

REFERENCES

1 **Dunn W**, Schwimmer JB. The obesity epidemic and nonalcoholic fatty liver disease in children. *Curr Gastroenterol Rep* 2008; **10**: 67-72

2 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669

3 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140

4 **Tominaga K**, Kurata JH, Chen YK, Fujimoto E, Miyagawa S, Abe I, Kusano Y. Prevalence of fatty liver in Japanese children and relationship to obesity. An epidemiological ultrasonographic survey. *Dig Dis Sci* 1995; **40**: 2002-2009

5 **Strauss RS**, Barlow SE, Dietz WH. Prevalence of abnormal serum aminotransferase values in overweight and obese adolescents. *J Pediatr* 2000; **136**: 727-733

6 **Park HS**, Han JH, Choi KM, Kim SM. Relation between elevated serum alanine aminotransferase and metabolic syndrome in Korean adolescents. *Am J Clin Nutr* 2005; **82**: 1046-1051

7 **Schwimmer JB**, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006; **118**: 1388-1393

8 **Tominaga K**, Fujimoto E, Suzuki K, Hayashi M, Ichikawa M, Inaba Y. Prevalence of non-alcoholic fatty liver disease in children and relationship to metabolic syndrome, insulin resistance, and waist circumference. *Environ Health Prev Med* 2009; **14**: 142-149

9 **Alavian SM**, Mohammad-Alizadeh AH, Esna-Ashari F, Ardalan G, Hajarizadeh B. Non-alcoholic fatty liver disease

prevalence among school-aged children and adolescents in Iran and its association with biochemical and anthropometric measures. *Liver Int* 2009; **29**: 159-163
10 **Tazawa Y**, Noguchi H, Nishinomiya F, Takada G. Serum alanine aminotransferase activity in obese children. *Acta Paediatr* 1997; **86**: 238-241
11 **Franzese A**, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; **42**: 1428-1432
12 **Guzzaloni G**, Grugni G, Minocci A, Moro D, Morabito F. Liver steatosis in juvenile obesity: correlations with lipid profile, hepatic biochemical parameters and glycemic and insulinemic responses to an oral glucose tolerance test. *Int J Obes Relat Metab Disord* 2000; **24**: 772-776
13 **Chan DF**, Li AM, Chu WC, Chan MH, Wong EM, Liu EK, Chan IH, Yin J, Lam CW, Fok TF, Nelson EA. Hepatic steatosis in obese Chinese children. *Int J Obes Relat Metab Disord* 2004; **28**: 1257-1263
14 **Flores-Calderón J**, Gómez-Díaz RA, Rodríguez-Gómez G, Morán-Villota S. Frequency of increased aminotransferases levels and associated metabolic abnormalities in obese and overweight children of an elementary school in Mexico City. *Ann Hepatol* 2005; **4**: 279-283
15 **Louthan MV**, Theriot JA, Zimmerman E, Stutts JT, McClain CJ. Decreased prevalence of nonalcoholic fatty liver disease in black obese children. *J Pediatr Gastroenterol Nutr* 2005; **41**: 426-429
16 **Quirós-Tejeira RE**, Rivera CA, Ziba TT, Mehta N, Smith CW, Butte NF. Risk for nonalcoholic fatty liver disease in Hispanic youth with BMI > or =95th percentile. *J Pediatr Gastroenterol Nutr* 2007; **44**: 228-236
17 **Rocha R**, Cotrim HP, Bitencourt AG, Barbosa DB, Santos AS, Almeida Ade M, Cunha B, Guimarães I. Nonalcoholic fatty liver disease in asymptomatic Brazilian adolescents. *World J Gastroenterol* 2009; **15**: 473-477
18 **Denzer C**, Thiere D, Muche R, Koenig W, Mayer H, Kratzer W, Wabitsch M. Gender-specific prevalences of fatty liver in obese children and adolescents: roles of body fat distribution, sex steroids, and insulin resistance. *J Clin Endocrinol Metab* 2009; **94**: 3872-3881
19 **Papandreou D**, Rousso I, Economou I, Makedou A, Moudiou T, Malindretos P, Pidonia I, Pantoleon A, Mavromichalis I. Is there any association between high-density lipoprotein, insulin resistance and non-alcoholic fatty liver disease in obese children? *Int J Food Sci Nutr* 2009; **60**: 312-318
20 **Papandreou D**, Rousso I, Mavromichalis I. Update on non-alcoholic fatty liver disease in children. *Clin Nutr* 2007; **26**: 409-415
21 **Loomba R**, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology* 2009; **50**: 1282-1293
22 **Patton HM**, Sirlin C, Behling C, Middleton M, Schwimmer JB, Lavine JE. Pediatric nonalcoholic fatty liver disease: a critical appraisal of current data and implications for future research. *J Pediatr Gastroenterol Nutr* 2006; **43**: 413-427
23 **Neuschwander-Tetri BA**, Unalp A, Creer MH. Influence of local reference populations on upper limits of normal for serum alanine aminotransferase levels. *Arch Intern Med* 2008; **168**: 663-666
24 **Fishbein MH**, Miner M, Mogren C, Chalekson J. The spectrum of fatty liver in obese children and the relationship of serum aminotransferases to severity of steatosis. *J Pediatr Gastroenterol Nutr* 2003; **36**: 54-61
25 **Burgert TS**, Taksali SE, Dziura J, Goodman TR, Yeckel CW, Papademetris X, Constable RT, Weiss R, Tamborlane WV, Savoye M, Seyal AA, Caprio S. Alanine aminotransferase levels and fatty liver in childhood obesity: associations with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006; **91**: 4287-4294
26 **Pacifico L**, Celestre M, Anania C, Paolantonio P, Chiesa C, Laghi A. MRI and ultrasound for hepatic fat quantification: relationships to clinical and metabolic characteristics of pediatric nonalcoholic fatty liver disease. *Acta Paediatr* 2007; **96**: 542-547
27 **Canalias F**, Camprubí S, Sánchez M, Gella FJ. Metrological traceability of values for catalytic concentration of enzymes assigned to a calibration material. *Clin Chem Lab Med* 2006; **44**: 333-339
28 **Nadeau KJ**, Ehlers LB, Zeitler PS, Love-Osborne K. Treatment of non-alcoholic fatty liver disease with metformin versus lifestyle intervention in insulin-resistant adolescents. *Pediatr Diabetes* 2009; **10**: 5-13
29 **Kawasaki T**, Hashimoto N, Kikuchi T, Takahashi H, Uchiyama M. The relationship between fatty liver and hyperinsulinemia in obese Japanese children. *J Pediatr Gastroenterol Nutr* 1997; **24**: 317-321
30 **Nadeau KJ**, Klingensmith G, Zeitler P. Type 2 diabetes in children is frequently associated with elevated alanine aminotransferase. *J Pediatr Gastroenterol Nutr* 2005; **41**: 94-98
31 **Bravo AA**, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; **344**: 495-500
32 **Maharaj B**, Maharaj RJ, Leary WP, Cooppan RM, Naran AD, Pirie D, Pudifin DJ. Sampling variability and its influence on the diagnostic yield of percutaneous needle biopsy of the liver. *Lancet* 1986; **1**: 523-525
33 **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
34 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906
35 **Merriman RB**, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, Bass NM. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. *Hepatology* 2006; **44**: 874-880
36 **Amarapurka DN**, Amarapurkar AD, Patel ND, Agal S, Baigal R, Gupte P, Pramanik S. Nonalcoholic steatohepatitis (NASH) with diabetes: predictors of liver fibrosis. *Ann Hepatol* 2006; **5**: 30-33
37 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854
38 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649
39 **Nobili V**, Marcellini M, Devito R, Ciampalini P, Piemonte F, Comparcola D, Sartorelli MR, Angulo P. NAFLD in children: a prospective clinical-pathological study and effect of lifestyle advice. *Hepatology* 2006; **44**: 458-465
40 **Patton HM**, Lavine JE, Van Natta ML, Schwimmer JB, Kleiner D, Molleston J. Clinical correlates of histopathology in pediatric nonalcoholic steatohepatitis. *Gastroenterology* 2008; **135**: 1961-1971.e2
41 **Schwimmer JB**, Deutsch R, Rauch JB, Behling C, Newbury R, Lavine JE. Obesity, insulin resistance, and other clinicopathological correlates of pediatric nonalcoholic fatty liver disease. *J Pediatr* 2003; **143**: 500-505
42 **Manco M**, Marcellini M, Devito R, Comparcola D, Sartorelli MR, Nobili V. Metabolic syndrome and liver histology in paediatric non-alcoholic steatohepatitis. *Int J Obes (Lond)* 2008; **32**: 381-387
43 **Carter-Kent C**, Yerian LM, Brunt EM, Angulo P, Kohli R, Ling SC, Xanthakos SA, Whitington PF, Charatcharoenwitthaya P, Yap J, Lopez R, McCullough AJ, Feldstein AE. Nonalcoholic

steatohepatitis in children: a multicenter clinicopathological study. *Hepatology* 2009; **50**: 1113-1120
44 **Fracanzani AL**, Valenti L, Fargion S. Risk of severe liver disease in NAFLD with normal ALT levels: a pediatric report. *Hepatology* 2008; **48**: 2088
45 **Fracanzani AL**, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, Bertelli C, Fatta E, Bignamini D, Marchesini G, Fargion S. Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. *Hepatology* 2008; **48**: 792-798
46 **Manco M**, Alisi A, Nobili V. Risk of severe liver disease in NAFLD with normal ALT levels: a pediatric report. *Hepatology* 2008; **48**: 2087-2088; author reply 2088
47 **Roberts EA**. Pediatric nonalcoholic fatty liver disease (NAFLD): a "growing" problem? *J Hepatol* 2007; **46**: 1133-1142
48 **Ciba I**, Widhalm K. The association between non-alcoholic fatty liver disease and insulin resistance in 20 obese children and adolescents. *Acta Paediatr* 2007; **96**: 109-112
49 **Targher G**, Franchini M, Guidi GC, Muggeo M, Lippi G. Alanine aminotransferase as an independent predictor of incident nonalcoholic fatty liver disease. *Clin Chem* 2007; **53**: 1159; author reply 1159-1161
50 **Adams LA**, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ* 2005; **172**: 899-905
51 **Sundaram SS**, Zeitler P, Nadeau K. The metabolic syndrome and nonalcoholic fatty liver disease in children. *Curr Opin Pediatr* 2009; **21**: 529-535
52 **Speliotes EK**. Genetics of common obesity and nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1492-1495
53 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845
54 **Struben VM**, Hespenheide EE, Caldwell SH. Nonalcoholic steatohepatitis and cryptogenic cirrhosis within kindreds. *Am J Med* 2000; **108**: 9-13
55 **Willner IR**, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961
56 **Schwimmer JB**, Celedon MA, Lavine JE, Salem R, Campbell N, Schork NJ, Shiehmorteza M, Yokoo T, Chavez A, Middleton MS, Sirlin CB. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 1585-1592
57 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395
58 **Petta S**, Muratore C, Craxì A. Non-alcoholic fatty liver disease pathogenesis: the present and the future. *Dig Liver Dis* 2009; **41**: 615-625
59 **Wilfred de Alwis NM**, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. *Semin Liver Dis* 2007; **27**: 44-54
60 **Namikawa C**, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, Akisawa N, Saibara T, Hiroi M, Enzan H, Onishi S. Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J Hepatol* 2004; **40**: 781-786
61 **Gambino R**, Cassader M, Pagano G, Durazzo M, Musso G. Polymorphism in microsomal triglyceride transfer protein: a link between liver disease and atherogenic postprandial lipid profile in NASH? *Hepatology* 2007; **45**: 1097-1107
62 **Musso G**, Gambino R, De Michieli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. *Hepatology* 2008; **47**: 1167-1177
63 **Lalazar A**, Wong L, Yamasaki G, Friedman SL. Early genes induced in hepatic stellate cells during wound healing. *Gene* 1997; **195**: 235-243
64 **Ratziu V**, Lalazar A, Wong L, Dang Q, Collins C, Shaulian E, Jensen S, Friedman SL. Zf9, a Kruppel-like transcription factor up-regulated in vivo during early hepatic fibrosis. *Proc Natl Acad Sci USA* 1998; **95**: 9500-9505
65 **Miele L**, Beale G, Patman G, Nobili V, Leathart J, Grieco A, Abate M, Friedman SL, Narla G, Bugianesi E, Day CP, Reeves HL. The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. *Gastroenterology* 2008; **135**: 282-291.e1
66 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465
67 **Liou I**, Kowdley KV. Natural history of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40** Suppl 1: S11-S16
68 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-S10
69 **Harrison SA**, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003; **98**: 2042-2047
70 **Fassio E**, Alvarez E, Domínguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology* 2004; **40**: 820-826
71 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138
72 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873
73 **Angulo P**. GI epidemiology: nonalcoholic fatty liver disease. *Aliment Pharmacol Ther* 2007; **25**: 883-889
74 **García-Monzón C**, Martín-Pérez E, Iacono OL, Fernández-Bermejo M, Majano PL, Apolinario A, Larrañaga E, Moreno-Otero R. Characterization of pathogenic and prognostic factors of nonalcoholic steatohepatitis associated with obesity. *J Hepatol* 2000; **33**: 716-724
75 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419
76 **Lee RG**. Nonalcoholic steatohepatitis: a study of 49 patients. *Hum Pathol* 1989; **20**: 594-598
77 **Powell EE**, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11**: 74-80
78 **Evans CD**, Oien KA, MacSween RN, Mills PR. Non-alcoholic steatohepatitis: a common cause of progressive chronic liver injury? *J Clin Pathol* 2002; **55**: 689-692
79 **Dam-Larsen S**, Franzmann M, Andersen IB, Christoffersen P, Jensen LB, Sørensen TI, Becker U, Bendtsen F. Long term prognosis of fatty liver: risk of chronic liver disease and death. *Gut* 2004; **53**: 750-755
80 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238
81 **Teli MR**, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995; **22**: 1714-1719
82 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121
83 **Ong JP**, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *J Hepatol* 2008; **49**: 608-612
84 **Kinugasa A**, Tsunamoto K, Furukawa N, Sawada T, Kusunoki T, Shimada N. Fatty liver and its fibrous changes found in simple obesity of children. *J Pediatr Gastroenterol Nutr* 1984; **3**: 408-414

85 **Molleston JP**, White F, Teckman J, Fitzgerald JF. Obese children with steatohepatitis can develop cirrhosis in childhood. *Am J Gastroenterol* 2002; **97**: 2460-2462
86 **Suzuki D**, Hashimoto E, Kaneda K, Tokushige K, Shiratori K. Liver failure caused by non-alcoholic steatohepatitis in an obese young male. *J Gastroenterol Hepatol* 2005; **20**: 327-329
87 **Baldridge AD**, Perez-Atayde AR, Graeme-Cook F, Higgins L, Lavine JE. Idiopathic steatohepatitis in childhood: a multicenter retrospective study. *J Pediatr* 1995; **127**: 700-704
88 **Jonas MM**, Krawczuk LE, Kim HB, Lillehei C, Perez-Atayde A. Rapid recurrence of nonalcoholic fatty liver disease after transplantation in a child with hypopituitarism and hepatopulmonary syndrome. *Liver Transpl* 2005; **11**: 108-110
89 **Jankowska I**, Socha P, Pawlowska J, Teisseyre M, Gliwicz D, Czubkowski P, Kalicinski P, Cielecka-Kuszyk J, Socha J. Recurrence of non-alcoholic steatohepatitis after liver transplantation in a 13-yr-old boy. *Pediatr Transplant* 2007; **11**: 796-798
90 **Adams LA**, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology* 2004; **39**: 909-914
91 **Feldstein AE**, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of non-alcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut* 2009; **58**: 1538-1544
92 **Rashid M**, Roberts EA. Nonalcoholic steatohepatitis in children. *J Pediatr Gastroenterol Nutr* 2000; **30**: 48-53
93 **Schwimmer JB**, McGreal N, Deutsch R, Finegold MJ, Lavine JE. Influence of gender, race, and ethnicity on suspected fatty liver in obese adolescents. *Pediatrics* 2005; **115**: e561-e565
94 **Manton ND**, Lipsett J, Moore DJ, Davidson GP, Bourne AJ, Couper RT. Non-alcoholic steatohepatitis in children and adolescents. *Med J Aust* 2000; **173**: 476-479
95 **Kirsch R**, Clarkson V, Shephard EG, Marais DA, Jaffer MA, Woodburne VE, Kirsch RE, Hall Pde L. Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. *J Gastroenterol Hepatol* 2003; **18**: 1272-1282
96 **Cook JS**, Hoffman RP, Stene MA, Hansen JR. Effects of maturational stage on insulin sensitivity during puberty. *J Clin Endocrinol Metab* 1993; **77**: 725-730
97 **Roemmich JN**, Clark PA, Lusk M, Friel A, Weltman A, Epstein LH, Rogol AD. Pubertal alterations in growth and body composition. VI. Pubertal insulin resistance: relation to adiposity, body fat distribution and hormone release. *Int J Obes Relat Metab Disord* 2002; **26**: 701-709
98 **Goran MI**, Gower BA. Longitudinal study on pubertal insulin resistance. *Diabetes* 2001; **50**: 2444-2450
99 **Stranges S**, Dorn JM, Muti P, Freudenheim JL, Farinaro E, Russell M, Nochajski TH, Trevisan M. Body fat distribution, relative weight, and liver enzyme levels: a population-based study. *Hepatology* 2004; **39**: 754-763
100 **Ellis KJ**. Body composition of a young, multiethnic, male population. *Am J Clin Nutr* 1997; **66**: 1323-1331
101 **Raitakari OT**, Porkka KV, Rönnemaa T, Knip M, Uhari M, Akerblom HK, Viikari JS. The role of insulin in clustering of serum lipids and blood pressure in children and adolescents. The Cardiovascular Risk in Young Finns Study. *Diabetologia* 1995; **38**: 1042-1050
102 **Jiang X**, Srinivasan SR, Webber LS, Wattigney WA, Berenson GS. Association of fasting insulin level with serum lipid and lipoprotein levels in children, adolescents, and young adults: the Bogalusa Heart Study. *Arch Intern Med* 1995; **155**: 190-196
103 **Steinberger J**, Moorehead C, Katch V, Rocchini AP. Relationship between insulin resistance and abnormal lipid profile in obese adolescents. *J Pediatr* 1995; **126**: 690-695
104 **Sinaiko AR**, Gomez-Marin O, Prineas RJ. Relation of fasting insulin to blood pressure and lipids in adolescents and parents. *Hypertension* 1997; **30**: 1554-1559
105 **Jiang X**, Srinivasan SR, Bao W, Berenson GS. Association of fasting insulin with blood pressure in young individuals. The Bogalusa Heart Study. *Arch Intern Med* 1993; **153**: 323-328
106 **Taittonen L**, Uhari M, Nuutinen M, Turtinen J, Pokka T, Akerblom HK. Insulin and blood pressure among healthy children. Cardiovascular risk in young Finns. *Am J Hypertens* 1996; **9**: 194-199
107 **Sinha R**, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, Savoye M, Rieger V, Taksali S, Barbetta G, Sherwin RS, Caprio S. Prevalence of impaired glucose tolerance among children and adolescents with marked obesity. *N Engl J Med* 2002; **346**: 802-810
108 **Patton HM**, Yates K, Unalp-Arida A, Behling CA, Huang TT, Rosenthal P, Sanyal AJ, Schwimmer JB, Lavine JE. Association Between Metabolic Syndrome and Liver Histology Among Children With Nonalcoholic Fatty Liver Disease. *Am J Gastroenterol* 2010; Epub ahead of print
109 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187
110 **Haukeland JW**, Konopski Z, Linnestad P, Azimy S, Marit Løberg E, Haaland T, Birkeland K, Bjøro K. Abnormal glucose tolerance is a predictor of steatohepatitis and fibrosis in patients with non-alcoholic fatty liver disease. *Scand J Gastroenterol* 2005; **40**: 1469-1477
111 **Angulo P**, Alba LM, Petrovic LM, Adams LA, Lindor KD, Jensen MD. Leptin, insulin resistance, and liver fibrosis in human nonalcoholic fatty liver disease. *J Hepatol* 2004; **41**: 943-949
112 **Pinzani M**, Vizzutti F, Arena U, Marra F. Technology Insight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 95-106
113 **Nobili V**, Vizzutti F, Arena U, Abraldes JG, Marra F, Pietrobattista A, Fruhwirth R, Marcellini M, Pinzani M. Accuracy and reproducibility of transient elastography for the diagnosis of fibrosis in pediatric nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 442-448
114 **Nobili V**, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology* 2009; **136**: 160-167
115 **Brockow K**, Steinkraus V, Rinninger F, Abeck D, Ring J. Acanthosis nigricans: a marker for hyperinsulinemia. *Pediatr Dermatol* 1995; **12**: 323-326
116 **Stuart CA**, Gilkison CR, Smith MM, Bosma AM, Keenan BS, Nagamani M. Acanthosis nigricans as a risk factor for non-insulin dependent diabetes mellitus. *Clin Pediatr (Phila)* 1998; **37**: 73-79
117 **Lavine JE**, Schwimmer JB. Nonalcoholic fatty liver disease in the pediatric population. *Clin Liver Dis* 2004; **8**: 549-558, viii-ix
118 **Barlow SE**. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics* 2007; **120** Suppl 4: S164-S192
119 **Lee DH**, Ha MH, Kim JH, Christiani DC, Gross MD, Steffes M, Blomhoff R, Jacobs DR Jr. Gamma-glutamyltransferase and diabetes--a 4 year follow-up study. *Diabetologia* 2003; **46**: 359-364
120 **Kim DJ**, Noh JH, Cho NH, Lee BW, Choi YH, Jung JH, Min YK, Lee MS, Lee MK, Kim KW. Serum gamma-glutamyltransferase within its normal concentration range is related to the presence of diabetes and cardiovascular risk factors. *Diabet Med* 2005; **22**: 1134-1140
121 **Kang YH**, Min HK, Son SM, Kim IJ, Kim YK. The association of serum gamma glutamyltransferase with components of the metabolic syndrome in the Korean adults. *Diabetes Res Clin Pract* 2007; **77**: 306-313

122 **Day CP**. From fat to inflammation. *Gastroenterology* 2006; **130**: 207-210

123 **Cotler SJ**, Kanji K, Keshavarzian A, Jensen DM, Jakate S. Prevalence and significance of autoantibodies in patients with non-alcoholic steatohepatitis. *J Clin Gastroenterol* 2004; **38**: 801-804

124 **Mehta SR**, Thomas EL, Bell JD, Johnston DG, Taylor-Robinson SD. Non-invasive means of measuring hepatic fat content. *World J Gastroenterol* 2008; **14**: 3476-3483

125 **Kawata R**, Sakata K, Kunieda T, Saji S, Doi H, Nozawa Y. Quantitative evaluation of fatty liver by computed tomography in rabbits. *AJR* 1984; **142**: 741-746

126 **Ricci C**, Longo R, Gioulis E, Bosco M, Pollesello P, Masutti F, Crocè LS, Paoletti S, de Bernard B, Tiribelli C, Dalla Palma L. Noninvasive in vivo quantitative assessment of fat content in human liver. *J Hepatol* 1997; **27**: 108-113

127 **Park SH**, Kim PN, Kim KW, Lee SW, Yoon SE, Park SW, Ha HK, Lee MG, Hwang S, Lee SG, Yu ES, Cho EY. Macrovesicular hepatic steatosis in living liver donors: use of CT for quantitative and qualitative assessment. *Radiology* 2006; **239**: 105-112

128 **Jacobs JE**, Birnbaum BA, Shapiro MA, Langlotz CP, Slosman F, Rubesin SE, Horii SC. Diagnostic criteria for fatty infiltration of the liver on contrast-enhanced helical CT. *AJR* 1998; **171**: 659-664

129 **Siegelman ES**, Rosen MA. Imaging of hepatic steatosis. *Semin Liver Dis* 2001; **21**: 71-80

130 **Joseph AE**, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. *Clin Radiol* 1991; **43**: 26-31

131 **Foster KJ**, Dewbury KC, Griffith AH, Wright R. The accuracy of ultrasound in the detection of fatty infiltration of the liver. *Br J Radiol* 1980; **53**: 440-442

132 **Debongnie JC**, Pauls C, Fievez M, Wibin E. Prospective evaluation of the diagnostic accuracy of liver ultrasonography. *Gut* 1981; **22**: 130-135

133 **Saverymuttu SH**, Joseph AE, Maxwell JD. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br Med J (Clin Res Ed)* 1986; **292**: 13-15

134 **Ryan CK**, Johnson LA, Germin BI, Marcos A. One hundred consecutive hepatic biopsies in the workup of living donors for right lobe liver transplantation. *Liver Transpl* 2002; **8**: 1114-1122

135 **Mottin CC**, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, Repetto G. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. *Obes Surg* 2004; **14**: 635-637

136 **Strauss S**, Gavish E, Gottlieb P, Katsnelson L. Interobserver and intraobserver variability in the sonographic assessment of fatty liver. *AJR* 2007; **189**: W320-W323

137 **Needleman L**, Kurtz AB, Rifkin MD, Cooper HS, Pasto ME, Goldberg BB. Sonography of diffuse benign liver disease: accuracy of pattern recognition and grading. *AJR* 1986; **146**: 1011-1015

138 **Fishbein M**, Castro F, Cheruku S, Jain S, Webb B, Gleason T, Stevens WR. Hepatic MRI for fat quantitation: its relationship to fat morphology, diagnosis, and ultrasound. *J Clin Gastroenterol* 2005; **39**: 619-625

139 **Fishbein MH**, Gardner KG, Potter CJ, Schmalbrock P, Smith MA. Introduction of fast MR imaging in the assessment of hepatic steatosis. *Magn Reson Imaging* 1997; **15**: 287-293

140 **Fishbein MH**, Stevens WR. Rapid MRI using a modified Dixon technique: a non-invasive and effective method for detection and monitoring of fatty metamorphosis of the liver. *Pediatr Radiol* 2001; **31**: 806-809

141 **Promrat K**, Lutchman G, Uwaifo GI, Freedman RJ, Soza A, Heller T, Doo E, Ghany M, Premkumar A, Park Y, Liang TJ, Yanovski JA, Kleiner DE, Hoofnagle JH. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology* 2004; **39**: 188-196

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.289

World J Hepatol 2010 July 27; 2(7): 289-291
ISSN 1948-5182 (online)

CASE REPORT

Paraparesis caused by transarterial chemoembolization: A case report

Kashif Tufail, Victor Araya, Ashaur Azhar, David Hertzog, Kamran Khanmoradi, Jorge Ortiz

Kashif Tufail, Victor Araya, Ashaur Azhar, Department of Hepatology, Albert Einstein Medical Center, Philadelphia, PA 19141, United States
David Hertzog, Department of Radiology, Albert Einstein Medical Center, Philadelphia, PA 19141, United States
Kamran Khanmoradi, Jorge Ortiz, Department of Transplant Surgery, Albert EinsteinMedical Center, Philadelphia, PA 19141, United States
Author contributions: Tufail K, Araya V, Azhar A, Hertzog D, Khanmoradi K and Ortiz J contributed equally to this work and were responsible for formatting, editing, technical assistance, literature search and writing; Tufail K, Araya V and Ortiz JA were responsible for the concept and manuscript writing; and Hertzog D edited and formatted figures and provided valuable information regarding the TACE procedure in this patient.
Correspondence to: Kashif Tufail, MD, FACP, Department of Hepatology, Albert Einstein Medical Center, 5501 Old York Road, Klein 509, Philadelphia, PA 19141, United States. kashif_tufail@yahoo.com
Telephone: +1-215-4568242 Fax: +1-215-4568058
Received: April 23, 2010 Revised: June 29, 2010
Accepted: July 6, 2010
Published online: July 27, 2010

Abstract

Transarterial chemoembolization (TACE) is an effective modality for the treatment of Hepatocellular Carcinoma. It is used to treat small tumors and to downstage large tumors to meet liver transplant criteria. TACE can be associated with multiple side effects, including fever, right upper quadrant pain, nausea, vomiting, hepatic failure, hepatic encephalopathy, cholecystitis and pancreatitis. Neurological complications after TACE are rare, usually caused by cerebral embolism, and confirmed by means of imaging studies. Spinal cord ischemia secondary to TACE is extremely rare and can lead to significant morbidity. We report a case of paraparesis caused by TACE with normal imaging and nerve conduction studies, suggestive of localized vasculitis.

Key words: Transarterial chemoembolization; Paraparesis; Spinal cord ischemia; Hepatocellular carcinoma

Peer reviewers: Giuseppe R Nigri, MD, PhD, FACS, Assistant Professor, Department of Surgery, University of Rome La Sapienza, II School of Medicine, St. Andrea Hospital, Rome, Italy; Martin Loss, MD, Associate Professor, Klinik und Poliklinik für Chirurgie, Universitätsklinikum Regensburg, Franz-Josef-Strauss-Allee 11, Regensburg 93053, Germany

Tufail K, Araya V, Azhar A, Hertzog D, Khanmoradi K, Ortiz J. Paraparesis caused by transarterial chemoembolization: A case report. *World J Hepatol* 2010; 2(7): 289-291 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/289.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.289

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide, and is the third most common cause of cancer-related death[1]. It is the fastest growing cause of cancer-related deaths in males in the United States[2]. Only 30%-40% of patients with HCC are diagnosed at a stage where curative therapy can be offered[3]. In appropriate candidates, liver resection and transplantation are associated with long-term response and improved survival. The remaining 60%-70% already have advanced HCC and are eligible only for palliative interventions, which include transarterial chemoembolization (TACE), ablation, radiation, and systemic chemotherapy. TACE is also used to treat small tumors, and to downstage large tumors to meet liver transplant criteria[4]. Although considered a relatively safe procedure, TACE may be associated with fever, right upper quadrant pain, nausea and transaminitis, known as post-embolization syndrome. The complication rate after TACE is reported to be 2.9%[5]. Examples include hepatic failure, cholecystitis,

pancreatitis, hepatic encephalopathy, common bile duct stricture, biloma, tumor lysis syndrome, pulmonary and cerebral embolism. Neurological deficits after TACE are very rare, usually caused by cerebral embolism of iodized oil (lipoidal), and confirmed by abnormal findings on imaging. We report a case of paraparesis caused by TACE and normal imaging of brain, thoracic and lumbosacral spine, and normal electromyography and nerve conduction studies.

CASE REPORT

A 45 year-old African American male with hepatitis C-related cirrhosis underwent open microwave ablation of a single 5.6 cm right hepatic lobe HCC. Follow-up MRI revealed a residual tumor, which was treated by TACE of the right hepatic artery, but follow up imaging revealed a persistent residual tumor. Hepatic angiogram confirmed that part of the tumor was supplied by the right inferior phrenic artery. The patient underwent repeat TACE, in which the right hepatic (Figure 1A) and inferior phrenic arteries (Figure 1B) were successfully chemoembolized with doxorubicin, cisplatin and mitomycin.

The patient developed bilateral lower extremity weakness eight hours after the procedure. His strength was 3/5 in the right and 4/5 in the left lower extremity. This was associated with numbness in the left anterior thigh and scrotum, together with urinary retention. Reflexes were 2+ bilaterally and there was no loss of rectal tone. MRI of the brain, thoracic, lumbar and sacral spine did not reveal stroke, mass or cord compression. Electromyography and nerve conduction studies were all within normal limits. The patient was diagnosed with localized vasculitis of the branches of the anterior spinal artery, caused by the chemotherapy. An angiogram revealed the anastomoses between the branches of the right inferior phrenic artery and intercostal arteries, confirmed by the filling of the anterior spinal artery, which was believed to be the route of the embolism causing the injury to the spinal cord (Figure 2). The patient was started on high dose steroids, which were then systematically reduced over a period of one mo. Aggressive physical therapy was also instituted. His symptoms markedly improved and he was able to walk without assistance. The patient underwent liver transplantation two months later and remained free of neurological complications.

DISCUSSION

Neurological complications occur rarely in association with TACE, and are usually due to lipoidal embolism in the central nervous system. Focal symptoms develop in the area of distribution of the affected vessel, which can be confirmed by imaging studies. Spinal cord injury is an extremely rare complication after TACE. Our patient developed bilateral lower extremity weakness following the procedure, associated with numbness in the left anterior thigh, together with urinary retention. All these

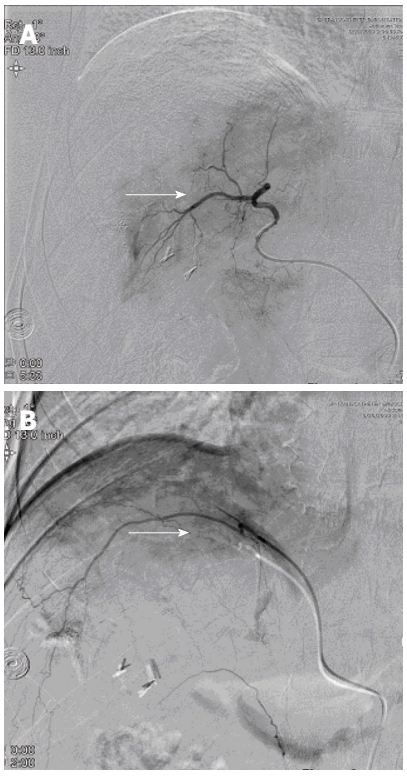


Figure 1 Selective catheterization. A: Right hepatic artery; B: Lateral branch of right inferior phrenic artery.

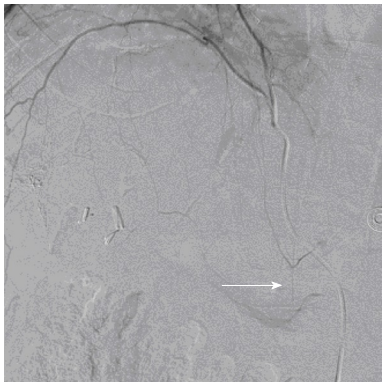


Figure 2 Anterior spinal artery filling *via* collaterals.

symptoms can be explained by ischemia to the lumbar spinal cord in the region of L1-L3. Since the MRI/MRA of the brain and spinal cord did not show any infarction or cord compression, we surmise that the symptoms occurred due to localized vasculitis caused by one of the chemotherapeutic agents used during TACE.

Neurological complications after TACE usually occur after the second or third session, which can be explained by hepatic artery injury or decreased flow at the previous TACE site, leading to collateral recruitment[6]. Our patient had previously undergone one initial TACE procedure of the right hepatic artery without any complications. The second session of TACE involved the right inferior phrenic artery, which supplied part of the tumor and resulted in this complication.

The inferior phrenic artery is the most common source of extra-hepatic collateral blood supply for HCC[7]. The right and left inferior phrenic arteries usually arise from the aorta above the celiac artery, and each divides

into medial and lateral branches. They are the main source of blood supply to the diaphragm, and also send a few branches to adrenal glands, and, occasionally, to the liver and spleen. The medial branch joins with its fellow of the opposite side, and with the musculophrenic and pericardiophrenic arteries, whereas the lateral branch joins with the lower intercostal arteries and musculophrenic arteries. We believe that the anastomosis between the lateral branches of the right inferior phrenic artery and intercostal arteries which supply the spinal cord through the anterior spinal artery, was the route of the embolism which caused the injury to the spinal cord in our patient. Few cases of spinal cord injury after TACE causing paraplegia or paraparesis, sensory loss, and urinary retention, confirmed by imaging studies and usually caused by communication through intercostal arteries have been reported[8]. Although the spinal cord injury in these case reports was seen on MRI, it was not classic for ischemia, and was found to be steroid-responsive. In our patient, since the extensive workup for paraparesis had been essentially unremarkable, with normal imaging, electromyography, and nerve conduction studies, the diagnosis of localized vasculitis was made, and treatment with steroids resulted in the resolution of symptoms.

In conclusion, we have reported a case of paraparesis following TACE with normal imaging, and its improvement with steroids, suggesting localized vasculitis. This emphasizes the fact that TACE and other loco-regional therapies can be associated with serious side effects which may lead to significant morbidity, and could jeopardize liver transplant candidacy. Physicians should be aware of these risks and appropriate consent should be obtained.

REFERENCES

1 **Parkin DM**. Global cancer statistics in the year 2000. *Lancet Oncol* 2001; **2**: 533-543

2 Cancer Facts & Figures. American Cancer Society. Atlanta: Georgia, 2008

3 **Bruix J**, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. *Hepatology* 2002; **35**: 519-524

4 **Chapman WC**, Majella Doyle MB, Stuart JE, Vachharajani N, Crippin JS, Anderson CD, Lowell JA, Shenoy S, Darcy MD, Brown DB. Outcomes of neoadjuvant transarterial chemoembolization to downstage hepatocellular carcinoma before liver transplantation. *Ann Surg* 2008; **248**: 617-625

5 **Pietrosi G**, Miraglia R, Luca A, Vizzini GB, Fili' D, Riccardo V, D'Antoni A, Petridis I, Maruzzelli L, Biondo D, Gridelli B. Arterial chemoembolization/embolization and early complications after hepatocellular carcinoma treatment: a safe standardized protocol in selected patients with Child class A and B cirrhosis. *J Vasc Interv Radiol* 2009; **20**: 896-902

6 **Lee KH**, Sung KB, Lee DY, Park SJ, Kim KW, Yu JS. Transcatheter arterial chemoembolization for hepatocellular carcinoma: anatomic and hemodynamic considerations in the hepatic artery and portal vein. *Radiographics* 2002; **22**: 1077-1091

7 **Gwon DI**, Ko GY, Yoon HK, Sung KB, Lee JM, Ryu SJ, Seo MH, Shim JC, Lee GJ, Kim HK. Inferior phrenic artery: anatomy, variations, pathologic conditions, and interventional management. *Radiographics* 2007; **27**: 687-705

8 **Kim JH**, Yeon JE, Jong YK, Seo WK, Cha IH, Seo TS, Park JJ, Kim JS, Bak YT, Byun KS. Spinal cord injury subsequent to transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. *Dig Liver Dis* 2010; **42**: 67-70

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i7.292

World J Hepatol 2010 July 27; 2(7): 292-294
ISSN 1948-5182 (online)

CASE REPORT

Resection of a rapid-growing 40-cm giant liver hemangioma

Andreas JM Koszka, Fabio G Ferreira, Caio GG de Aquino, Maurício A Ribeiro, André S Gallo, Elisa MC Aranzana, Luiz A Szutan

Andreas JM Koszka, Fabio G Ferreira, Caio GG de Aquino, Maurício A Ribeiro, André S Gallo, Elisa MC Aranzana, Luiz A Szutan, Department of Surgery, Liver and Portal Hypertension Group, Santa Casa School of Medical Sciences, Sao Paulo, Cep 01221-900, Brazil

Author contributions: Koszka AJM, Ferreira FG, Aquino C GG, Ribeiro MA, Gallo AS, Aranzana EMC and Szutan LA made substantial contributions to publication. Since everyone is a member of the Liver and Portal Hypertension Group at our institution, some have participated in the operation and others attended the patient during follow-up.

Correspondence to: Andreas JM Koszka, MD, Department of Surgery, Liver and Portal Hypertension Group, Santa Casa School of Medical Sciences, Sao Paulo 1948-5182, Brazil. andrekoszka@yahoo.com.br

Telephone: +55-11-21767270 Fax: +55-11-33378164

Received: March 2, 2010 Revised: June 16, 2010
Accepted: June 23, 2010
Published online: July 27, 2010

Abstract

Hemangiomas are the most frequent benign tumors of the liver. Most hemangiomas are asymptomatic and therefore largely diagnosed only in routine screening tests. Usually they are small and require no specific treatment. In some situations they can reach great dimensions, causing some discomfort to the patient. Resection of liver hemangioma is indicated in cases of great dimension tumors causing symptoms such as pain, nausea or bloating caused by compression of adjacent organs. We report a case of a rare giant hemangioma with rapid growth in short time: a 50 year old female reported to our institution with a 40 cm giant liver hemangioma and then underwent a left hepatectomy.

Key words: Liver benign neoplasms; Giant liver Hemangioma; Liver surgery

Peer reviewers: Lars Mueller, MD, PhD, Pediatric Hematology/Oncology, Children's Hospital Boston, MA 02115, United States; Boon Hun Yong, Associate Professor, Department of Anaesthesiology, University of Hong Kong, Hong Kong, China

Koszka AJM, Ferreira FG, Aquino CGG, Ribeiro MA, Gallo AS, Aranzana EMC, Szutan LA. Resection of a rapid-growing 40-cm giant liver hemangioma. *World J Hepatol* 2010; 2(7): 292-294 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i7/292.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i7.292

INTRODUCTION

Hemangiomas are the most frequent benign tumors of the liver with an incidence ranging from 5% to 20% and women accounting for the largest part. Most hemangiomas are asymptomatic and therefore largely diagnosed only in routine screening tests. Usually they are small and require no specific treatment[1-5].

In some situations they can reach great dimensions, causing some discomfort to the patient. There is no consensus in the literature regarding the minimum size a hemangioma is considered as giant but in our service we use the limit of 10 cm. Rare cases of rupture followed by massive bleeding and Kassabach-Merrit's syndrome with serious complications have been reported.

Resection of liver hemangioma is indicated in cases of great dimension tumors causing symptoms such as pain, nausea or bloating by compression of adjacent organs[6-9]. Resection is also advisable in a controversial diagnosis where malignancy cannot be fully eliminated[4-7].

CASE REPORT

We report a case of a rare giant hemangioma with rapid growth in a short time: a 50-year old female being monitored for 7 years since a liver hemangioma was incidentally diagnosed.

At that time, the lesion already measured 20 cm in its largest axis (Figure 1A). In the last 4 mo, she presented

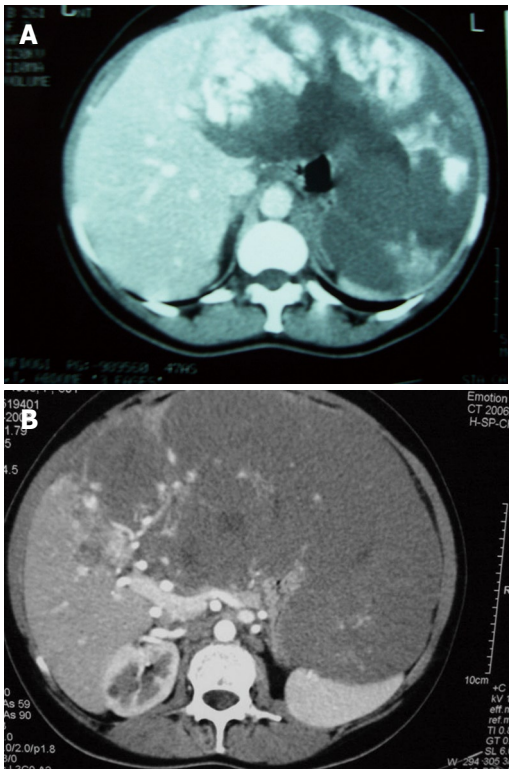


Figure 1 Computed tomography scan of a patients with a liver hemangioma. A: The first scan performed 7 years ago, showing an oversized hemangioma on the left hepatic lobe; B: During the symptoms worsening a second CT-Scan showed tumor growth. The lesion reached the central portions of the liver.

with symptoms such as a dry cough and in the last 2 mo, abdominal pain. CT scan evidenced an increase of 10 cm in the lesion in the left lobe with compression of the diaphragm, reaching 30 cm in its largest axis (Figure 1B).

An arterial embolization procedure was attempted in order to restrict the hemangioma growing. It brought no benefits but worsened the symptoms.

The patient then underwent a left hepatectomy. We usually access the abdominal cavity through a Chevron incision to perform hepatectomies. After liver mobilization was done, a great deformity in the vascular anatomy of the portal and supra hepatic veins was identified, mainly due to the compression caused by the huge hemangioma (Figure 2A). The left hepatic vein was involved by the tumor and had to be ligated. For a safe parenchymal transection, blood inflow and outflow control to the liver was performed. Pringle maneuver and the infra and supra hepatic vena cava were laced but not clamped. The liver transection was performed with kellyclasy and silkclasy[10] and hemostasis was achieved with cauterization. Figure 2B shows the final aspect of the resection. The anatomopathological findings confirmed the diagnosis of a 40 cm hemangioma in its largest axis (Figure 2C). The patient is now asymptomatic at 6 mo post-operation.

DISCUSSION

After a review of our patient database, we found that the

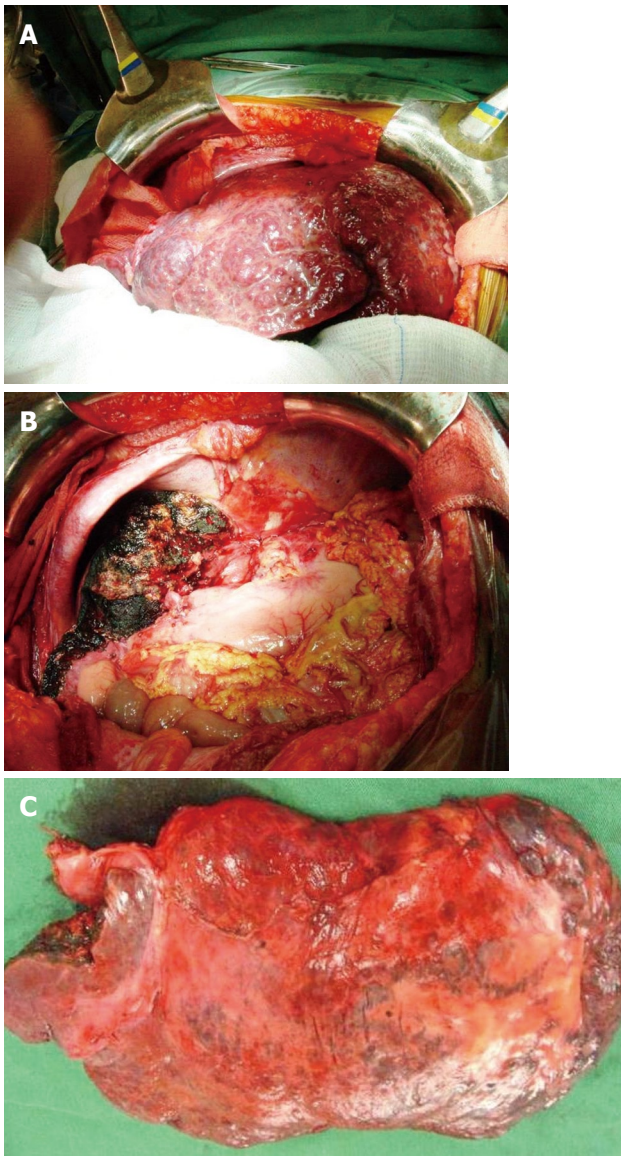


Figure 2 Surgical treatment of the patient with a liver hemangioma. A: After the laparotomy, a giant hemangioma was identified, and this lesion led to a great anatomical deformity in the liver; B: After the resection, the remnant liver in the cleared abdominal cavity; C: The 40 cm-giant liver hemangioma was sent to anatomical and pathological analysis.

number of diagnosed liver hemangiomas greater than 10 cm is very low. The few patients with giant hemangiomas do not present with any signs of tumor growing at follow up. Therefore, resection of liver hemangiomas is quite restricted in our service; the main argument against the resection is the need of submitting a patient with a benign disease to such a major surgery. Interestingly, this was the only report of a size changing hemangioma in our experience.

The literature shows that arterial embolization of the lesion to contain the tumor growth is not effective unless the hemangioma is a lesion with a clear arterial blood supply[11]. At first we assumed that this hemangioma could have an arterial supply that would justify such a rapid size enhancement. The arterial embolization was the initial

therapeutic approach but unfortunately the procedure did not help and, due to necrosis, worsened the pain for the patient.

Discussions on resection of hemangiomas include procedures on the so-called giant tumors or in those that result in symptoms to the patient. Reports on surgery due to hemangioma growth are rare[6].

In this report, our attention is drawn to the speed of the hemangioma increase, leading to abdominal pain and cough due to diaphragm compression. In our service, we maintain clinical follow-up of hemangiomas in asymptomatic patients regardless the size. Nevertheless, due to the particular evolution of this case (rapid growth and the tumor necrosis as a consequence of the arterial embolization), we decided to carry out the resection despite the consequent complications involved in this kind of procedure.

REFERENCES

1 **Fu XH**, Lai EC, Yao XP, Chu KJ, Cheng SQ, Shen F, Wu MC, Lau WY. Enucleation of liver hemangiomas: is there a difference in surgical outcomes for centrally or peripherally located lesions? *Am J Surg* 2009; **198**: 184-187

2 **Singh RK**, Kapoor S, Sahni P, Chattopadhyay TK. Giant haemangioma of the liver: is enucleation better than resection? *Ann R Coll Surg Engl* 2007; **89**: 490-493

3 **Romano F**, Messinesi G, Tana F, Uggeri F. Recurrent giant hemangioma causing severe respiratory distress. *Dig Dis Sci* 2007; **52**: 3526-3529

4 **Erdogan D**, Busch OR, van Delden OM, Bennink RJ, ten Kate FJ, Gouma DJ, van Gulik TM. Management of liver hemangiomas according to size and symptoms. *J Gastroenterol Hepatol* 2007; **22**: 1953-1958

5 **Hazinedaroglu SM**, Kayaoglu HA, Ali Yerdel M. Enucleation of centrally located giant hepatic hemangioma: report of two cases. *Dig Dis Sci* 2006; **51**: 1213-1217

6 **Herman P**, Costa ML, Machado MA, Pugliese V, D'Albuquerque LA, Machado MC, Gama-Rodrigues JJ, Saad WA. Management of hepatic hemangiomas: a 14-year experience. *J Gastrointest Surg* 2005; **9**: 853-859

7 **Hamaloglu E**, Altun H, Ozdemir A, Ozenc A. Giant liver hemangioma: therapy by enucleation or liver resection. *World J Surg* 2005; **29**: 890-893

8 **Demircan O**, Demiryurek H, Yagmur O. Surgical approach to symptomatic giant cavernous hemangioma of the liver. *Hepatogastroenterology* 2005; **52**: 183-186

9 **Lerner SM**, Hiatt JR, Salamandra J, Chen PW, Farmer DG, Ghobrial RM, Busuttil RW. Giant cavernous liver hemangiomas: effect of operative approach on outcome. *Arch Surg* 2004; **139**: 818-821; discussion 821-823

10 **Herman P**, Machado MA, Machado MC. Silkclasy: a simple way for liver transection during anatomic hepatectomies. *J Surg Oncol* 2007; **95**: 86-89

11 **Vassiou K**, Rountas H, Liakou P, Arvanitis D, Fezoulidis I, Tepetes K. Embolization of a giant hepatic hemangioma prior to urgent liver resection. Case report and review of the literature. *Cardiovasc Intervent Radiol* 2007; **30**: 800-802

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 July 27; 2(7): I
ISSN 1948-5182 (online)

Acknowledgments to reviewers of *World Journal of Hepatology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Hepatology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Nabil Mohie Abdel-Hamid, Professor, Chairman of Biochemistry, Department, Pharmacy College, Minia University, Al Minya 61519, Egypt

Stephen Lam Chan, MD, Department of Clinical Oncology, The Chinese University of Hong Kong, Prince of Wales Hospital, 30-32 Ngan Street, Shatin, New Territories, Hong Kong, China

Papandreou Dimitrios, PhD, MD, RD, Assistant Professor of Nutrition, Department of Health Science, University of Nicosia, Cyprus; Head of Pediatric Obesity Unit, Aristotle University of Thessaloniki, School of Medicine, Ahepa General Hospital, P. Mela 22 GR 54622, Greece

Regina Coeli dos Santos Goldenberg, Professor, Department of Carlos Chagas filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro 21941-902, Brazil

Henning Gronbaek, MD, PhD, Department of Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, Aarhus C8000, Denmark

Nattiya Hirankarn, MD, Associated Professor, Immunology Unit, Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Rama 4 road, Bangkok 10330, Thailand

Wan Yee Joseph Lau, MD, Professor, Clinical Sciences Building, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, China

Martin Loss, MD, Associate Professor, Klinik und Poliklinik für Chirurgie, Universitätsklinikum Regensburg, Franz-Josef-Strauss-Allee 11, Regensburg 93053, Germany

Hitoshi Maruyama, MD, Department of Medicine and Clinical Oncology, Chiba University Graduate School of Medicine, 1-8-1, Inohana, Chuou-ku, Chiba 260-8670, Japan

Zenichi Morise, MD, PhD, Department of Surgery, Fujita Health University School of Medicine, 1-98 Dengakugakubo Kutsukakecho, Toyoake, AICHI 470-1192, Japan

Lars Mueller, MD, PhD, Children's Hospital Boston, Pediatric Hematology/Oncology, 300 Longwood Avenue, Karp Family Research Building, 08125.4, Boston, MA 02115, United States

Giuseppe R Nigri, MD, PhD, FACS, Assistant Professor, Department of Surgery, University of Rome La Sapienza, II School of Medicine, St. Andrea Hospital, Rome, Italy

Waka Ohishi, MD, PhD, Senior Scientist, Chief, Division of Clinical Laboratories, Department of Clinical Studies, Radiation Effects Research Foundation, Hiroshima 732-0815, Japan

Xun-Di Xu, MD, PhD, Department of Gastroenterological Surgery, Xiangya 2nd Hospital, Central South University, Renmin Zhong Road 139, Changsha 410011, Hunan Province, China

Boon Hun Yong, Associate Professor, Department of Anaesthesiology, University of Hong Kong, Hong Kong, China

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 July 27; 2(7): I
ISSN 1948-5182 (online)

Meetings

Events Calendar 2010

January 25-26
Tamilnadu, India
International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

September 10-12
Montreal, Canada
International Liver Association's Fourth Annual Conference

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
Prague, Czech Republic
Prague Hepatology Meeting 2010

September 23-26
Prague, Czech Republic
The 1st World Congress on Controversies in Gastroenterology & Liver Diseases

October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

October 23-27
Barcelona, Spain
18th United European Gastroenterology Week

October 29-November 02
Boston, Massachusetts, United States
The Liver Meeting® 2010--AASLD's 61st Annual Meeting

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
www.wjgnet.com

World J Hepatol 2010 July 27; 2(7): I-V
ISSN 1948-5182 (online)

Instructions to authors

GENERAL INFORMATION

World Journal of Hepatology (*World J Hepatol, WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, openaccess, peer-reviewed journal supported by an editorial board of 572 experts in hepatology from 45 countries..

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJH* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJH* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJH* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

The major task of *WJH* is to report rapidly the most recent results in basic and clinical research on hepatology, including: liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology.

The columns in the issues of *WJH* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in hepatology; (9) Brief Article: To briefly report the novel and innovative findings in hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJH*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in hepatology.

CSSN
ISSN 1948-5182 (online)

Published by
Beijing Baishideng BioMed Scientific Co., Ltd.

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 Beijing Baishideng BioMed Scientific Co., Ltd, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-5182 office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjh@wjgnet.com, or by telephone: +86-10-59080038. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJH*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original

contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107113351.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World*

J Gastroenterol 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 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/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107115140.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.*

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJH*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white

photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Hepatology

Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080038
Fax: +86-10-85381893
E-mail: wjh@wjgnet.com
http://www.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 or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1948-5182/g_info_20100107114726.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/1948-5182/g_info_20100107114601.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJH will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.