ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2014 July 27; 6(7): 453-537

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

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

Editorial Board

2014-2017

The *World Journal of Hepatology* Editorial Board consists of 468 members, representing a team of worldwide experts in hepatology. They are from 53 countries, including Algeria (1), Argentina (6), Armenia (1), Australia (1), Austria (4), Bangladesh (2), Belgium (3), Botswana (2), Brazil (13), Bulgaria (2), Canada (3), Chile (1), China (98), Czech Repoublic (1), Denmark (2), Egypt (12), France (6), Germany (19), Greece (11), Hungary (5), India (15), Indonesia (2), Iran (4), Israel (1), Italy (51), Japan (35), Jordan (1), Malaysia (2), Mexico (3), Moldova (1), Netherlands (3), Nigeria (1), Pakistan (1), Philippines (2), Poland (1), Portugal (2), Qatar (1), Romania (6), Russia (2), Saudi Arabia (4), Singapore (1), South Korea (11), Spain (20), Sri Lanka (1), Sudan (1), Sweden (1), Switzerland (1), Thailand (4), Turkey (21), Ukraine (3), United Kingdom (17), and United States (56).

China

Guang-Wen Cao, *Shanghai*
En-Qiang Chen, *Chengdu*
Gong-Ying Chen, *Hangzhou*
Jin-lian Chen, *Shanghai*
Jun Chen, *Changsha*
Alfred Cheng, *Hong Kong*
Chun-Ping Cui, *Beijing*
Shuang-Suo Dang, *Xi'an*
Ming-Xing Ding, *Jinhua*
Zhi-Jun Duang, *Dalian*
He-Bin Fan, *Wuhan*
Xiao-Ming Fan, *Shanghai*
James Yan Yue Fung, *Hong Kong*
Yi Gao, *Guangzhou*
Zuo-Jiong Gong, *Wuhan*
Zhi-Yong Guo, *Guangzhou*
Shao-Liang Han, *Wenzhou*
Tao Han, *Tianjin*
Jin-Yang He, *Guangzhou*
Ming-Liang He, *Hong Kong*
Can-Hua Huang, *Chengdu*
Bo Jin, *Beijing*
Shan Jin, *Hohhot*
Hui-Qing Jiang, *Shijiazhuang*
Wan-Yee Joseph Lau, *Hong Kong*
Guo-Lin Li, *Changsha*
Jin-Jun Li, *Shanghai*
Qiang Li, *Jinan*
Sheng Li, *Jinan*
Zong-Fang Li, *Xi'an*
Xu Li, *Guangzhou*
Xue-Song Liang, *Shanghai*
En-Qi Liu, *Xi'an*
Pei Liu, *Shenyang*
Zhong-Hui Liu, *Changchun*
Guang-Hua Luo, *Changzhou*
Yi Lv, *Xi'an*
Guang-Dong Pan, *Liuzhou*
Wen-Sheng Pan, *Hangzhou*
Jian-Min Qin, *Shanghai*
Wai-Kay Seto, *Hong Kong*
Hong Shen, *Changsha*
Xiao Su, *Shanghai*
Li-Ping Sun, *Beijing*
Wei-Hao Sun, *Nanjing*
Xue-Ying Sun, *Harbin*
Hua Tang, *Tianjin*
Ling Tian, *Shanghai*
Eric Tse, *Hong Kong*
Guo-Ying Wang, *Changzhou*
Yue Wang, *Beijing*
Shu-Qiang Wang, *Chengdu*
Mary MY Waye, *Hong Kong*
Hong-Shan Wei, *Beijing*
Danny Ka-Ho Wong, *Hong Kong*
Grace Lai-Hung Wong, *Hong Kong*
Bang-Fu Wu, *Dongguan*
Feng Wu, *Chongqing*
Xiong-Zhi Wu, *Tianjin*
Chun-Fang Xu, *Suzhou*
Rui-An Xu, *Quanzhou*
Rui-Yun Xu, *Guangzhou*
Wei-Li Xu, *Shijiazhuang*
Shi-Ying Xuan, *Qingdao*
Ming-Xian Yan, *Jinan*
Lv-Nan Yan, *Chengdu*
Jin Yang, *Hangzhou*
Ji-Hong Yao, *Dalian*
Winnie Yeo, *Hong Kong*
Zheng Zeng, *Beijing*
Qi Zhang, *Hangzhou*
Shi-Jun Zhang, *Guangzhou*
Xiao-Lan Zhang, *Shijiazhuang*
Xiao-Yong Zhang, *Guangzhou*
Xin-Chen Zhang, *Harbin*
Yong Zhang, *Xi'an*
Hong-Chuan Zhao, *Hefei*
Ming-Hua Zheng, *Wenzhou*
Yu-Bao Zheng, *Guangzhou*
Ren-Qian Zhong, *Shanghai*
Fan Zhu, *Wuhan*
Xiao Zhu, *Dongguan*

Czech Repoublic

Kamil Vyslouzil, *Olomouc*

Denmark

Henning Gronbaek, *Aarhus*
Christian Mortensen, *Hvidovre*

Egypt

Ihab T Abdel-Raheem, *Damanhour*
NGB G Bader EL Din, *Cairo*
Hatem Elalfy, *Mansoura*
Mahmoud M El-Bendary, *Mansoura*
Mona El SH El-Raziky, *Cairo*
Mohammad El-Sayed, *Cairo*
Yasser M Fouad, *Minia*
Mohamed AA Metwally, *Benha*
Hany Shehab, *Cairo*
Mostafa M Sira, *Shebin El-koom*
Ashraf Taye, *Minia*
MA Ali Wahab, *Mansoura*

France

Laurent Alric, *Toulouse*
Sophie Conchon, *Nantes*
Daniel J Felmlee, *Strasbourg*
Herve Lerat, *Creteil*
Dominique Salmon, *Paris*
Jean-Pierre Vartanian, *Paris*

Germany

Laura E Buitrago-Molina, *Hannover*
Enrico N De Toni, *Munich*
Oliver Ebert, *Muenchen*
Rolf Gebhardt, *Leipzig*
Janine V Hartl, *Regensburg*
Sebastian Hinz, *Kiel*
Benjamin Juntermanns, *Essen*
Roland Kaufmann, *Jena*
Viola Knop, *Frankfurt*
Veronika Lukacs-Kornek, *Homburg*
Benjamin Maasoumy, *Hannover*
Jochen Mattner, *Erlangen*
Nadja M Meindl-Beinker, *Mannheim*
Ulf P Neumann, *Aachen*
Margarete Odenthal, *Cologne*
Yoshiaki Sunami, *Munich*
Christoph Roderburg, *Aachen*
Frank Tacke, *Aachen*
Yuchen Xia, *Munich*

Greece

Alex P Betrosian, *Athens*
George N Dalekos, *Larissa*
Ioanna K Delladetsima, *Athens*
Nikolaos K Gatselis, *Larissa*
Stavros Gourgiotis, *Athens*
Christos G Savopoulos, *Thessaloniki*
Tania Siahanidou, *Athens*
Emmanouil Sinakos, *Thessaloniki*
Nikolaos G Symeonidi, *Thessaloniki*
Konstantinos C Thomopoulos, *Larissa*
Konstantinos Tziomalos, *Thessaloniki*

Hungary

Gabor Banhegyi, *Budapest*
Peter L Lakatos, *Budapest*
Maria Papp, *Debrecen*
Ferenc Sipos, *Budapest*
Zsolt J Tulassay, *Budapest*

India

Deepak N Amarapurkar, *Mumbai*
Girish M Bhopale, *Pune*
Sibnarayan Datta, *Tezpur*
Nutan D Desai, *Mumbai*
Sorabh Kapoor, *Mumbai*
Jaswinder S Maras, *New Delhi*
Nabeen C Nayak, *New Delhi*
C Ganesh Pai, *Manipal*
Amit Pal, *Chandigarh*
K Rajeshwari, *New Delhi*
Anup Ramachandran, *Vellore*
D Nageshwar Reddy, *Hyderabad*
Shivaram P Singh, *Cuttack*
Ajith TA, *Thrissur*
Balasubramaniyan Vairappan, *Pondicherry*

Indonesia

Cosmas RA Lesmana, *Jakarta*
Neneng Ratnasari, *Yogyakarta*

Iran

Seyed M Jazayeri, *Tehran*
Sedigheh Kafi-Abad, *Tehran*
Iradj Maleki, *Sari*
Fakhraddin Naghibalhossaini, *Shiraz*

Israel

Stephen DH Malnick, *Rehovot*

Italy

Francesco Angelico, *Rome*

Alfonso W Avolio, *Rome*
Francesco Bellanti, *Foggia*
Marcello Bianchini, *Modena*
Guglielmo Borgia, *Naples*
Mauro Borzio, *Milano*
Enrico Brunetti, *Pavia*
Valeria Cento, *Roma*
Beatrice Conti, *Rome*
Francesco D'Amico, *Padova*
Samuele De Minicis, *Fermo*
Fabrizio De Ponti, *Bologna*
Giovan Giuseppe Di Costanzo, *Napoli*
Luca Fabris, *Padova*
Giovanna Ferraioli, *Pavia*
Andrea Galli, *Florencee*
Matteo Garcovich, *Rome*
Edoardo G Giannini, *Genova*
Rossano Girometti, *Udine*
Alessandro Granito, *Bologna*
Alberto Grassi, *Rimini*
Alessandro Grasso, *Savona*
Francesca Guerrieri, *Rome*
Quirino Lai, *Aquila*
Andrea Lisotti, *Bologna*
Marcello F Maida, *Palermo*
Lucia Malaguarnera, *Catania*
Andrea Mancuso, *Palermo*
Luca Maroni, *Ancona*
Francesco Marotta, *Milano*
Pierluigi Marzuillo, *Naples*
Sara Montagnese, *Padova*
Giuseppe Nigri, *Rome*
Claudia Piccoli, *Foggia*
Camillo Porta, *Pavia*
Chiara Raggi, *Rozzano (MI)*
Maria Rendina, *Bari*
Maria Ripoli, *San Giovanni Rotondo*
Kryssia I Rodriguez-Castro, *Padua*
Raffaella Romeo, *Milan*
Amedeo Sciarra, *Milano*
Antonio Solinas, *Sassari*
Aurelio Sonzogni, *Bergamo*
Giovanni Squadrito, *Messina*
Salvatore Sutti, *Novara*
Valentina Svicher, *Rome*
Luca Toti, *Rome*
Elvira Verduci, *Milan*
Umberto Vespasiani-Gentilucci, *Rome*
Maria A Zocco, *Rome*

Japan

Yasuhiro Asahina, *Tokyo*
Nabil AS Eid, *Takatsuki*
Kenichi Ikejima, *Tokyo*
Shoji Ikuo, *Kobe*
Yoshihiro Ikura, *Takatsuki*
Shinichi Ikuta, *Nishinomiya*
Kazuaki Inoue, *Yokohama*
Toshiya Kamiyama, *Sapporo*
Takanobu Kato, *Tokyo*
Saiho Ko, *Nara*
Haruki Komatsu, *Sakura*
Masanori Matsuda, *Chuo-city*
Yasunobu Matsuda, *Niigata*
Yoshifumi Nakayama, *Kitakyushu*
Taichiro Nishikawa, *Kyoto*
Satoshi Oeda, *Saga*
Kenji Okumura, *Urayasu*
Michitaka Ozaki, *Sapporo*
Takahiro Sato, *Sapporo*
Junichi Shindoh, *Tokyo*
Ryo Sudo, *Yokohama*
Atsushi Suetsugu, *Gifu*
Haruhiko Sugimura, *Hamamatsu*
Reiji Sugita, *Sendai*
Koichi Takaguchi, *Takamatsu*
Shinji Takai, *Takatsuki*
Akinobu Takaki, *Okayama*
Yasuhito Tanaka, *Nagoya*
Takuji Tanaka, *Gifu City*
Atsunori Tsuchiya, *Niigata*
Koichi Watashi, *Tokyo*
Hiroshi Yagi, *Tokyo*
Taro Yamashita, *Kanazawa*
Shuhei Yoshida, *Chiba*
Hitoshi Yoshiji, *Kashihara*

Jordan

Kamal E Bani-Hani, *Zarqa*

Malaysia

Peng Soon Koh, *Kuala Lumpur*
Yeong Yeh Lee, *Kota Bahru*

Mexico

Francisco J Bosques-Padilla, *Monterrey*
María de F Higuera-de la Tijera, *Mexico City*
José A Morales-Gonzalez, *México City*

Moldova

Angela Peltec, *Chishinev*

Netherlands

Wybrich R Cnossen, *Nijmegen*
Frank G Schaap, *Maastricht*
Fareeba Sheedfar, *Groningen*

Nigeria

CA Asabamaka Onyekwere, *Lagos*

Pakistan

Bikha Ram Devrajani, *Jamshoro*

Philippines

Janus P Ong, *Pasig*
JD Decena Sollano, *Manila*

Poland

Jacek Zielinski, *Gdansk*

Portugal

Rui T Marinho, *Lisboa*
Joao B Soares, *Braga*

Qatar

Reem Al Olaby, *Doha*

Romania

Bogdan Dorobantu, *Bucharest*
Liana Gheorghe, *Bucharest*
George S Gherlan, *Bucharest*
Romeo G Mihaila, *Sibiu*
Bogdan Procopet, *Cluj-Napoca*
Streba T Streba, *Craiova*

Russia

Anisa Gumerova, *Kazan*
Pavel G Tarazov, *St.Petersburg*

Saudi Arabia

Abdulrahman A Aljumah, *Riyadh*
Ihab MH Mahmoud, *Riyadh*
Ibrahim Masoodi, *Riyadh*
Mhoammad K Parvez, *Riyadh*

Singapore

Ser Yee Lee, *Singapore*

South Korea

Young-Hwa Chung, *Seoul*
Dae-Won Jun, *Seoul*
Bum-Joon Kim, *Seoul*
Do Young Kim, *Seoul*
Ji Won Kim, *Seoul*
Moon Young Kim, *Wonu*
Mi-Kyung Lee, *Suncheon*
Kwan-Kyu Park, *Daegu*
Young Nyun Park, *Seoul*
Jae-Hong Ryoo, *Seoul*
Jong Won Yun, *Kyungsan*

Spain

Ivan G Marina, *Madrid*
Juan G Acevedo, *Barcelona*
Javier Ampuero, *Sevilla*
Jaime Arias, *Madrid*
Andres Cardenas, *Barcelona*
Agustin Castiella, *Mendaro*
Israel Fernandez-Pineda, *Sevilla*
Rocio Gallego-Duran, *Sevilla*
Rita Garcia-Martinez, *Barcelona*

World Journal of Hepatology

Contents

Monthly Volume 6 Number 7 July 27, 2014

World Journal of Hepatology
Volume 6 Number 7 July 27, 2014

Contents

AIM AND SCOPE

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJH covers topics concerning 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. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

INDEXING/ABSTRACTING

World Journal of Hepatology is now indexed in PubMed Central, PubMed, Digital Object Identifier, Directory of Open Access Journals, and Scopus.

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

Xiu-Xia Song, Vice Director
World Journal of Hepatology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
July 27, 2014

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm

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

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.453

World J Hepatol 2014 July 27; 6(7): 453-463
ISSN 1948-5182 (online)

REVIEW

Colorectal hepatic metastasis: Evolving therapies

Francisco Igor B Macedo, Tafadzwa Makarawo

Francisco Igor B Macedo, Tafadzwa Makarawo, Department of Surgery, Providence Hospital and Medical Centers, Southfield, MI 48075, United States
Author contributions: Macedo FI and Makarawo T contributed equally in all steps of this paper.
Correspondence to: Francisco Igor B Macedo, MD, Department of Surgery, Providence Hospital and Medical Centers, 16001 W Nine Mile Rd, Southfield, MI 48075, United States. franciscoigor.macedo@stjohn.org
Telephone: +1-786-9994754 Fax: +1-786-9994754
Received: November 28, 2013 Revised: February 23, 2014
Accepted: May 31, 2014
Published online: July 27, 2014

Abstract

The approach for colorectal hepatic metastasis has advanced tremendously over the past decade. Multidrug chemotherapy regimens have been successfully introduced with improved outcomes. Concurrently, adjunct multimodal therapies have improved survival rates, and increased the number of patients eligible for curative liver resection. Herein, we described major advancements of surgical and oncologic management of such lesions, thereby discussing modern chemotherapeutic regimens, adjunct therapies and surgical aspects of liver resection.

Key words: Colorectal cancer; Hepatic metastasis; Hepatectomy; Survival; Chemotherapy; 5-fluorouracil leucovorin and oxaliplatin

Core tip: The management of colorectal hepatic metastasis is complex, and should involve a multidisciplinary tumor board involving specialized medical and surgical oncologists. Although liver resection still remains as the key step in the management of liver metastasis, the introduction of new chemotherapeutic regimens and recent adjunct therapies, including radiofrequency ablation, cryotherapy and radioembolization improved patient care, and prolonged survival in patients with unresectable disease.

Macedo FI, Makarawo T. Colorectal hepatic metastasis: Evolving therapies. *World J Hepatol* 2014; 6(7): 453-463 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/453.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.453

INTRODUCTION

Colon cancer is the third most common malignancy in the United States, and comprising around 10% of all cancer-related mortality[1]. Most disease-related mortality is associated with metastatic disease. Approximately 25% of patients is diagnosed with metastases at initial presentation, and around 50% will present metastases during the clinical management of the disease[2,3]. The survival for untreated colorectal hepatic metastasis (CHM) are dismal with medial survival estimated in only 6 to 9 mo[4].

Although liver resection still remains as the most important modality in the treatment of CHM, the introduction of recent adjunct therapies, including radiofrequency ablation (RFA), cryotherapy and radioembolization improved patient care, and prolonged survival in patients with unresectable disease. Concurrently, the evolution of chemotherapy with the introduction of multidrug therapy optimized response rates, and expanded the number of surgical candidates for curative liver resection. Herein, we describe the current management of CHM, thereby discussing major advancements in chemotherapeutic regimens, adjunct therapies and surgical technique, and describe paradigm changes in resectability and outcomes.

DETERMINATION OF STRATEGY

The management of CHM is complex, and should involve a multidisciplinary tumor board including oncologists, radiologists, colorectal and hepatobiliary surgeons. Clinical and laboratory suspicion of metastasis should be routinely confirmed by radiological imaging. Options

available include computed tomography (CT), ultrasound, fluorodeoxyglucose-positron emission tomography (PET), and magnetic resonance imaging (MRI). Multi-detector CT is widely available, and is routinely used for detection of CHM[5]. MRI is being used more commonly, and provides better visualization of liver lesions as compared to CT by some experts[6]. PET scan is usually associated with CT (PET-CT), and is superior to CT or MRI for identification of equivocal lesions, metastases, and local recurrence, prior to resection of metastatic disease[7-10].

Several prognostic factors should be considered during definition of therapeutic strategy, including: staging of the primary tumor, interval diagnosis between the primary and metastatic lesions, number and size of metastases, presence of surgical margins and extrahepatic recurrence, and elevated biochemical markers such as carcinoembrionic antigen, alkaline phosphatase, and albumin[11-15]. The most important decision for definition of the therapeutic plan is defined based on resectability of metastatic disease. Patients should be stratified as suitable for resection, potentially resectable after chemotherapy and/or adjunct therapies, and those with unresectable disease.

MANAGEMENT OF RESECTABLE DISEASE

Liver resection continues to be the most crucial step in the management of CHM, potentially offering definitive treatment to a subset of patients. The use of chemotherapy is used as an adjunct therapy, thereby enhancing the 5-year survival at approximately 37%-58%[16,17]. Assessment of resectability is based on the volume of future remnant liver with adequate vascular inflow and outflow and biliary drainage[18]. For patients with normal liver function, 20% of remnant tissue is required, whereas in the presence of steatosis and cirrhosis, 30% and 40% of residual liver is necessary, respectively. Negative margins of 1-cm is associated with improved outcomes, and is currently recommended by most experts[19,20]. Contraindications to resection include uncontrollable extrahepatic disease, extensive lymph node involvement, including retroperitoneal or mediastinal nodes, bone or central nervous system metastases[21]. Local predictors of unresectability are determined by hepatic vascular involvement, and bilaterally, that would leave an inadequate functional liver remnant. Perioperative combination with chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) regimen given 3 mo prior and 3 mo following resection of metastases enhances survival by 8% at 3 years[22]. Neoadjuvant chemotherapy for patients with resectable liver metastases is still under investigation, and currently, remains controversial. Another topic of major debate is regarding the timing of the colectomy relative to the hepatectomy in cases of synchronous CHM. Typically, the primary colorectal cancer (CRC) is resected first, however in select cases where the liver disease is marginally resectable and primary CRC is small, the liver resection may be considered as initial approach to avoid progression of CHM. Combined resections are associated with shorter hospital stay and less morbidity, with similar 5-year survival and technically more challenging[23].

MANAGEMENT OF POTENTIALLY RESECTABLE DISEASE

Initially unresectable liver metastases can become resectable after being downsized by neoadjuvant chemotherapy, and, in such cases, resection may be advocated. Bismuth *et al*[24] reported the first experience with downstaging of unresectable lesions to resectable. They found similar outcomes to those patients with initially resectable lesions[25]. Nuzzo *et al*[26] found similar operative complications, and 3-year overall survival between initially resectable patients and those with initially unresectable but downstaged lesions. Subsequent reports showed conversion rates between 30%-50% with the combination of hepatic artery infusional fluoxuridine with systemic chemotherapy[27,28]. In these patients, response to initial chemotherapy appears to be a predictor of outcome[29].

Initial experience with addition of a vascular endothelial growth factor (VEGF) or epidermal growth factor receptor (EGFR) target agent (bevacizumab or cetuximab, respectively) is associated with higher resection rates in patients with initially unresectable disease. Resection is usually performed 5-8 wk after the last chemotherapy cycle with cetuximab or bevacizumab, respectively. The decision for resectability in these patients is often challenging, and involves a multidisciplinary team, depending on the experience of hepatobiliary surgeon and assessment for sufficient remnant liver. Many surgeons and oncologists would offer resection as soon as the lesion has become resectable, whereas others usually continue chemotherapy for 4 to 9 mo regardless of the response[30].

Several techniques have been recently introduced aiming at downsizing metastatic disease and improving resectability, including radioembolization, intra-arterial chemotherapy, and local ablation techniques, especially radiofrequency ablation. These adjunct modalities will be discussed separately.

MANAGEMENT OF UNRESECTABLE DISEASE

The majority of patients with CRC and concurrent metastasis has unresectable disease. However, due advances in systemic therapy, the survival of these patients is progressively improving[31]. The median survival is improved, estimated in up to 24 mo.

The approach for unresectable metastatic disease with synchronous CRC is still controversial. Resection of the bowel cancer initially is associated with precise definition of nodal and peritoneal status, prevention of local complications, the theoretical advantage of reduced total-body tumor load as well as psychological benefits for the patient[11]. However, the chemotherapy-first approach

is considered better by other experts due to the avoidance of postoperative morbidity and mortality, potential downstaging of unresectable CHM to resectability, and data showing equivalent survival benefits[11].

Monoclonal therapy against VEGF and EGFR should be considered especially in refractory cases, and will be further discussed in this review. For non-curative therapy of CHM, in addition to using the standard FOLFOX or FOLFIRI chemotherapy regimens, single agent strategies have been used with survival benefits as evidenced by the MRC FOCUS (using 5-FU-LV) and CAIRO (using capecitabine) trials[32-34].

TREATMENT MODALITIES

Resection

Surgery is the key step in the management of patients with CHM and represents the only chance for cure. Resection of CHM is considered a relatively safe operation with an operative mortality less than 5% by most recent series[30,35,36]. In high volume centers, median hospital stay ranges between 5 and 10 d for minor and major resections[36,37]. With increased outcomes, hepatectomies are now safely performed in elderly patients[38].

In cases of multiple, bilateral CHM, surgical options include: parenchyma-sparing approaches, and two-stage hepatectomy. In a two-stage operation, a portion of the liver disease is removed, and the contralateral portal vein is occluded, followed by 1 to 3 mo interval to allow for hypertrophy of the remaining liver and a curative-intent, second-stage hepatectomy. In such cases, the portal vein is occluded intraoperatively or subsequently by percutaneous embolization. Most experts perform minor segment resection first followed by resection of major liver. The minor-first approach spares the patient with progressive disease to undergo a major hepatectomy.

Within 2 years, most patients developed a recurrence[11,39]. Approximately 40% of them are eligible to undergo reoperation. The 5-year survival after first and second hepatectomies was 47% and 32%, respectively[40].

The experience with laparoscopic resection of CHM is yet minimal. Buell *et al*[41] and Mala *et al*[42] demonstrated tumor clearance, feasibility and safety of laparoscopic liver resection in 31 and 42 patients with CHM, respectively[41,42]. Long-term outcomes compared to open approach remains unknown.

CHEMOTHERAPY

Although chemotherapy plays a vital role in managing resectable and unresectable CHM, the timing of delivery is still controversial. For resectable disease, delivery of chemotherapy may be offered before colon resection (pre-operative), after colon resection but before liver resection (peri-operative) or after both resections (post-operative).

Pre- and peri-operative chemotherapy for resectable disease

For patients with potentially resectable CHM, response to chemotherapy has become an important adjunct in deciding whether to proceed with surgery. Typically, most tumors either reduce in size or remain unchanged following chemotherapy[22,43-46].

The recommended approach of delivering neoadjuvant chemotherapy to patients with resectable CHM consists of a 2-3 mo course of FOLFOX in order to limit chemotherapy-induced liver injury[46]. Chemotherapy application is considered safe to be used in patients with intact colorectal tumors[47]. In order to avoid difficulties locating both colorectal tumors and CHM that respond well to systemic chemotherapy, it would be prudent to mark the lesions before initiation of therapy, typically done using India ink tattoo or metallic coils placed by interventional radiology[48]. The disadvantages of pre-operative chemotherapy application include the development of new extrahepatic lesions[49] as well as a possible increased incidence of post-operative sequelae[22].

Adjuvant chemotherapy

The application of 5-FU-based chemotherapy post-CHM resection is established in most clinical practice despite prospective data limited to only two studies[50-52]. Pooled analysis of these two trials demonstrated a trend towards longer disease-free survival but no difference in median progression-free survival or overall survival. At present, there is no role for irinotecan-containing chemotherapy regimen (FOLFIRI) following hepatic resection with no benefit demonstrated when compared to 5-FU based regimens[53].

The application of systemic chemotherapy for CHM is associated with hepatotoxicity, a sequelae that has been recognized to increase the risk of peri- and post-operative mortality for CHM resection candidates. Amongst these hepatotoxic sequelae are hepatic steatosis seen in 30%-47% of patients on 5-FU[17], non-alcoholic steatohepatitis (NASH) in 12%-25% of patients on irinotecan[18] and sinusoidal dilation in 78% of patient on oxaliplatin[37]. The impact of these hepatotoxic effects is somewhat varied, although it is clear that the irinotecan-associated NASH appears to be the most significant with established evidence of increased post-operative mortality due to liver failure. Although previously the recognition of these adverse reactions was the domain of oncologists, the significant impact on post-operative outcomes has made it imperative for surgeons to be mindful of them too before considering operative intervention.

MOLECULAR TARGETED THERAPIES

Monoclonal antibodies against VEGF and EGFR have added an additional therapeutic option for treatment in select patients when used in combination with chemotherapy. Evidence of the therapeutic benefit of this treatment modality was initially found using the anti-VEGF monoclonal antibody, bevacizumab, with findings of improved survival when used in combination with therapy of IFL (irinotecan, 5-FU and leucovorin)[54]. Additional studies have demonstrated similar benefits in response

rate, disease-free progression and overall survival of using bevacizumab in combination with 5-FU/LV alone and FOLFOX in the first-line and second-line settings respectively[29]. Bevacizumab has, however, been associated with a number of complications, most notably gastrointestinal perforation, risk of bleeding and wound healing problems. As a result, the use of this modality requires careful monitoring, with treatment withheld for 6-8 wk prior to resection[55,56].

Panitumumab and cetuximab are EGFR inhibitors that have also demonstrated benefits in treating patients with metastatic CRC. Benefits have particularly been found using cetuximab in chemorefractory patients, improving survival compared to standard therapies[57]. Indeed, similar to bevacizumab, cetuximab appears to have superior effects when used in combination with[29]. It also appears that EGFR inhibitors are most effective for non-mutated (wild-type) K-ras colorectal tumors[55]. The side-effect profile for anti-EGFR antibodies is less extensive, limited to acneiform rash and hypomagnesemia and allergic reactions with cetuximab only[29] and no significant hepatotoxic effects seen thus far.

ADJUNCT THERAPIES

With the role of surgical resection for CHM widely accepted, the roles of non-operative liver directed therapies continue to evolve. With numerous new adjunctive therapies coming to the fore in recent years producing encouraging outcomes (including downstaging of CHM and increasing survival), the decision to integrate these options into current practice is challenging. Broadly speaking, there are three non-operative, liver directed therapies in use; intra-arterial therapies, ablative therapies, and radiotherapies.

INTRA-ARTERIAL THERAPIES

The role of intra-arterial therapies continues to evolve. The delivery of intra-arterial therapies uses the principal that hepatic metastases deriving their blood supply from hepatic arteries[58,59]. Therefore, intra-arterial therapy enhances drug delivery to hepatic tumors, maximizing local tumor therapy and limiting systemic therapy with its side-effects.

Hepatic arterial infusion chemotherapy

The hepatic arterial infusion (HAI) modality delivers chemotherapy directly to the liver *via* intra-abdominal catheters or infusion pumps cannulating the gastroduodenal artery[60,61]. An intimate understanding of hepatobiliary anatomy by surgeons is required to avoid placement of these catheters within aberrant anatomy leading to organ underperfusion with associated peptic ulceration, pancreatitis or biliary sclerosis[62]. The complex technical skills for correct placement of these infusion pumps requires experience often attainable at high volume centers. The delivery of HAI may be initiated as soon as the first post-operative week provided the patient has recovered well. In the United States, the chemotherapeutic agent most commonly used for HAI is fluoxuridine (FUDR) due to its high uptake by the liver limiting systemic toxic effects[63], although the low toxicity benefit may be lost by concomitant systemic chemotherapy use[64]. Dexamethasone has been delivered in conjunction with FUDR HAI-therapy, reducing biliary sclerosis, increasing tumor response rate and patient survival[65]. In Europe, 5-FU based HAI chemotherapy has also been used with some success.

HAI in unresectable disease

The role of HAI chemotherapy in unresectable disease is yet to be defined. This is largely due to inconclusive evidence from trials regarding patient outcomes[66]. On one hand, HAI has been found to produce higher tumor response rates than systemic therapy alone, but on the other no significant survival advantage has been found *via* the numerous randomized trials performed so far[67]. The application of combination therapy of HAI and systemic chemotherapy as second-line therapy following failed conventional chemotherapy[68] or to downstage initially unresectable CHM[28] have been suggested roles for HAI.

HAI as adjuvant therapy

The evidence supporting adjuvant HAI-therapy is even less established. To date, there has only been evidence from a single RCT that demonstrated a significant survival advantage applying HAI chemotherapy over systemic chemotherapy in the adjuvant setting[69]. This subject is therefore under ongoing scrutiny in current studies assessing HAI chemotherapy *vs* modern chemotherapy regimens.

Radioembolization

Radioembolization (or selective internal radiation therapy; SIRT) delivers high-energy beta-emitting radiation locally to CHM, delivering its effects specifically on tumor vasculature and minimizing collateral hepatic damage[70]. At present, this modality is delivered *via* two forms; Yttrium-90 (^{90}Y)-labeled resin microspheres (SIR-Spheres®; Sirtex Medical, Sydney, Australia) and ^{90}Y-labeled glass microspheres (Therasphere®; MDS Nordion, Ottawa, Canada). Radioembolization therapy is performed by injecting radioactive microspheres designed to embolize into small vessels around the metastases *via* branches of the hepatic artery, usually using a percutaneous femoral approach and fluoroscopic monitoring[71].

The current benefits with radioembolization using ^{90}Y microspheres have been reduced tumor load of unresectable CHM particularly if refractory to conventional chemotherapy. Indeed, combing radioembolization with chemotherapy has produced longer tumor suppression compared to chemotherapy alone[72]. The results of the recently ended SIRFLOX trial evaluating the efficacy of first-line therapy of FOLFOX6 combined with SIR-Spheres® *vs* FOLFOX6 alone will hopefully provide ad-

ditional evidence in favor of this treatment strategy in patients with unresectable CHM[73,74].

Further, trials have also demonstrated similar benefits of ^{90}Y microspheres used in combination with other treatment modalities like HAI therapy, demonstrating a superior time to progression compared to HAI alone[73,74].

The evidence supporting the use of ^{90}Y glass microspheres in CHM is less extensive with limited research demonstrating CHM tumor regression in upto 88% of patients with chemo-refractory tumors treated with ^{90}Y glass microspheres[75]. The further assessment of ^{90}Y-glass microspheres as salvage therapy continues to be evaluated with an ongoing phase Ⅲ multicenter randomized trial (EPOCH trial) which will hopefully provide corroborative evidence in support of this modality[17]. The long-term toxicity effects of radioembolization techniques are yet to elucidated.

Chemoembolization

Chemoembolization [or transcatheter arterial chemoembolization (TACE)] is a form of transarterial therapy that also utilizes the principal of liver tumors' predominantly arterial supply, allowing for regional therapy to the tumors. Similar to HAI, TACE is delivered using selective angiographic techniques by injection of chemotherapeutic drug combined with embolic material resulting in selective ischemic and chemotherapeutic effects on the CHM[76].

At present, there is no standard approach to delivering TACE therapy, although the application of a newer approach, drug-eluting beads composed of irinotecan (DEBIRI®; Biocompatibles United Kingdom Ltd, Farnham, United Kingdom) is gaining wider acceptance through ongoing clinical trials[77-79]. Irinotecan is preferentially used in this modality due to its properties allowing for application to the beads. Administration of DEBIRI® occurs *via* a selective arterial catheter, depositing the beads adjacent to the CHM tumors. This allows for slow release of irinotecan locally to the tumors.

Although DEBIRI® is presently not approved by the United States Food and Drug Administration, there are promising early results on its efficacy and safety. Available clinical trials suggest that DEBIRI® treatment may be associated with a median survival time of 15-25 mo, which is broadly equivalent to the outcomes achieved for unresectable CHM with the use of best-practice systemic chemotherapy[76]. In addition, the majority of patients that had responded to TACE treatment had failed first-line chemotherapy regimens[76]. Further evidence from additional trials[78,80-82] have also found successful downstaging of unresectable CHM to resectable status with most trials describing minimal toxicity effects[76].

It must be mentioned that the majority of the available trials to date have methodological flaws, and their conclusions must be interpreted with caution. To address the lack of high-quality randomized comparative trials assessing DEBIRI® use, there is ongoing research to evaluate its benefits when used in combination with systemic chemotherapy.

ABLATION TECHNIQUES

Ablation techniques aim to induce local destruction of the CHM. At present, the exact role of ablative techniques in the treatment of CHM is unclear, although there have been suggestions that its roles may include to reduce tumor size minimizing the extent of liver resection required, adjunctive therapy for patients either unfit for surgery or with unresectable disease. Ablative approaches can be subdivided into cryoablation, RFA and microwave ablation.

Cryoablation

Cryoablation was the first thermal ablative modality attempted to treat unresectable hepatic malignancies[83]. Cryoablation (or cryosurgery) is induced by local delivery of liquid nitrogen or argon on a probe tip to the CHM, resulting in tumor destruction by intracellular ice crystals that form from the rapid cooling. The "iceball" that forms around the tip of the probe can be measured by real-time intraoperative ultrasound although there has been some suggestion that the tissue furthest away from the tip may not be cooled sufficiently to cause tissue destruction[17].

Cryotherapy applications

Cryoablation application appears to vary between institutions. In general, its primary use has been for the ablation of unresectable CHM. Despite initial thoughts that cryoablation could be used in patients with resectable CHM, high tumor recurrence following cryosurgery has tempered this enthusiasm. So far, previous research has demonstrated a modest 5 year survival of 26% but also low mortality rates of less than 5% following cryotherapy for CHM[84]. Cryoablation used in combination with surgery has also been shown to produce similar survival benefits to surgery alone in patients with initially unresectable CHM[85].

The application of cryotherapy to the remnant liver resection margins (edge cryotherapy) remains undecided. Although some authors have reported the decreased application of edge cryotherapy due to report higher complication rates than hepatic resection alone[17], other institutions have reported positive outcomes with this approach, finding potential cure of up to 13% of advanced unresectable CHM compared with resection alone.

Additional benefits of cryosurgery include its facility in treating bilobar CHM or recurrent hepatic tumors following resection in addition to evidence from animal models that shows decreased secretion of factors that stimulate growth of occult micrometastases following cryotherapy compared to post-surgical resection[86]. One of the shortcomings of cryoablation is its poor ability to destroy tumors next to larger blood vessels due to the "heat-sinking" effect[87], resulting in recurrence rates as high as 44%. Another disadvantage of this modality is that for unclear physiologic reasons, patients may suffer from a systematic

inflammatory response (cryoshock phenomenon)[82,88,89] associated with periprocedural deaths[88,89].

RFA

By far, the most extensively evaluated ablative approach is RFA. RFA is the most widely applied ablative modality due to ease and safety of application and inexpense of equipment[17]. This modality is applied by placing needles within and adjacent to CHM through which alternating electrical current is delivered at radiofrequency range generating heat to desiccate the tumors[90,91].

Application

Although RFA is in widespread use across many institutions internationally, a paucity of randomized controlled trials up to now has prevented the development of a consistent approach to its use. Indeed, to date, there are no RCTs comparing surgical resection with RFA in resectable CHM, a study that at present seems inconceivable and unethical considering established survival data from surgical resection. At present, most evidence from the retrospective studies available comparing RFA and resection has demonstrated the inferiority of RFA compared to surgical resection with increased local recurrence rates (16%-60% *vs* 0%-24%) and worse long-term survival[91,92].

At present, RFA is being used to treat unresectable CHM only, with no extrahepatic metastatic disease[93]. Tumors amenable to successful treatment with RFA have typically been solitary CHM or a few which are not close to large hepatic vessels[93]. Tumor size in particular has been limited to 3-cm due to the circumferential rim of ablation currently delivered by ablation probes being approximately 4-cm in diameter, a limitation that may be addressed with advancement of the technology. Overlapping ablations can be used to treat larger tumors although this has been associated with less successful complete ablation[94]. The presence of large blood vessels limits RFA efficacy because their high blood flow acts a "heat sink", protecting adjacent cells from thermal ablation[17].

RFA is delivered *via* open, laparoscopic or percutaneous approaches[93]. The application of ultrasound, CT and MRI are particularly important to guide the needle in the percutaneous approach while intraoperative ultrasound is an additional adjunct used to directly visualize the tumor in the operative approaches. It appears at present that RFA *via* laparotomy is associated with the lowest recurrence rate followed by laparoscopy, and finally by percutaneous approach. The trade-off of using the least invasive percutaneous approach must be weighed up against poor tumor visualization increasing the potential for recurrence. The surgical approaches are typically applied at the time of primary or hepatic metastasis tumor resection.

In addition to the aforementioned advantages of RFA, it has a relatively lower morbidity profile of < 10% independent of the approach used for delivery being surgical or percutaneous[95]. Amongst the complications that have been seen, thermal injury (bowel and biliary injury), mechanical (biliary and vessel injury) and septic (abscess and peritonitis) have been the most widely reported. A more infrequent presentation of post-ablative syndrome where patients suffer from self-limiting constitutional upset including malaise, febrile episodes, myalgia, nausea and vomiting has also been reported[93].

Microwave ablation

Microwave ablation (MWA) is a more recently developed technique used for CHM. MWA is applied *via* a microwave probe delivered into the tumor *via* image-guided percutaneous, or ultrasound guided surgical approaches. *Via* these probes, microwave radiation between 900 MHz and 2.4 GHz is delivered that causes polarized water molecules within the tissue to oscillate generating friction that produces heat that destroys tissue by coagulative necrosis[96].

MWA application

As this modality is relatively new, the evidence of its efficacy is limited and has included too many different liver tumor types particularly hepatocellular carcinoma. The exact application of MWA for CHM is therefore still unclear. Although reported local recurrence rates have been extremely variable ranging from 3% to 50%, encouraging evidence from the largest series reported rates as low as 3% and 6%[97,98]. Further research would therefore provide the evidence to define its role as an ablative therapy in CHM management.

The purported advantages of MWA have been the more extensive nature of tissue destruction created by the heating mechanism generated by this technique. This mechanism also appears to be less prone to the "heat-sink" effect seen with RFA therapy[99]. There has also been suggestion that intra-operative hepatic inflow occlusion (Pringle maneuver) increases the size of ablated lesions[100]. Further, there appears to be reduced occurrence of charring using MWA and it creates larger ablation zones up to 6 cm away more rapidly than RFA[96]. Interestingly, there is now growing interest over a further method of cell death induced by microwaves characterized by normal-looking but non-viable cells. If indeed this is correct, this would have important implications in the post-procedure observation of the ablated tumors, requiring likely routine histopathology to differentiate seemingly viable tumor from completely ablated ones.

The complication rates from MWA range from 6% to 30%, most often associated with cases where laparotomy and additional procedures had been performed[90,97,98]. There are at present concerns of potential inadvertent injury to surrounding organs due to the higher energy generated by this modality.

STEREOTACTIC BODY RADIOTHERAPY

Stereotactic body radiotherapy (SBRT) is another newer technology that has generated growing interest for use in ablating CHM[101]. Unlike external beam radiation therapy (EBRT) which had previously been abandoned for use

in liver tumors due to the narrow therapeutic window between tumoricidial and hepatoxic effects, SBRT uses more modern technology that allows for safe treatment delivery in lung and liver with hypofractionation[101].

Application

SBRT is based on techniques used in stereotactic radiosurgery for brain tumors[101]. In this modality, the tumor location is identified using four-dimensional imaging that maps the target area accounting for patient movements during breathing. Gold seeds called fiducials are then placed within the tumor, which guide treatment. Using the predetermined tumor coordinates, high-dose radiation is delivered over a relatively shorter duration compared to conventional EBRT.

Although encouraging evidence of tumor local control rates as high as > 90% have been demonstrated in lung tumors using SBRT[102,103], its application in liver tumors specifically CHM is still under scrutiny with few well-designed studies presently available in current literature. The optimum radiation dosage is also undetermined, although it appears that a higher dose of up to 60 Gy is most effective, eliminating high local progression rates seen at lower doses[104], maximizing tumor response rate (up to 90%) and 2-year local control rate of 100%.

Although the treatment is focused, it does not eliminate surrounding toxicity. Specifically, acute gastrointestinal and liver toxicity in addition to chest wall pain have been reported side effects of the therapy. In addition, and more importantly, although there is some early evidence of local tumor control with SBRT, it is not yet been demonstrated to significantly impact survival.

However, the encouraging early results have lead to the assertion that SBRT be considered as an option in patients not offered surgery after chemotherapy to locally ablate their CHM[101].

CONCLUSION

The management of CHM is complex, and should involve a multidisciplinary tumor board involving specialized medical and surgical oncologists. Although overall survival has increased tremendously over the last 5 years with the introduction of adjunct therapies, more efficient chemotherapeutic regimens still need to be discovered. Concurrently, the criteria for resection is much more liberal and should be based on functional remnant liver volume. Even in situations where multiple, bilobar liver metastases are present, resection may be a considered option. Both basic studies and prospective trials are necessary to further understand the molecular aspects of colorectal hepatic metastasis, and therefore improve outcomes.

REFERENCES

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

2 **Steele G**, Ravikumar TS. Resection of hepatic metastases from colorectal cancer. Biologic perspective. *Ann Surg* 1989; **210**: 127-138 [PMID: 2667471 DOI: 10.1097/00000658-198908000-00001]

3 **Mohammad WM**, Balaa FK. Surgical management of colorectal liver metastases. *Clin Colon Rectal Surg* 2009; **22**: 225-232 [PMID: 21037813 DOI: 10.1055/s-0029-1242462]

4 **Scheele J**, Stangl R, Altendorf-Hofmann A. Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. *Br J Surg* 1990; **77**: 1241-1246 [PMID: 2253003 DOI: 10.1002/bjs.1800771115]

5 **Kamel IR**, Fishman EK. Recent advances in CT imaging of liver metastases. *Cancer J* 2004; **10**: 104-120 [PMID: 15130270 DOI: 10.1097/00130404-200403000-00006]

6 **Braga L**, Guller U, Semelka RC. Modern hepatic imaging. *Surg Clin North Am* 2004; **84**: 375-400 [PMID: 15062651 DOI: 10.1016/S0039-6109(03)00227-5]

7 **Adams RB**, Aloia TA, Loyer E, Pawlik TM, Taouli B, Vauthey JN. Selection for hepatic resection of colorectal liver metastases: expert consensus statement. *HPB* (Oxford) 2013; **15**: 91-103 [PMID: 23297719 DOI: 10.1111/j.1477-2574.2012.00557.x]

8 **Chen LB**, Tong JL, Song HZ, Zhu H, Wang YC. (18)F-DG PET/CT in detection of recurrence and metastasis of colorectal cancer. *World J Gastroenterol* 2007; **13**: 5025-5029 [PMID: 17854148]

9 **Schmidt GP**, Baur-Melnyk A, Haug A, Utzschneider S, Becker CR, Tiling R, Reiser MF, Hermann KA. Whole-body MRI at 1.5 T and 3 T compared with FDG-PET-CT for the detection of tumour recurrence in patients with colorectal cancer. *Eur Radiol* 2009; **19**: 1366-1378 [PMID: 19190917 DOI: 10.1007/s00330-008-1289-y]

10 **Truant S**, Huglo D, Hebbar M, Ernst O, Steinling M, Pruvot FR. Prospective evaluation of the impact of [18F]fluoro-2-deoxy-D-glucose positron emission tomography of resectable colorectal liver metastases. *Br J Surg* 2005; **92**: 362-369 [PMID: 15672427 DOI: 10.1002/bjs.4843]

11 **Haddad AJ**, Bani Hani M, Pawlik TM, Cunningham SC. Colorectal liver metastases. *Int J Surg Oncol* 2011; **2011**: 285840 [PMID: 22312501 DOI: 10.1155/2011/285840]

12 **Fong Y**, Fortner J, Sun RL, Brennan MF, Blumgart LH. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer: analysis of 1001 consecutive cases. *Ann Surg* 1999; **230**: 309-318; discussion 318-321 [PMID: 10493478 DOI: 10.1097/00000658-199909000-00004]

13 **Nordlinger B**, Guiguet M, Vaillant JC, Balladur P, Boudjema K, Bachellier P, Jaeck D. Surgical resection of colorectal carcinoma metastases to the liver. A prognostic scoring system to improve case selection, based on 1568 patients. Association Française de Chirurgie. *Cancer* 1996; **77**: 1254-1262 [PMID: 8608500]

14 **Schindl M**, Wigmore SJ, Currie EJ, Laengle F, Garden OJ. Prognostic scoring in colorectal cancer liver metastases: development and validation. *Arch Surg* 2005; **140**: 183-189 [PMID: 15724001 DOI: 10.1001/archsurg.140.2.183]

15 **Iwatsuki S**, Dvorchik I, Madariaga JR, Marsh JW, Dodson F, Bonham AC, Geller DA, Gayowski TJ, Fung JJ, Starzl TE. Hepatic resection for metastatic colorectal adenocarcinoma: a proposal of a prognostic scoring system. *J Am Coll Surg* 1999; **189**: 291-299 [PMID: 10472930 DOI: 10.1016/S1072-7515(99)00089-7]

16 **Choti MA**, Sitzmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Schulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. *Ann Surg* 2002; **235**: 759-766 [PMID: 12035031 DOI: 10.1097/00000658-200206000-00002]

17 **Abdalla EK**, Vauthey JN, Ellis LM, Ellis V, Pollock R, Broglio KR, Hess K, Curley SA. Recurrence and outcomes following hepatic resection, radiofrequency ablation, and combined resection/ablation for colorectal liver metastases. *Ann Surg*

2004; **239**: 818-825; discussion 825-827 [PMID: 15166961 DOI: 10.1097/01.sla.0000128305.90650.71]
18 **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]
19 **Cady B**, Jenkins RL, Steele GD, Lewis WD, Stone MD, McDermott WV, Jessup JM, Bothe A, Lalor P, Lovett EJ, Lavin P, Linehan DC. Surgical margin in hepatic resection for colorectal metastasis: a critical and improvable determinant of outcome. *Ann Surg* 1998; **227**: 566-571 [PMID: 9563547 DOI: 10.1097/00000658-199804000-00019]
20 **Scheele J**, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. *World J Surg* 1995; **19**: 59-71 [PMID: 7740812 DOI: 10.1007/BF00316981]
21 **Garden OJ**, Rees M, Poston GJ, Mirza D, Saunders M, Ledermann J, Primrose JN, Parks RW. Guidelines for resection of colorectal cancer liver metastases. *Gut* 2006; **55** Suppl 3: iii1-iii8 [PMID: 16835351 DOI: 10.1136/gut.2006.098053]
22 **Nordlinger B**, Sorbye H, Glimelius B, Poston GJ, Schlag PM, Rougier P, Bechstein WO, Primrose JN, Walpole ET, Finch-Jones M, Jaeck D, Mirza D, Parks RW, Collette L, Praet M, Bethe U, Van Cutsem E, Scheithauer W, Gruenberger T. Perioperative chemotherapy with FOLFOX4 and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC Intergroup trial 40983): a randomised controlled trial. *Lancet* 2008; **371**: 1007-1016 [PMID: 18358928 DOI: 10.1016/S0140-6736(08)60455-9]
23 **Hillingsø JG**, Wille-Jørgensen P. Staged or simultaneous resection of synchronous liver metastases from colorectal cancer--a systematic review. *Colorectal Dis* 2009; **11**: 3-10 [PMID: 18637099 DOI: 10.1111/j.1463-1318.2008.01625.x]
24 **Bismuth H**, Adam R, Lévi F, Farabos C, Waechter F, Castaing D, Majno P, Engerran L. Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. *Ann Surg* 1996; **224**: 509-520; discussion 520-522 [PMID: 8857855 DOI: 10.1097/00000658-199610000-00009]
25 **Adam R**, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, Giacchetti S, Paule B, Kunstlinger F, Ghémard O, Levi F, Bismuth H. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004; **240**: 644-657; discussion 657-658 [PMID: 15383792]
26 **Nuzzo G**, Giuliante F, Ardito F, Vellone M, Pozzo C, Cassano A, Giovannini I, Barone C. Liver resection for primarily unresectable colorectal metastases downsized by chemotherapy. *J Gastrointest Surg* 2007; **11**: 318-324 [PMID: 17458605 DOI: 10.1007/s11605-006-0070-2]
27 **Clavien PA**, Selzner N, Morse M, Selzner M, Paulson E. Downstaging of hepatocellular carcinoma and liver metastases from colorectal cancer by selective intra-arterial chemotherapy. *Surgery* 2002; **131**: 433-442 [PMID: 11935134 DOI: 10.1067/msy.2002.122374]
28 **Kemeny NE**, Melendez FD, Capanu M, Paty PB, Fong Y, Schwartz LH, Jarnagin WR, Patel D, D'Angelica M. Conversion to resectability using hepatic artery infusion plus systemic chemotherapy for the treatment of unresectable liver metastases from colorectal carcinoma. *J Clin Oncol* 2009; **27**: 3465-3471 [PMID: 19470932 DOI: 10.1200/JCO.2008.20.1301]
29 **Van Cutsem E**, Nordlinger B, Cervantes A. Advanced colorectal cancer: ESMO Clinical Practice Guidelines for treatment. *Ann Oncol* 2010; **21** Suppl 5: v93-v97 [PMID: 20555112 DOI: 10.1093/annonc/mdq222]
30 **Ito K**, Govindarajan A, Ito H, Fong Y. Surgical treatment of hepatic colorectal metastasis: evolving role in the setting of improving systemic therapies and ablative treatments in the 21st century. *Cancer J* 2010; **16**: 103-110 [PMID: 20404606 DOI: 10.1097/PPO.0b013e3181d7e8e5]
31 **Schwarz RE**, Berlin JD, Lenz HJ, Nordlinger B, Rubbia-Brandt L, Choti MA. Systemic cytotoxic and biological therapies of colorectal liver metastases: expert consensus statement. *HPB* (Oxford) 2013; **15**: 106-115 [PMID: 23297721 DOI: 10.1111/j.1477-2574.2012.00558.x]
32 **Falcone A**, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, Crinò L, Benedetti G, Evangelista W, Fanchini L, Cortesi E, Picone V, Vitello S, Chiara S, Granetto C, Porcile G, Fioretto L, Orlandini C, Andreuccetti M, Masi G. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J Clin Oncol* 2007; **25**: 1670-1676 [PMID: 17470860 DOI: 10.1200/JCO.2006.09.0928]
33 **Seymour MT**, Maughan TS, Ledermann JA, Topham C, James R, Gwyther SJ, Smith DB, Shepherd S, Maraveyas A, Ferry DR, Meade AM, Thompson L, Griffiths GO, Parmar MK, Stephens RJ. Different strategies of sequential and combination chemotherapy for patients with poor prognosis advanced colorectal cancer (MRC FOCUS): a randomised controlled trial. *Lancet* 2007; **370**: 143-152 [PMID: 17630037 DOI: 10.1016/S0140-6736(07)61087-3]
34 **Koopman M**, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FL, de Jong RS, Rodenburg CJ, Vreugdenhil G, Loosveld OJ, van Bochove A, Sinnige HA, Creemers GJ, Tesselaar ME, Slee PH, Werter MJ, Mol L, Dalesio O, Punt CJ. Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. *Lancet* 2007; **370**: 135-142 [PMID: 17630036 DOI: 10.1016/S0140-6736(07)61086-1]
35 **Fernandez FG**, Drebin JA, Linehan DC, Dehdashti F, Siegel BA, Strasberg SM. Five-year survival after resection of hepatic metastases from colorectal cancer in patients screened by positron emission tomography with F-18 fluorodeoxyglucose (FDG-PET). *Ann Surg* 2004; **240**: 438-447; discussion 447-450 [PMID: 15319715 DOI: 10.1097/01.sla.0000138076.72547.b1]
36 **Kato T**, Yasui K, Hirai T, Kanemitsu Y, Mori T, Sugihara K, Mochizuki H, Yamamoto J. Therapeutic results for hepatic metastasis of colorectal cancer with special reference to effectiveness of hepatectomy: analysis of prognostic factors for 763 cases recorded at 18 institutions. *Dis Colon Rectum* 2003; **46**: S22-S31 [PMID: 14530655]
37 **Minagawa M**, Makuuchi M, Torzilli G, Takayama T, Kawasaki S, Kosuge T, Yamamoto J, Imamura H. Extension of the frontiers of surgical indications in the treatment of liver metastases from colorectal cancer: long-term results. *Ann Surg* 2000; **231**: 487-499 [PMID: 10749608 DOI: 10.1097/00000658-200004000-00006]
38 **Fong Y**, Blumgart LH, Fortner JG, Brennan MF. Pancreatic or liver resection for malignancy is safe and effective for the elderly. *Ann Surg* 1995; **222**: 426-434; discussion 434-437 [PMID: 7574924]
39 **de Jong MC**, Pulitano C, Ribero D, Strub J, Mentha G, Schulick RD, Choti MA, Aldrighetti L, Capussotti L, Pawlik TM. Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients. *Ann Surg* 2009; **250**: 440-448 [PMID: 19730175]
40 **de Jong MC**, Mayo SC, Pulitano C, Lanella S, Ribero D, Strub J, Hubert C, Gigot JF, Schulick RD, Choti MA, Aldrighetti L, Mentha G, Capussotti L, Pawlik TM. Repeat curative intent liver surgery is safe and effective for recurrent colorectal liver metastasis: results from an international multi-institutional analysis. *J Gastrointest Surg* 2009; **13**: 2141-2151 [PMID: 19795176 DOI: 10.1007/s11605-009-1050-0]
41 **Buell JF**, Thomas MT, Rudich S, Marvin M, Nagubandi R, Ravindra KV, Brock G, McMasters KM. Experience with more than 500 minimally invasive hepatic procedures. *Ann Surg* 2008; **248**: 475-486 [PMID: 18791368]

42 **Mala T**, Edwin B, Rosseland AR, Gladhaug I, Fosse E, Mathisen O. Laparoscopic liver resection: experience of 53 procedures at a single center. *J Hepatobiliary Pancreat Surg* 2005; **12**: 298-303 [PMID: 16133696 DOI: 10.1007/s00534-005-0974-3]

43 **Chua TC**, Saxena A, Liauw W, Kokandi A, Morris DL. Systematic review of randomized and nonrandomized trials of the clinical response and outcomes of neoadjuvant systemic chemotherapy for resectable colorectal liver metastases. *Ann Surg Oncol* 2010; **17**: 492-501 [PMID: 19856028 DOI: 10.1245/s10434-009-0781-1]

44 **Adam R**, Pascal G, Castaing D, Azoulay D, Delvart V, Paule B, Levi F, Bismuth H. Tumor progression while on chemotherapy: a contraindication to liver resection for multiple colorectal metastases? *Ann Surg* 2004; **240**: 1052-1061; discussion 1061-1064 [PMID: 15570210 DOI: 10.1097/01.sla.0000145964.08365.01]

45 **Gallagher DJ**, Zheng J, Capanu M, Haviland D, Paty P, Dematteo RP, D'Angelica M, Fong Y, Jarnagin WR, Allen PJ, Kemeny N. Response to neoadjuvant chemotherapy does not predict overall survival for patients with synchronous colorectal hepatic metastases. *Ann Surg Oncol* 2009; **16**: 1844-1851 [PMID: 19224284 DOI: 10.1245/s10434-009-0348-1]

46 **Karoui M**, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, Rougier P, Nordlinger B. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; **243**: 1-7 [PMID: 16371728 DOI: 10.1097/01.sla.0000193603.26265.c3]

47 **Abdalla EK**, Bauer TW, Chun YS, D'Angelica M, Kooby DA, Jarnagin WR. Locoregional surgical and interventional therapies for advanced colorectal cancer liver metastases: expert consensus statements. *HPB* (Oxford) 2013; **15**: 119-130 [PMID: 23297723 DOI: 10.1111/j.1477-2574.2012.00597.x]

48 **Zalinski S**, Abdalla EK, Mahvash A, Vauthey JN. A marking technique for intraoperative localization of small liver metastases before systemic chemotherapy. *Ann Surg Oncol* 2009; **16**: 1208-1211 [PMID: 19214636 DOI: 10.1245/s10434-009-0328-5]

49 **Mitry E**, Fields AL, Bleiberg H, Labianca R, Portier G, Tu D, Nitti D, Torri V, Elias D, O'Callaghan C, Langer B, Martignoni G, Bouché O, Lazorthes F, Van Cutsem E, Bedenne L, Moore MJ, Rougier P. Adjuvant chemotherapy after potentially curative resection of metastases from colorectal cancer: a pooled analysis of two randomized trials. *J Clin Oncol* 2008; **26**: 4906-4911 [PMID: 18794541 DOI: 10.1200/JCO.2008.17.3781]

50 **Langer B**, Bleiberg H, Labianca R, Shepherd L, Nitti D, Marsoni S. Fluorouracil (FU) plus l-leucovorin (l-LV) versus observation after potentially curative resection of liver or lung metastases from colorectal cancer (CRC): results of the ENG (EORTC/NCIC CTG/GIVIO) randomized trial. *Proc Am Soc Clin Oncol* 2002; **21**: 592

51 **Portier G**, Elias D, Bouche O, Rougier P, Bosset JF, Saric J, Belghiti J, Piedbois P, Guimbaud R, Nordlinger B, Bugat R, Lazorthes F, Bedenne L. Multicenter randomized trial of adjuvant fluorouracil and folinic acid compared with surgery alone after resection of colorectal liver metastases: FFCD ACHBTH AURC 9002 trial. *J Clin Oncol* 2006; **24**: 4976-4982 [PMID: 17075115 DOI: 10.1200/JCO.2006.06.8353]

52 **Reddy SK**, Zorzi D, Lum YW, Barbas AS, Pawlik TM, Ribero D, Abdalla EK, Choti MA, Kemp C, Vauthey JN, Morse MA, White RR, Clary BM. Timing of multimodality therapy for resectable synchronous colorectal liver metastases: a retrospective multi-institutional analysis. *Ann Surg Oncol* 2009; **16**: 1809-1819 [PMID: 18979139 DOI: 10.1245/s10434-008-0181-y]

53 **Ychou M**, Hohenberger W, Thezenas S, Navarro M, Maurel J, Bokemeyer C, Shacham-Shmueli E, Rivera F, Kwok-Keung Choi C, Santoro A. A randomized phase III study comparing adjuvant 5-fluorouracil/folinic acid with FOLFIRI in patients following complete resection of liver metastases from colorectal cancer. *Ann Oncol* 2009; **20**: 1964-1970 [PMID: 19567451 DOI: 10.1093/annonc/mdp236]

54 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435 DOI: 10.1056/NEJMoa032691]

55 **Normanno N**, Tejpar S, Morgillo F, De Luca A, Van Cutsem E, Ciardiello F. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol* 2009; **6**: 519-527 [PMID: 19636327]

56 **Kabbinavar F**, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol* 2003; **21**: 60-65 [PMID: 12506171]

57 **Jonker DJ**, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C, Moore MJ. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007; **357**: 2040-2048 [PMID: 18003960 DOI: 10.1056/NEJMoa071834]

58 **Lucke B**, Breedis C, Woo ZP, Berwick L, Nowell P. Differential growth of blood-borne metastatic tumors in liver and lung (experiments with rabbit V-2 carcinoma). *Am J Pathol* 1951; **27**: 729-730 [PMID: 14846956]

59 **Ackerman NB**. The blood supply of experimental liver metastases. IV. Changes in vascularity with increasing tumor growth. *Surgery* 1974; **75**: 589-596 [PMID: 4840805]

60 **Ensminger WD**. Intrahepatic arterial infusion of chemotherapy: pharmacologic principles. *Semin Oncol* 2002; **29**: 119-125 [PMID: 11951209 DOI: 10.1053/sonc.2002.31679]

61 **Allen PJ**, Stojadinovic A, Ben-Porat L, Gonen M, Kooby D, Blumgart L, Paty P, Fong Y. The management of variant arterial anatomy during hepatic arterial infusion pump placement. *Ann Surg Oncol* 2002; **9**: 875-880 [PMID: 12417509 DOI: 10.1007/BF02557524]

62 **Stratmann SL**. Hepatic artery chemotherapy in the management of colorectal metastases. *Proc* (Bayl Univ Med Cent) 2002; **15**: 376-379 [PMID: 16333468]

63 **Sigurdson ER**, Ridge JA, Kemeny N, Daly JM. Tumor and liver drug uptake following hepatic artery and portal vein infusion. *J Clin Oncol* 1987; **5**: 1836-1840 [PMID: 3681370]

64 **Kemeny N**, Capanu M, D'Angelica M, Jarnagin W, Haviland D, Dematteo R, Fong Y. Phase I trial of adjuvant hepatic arterial infusion (HAI) with floxuridine (FUDR) and dexamethasone plus systemic oxaliplatin, 5-fluorouracil and leucovorin in patients with resected liver metastases from colorectal cancer. *Ann Oncol* 2009; **20**: 1236-1241 [PMID: 19233901 DOI: 10.1093/annonc/mdn769]

65 **Kemeny N**, Seiter K, Conti JA, Cohen A, Bertino JR, Sigurdson ER, Botet J, Chapman D, Mazumdar M, Budd AJ. Hepatic arterial floxuridine and leucovorin for unresectable liver metastases from colorectal carcinoma. New dose schedules and survival update. *Cancer* 1994; **73**: 1134-1142 [PMID: 8313315 DOI: 10.1002/1097-0142(19940215)73:4<1134::AID-CNCR2820730403>3.0.CO;2-V]

66 **Mocellin S**, Pilati P, Lise M, Nitti D. Meta-analysis of hepatic arterial infusion for unresectable liver metastases from colorectal cancer: the end of an era? *J Clin Oncol* 2007; **25**: 5649-5654 [PMID: 18065736 DOI: 10.1200/JCO.2007.12.1764]

67 **Kemeny NE**, Niedzwiecki D, Hollis DR, Lenz HJ, Warren RS, Naughton MJ, Weeks JC, Sigurdson ER, Herndon JE, Zhang C, Mayer RJ. Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal cancer: a randomized trial of efficacy, quality of life, and molecular markers (CALGB 9481). *J Clin Oncol* 2006; **24**: 1395-1403 [PMID: 16505413 DOI: 10.1200/JCO.2005.03.8166]

68 **Kemeny N**, Gonen M, Sullivan D, Schwartz L, Benedetti F, Saltz L, Stockman J, Fong Y, Jarnagin W, Bertino J, Tong W,

Paty P. Phase I study of hepatic arterial infusion of floxuridine and dexamethasone with systemic irinotecan for unresectable hepatic metastases from colorectal cancer. *J Clin Oncol* 2001; **19**: 2687-2695 [PMID: 11352961]

69 **Kemeny N**, Huang Y, Cohen AM, Shi W, Conti JA, Brennan MF, Bertino JR, Turnbull AD, Sullivan D, Stockman J, Blumgart LH, Fong Y. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. *N Engl J Med* 1999; **341**: 2039-2048 [PMID: 10615075 DOI: 10.1056/NEJM199912303412702]

70 **Kennedy AS**, Nutting C, Coldwell D, Gaiser J, Drachenberg C. Pathologic response and microdosimetry of (90)Y microspheres in man: review of four explanted whole livers. *Int J Radiat Oncol Biol Phys* 2004; **60**: 1552-1563 [PMID: 15590187 DOI: 10.1016/j.ijrobp.2004.09.004]

71 **Gulec SA**, Pennington K, Wheeler J, Barot TC, Suthar RR, Hall M, Schwartzentruber D. Yttrium-90 microsphere-selective internal radiation therapy with chemotherapy (chemoSIRT) for colorectal cancer liver metastases: an in vivo double-arm-controlled phase II trial. *Am J Clin Oncol* 2013; **36**: 455-460 [PMID: 22643569 DOI: 10.1097/COC.0b013e3182546c50]

72 **Wasan H**, Kennedy A, Coldwell D, Sangro B, Salem R. Integrating radioembolization with chemotherapy in the treatment paradigm for unresectable colorectal liver metastases. *Am J Clin Oncol* 2012; **35**: 293-301 [PMID: 21278562 DOI: 10.1097/COC.0b013e3182005747]

73 **Gray B**, Van Hazel G, Hope M, Burton M, Moroz P, Anderson J, Gebski V. Randomised trial of SIR-Spheres plus chemotherapy vs. chemotherapy alone for treating patients with liver metastases from primary large bowel cancer. *Ann Oncol* 2001; **12**: 1711-1720 [PMID: 11843249 DOI: 10.1023/A:1013569329846]

74 **Cosimelli M**, Golfieri R, Cagol PP, Carpanese L, Sciuto R, Maini CL, Mancini R, Sperduti I, Pizzi G, Diodoro MG, Perrone M, Giampalma E, Angelelli B, Fiore F, Lastoria S, Bacchetti S, Gasperini D, Geatti O, Izzo F. Multi-centre phase II clinical trial of yttrium-90 resin microspheres alone in unresectable, chemotherapy refractory colorectal liver metastases. *Br J Cancer* 2010; **103**: 324-331 [PMID: 20628388 DOI: 10.1038/sj.bjc.6605770]

75 **Lewandowski RJ**, Thurston KG, Goin JE, Wong CY, Gates VL, Van Buskirk M, Geschwind JF, Salem R. 90Y microsphere (TheraSphere) treatment for unresectable colorectal cancer metastases of the liver: response to treatment at targeted doses of 135-150 Gy as measured by [18F]fluorodeoxyglucose positron emission tomography and computed tomographic imaging. *J Vasc Interv Radiol* 2005; **16**: 1641-1651 [PMID: 16371530 DOI: 10.1097/01.RVI.0000179815.44868.66]

76 **Richardson AJ**, Laurence JM, Lam VW. Transarterial chemoembolization with irinotecan beads in the treatment of colorectal liver metastases: systematic review. *J Vasc Interv Radiol* 2013; **24**: 1209-1217 [PMID: 23885916 DOI: 10.1016/j.jvir.2013.05.055]

77 **Fiorentini G**, Aliberti C, Tilli M, Mulazzani L, Graziano F, Giordani P, Mambrini A, Montagnani F, Alessandroni P, Catalano V, Coschiera P. Intra-arterial infusion of irinotecan-loaded drug-eluting beads (DEBIRI) versus intravenous therapy (FOLFIRI) for hepatic metastases from colorectal cancer: final results of a phase III study. *Anticancer Res* 2012; **32**: 1387-1395 [PMID: 22493375]

78 **Martin RC**, Scoggins CR, Tomalty D, Schreeder M, Metzger T, Tatum C, Sharma V. Irinotecan drug-eluting beads in the treatment of chemo-naive unresectable colorectal liver metastasis with concomitant systemic fluorouracil and oxaliplatin: results of pharmacokinetics and phase I trial. *J Gastrointest Surg* 2012; **16**: 1531-1538 [PMID: 22528576 DOI: 10.1007/s11605-012-1892-8]

79 **Fiorentini G**, Poddie DB, Cantore M, Giovanis P, Guadagni S, De Giorgi U, Cariello A, Dazzi C, Turci D. Locoregional therapy for liver metastases from colorectal cancer: the possibilities of intraarterial chemotherapy, and new hepatic-directed modalities. *Hepatogastroenterology* 2001; **48**: 305-312 [PMID: 11379296]

80 **Bower M**, Metzger T, Robbins K, Tomalty D, Válek V, Boudný J, Andrasina T, Tatum C, Martin RC. Surgical downstaging and neo-adjuvant therapy in metastatic colorectal carcinoma with irinotecan drug-eluting beads: a multi-institutional study. *HPB* (Oxford) 2010; **12**: 31-36 [PMID: 20495642 DOI: 10.1111/j.1477-2574.2009.00117.x]

81 **Thirion P**, Michiels S, Pignon JP, Buyse M, Braud AC, Carlson RW, O'Connell M, Sargent P, Piedbois P. Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: an updated meta-analysis. *J Clin Oncol* 2004; **22**: 3766-3775 [PMID: 15365073 DOI: 10.1200/JCO.2004.03.104]

82 **Seifert JK**, Junginger T, Morris DL. A collective review of the world literature on hepatic cryotherapy. *J R Coll Surg Edinb* 1998; **43**: 141-154 [PMID: 9654872]

83 **Ng KM**, Chua TC, Saxena A, Zhao J, Chu F, Morris DL. Two decades of experience with hepatic cryotherapy for advanced colorectal metastases. *Ann Surg Oncol* 2012; **19**: 1276-1283 [PMID: 21913018 DOI: 10.1245/s10434-011-2025-4]

84 **Seifert JK**, Springer A, Baier P, Junginger T. Liver resection or cryotherapy for colorectal liver metastases: a prospective case control study. *Int J Colorectal Dis* 2005; **20**: 507-520 [PMID: 15973545 DOI: 10.1007/s00384-004-0723-0]

85 **Rivoire M**, De Cian F, Meeus P, Négrier S, Sebban H, Kaemmerlen P. Combination of neoadjuvant chemotherapy with cryotherapy and surgical resection for the treatment of unresectable liver metastases from colorectal carcinoma. *Cancer* 2002; **95**: 2283-2292 [PMID: 12436433 DOI: 10.1002/cncr.10973]

86 **Allen PJ**, D'Angelica M, Hodyl C, Lee J, You YJ, Fong Y. The effects of hepatic cryosurgery on tumor growth in the liver. *J Surg Res* 1998; **77**: 132-136 [PMID: 9733599 DOI: 10.1006/jsre.1998.5365]

87 **Bhardwaj N**, Strickland AD, Ahmad F, Atanesyan L, West K, Lloyd DM. A comparative histological evaluation of the ablations produced by microwave, cryotherapy and radiofrequency in the liver. *Pathology* 2009; **41**: 168-172 [PMID: 19152189 DOI: 10.1080/00313020802579292]

88 **Seifert JK**, Morris DL. World survey on the complications of hepatic and prostate cryotherapy. *World J Surg* 1999; **23**: 109-113; discussion 113-114 [PMID: 9880417 DOI: 10.1007/PL00013173]

89 **Primrose JN**. Treatment of colorectal metastases: surgery, cryotherapy, or radiofrequency ablation. *Gut* 2002; **50**: 1-5 [PMID: 11772955 DOI: 10.1136/gut.50.1.1]

90 **Rocha FG**, D'Angelica M. Treatment of liver colorectal metastases: role of laparoscopy, radiofrequency ablation, and microwave coagulation. *J Surg Oncol* 2010; **102**: 968-974 [PMID: 21166000 DOI: 10.1002/jso.21720]

91 **Hompes D**, Prevoo W, Ruers T. Radiofrequency ablation as a treatment tool for liver metastases of colorectal origin. *Cancer Imaging* 2011; **11**: 23-30 [PMID: 21435988]

92 **Stang A**, Fischbach R, Teichmann W, Bokemeyer C, Braumann D. A systematic review on the clinical benefit and role of radiofrequency ablation as treatment of colorectal liver metastases. *Eur J Cancer* 2009; **45**: 1748-1756 [PMID: 19356924 DOI: 10.1016/j.ejca.2009.03.012]

93 **Wong SL**, Mangu PB, Choti MA, Crocenzi TS, Dodd GD, Dorfman GS, Eng C, Fong Y, Giusti AF, Lu D, Marsland TA, Michelson R, Poston GJ, Schrag D, Seidenfeld J, Benson AB. American Society of Clinical Oncology 2009 clinical evidence review on radiofrequency ablation of hepatic metastases from colorectal cancer. *J Clin Oncol* 2010; **28**: 493-508 [PMID: 19841322 DOI: 10.1200/JCO.2009.23.4450]

94 **Jiang HC**, Liu LX, Piao DX, Xu J, Zheng M, Zhu AL, Qi SY, Zhang WH, Wu LF. Clinical short-term results of radiofrequency ablation in liver cancers. *World J Gastroenterol* 2002; **8**: 624-630 [PMID: 12174368]

95 **Mulier S**, Mulier P, Ni Y, Miao Y, Dupas B, Marchal G, De Wever I, Michel L. Complications of radiofrequency coagulation of liver tumours. *Br J Surg* 2002; **89**: 1206-1222 [PMID: 12296886 DOI: 10.1046/j.1365-2168.2002.02168.x]
96 **Pathak S**, Jones R, Tang JM, Parmar C, Fenwick S, Malik H, Poston G. Ablative therapies for colorectal liver metastases: a systematic review. *Colorectal Dis* 2011; **13**: e252-e265 [PMID: 21689362 DOI: 10.1111/j.1463-1318.2011.02695.x]
97 **Iannitti DA**, Martin RC, Simon CJ, Hope WW, Newcomb WL, McMasters KM, Dupuy D. Hepatic tumor ablation with clustered microwave antennae: the US Phase II trial. *HPB* (Oxford) 2007; **9**: 120-124 [PMID: 18333126 DOI: 10.1080/13651820701222677]
98 **Martin RC**, Scoggins CR, McMasters KM. Safety and efficacy of microwave ablation of hepatic tumors: a prospective review of a 5-year experience. *Ann Surg Oncol* 2010; **17**: 171-178 [PMID: 19707829 DOI: 10.1245/s10434-009-0686-z]
99 **Wright AS**, Sampson LA, Warner TF, Mahvi DM, Lee FT. Radiofrequency versus microwave ablation in a hepatic porcine model. *Radiology* 2005; **236**: 132-139 [PMID: 15987969 DOI: 10.1148/radiol.2361031249]
100 **Shibata T**, Niinobu T, Ogata N. Comparison of the effects of in-vivo thermal ablation of pig liver by microwave and radiofrequency coagulation. *J Hepatobiliary Pancreat Surg* 2000; **7**: 592-598 [PMID: 11180892 DOI: 10.1007/s005340070009]
101 **Chang DT**, Swaminath A, Kozak M, Weintraub J, Koong AC, Kim J, Dinniwell R, Brierley J, Kavanagh BD, Dawson LA, Schefter TE. Stereotactic body radiotherapy for colorectal liver metastases: a pooled analysis. *Cancer* 2011; **117**: 4060-4069 [PMID: 21432842 DOI: 10.1002/cncr.25997]
102 **Baumann P**, Nyman J, Hoyer M, Wennberg B, Gagliardi G, Lax I, Drugge N, Ekberg L, Friesland S, Johansson KA, Lund JA, Morhed E, Nilsson K, Levin N, Paludan M, Sederholm C, Traberg A, Wittgren L, Lewensohn R. Outcome in a prospective phase II trial of medically inoperable stage I non-small-cell lung cancer patients treated with stereotactic body radiotherapy. *J Clin Oncol* 2009; **27**: 3290-3296 [PMID: 19414667 DOI: 10.1200/JCO.2008.21.5681]
103 **Timmerman R**, Paulus R, Galvin J, Michalski J, Straube W, Bradley J, Fakiris A, Bezjak A, Videtic G, Johnstone D, Fowler J, Gore E, Choy H. Stereotactic body radiation therapy for inoperable early stage lung cancer. *JAMA* 2010; **303**: 1070-1076 [PMID: 20233825 DOI: 10.1001/jama.2010.261]
104 **van der Pool AE**, Méndez Romero A, Wunderink W, Heijmen BJ, Levendag PC, Verhoef C, Ijzermans JN. Stereotactic body radiation therapy for colorectal liver metastases. *Br J Surg* 2010; **97**: 377-382 [PMID: 20095016 DOI: 10.1002/bjs.6895]

P- Reviewer: Higuera-de la Tijera MF, Ogura T, Tan CH
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.464

World J Hepatol 2014 July 27; 6(7): 464-476
ISSN 1948-5182 (online)

REVIEW

Juvenile autoimmune hepatitis: Spectrum of the disease

Giuseppe Maggiore, Silvia Nastasio, Marco Sciveres

Giuseppe Maggiore, Silvia Nastasio, Department of Clinical and Experimental Medicine, Pediatric Gastroenterology, University Hospital of Pisa, 56127 Pisa, Italy
Marco Sciveres, Pediatric Hepatology and Pediatric Liver Transplantation, UPMC Ismett, 90100 Palermo, Italy
Author contributions: All authors equally contributed to this paper.
Correspondence to: Giuseppe Maggiore, MD, Department of Clinical and Experimental Medicine, Pediatric Gastroenterology, University Hospital of Pisa, Via Roma 67, 56127 Pisa, Italy. giuseppe.maggiore@med.unipi.it
Telephone: + 39-50-992639 Fax: + 39-50-993044
Received: November 7, 2013 Revised: May 19, 2014
Accepted: May 29, 2014
Published online: July 27, 2014

Abstract

Juvenile autoimmune hepatitis (JAIH) is a progressive inflammatory liver disease, affecting mainly young girls, from infancy to late adolescence, characterized by active liver damage, as shown by high serum activity of aminotransferases, by elevated immunoglobulin G levels, high titers of serum non organ-specific and organ-specific autoantibodies, and by interface hepatitis on liver biopsy. It is a multifactorial disease of unknown etiology in which environmental factors act as a trigger in genetically predisposed individuals. Two types of JAIH are identified according to the autoantibody panel detected at diagnosis: AIH-1, characterized by the presence of anti-smooth muscle antibody and/or antinuclear antibody and AIH-2, by anti-liver-kidney microsomal antibody type 1 and/or by the presence of anti-liver cytosol type 1 antibody. Epidemiological distribution, genetic markers, clinical presentation and pattern of serum cytokines differentiate the two types of AIH suggesting possible pathogenetic mechanisms. The most effective therapy for AIH is pharmacological suppression of the immune response. Treatment should be started as soon as the diagnosis is made to avoid severe liver damage and progression of fibrosis. The aim of this review is to outline the most significant and peculiar features of JAIH, based largely on our own personal database and on a review of current literature.

Key words: Juvenile autoimmune hepatitis; Autoimmune hepatitis; Autoantibodies; Autoimmune liver disease; Chronic hepatitis; Acute liver failure

Core tip: Juvenile autoimmune hepatitis is an inflammatory liver disease affecting mainly young girls from infancy to late adolescence, characterized by active liver damage, elevated immunoglobulin G levels, high titers of serum non organ-specific and organ-specific autoantibodies, and interface hepatitis on liver biopsy. Two types are identified according to the autoantibody panel, with differences in the epidemiological distribution, genetic markers and clinical presentation. The most effective therapy for autoimmune hepatitis is pharmacological suppression of the immune response. Treatment should be started as soon as the diagnosis is made to avoid severe liver damage and progression of fibrosis.

Maggiore G, Nastasio S, Sciveres M. Juvenile autoimmune hepatitis: Spectrum of the disease. *World J Hepatol* 2014; 6(7): 464-476 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/464.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.464

INTRODUCTION

Autoimmune hepatitis (AIH) results from an autoimmune attack of the liver parenchyma. The term "autoimmune hepatitis" was first employed by Mackay *et al*[1] in 1965 to describe "a persistent liver disease, with highly elevated levels of serum transaminase, sometimes over 1000 units, elevated serum gamma globulins, up to 6.0 g per 100 mL, piecemeal necrosis on liver biopsy with diffuse lymphoid infiltration and fibrosis, progressing to cirrhosis, various autoantibodies reactions in the serum,

improving with immunosuppressive drugs". The pattern of the serum autoantibodies that characterize patients with AIH led to a classification of this disease[2,3].

In children, after anecdotal reports of cases of chronic hepatitis and hypergammaglobulinaemia in the sixties[4], it was clearly shown that about half of the children with the histological features of chronic active hepatitis had hypergammaglobulinemia and high titers of serum autoantibodies[5] and that these patients responded in most cases to prednisone and azathioprine treatment, therefore suggesting an autoimmune mechanism[6,7]. In the same period a peculiar form of autoimmune hepatitis, now called AIH-2 was described as a distinct entity in children[8] and later confirmed in adults[9].

The aim of this review is to outline the most significant and peculiar features of juvenile AIH (JAIH), based largely from our own personal database; additionally we searched PubMed with the term of "juvenile autoimmune hepatitis", "autoimmune hepatitis", "epidemiology", "pathogenesis" and "treatment", filtered for age "birth-18 years".

DEFINITION

AIH is a liver disease of unknown origin, pathogenetically characterized by an inflammatory liver disease, as shown by high serum activity of aminotransferases, by elevated immunoglobulin G levels, high titers of serum non organ-specific and organ-specific autoantibodies, and by interface hepatitis on liver biopsy[10]. It affects mainly young girls and spontaneously progresses to severe liver damage. Immunosuppressive therapy, which should be started as soon as diagnosis is made, induces clinical and biochemical remission in most treated patients. If untreated, cirrhosis and terminal liver failure may rapidly occur[6].

EPIDEMIOLOGY

AIH can be diagnosed at any age in both sexes. Mean annual prevalence in European adults ranges from 11.6 per 100 individuals to 17 per 100[11,12] with point prevalence in homogeneous populations, such as Alaskan natives, of 42.9 per 100 individuals[13]. Epidemiological data on JAIH are largely incomplete. There is only one recent report on the incidence and prevalence of JAIH, which was conducted in Utah, United States. In this study, the incidence and prevalence of JAIH was reported to be 0.4 and 3.0 cases per 100000 children, respectively[14]. AIH-1 is the more common type of AIH, which also affects adults and often presents at puberty, while AIH-2 is typical of pediatric age, presenting at a younger age, and even during infancy[8,15].

PATHOGENESIS

AIH is a multifactorial disease of unknown etiology. Environmental factors act as a trigger with self-perpetuating liver inflammation in predisposed individuals who carry a complex genetic background. Moreover, a defective immunoregulatory function, possibly genetically related, fails to control autoreactive clones and let the disease become clinically evident. The histological picture of interface hepatitis, in which a mononuclear and plasma cell infiltrate, which originates in the portal tracts, and disrupts the parenchymal limiting plate, morphologically illustrates this process. Among the inflammatory cells, activated T lymphocytes, positive for the $CD4^+$ helper/inducer phenotype, predominate. These cells are believed to recognize self-antigens on the hepatocyte surface and to trigger the autoimmune liver damage[16].

Genetics

Main susceptibility HLA alleles for AIH-1 in Europe and North America were found to be *DRB1*0401* and *DRB1*0301*. The presence of these alleles confers an increased risk of developing AIH-1 and influences some features of the disease[17]. Geographic variation of the genetic predisposition to AIH-1 exists: in some countries such as Japan, Mexico and Argentina, DR3 haplotype is poorly represented in the general population, and the principal susceptibility alleles for AIH-1 are *DRB1*0404* and *DRB1*0405*[18-20]. European children display the typical pattern for AIH-1 of Caucasian patients with a significant prevalence of *DRB1*0301* and *DRB3*0101*[21].

Knowledge of the genetic background of AIH-2 is limited. In Europe, *DRB1*03* and *DQB1*02* alleles may have an important role, whereas other studies reported an increased frequency of *DRB1*07*, *DRB4*01* and *DQB1*06*. In a pediatric population from Brazil, a significant increase of *DRB1*07*, *DRB4* and *DQB1*02* was observed. Moreover, *HLA-DRB1*07* allele was found significantly associated with the presence of anti-liver-kidney microsomal antibody type 1 (LKM-1) alone and *HLA-DRB1*03* allele with anti-liver cytosol type 1 antibody (LC-1)[22-24].

A partial deficiency of HLA class Ⅲ complement component C4, genetically determined, has been associated with JAIH[25].

Environmental factors

A number of drugs may cause unpredictable, dose-independent, immune-mediated liver damage. Autoimmune hepatitis related to halothane, tienilic acid, dihydralazine and minocycline are typically associated with LKM autoantibodies even though the molecular targets are different from AIH-2 (*i.e.*, CYP2E1 for halothane and CYP2C9 for tienilic acid)[26].

Several viruses have been proposed as triggering factors for AIH such as HAV, measles, EBV or HSV, based on clinical or epidemiological criteria[27]. CYP2D6, the specific target of LKM-1 antibodies, shows epitopes that cross-react with homologous region of HCV, CMV and HSV[28]. Although definite evidence supporting this mechanism is lacking, it is conceivable that, infections with otherwise common viruses might lead, within a per-

missive genetic background, to break tolerance to self-antigens like CYP2D6, which could also be expressed, under particular conditions, on the hepatocyte surface[29].

Autoimmune reaction as a defect of regulatory function

A defect in a subpopulation of T lymphocytes regulating the immune response to liver- antigens expressed on the hepatocyte membrane has been reported in patients with AIH-1[30]. This T-cell subpopulation, bearing the interleukin 2 receptor α-chain ($CD25^+$) and known as functional regulatory T-cells (T-regs), has been extensively studied as the putative main subset of regulatory cells for immune tolerance maintenance. In AIH patients, T-regs lymphocytes were found to be defective in number[16]. Moreover, functional studies have suggested that these cells are defective in promoting secretion of regulatory cytokines by their targets and in regulating $CD4^+$ and $CD8^+$ T-cell proliferation and interferon-gamma production and that they are unable to restrain monocyte activation and function[16]. However, using different markers, such as FOXP3, to identify T-regs lymphocytes, their role, in AIH, as the main immunoregulatory cells was recently challenged[31,32].

In AIH-2, the principal autoantigen (CYP2D6) is known, and the dominant epitopes target of the B and T-cell immune responses are also well characterized. On this basis, generation and expansion of HLA-restricted specific T-reg lymphocytes has been attempted and their immunomodulatory properties have been described *in vitro*[33]. Targeted immunotherapy with autologous infusion of *ex-vivo* expanded T-regs was demonstrated to induce remission of experimental AIH of mice[34].

Animal models

Advances in understanding the pathogenesis of AIH has been limited by the lack of accurate animal models. Murine models have been generated through DNA immunization with a chimeric fusion protein containing human CYP2D6 and human forminotransferase cyclodeaminase, the two self antigens of type 2 AIH, together with the extracellular region of mouse, cytotoxic T-lymphocyte antigen 4, as an immunological modulator[34,35]. Another model for AIH-2 uses CYP2D6 transgenic mice and tolerance mechanisms are overrun with the use of an adenovirus-CYP2D6 vector[36]. Immunized or infected mice developed chronic histological changes in the liver close to interface hepatitis, resembling those of AIH, with the development of a specific immune response with the production of anti-LKM1 and anti-LC-1 antibodies. A third animal model was created without the use of active immunization against xenopeptides, but using a transgenic mouse expressing chicken ovalbumin on the hepatocyte surface[37].

CLINICAL FEATURES

A specific autoantibody panel identifies two types of AIH: the presence of anti-smooth muscle antibody (SMA) and/or antinuclear antibody (ANA), in AIH-1[21,38], and LKM-1 and/or LC-1, in AIH-2[8,39]. Epidemiological distribution, genetic markers, clinical presentation and pattern of serum cytokines differentiate the two types of AIH suggesting possible pathogenetic mechanisms[40]. AIH-1 presents at any age, from infancy to the elderly, and in both sexes, while AIH-2 presents almost exclusively in childhood, with a very high incidence in females[8,39]. Patients with AIH-2 present at younger age that AIH-1, and are at higher risk to develop an acute liver failure[41]. Hypergammaglobulinemia is common in AIH-1, but it can be absent in AIH-2[8,38]. Moreover, AIH-2 is almost never associated with evidence of bile duct lesions while bile duct lesion is commonly observed in AIH-1[38]. Extra hepatic diseases of autoimmune mechanism are frequently observed in patients with both types of AIH. Autoimmune thyroid diseases (Grave's and Hashimoto diseases) and autoimmune skin diseases such as vitiligo or alopecia are more frequently observed in AIH-2[8,38].

Three patterns of clinical onset characterize JAIH: (1) Acute onset with anorexia, nausea, vomiting and abdominal pain followed by jaundice, eventually suggesting an acute viral hepatitis, is the most frequent. In particular, patients, with AIH-2, are at higher risk than AIH-1 to develop acute liver failure with encephalopathy; (2) Insidious onset with progressive fatigue, anorexia, and intermittent jaundice lasting for several months/years before diagnosis, can be observed in about a third of patients. All these patients have clinical evidence of chronic liver disease and/or of cirrhosis at diagnosis; and (3) About 10% of patients may be asymptomatic when the liver disease is serendipitously discovered by the finding of clinical signs of chronic liver disease or by an increase of aminotransferase activity.

In a few patients, JAIH may reveal itself with symptomatic portal hypertension or with symptoms related to an extrahepatic autoimmune disease such as autoimmune thrombocytopenia, autoimmune haemolytic anemia, diabetes type 1, autoimmune thyroiditis, vitiligo, cutaneous vasculitis, uveitis, glomerulonephritis, juvenile chronic arthritis, systemic lupus erythematosus, Sjögren's syndrome, celiac disease and inflammatory bowel disease[8,15,38].

LABORATORY FEATURES

At diagnosis, the "activity" of the liver disease can be documented by the presence of an almost constant increase of liver enzyme, in particular of serum transaminase activity that may increase up to 50 times or more the upper normal limit, while gamma glutamyltransferase (GGT) activity may be normal or only slightly elevated. An increase of GGT should suggest bile duct damage as in the case of autoimmune hepatitis/cholangitis overlap syndrome. Serum gamma globulins and immunoglobulins G are usually elevated, sometimes markedly, up to 6-8 g/L. Serum albumin may be normal in absence of liver function impairment and ascites. Serum immunoglobulin A deficiency and/or genetically determined low levels of

C4 can be observed in AIH-2[8,25]. Prolonged prothrombin time suggests severe liver function impairment.

Autoantigens and autoantibodies

Recognition of pathogenetic autoantigens in AIH might be one of the key factors to develop an etiologic-based therapy. Unfortunately most of the antigens recognized by autoantibodies detected in AIH are either non organ-specific or intracellular molecules, unlikely involved in triggering autoimmune reaction. The most studied candidate autoantigens are the asialoglycoproteins receptor (ASGP-R) for type AIH-1 and the cytochrome P4502D6 (CYP2D6) for AIH-2.

The ASGP-R is an organ-specific antigen expressed in the hepatocyte membrane. Even if several experimental studies had been published, its role in pathogenesis of AIH is still controversial[42]. Both peripheral and infiltrating lymphocytes collected from adult and pediatric patients with AIH show a proliferative response to human ASGP-R[43], and a lack in T-suppressing function of $CD4^+$ T-cells specific for ASGP-R and corrigible by immunosuppressive therapy, has been described both in patients and in their healthy relatives.

Seven isoforms of cytochrome P450 are expressed in human liver and all of these isoforms are targets of LKM reactivity in different types of autoimmune, viral or drug induced liver disease. CYP2D6 is an intracellular enzyme active in detoxification of several drugs and is the molecular target of AIH-2[44]. By effect of some cytokines, CYP2D6 can be expressed on hepatocytes surface becoming a potential target for autoreactive T-cells[29].

Detection of serum non-organ-specific autoantibodies (ANA, SMA and LKM-1) known to be associated with autoimmune liver diseases is a critical component of diagnostic criteria developed by International Autoimmune Hepatitis Group[2,3]. Their assessment should preferably be performed by indirect immunofluorescence (IIF) on frozen section of rat liver, kidney and stomach, and the presence of ANA/SMA and LKM-1 is virtually mutually exclusive. Sera screened positive for ANA/SMA should be further examined to assess the pattern of nuclear staining by the use of HEp2 cell monolayers, or to define the target of the SMA reaction[45].

The autoantibody profile does not markedly vary in the course of AIH with the exception of ANA reactivity that can be detected "*de novo*" in both subgroups of AIH. Autoantibodies titers varies during the course of the disease usually reducing in titer in case of remission, but also independently[46,47].

Autoantibody titers are not predictive of biochemical or histological remission. High titers at onset do not suggest a more aggressive disease and their disappearance from serum is not predictive of a better disease control during treatment or of a sustained remission in case of discontinuation of treatment.

Antinuclear and anti smooth muscle antibody, the serological hallmark of AIH-1, are usually present at high ($\geqslant$ 1:100) titer, but they are not specific of AIH. ANA and SMA can, in fact, be detected in other liver diseases (viral or drug induced hepatitis, steatohepatitis and hepatocellular carcinoma) and also in non-hepatic disorders, however at lower titers.

Various patterns of ANA staining can be observed: homogeneous (60%) and speckled (15%-25%) are the most frequent, however they are not considered of clinical importance and they may vary in the same patient, during treatment. Several nuclear antigens have been identified, as a target of ANA reactivity: single and double-stranded DNA, histones, chromatin, ribonucleoprotein complexes, cyclin A and centromere, but no single AIH specific antigen has been detected so far. In AIH-1, ANA can be detected either alone or in conjunction with SMA. In children, ANA is considered positive when the titer is $\geqslant$ 1:40, however since ANA reactivity at low titer can be frequently found in children we suggest raising the positivity cut-off to at least to 1:100. Moreover, Anti dsDNA antibodies can be detected in 25% of ANA-reactive AIH-1 patients[46].

When using rat stomach as substrate for SMA: uniform IIF stain of the muscolaris mucosa, blood vessels walls (V) and parietal cell occurs. With rat kidney tissue, staining of the mesangial area of glomeruli (G) and of proximal renal tubular cells (T) also occurs. "VG" and "VGT" staining patterns are the most frequent IIF patterns encountered in AIH. SMA reactivity usually stains structural components of the cytoskeleton such as desmin and troponin. In AIH SMA reactivity is directed against filamentous (F) actin. Anti-F-actin can be detected using cultured human fibroblast or HEp2 cells. Anti-F-actin specificity is higher than SMA but anti-F-actin antibodies may be found also in viral infection, connective tissue disease and celiac disease.

Anti-LKM-1 serum reactivity defines the AIH-2, the most common type of JAIH occurring in infancy and childhood[8]. LKM-1 are present in 30%-70% of sera of patients with AIH-2 with anti-LC-1 antibody. Occasional patients with both ANA and LKM-1 have been defined as AIH-2.

LKM-1 stains hepatocytes and the proximal renal tubular cells (P3 portion) of liver and kidney sections in mice. Occasional staining of the distal renal tubules usually generates confusion with anti-mitochondrial autoantibody (AMA). AMA positivity in children is rare and Primary Biliary Cirrhosis is exceptional in pediatric age.

Anti-LC-1 is an organ-specific antibody, which homogeneously stains, in IIF, the cytoplasm of the hepatocytes, sparing the perilobular layer of central veins and without staining of the proximal renal tubules[48]. LC-1 can also be detected with both immunodiffusion and immunoblotting. LC-1 antibody reacts with forminotransferase cyclodeaminase, a 58-62 Kd liver specific antigen[49] and together with LKM-1, characterizes AIH-2. In fact LC1 reactivity can be found associated with LKM-1 in about 50% of AIH-2, but LC-1 may characterize on its own, as a sole autoantibody children with AIH-2[39].

The simultaneous presence of LKM-1 may obscure

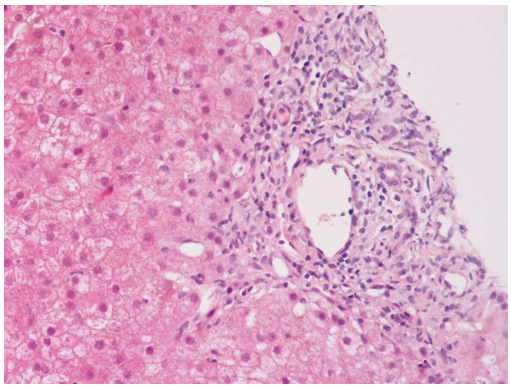

Figure 1 Interface hepatitis with piecemeal necrosis and lymphocyte spillover across the limiting plate.

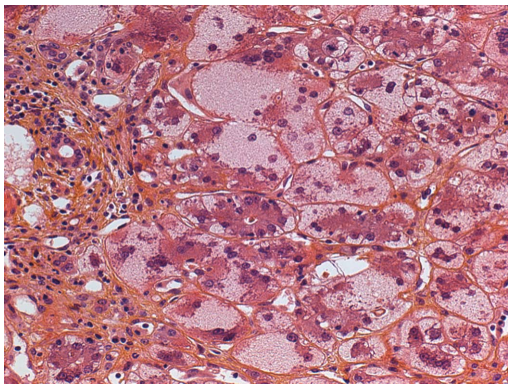

Figure 2 Liver biopsy of a 6 mo old infant with autoimmune hemolytic anemia showing diffuse giant cell transformation and moderate inflammatory portal infiltrate.

LC-1 IIF reactivity. In these cases it is necessary to use another method to detect the presence of LC-1 such as immunodiffusion, ELISA, Western blot or dot blot.

Anti-SLA is a non organ-specific antibody which target antigen is likely to be a 50 Kd protein identified as O-phosphoseryl-tRNA: selenocysteinyl-tRNA synthase. Anti-SLA is considered a specific marker for AIH-1 being present in 6% to 58% of adults and children with AIH-1, alone or in combination with SMA and/or ANA[50]. Its detection could be particularly useful in patients who are negative for conventional markers of the disease (ANA, SMA), but its diagnostic role in JAIH is not relevant.

Anti-human ASGPR is a species-specific, liver-specific autoantibody that can be detected in sera of patients with various inflammatory liver diseases, but predominantly in AIH. The absence of a commercialized assay restricts its use to few laboratories.

Anti-neutrophil cytoplasmic antibodies (ANCA) constitute a heterogeneous group of autoantibodies directed against various subcellular components of neutrophils or myeloid cells and their presence has been proven to be a reliable diagnostic tool in systemic vasculitis. They are routinely detected by IIF on ethanol fixed human neutrophils and commonly classified in cytoplasmic (cANCA), perinuclear (pANCA) and atypical (pANNA). Atypical pANCA are characterized by non-homogeneous labeling of the nuclear periphery together with multiple intranuclear fluorescent staining and have been reported in patients with autoimmune liver disease including sclerosing cholangitis associated with inflammatory colitis and in AIH-1.

LIVER HISTOLOGY

The International Autoimmune Hepatitis Group has affirmed the role of liver biopsy for the diagnosis of AIH[2,3]; liver biopsy is thus recommended in all patients suspected AIH unless there is a significant contraindication[51]. The histological hallmark of AIH is "interface hepatitis" (formerly called piecemeal necrosis) (Figure 1). A considerable amount of eosinophilic granulocytes can be observed within the portal infiltrate, especially in such cases associated to celiac disease[52].

In patients with AIH presenting as an acute liver disease, liver histology allows for differentiation between spontaneous exacerbation of a chronic liver disease ("acute-on-chronic") and a newly developed disease. In the latter case, centrilobular zone 3 necrosis is the most typical pattern[53]. Subsequent transition to the classic features of "interface hepatitis" usually occurs. Other possible liver biopsy findings in AIH include the presence of giant multinucleated hepatocytes[54]. Moreover, diffuse giant cell transformation characterizes a distinct form of AIH in infants, associated with autoimmune hemolytic anemia[55] (Figure 2).

Massive liver cell necrosis may be present in AIH with acute severe/fulminant onset and may be associated with bridging necrosis and/or with multilobular or panlobular necrosis. These histological findings support but do not constitute firm evidence for the diagnosis of AIH.

Biliary ducts are usually not affected in AIH and the presence of lymphocytic cholangitis, or that of a mixed inflammatory infiltrate surrounding and infiltrating the bile ducts, has received a negative diagnostic rating on the IAIHG diagnostic score[3]. However, the incidental presence of bile duct inflammatory changes has been recognized in patients with AIH responding to immunosuppressive treatment[56]. In children, bile duct inflammatory changes are common in AIH-1, but are very rare or absent in AIH-2.

Liver biopsy also provides information on prognosis, identifying the presence of cirrhosis. Cirrhosis may be present at diagnosis or rapidly develop in JAIH[57]. Cirrhosis is more frequent at diagnosis in AIH-1 than in AIH-2[58], however, concerning the diagnosis of cirrhosis, "blind" percutaneous liver biopsy has been demonstrated to be of low diagnostic sensitivity, since up to 50% of patients may not be correctly diagnosed[57].

DIAGNOSIS

JAIH has variable clinical manifestations and should be considered in the diagnostic work-up of any patient with

a cryptogenic liver disease. Diagnosis of JAIH basically relies on the exclusion of other possible known causes of the hepatic disease, such as chronic viral infections and Wilson's disease and by clinical biochemical and histological "positive" criteria. Diagnosis is not challenging when all the major clinical and biochemical elements of the disease are present, such as the occurrence of an autoimmune disease in the same patient, a biochemically "active" liver disease, an elevation of serum gamma globulins, presence in serum of autoantibodies known to characterize JAIH, and compatible histopathological features on liver biopsy. However, sometimes the diagnosis may become difficult and for this reason, in 1993, an international board of physicians published a set of criteria to identify patients as having either "definite" or "probable" autoimmune hepatitis[2]. Once used primarily for scientific and research purposes, this scoring system is now widely used in clinical practice after being reviewed in 1999[3].

A simplified scoring system has since been proposed based solely on four parameters (autoantibodies, IgG levels, liver histology and exclusion of viral hepatitis), and has been validated in adults with 88% sensitivity and 97% specificity[59]. When comparing both scoring system in adults, we see that the revised original scoring system has shown greater sensitivity for the diagnosis than the simplified scoring system (100% *vs* 95%), while the simplified score had greater specificity (90% *vs* 73%) and predictability (92% *vs* 82%) for AIH than the revised original system[60]. The original scoring system was assessed in children using the GGT/aminotransferase ratio instead of the alkaline phosphatase/aminotransferase ratio to improve its specificity[61]. When tested in children the simplified scoring system was not proved to be effective mainly because of its low sensitivity[62]. In conclusion, no validated scoring system for the diagnosis exists for JAIH[63]. In challenging cases, once Wilson's disease is excluded, and in absence of liver function failure, an immunosuppressive treatment should be attempted for at least 6 wk. A positive response to this treatment would suggest AIH. Moreover, relapse after immunosuppressive drug withdrawal is positively weighted in the IAIHG diagnostic criteria[2,3].

MANAGEMENT

The most effective therapy for JAIH is pharmacological suppression of the immune response. Treatment should be started as soon as the diagnosis is made to avoid severe liver damage and progression of fibrosis. Standard therapy includes a combination of prednisone and azathioprine[6] or occasionally prednisone as a monotherapy[6,7,21]. Prednisone or prednisolone is used at a higher dose that used in adults (2 mg/kg per day, up to a maximal daily dose of 60 mg/d in the adolescent) and azathioprine is administered starting from 1 mg/kg per day up to a maximum of 2.5 mg/kg per day. First line combination therapy including prednisone and azathioprine can be more effective than prednisone alone[64]. Moreover, the "steroid-sparing" effect of the azathioprine allows reducing more rapidly the steroid dose, thus tapering side effects related to the prolonged use of steroids at high dose.

The goal of the treatment is to obtain clinical and biochemical remission of the liver disease clinical signs with normalization of the "activity" of the disease (transaminase, gamma globulins) and of the liver function (prothrombin activity; INR). The definition of treatment-induced remission in JAIH should be stricter than that used in adult disease: the serum activity of aminotransferase should be maintained within the upper limit of normal, serum immunoglobulin G levels within the normal range for age, and serum autoantibodies absent or at very low titer[6,15,21]. Even clinical and biochemical remission do not always reflect histological resolution of inflammation, the proof of histological remission is not required. The rapidity and degree of response to treatment depends on the disease severity at onset. In JAIH, treatment is associated, in over 90% of cases, with a measurable clinical and laboratory response within 4 to 8 wk. Complete normalization of biochemical parameters may, however, take several months. On histopathological evaluation, the immunosuppressive treatment improves the fibrosis score, with an arrest in its progression into cirrhosis. Fibrosis control is mainly associated with regression of necroinflammatory activity[65]. Once remission is obtained, it must be maintained in the long term on the lowest possible dose of medication. Different therapeutic schedules of treatment discontinuation exist and should be tailored on individual patients. Prednisone is usually first decreased; the shift to alternate-day use of steroids may be suitable because of the lower incidence of side effect[66]. In cases of severe liver function impairment at diagnosis, liver function may further deteriorate despite an appropriate therapy. In these patients immunosuppressive therapy should be modified with the introduction of a third drug such as cyclosporine. In case of further non-response, the possibility of a liver transplant should be considered.

When complete remission is achieved, the goal of the immunosuppressive treatment is to maintain remission and to prevent relapse of the disease. Prednisone should be further reduced to the lowest dose that allows a biochemical remission. Alternate-day doses of prednisone associated to azathioprine are usually effective in maintaining remission. A relapse may occur at any time, the most frequent cause of a relapse is patient's non-compliance. It is questionable that a histological remission has to be demonstrated through a liver biopsy in patients with clinical and biochemical remission, since the presence of histological remission has not been shown to be sufficiently indicative of an absence of possibility of relapse in the case of further reduction of the immunosuppression[6]. Liver fibrosis rarely progresses in patients who maintain a persistent biochemical remission and it can even diminish during treatment. Duration of the immunosuppressive treatment before attempting discontinuation is unknown; stopping treatment within the first two years is usually followed by a relapse[6]. We suggest

that sustained remission should be maintained for at least five years, thereafter, in case of combined treatment of prednisone and azathioprine, prednisone is stopped and the patient is maintained on azathioprine monotherapy. Azathioprine monotherapy had been demonstrated to maintain remission in most patients with AIH[67]. Undetectable serum autoantibodies do not exclude the risk of relapse, but an increase of the titer of autoantibodies suggests caution in modifying the dose of immunosuppressive therapy.

Particular variant forms of AIH, as celiac disease-associated AIH on gluten free diet, might have a lower risk to relapse after treatment discontinuation[68]. In such cases a discontinuation attempt after less than 5 years of treatment could be justified.

Steroids mostly cause side effects of immunosuppressive therapy, including increase of food intake leading to moderate and reversible weight increase and a reduction of height growth. Severe side effects include obesity, growth failure, severe cosmetic changes, cutaneous striae, vertebral collapse, hyperglycemia, and cataracts, causing both visual impairment and, potentially, psychosis. Azathioprine is usually a safe drug, and cytopenia necessitating a dosage reduction is a rare event. Teratogenicity and oncogenicity issues resulting from azathioprine use in humans have not been conclusively demonstrated. Pregnancy should however be excluded in adolescent girls before starting treatment with azathioprine. However, if prolonged azathioprine treatment is needed, pregnancy has been demonstrated to be safe, in the long term, in young females with AIH[69,70]. During pregnancy, higher doses of prednisone may be an alternative option for those young women who prefer azathioprine withdrawal. However, vigilance is required at all times, and patients need careful monitoring, especially in the postpartum period, because of the possibility of relapses.

In case of non-response to conventional treatment or in the presence of severe side effects of corticosteroids the use of cyclosporine A is indicated. Cyclosporine A at a median dose of 5 mg/kg per day induces remission in children and adolescents with AIH with a initial target concentration in serum of cyclosporine of 200-250 ng/mL[71,72]. Cyclosporine treatment side effects including mild gingival hyperplasia and reversible irsutism in some patients, are usually well tolerated and disappear after reduction of the dose[73]. Normality of renal function should be verified before starting this drug. In the follow-up, once remission is obtained, the dose of cyclosporine can be reduced with a target concentration of 100 ng/mL or the patient may be shifted to conventional treatment.

In children who either did not tolerate azathioprine or did not respond to conventional treatment, mycophenolate-mofetil (MFM, 20 mg/kg per day) in addition to steroids therapy has been shown to induce and maintain remission[74]. Side effects of MFM include headache, diarrhea, dizziness, hair loss and neutropenia.

Budesonide, a steroid that is rapidly metabolized with low systemic exposure, in combination with azathioprine, has been recently shown in a trial including patients with JAIH to induce and maintain remission with fewer side effects than prednisone[75]. However, the low proportion of remission observed in this study compared to that reported in others pediatric studies using prednisone and azathioprine schedules, do not support its use as first-line treatment of JAIH[76]. Recently Rituximab, a monoclonal antibody against CD20, a B-lymphocyte surface antigen, has been successfully used in selected cases as a rescue therapy[77].

Liver transplantation should be considered as a therapeutic option for children and adolescents with JAIH and chronic end stage liver disease or in patients with acute liver failure at onset not responding to rescue immunosuppression. Five-year post-transplant survival in JAIH patients is scored at 86%[78].

LONG-TERM OUTCOME

Immunosuppressive treatment has convincingly altered the outcome of most patients with AIH[6,21,79-82]. Indeed according to previous prognostic studies on adults, 40% of patients with severe disease without treatment die within six months of diagnosis and cirrhosis eventually develops in at least 40% of untreated survivors[83,84].

On the other hand the 10-year survival rates among treated adults is 60% for those with cirrhosis on the initial liver biopsy[83,85] and more than 80% for those patients without cirrhosis at presentation[86].

The long-term outcome of JAIH still remains scarcely known, however, in case of full and prompt response to immunosuppressive therapy, the prognosis is usually satisfactory and most patients survive in the long-term with excellent quality of life and, in the majority of cases, on low dose immunosuppression.

In the five largest published series of children with AIH, overall survival rate in long-term treated patients exceeded 80% with a 5-year survival with native liver ranging between 67% and 87%; follow-up ranged from 4, 8 to 10 years[21,38,87-89].

The presence of cirrhosis on initial liver biopsy did not seem to impact long-term survival in children with AIH[87,88] while elevated total bilirubin and prolonged INR are independent risk factors of death and/or need of liver transplantation[21]. Immunosuppressive treatment requires to be prolonged in the long term in the majority of patients; however, sustained remission after treatment discontinuation has been reported in 13% to 20% of patients[21,87].

End-stage liver disease leading to liver transplantation has been reported to develop up to 14 years after diagnosis in 8% to 16% of children with JAIH compliant to immunosuppressive therapy and in absence of an evident biochemical relapse[21,87].

VARIANT FORMS OF JAIH

Giant cell hepatitis with autoimmune hemolytic anemia

Giant cell hepatitis with autoimmune hemolytic anemia is a rare entity described by Bernard *et al*[55] in 1981, pre-

senting in early childhood with severe progressive liver disease in combination with Coombs positive hemolytic anemia. The clinical course is usually aggressive leading to hepatic failure and death.

The mechanism of liver disease is not known, but an autoimmune process is believed to be responsible for this component of the disease as well. A study by Whitington *et al*[90] has recently provided evidence that systemic B cell autoimmunity might play a pathogenetic role even if autoantibodies are usually absent, histological features characteristic of auto-immune hepatitis are missing, and the disease is highly refractory to therapy that would usually be effective in AIH. Conventional immunosuppressive treatments (steroids, azathioprine, cyclophosphamide, cyclosporine, mycophenolate) are associated with high toxicity and often produce only partial or short-lasting remission[91]. Liver transplantation is associated with high rate of disease recurrence. In more recent studies the use of anti-CD20 monoclonal antibody (Rituximab) has been reported to be effective in patients with refractory hepatitis[92]. Intravenous immunoglobulins have been also reported to be efficacious in case of severe liver function impairment at onset or during a relapse in patients treated by multiple immunosuppression although their efficacy seems to be only temporary[93].

Autoantibody-negative autoimmune hepatitis

Cryptogenic hepatitis with autoimmune features in absence of detectable serum autoantibodies is described as "autoantibody-negative AIH"[94]. It affects a small proportion of adult patients presenting with a cryptogenic liver disease with acute or chronic presentation with the clinical, biochemical and histopathological features of AIH and responsive to immunosuppressive treatment[94-96]. The comprehensive international scoring system can support, but never override the clinical diagnosis pre-treatment, and non-standard serological markers should be sought in order to enhance diagnostic confidence[3,60,97]. A 3-mo treatment trial with corticosteroids should be considered in all candidates for the diagnosis, regardless of the serological findings[98,99]. This entity has been reported in children only in small series or in single case report[100].

Celiac disease associated-AIH

Celiac disease (CD) is common in patients with AIH, especially in children, as it has been shown in a previous Italian multicenter survey[52] and in more recent small pediatric series[68,101-103]. These studies reported a prevalence of CD in up to 19% of children with JAIH. The pathogenetic role of gluten in triggering AIH is uncertain, however, both types of AIH have been described in association with CD as well as autoantibody-negative AIH[52,100]. Liver damage, as evidenced by elevated aminotransferase activity, has been reported as being present from the first observation of celiac patients in some cases while in other, CD was diagnosed by a serological screening in patients with a known AIH[101,102]. Therefore all patients with AIH should be serological screened for CD and moreover all CD patients with clinical and/or biochemical signs of liver damage should be closely followed-up to exclude an AIH, especially in the case of persistent elevation of liver enzymes on a gluten free diet.

Children with co-existent CD seem to have an apparently more favorable response to treatment, suggesting a positive effect of gluten withdrawal on AIH co-existent with CD. Gluten withdrawal might potentiate the immunosuppressive effect of the immunosuppressive drugs, maintaining remission even when the treatment has been withdrawn[68,103].

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy-associated JAIH

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomal recessive disorder caused by mutations in autoimmune regulator gene (AIRE) inducing a loss in central immune tolerance, failure to eliminate autoreactive T cells in the thymus, and their escape to the periphery. APECED is characterized by an extremely variable pattern of destructive autoimmune reaction, mainly mediated by specific autoantibodies toward different endocrine and non-endocrine organs. Virtually, all tissues and organs may represent the target of the autoimmune attacks, thus leading to a wide spectrum of clinical features. The three main components of APECED are chronic mucocutaneous candidiasis, chronic hypoparathyroidism and Addison's disease. Generally chronic mucocutaneous candidiasis develops first and it is often followed by chronic hypoparathyroidism, before the age of 10 years, and later on by adrenal insufficiency. In addition to the main components, the spectrum of minor manifestations may include ectodermal dystrophy, other endocrinopathies, such as hypergonadotropic hypogonadism, insulin-dependent diabetes, autoimmune thyroiditis, and pituitary dysfunction. Moreover, skin diseases (vitiligo and alopecia) and gastrointestinal disorders (chronic atrophic gastritis, pernicious anemia) and particularly AIH, may be present. AIH shows a clinical phenotype akin to AIH-2 and it is present in 15%-20% of cases with CYP1A2 and CYP2A6 as a specific target antigens[104]. Heterozygous mutations of *AIRE* gene have been reported in children with AIH-1 suggesting a possible predisposition role[105].

De novo autoimmune hepatitis

De novo autoimmune hepatitis, after liver transplantation, was first described in 1998 by the group of King's College Hospital in London[106]. It is a form of late graft dysfunction characterized by abnormal liver function tests, high serum concentration of immunoglobulin, presence of autoantibodies, and histological features of interface hepatitis coupled with a rich plasma cell infiltrate[107]. This recently recognized entity affects patients transplanted for disorders other than AIH and usually of non-autoimmune nature. Since its first description several authors reported the occurrence of *de novo* AIH in children and adults

transplanted for non-autoimmune conditions[108-115].

The pathogenesis of *de novo* AIH is not yet defined and there are a variety of potential mechanisms leading to autoimmune liver disease post-transplant. Possible pathogenetic mechanism include the release of autoantigens from damaged tissue, as well as molecular mimicry, whereby exposure to viruses sharing amino acid sequences with autoantigens leads to cross-reactive immunity[106]. *De novo* AIH responds to treatment with corticosteroids and azathioprine allowing excellent graft and patient survival. Early recognition and appropriate management are therefore essential to avoid graft loss[107,116].

CONCLUSION

Juvenile AIH is a severe liver disease of childhood and adolescence progressing rapidly toward cirrhosis and severe liver function impairment unless immunosuppressive treatment is promptly started. Its clinical spectrum is broad: from asymptomatic liver damage to acute symptomatic and even severe hepatitis. Early diagnosis is mandatory but no scoring system of sufficient sensitivity exists. Serum autoantibodies are a relevant tool, but not essential for diagnosis and liver histology has distinct but non-pathognomonic features. The vast majority of treated patients responds to the immunosuppressive treatment, but relapses are frequent and mostly related to defective compliance to treatment. Long-term outcome studies on JAIH concerning the possibility of safely stopping the immunosuppressive treatment are needed for appropriate counseling to families and patients.

REFERENCES

1 **Mackay IR**, Weiden S, Hasker J. Autoimmune hepatitis. *Ann N Y Acad Sci* 1965; **124**: 767-780 [PMID: 5214838 DOI: 10.1111/j.1749-6632.1965.tb19000.x]

2 **Johnson PJ**, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology* 1993; **18**: 998-1005 [PMID: 8406375 DOI: 10.1002/hep.1840180435]

3 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938 [PMID: 10580593 DOI: 10.1016/S0168-8278(99)80297-9]

4 **Grossman A**, Rosenthal IM, Szanto PB. Chronic hepatitis with hypergammaglobulinemia in childhood. Report of eight cases. *Pediatrics* 1962; **29**: 933-947 [PMID: 13902289]

5 **Odièvre M**, Maggiore G, Homberg JC, Saadoun F, Couroucé AM, Yvart J, Hadchouel M, Alagille D. Seroimmunologic classification of chronic hepatitis in 57 children. *Hepatology* 1983; **3**: 407-409 [PMID: 6840686 DOI: 10.1002/hep.1840030320]

6 **Maggiore G**, Bernard O, Hadchouel M, Hadchouel P, Odievre M, Alagille D. Treatment of autoimmune chronic active hepatitis in childhood. *J Pediatr* 1984; **104**: 839-844 [PMID: 6726513 DOI: 10.1016/S0022-3476(84)80477-1]

7 **Vegnente A**, Larcher VF, Mowat AP, Portmann B, Williams R. Duration of chronic active hepatitis and the development of cirrhosis. *Arch Dis Child* 1984; **59**: 330-335 [PMID: 6721559 DOI: 10.1136/adc.59.4.330]

8 **Maggiore G**, Bernard O, Homberg JC, Hadchouel M, Alvarez F, Hadchouel P, Odièvre M, Alagille D. Liver disease associated with anti-liver-kidney microsome antibody in children. *J Pediatr* 1986; **108**: 399-404 [PMID: 3950819 DOI: 10.1016/S0022-3476(86)80880-0]

9 **Homberg JC**, Abuaf N, Bernard O, Islam S, Alvarez F, Khalil SH, Poupon R, Darnis F, Lévy VG, Grippon P. Chronic active hepatitis associated with antiliver/kidney microsome antibody type 1: a second type of "autoimmune" hepatitis. *Hepatology* 1987; **7**: 1333-1339 [PMID: 3679093 DOI: 10.1002/hep.1840070626]

10 **Maggiore G**, Sciveres M. Autoimmune hepatitis: a childhood disease. *Curr Pediatr Rev* 2005; **1**: 73-90 [DOI: 10.2174/1573396052953499]

11 **Boberg KM**. Prevalence and epidemiology of autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 635-647 [PMID: 12362572 DOI: 10.1016/S1089-3261(02)00021-1]

12 **Primo J**, Merino C, Fernández J, Molés JR, Llorca P, Hinojosa J. [Incidence and prevalence of autoimmune hepatitis in the area of the Hospital de Sagunto (Spain)]. *Gastroenterol Hepatol* 2004; **27**: 239-243 [PMID: 15056409 DOI: 10.1016/S0210-5705(03)70452-X]

13 **Hurlburt KJ**, McMahon BJ, Deubner H, Hsu-Trawinski B, Williams JL, Kowdley KV. Prevalence of autoimmune liver disease in Alaska Natives. *Am J Gastroenterol* 2002; **97**: 2402-2407 [PMID: 12358264 DOI: 10.1111/j.1572-0241.2002.06019.x]

14 **Deneau M**, Jensen MK, Holmen J, Williams MS, Book LS, Guthery SL. Primary sclerosing cholangitis, autoimmune hepatitis, and overlap in Utah children: epidemiology and natural history. *Hepatology* 2013; **58**: 1392-1400 [PMID: 23686586 DOI: 10.1002/hep.26454]

15 **Mieli-Vergani G**, Vergani D. Autoimmune liver diseases in children - what is different from adulthood? *Best Pract Res Clin Gastroenterol* 2011; **25**: 783-795 [PMID: 22117642 DOI: 10.1016/j.bpg.2011.10.007]

16 **Longhi MS**, Ma Y, Mieli-Vergani G, Vergani D. Aetiopathogenesis of autoimmune hepatitis. *J Autoimmun* 2010; **34**: 7-14 [PMID: 19766456 DOI: 10.1016/j.jaut.2009.08.010]

17 **Donaldson PT**, Czaja AJ. Genetic effects on susceptibility, clinical expression, and treatment outcome of type 1 autoimmune hepatitis. *Clin Liver Dis* 2002; **6**: 707-725 [PMID: 12362576 DOI: 10.1016/S1089-3261(02)00023-5]

18 **Yoshizawa K**, Ota M, Katsuyama Y, Ichijo T, Matsumoto A, Tanaka E, Kiyosawa K. Genetic analysis of the HLA region of Japanese patients with type 1 autoimmune hepatitis. *J Hepatol* 2005; **42**: 578-584 [PMID: 15763345 DOI: 10.1016/j.jhep.2004.12.019]

19 **Pando M**, Larriba J, Fernandez GC, Fainboim H, Ciocca M, Ramonet M, Badia I, Daruich J, Findor J, Tanno H, Cañero-Velasco C, Fainboim L. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology* 1999; **30**: 1374-1380 [PMID: 10573514 DOI: 10.1002/hep.510300611]

20 **Vázquez-García MN**, Aláez C, Olivo A, Debaz H, Pérez-Luque E, Burguete A, Cano S, de la Rosa G, Bautista N, Hernández A, Bandera J, Torres LF, Kershenobich D, Alvarez F, Gorodezky C. MHC class II sequences of susceptibility and protection in Mexicans with autoimmune hepatitis. *J Hepatol* 1998; **28**: 985-990 [PMID: 9672174 DOI: 10.1016/S0168-8278(98)80347-4]

21 **Gregorio GV**, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, Vergani D, Mieli-Vergani G. Autoimmune hepatitis in childhood: a 20-year experience. *Hepatology* 1997; **25**: 541-547 [PMID: 9049195 DOI: 10.1002/hep.510250308]

22 **Ma Y**, Bogdanos DP, Hussain MJ, Underhill J, Bansal S, Longhi MS, Cheeseman P, Mieli-Vergani G, Vergani D. Polyclonal T-cell responses to cytochrome P450IID6 are as-

sociated with disease activity in autoimmune hepatitis type 2. *Gastroenterology* 2006; **130**: 868-882 [PMID: 16530525 DOI: 10.1053/j.gastro.2005.12.020]
23 **Djilali-Saiah I**, Fakhfakh A, Louafi H, Caillat-Zucman S, Debray D, Alvarez F. HLA class II influences humoral autoimmunity in patients with type 2 autoimmune hepatitis. *J Hepatol* 2006; **45**: 844-850 [PMID: 17050030 DOI: 10.1016/j.jhep.2006.07.034]
24 **Cassinotti A**, Birindelli S, Clerici M, Trabattoni D, Lazzaroni M, Ardizzone S, Colombo R, Rossi E, Porro GB. HLA and autoimmune digestive disease: a clinically oriented review for gastroenterologists. *Am J Gastroenterol* 2009; **104**: 195-217; quiz 194, 218 [PMID: 19098870 DOI: 10.1038/ajg.2008.10]
25 **Vergani D**, Wells L, Larcher VF, Nasaruddin BA, Davies ET, Mieli-Vergani G, Mowat AP. Genetically determined low C4: a predisposing factor to autoimmune chronic active hepatitis. *Lancet* 1985; **2**: 294-298 [PMID: 2862466]
26 **Mizutani T**, Shinoda M, Tanaka Y, Kuno T, Hattori A, Usui T, Kuno N, Osaka T. Autoantibodies against CYP2D6 and other drug-metabolizing enzymes in autoimmune hepatitis type 2. *Drug Metab Rev* 2005; **37**: 235-252 [PMID: 15747502 DOI: 10.1081/DMR-28798]
27 **Vento S**, Cainelli F. Is there a role for viruses in triggering autoimmune hepatitis? *Autoimmun Rev* 2004; **3**: 61-69 [PMID: 14871651 DOI: 10.1016/S1568-9972(03)00053-3]
28 **Bogdanos DP**, Choudhuri K, Vergani D. Molecular mimicry and autoimmune liver disease: virtuous intentions, malign consequences. *Liver* 2001; **21**: 225-232 [PMID: 11454184 DOI: 10.1034/j.1600-0676.2001.021004225.x]
29 **Muratori L**, Parola M, Ripalti A, Robino G, Muratori P, Bellomo G, Carini R, Lenzi M, Landini MP, Albano E, Bianchi FB. Liver/kidney microsomal antibody type 1 targets CYP2D6 on hepatocyte plasma membrane. *Gut* 2000; **46**: 553-561 [PMID: 10716687 DOI: 10.1136/gut.46.4.553]
30 **Vento S**, Hegarty JE, Bottazzo G, Macchia E, Williams R, Eddleston AL. Antigen specific suppressor cell function in autoimmune chronic active hepatitis. *Lancet* 1984; **1**: 1200-1204 [PMID: 6202994 DOI: 10.1016/S0140-6736(84)91691-X]
31 **Ferri S**, Longhi MS, De Molo C, Lalanne C, Muratori P, Granito A, Hussain MJ, Ma Y, Lenzi M, Mieli-Vergani G, Bianchi FB, Vergani D, Muratori L. A multifaceted imbalance of T cells with regulatory function characterizes type 1 autoimmune hepatitis. *Hepatology* 2010; **52**: 999-1007 [PMID: 20683931 DOI: 10.1002/hep.23792]
32 **Peiseler M**, Sebode M, Franke B, Wortmann F, Schwinge D, Quaas A, Baron U, Olek S, Wiegard C, Lohse AW, Weiler-Normann C, Schramm C, Herkel J. FOXP3+ regulatory T cells in autoimmune hepatitis are fully functional and not reduced in frequency. *J Hepatol* 2012; **57**: 125-132 [PMID: 22425700 DOI: 10.1016/j.jhep.2012.02.029]
33 **Longhi MS**, Hussain MJ, Kwok WW, Mieli-Vergani G, Ma Y, Vergani D. Autoantigen-specific regulatory T cells, a potential tool for immune-tolerance reconstitution in type-2 autoimmune hepatitis. *Hepatology* 2011; **53**: 536-547 [PMID: 21274874 DOI: 10.1002/hep.24039]
34 **Lapierre P**, Béland K, Yang R, Alvarez F. Adoptive transfer of ex vivo expanded regulatory T cells in an autoimmune hepatitis murine model restores peripheral tolerance. *Hepatology* 2013; **57**: 217-227 [PMID: 22911361 DOI: 10.1002/hep.26023]
35 **Lapierre P**, Djilali-Saiah I, Vitozzi S, Alvarez F. A murine model of type 2 autoimmune hepatitis: Xenoimmunization with human antigens. *Hepatology* 2004; **39**: 1066-1074 [PMID: 15057911 DOI: 10.1002/hep.20109]
36 **Holdener M**, Hintermann E, Bayer M, Rhode A, Rodrigo E, Hintereder G, Johnson EF, Gonzalez FJ, Pfeilschifter J, Manns MP, Herrath Mv, Christen U. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. *J Exp Med* 2008; **205**: 1409-1422 [PMID: 18474629 DOI: 10.1084/jem.20071859]
37 **Buxbaum J**, Qian P, Allen PM, Peters MG. Hepatitis resulting from liver-specific expression and recognition of self-antigen. *J Autoimmun* 2008; **31**: 208-215 [PMID: 18513923 DOI: 10.1016/j.jaut.2008.04.015]
38 **Maggiore G**, Veber F, Bernard O, Hadchouel M, Homberg JC, Alvarez F, Hadchouel P, Alagille D. Autoimmune hepatitis associated with anti-actin antibodies in children and adolescents. *J Pediatr Gastroenterol Nutr* 1993; **17**: 376-381 [PMID: 8145091 DOI: 10.1097/00005176-199311000-00007]
39 **Bridoux-Henno L**, Maggiore G, Johanet C, Fabre M, Vajro P, Dommergues JP, Reinert P, Bernard O. Features and outcome of autoimmune hepatitis type 2 presenting with isolated positivity for anti-liver cytosol antibody. *Clin Gastroenterol Hepatol* 2004; **2**: 825-830 [PMID: 15354284 DOI: 10.1016/S1542-3565(04)00354-4]
40 **Maggiore G**, De Benedetti F, Massa M, Pignatti P, Martini A. Circulating levels of interleukin-6, interleukin-8, and tumor necrosis factor-alpha in children with autoimmune hepatitis. *J Pediatr Gastroenterol Nutr* 1995; **20**: 23-27 [PMID: 7884614]
41 **Maggiore G**, Porta G, Bernard O, Hadchouel M, Alvarez F, Homberg JC, Alagille D. Autoimmune hepatitis with initial presentation as acute hepatic failure in young children. *J Pediatr* 1990; **116**: 280-282 [PMID: 2299503 DOI: 10.1016/S0022-3476(05)82892-6]
42 **Rigopoulou EI**, Roggenbuck D, Smyk DS, Liaskos C, Mytilinaiou MG, Feist E, Conrad K, Bogdanos DP. Asialoglycoprotein receptor (ASGPR) as target autoantigen in liver autoimmunity: lost and found. *Autoimmun Rev* 2012; **12**: 260-269 [PMID: 22571878 DOI: 10.1016/j.autrev.2012.04.005]
43 **Löhr H**, Treichel U, Poralla T, Manns M, Meyer zum Büschenfelde KH. Liver-infiltrating T helper cells in autoimmune chronic active hepatitis stimulate the production of autoantibodies against the human asialoglycoprotein receptor in vitro. *Clin Exp Immunol* 1992; **88**: 45-49 [PMID: 1532926 DOI: 10.1111/j.1365-2249.1992.tb03037.x]
44 **Gueguen M**, Meunier-Rotival M, Bernard O, Alvarez F. Anti-liver kidney microsome antibody recognizes a cytochrome P450 from the IID subfamily. *J Exp Med* 1988; **168**: 801-806 [PMID: 2842431 DOI: 10.1084/jem.168.2.801]
45 **Vergani D**, Alvarez F, Bianchi FB, Cançado EL, Mackay IR, Manns MP, Nishioka M, Penner E. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. *J Hepatol* 2004; **41**: 677-683 [PMID: 15464251 DOI: 10.1016/j.jhep.2004.08.002]
46 **Johanet C**, Beleoken E, Ballot E. Autoantibodies in autoimmune hepatitis: antinuclear antibodies (ANA). *Clin Res Hepatol Gastroenterol* 2012; **36**: 394-396 [PMID: 22481089 DOI: 10.1016/j.clinre.2012.02.005]
47 **Couto CA**, Bittencourt PL, Porta G, Abrantes-Lemos CP, Carrilho FJ, Guardia BD, Cançado EL. Antismooth muscle and antiactin antibodies are indirect markers of histological and biochemical activity of autoimmune hepatitis. *Hepatology* 2014; **59**: 592-600 [PMID: 23929663 DOI: 10.1002/hep.26666]
48 **Martini E**, Abuaf N, Cavalli F, Durand V, Johanet C, Homberg JC. Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. *Hepatology* 1988; **8**: 1662-1666 [PMID: 3192182 DOI: 10.1002/hep.1840080632]
49 **Lapierre P**, Hajoui O, Homberg JC, Alvarez F. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. *Gastroenterology* 1999; **116**: 643-649 [PMID: 10029623 DOI: 10.1016/S0016-5085(99)70186-1]
50 **Johanet C**, Ballot E. Auto-antibodies in autoimmune hepatitis: anti-soluble liver antigen (SLA). *Clin Res Hepatol Gastroenterol* 2012; **36**: 244-246 [PMID: 22306052 DOI: 10.1016/j.clinre.2011.10.013]
51 **Gleeson D**, Heneghan MA. British Society of Gastroenterology (BSG) guidelines for management of autoimmune hepatitis. *Gut* 2011; **60**: 1611-1629 [PMID: 21757447 DOI: 10.1136/gut.2010.235259]

52 **Caprai S**, Vajro P, Ventura A, Sciveres M, Maggiore G. Autoimmune liver disease associated with celiac disease in childhood: a multicenter study. *Clin Gastroenterol Hepatol* 2008; **6**: 803-806 [PMID: 18258488 DOI: 10.1016/j.cgh.2007.12.002]

53 **Hofer H**, Oesterreicher C, Wrba F, Ferenci P, Penner E. Centrilobular necrosis in autoimmune hepatitis: a histological feature associated with acute clinical presentation. *J Clin Pathol* 2006; **59**: 246-249 [PMID: 16505273 DOI: 10.1136/jcp.2005.029348]

54 **Devaney K**, Goodman ZD, Ishak KG. Postinfantile giant-cell transformation in hepatitis. *Hepatology* 1992; **16**: 327-333 [PMID: 1639341 DOI: 10.1002/hep.1840160208]

55 **Bernard O**, Hadchouel M, Scotto J, Odièvre M, Alagille D. Severe giant cell hepatitis with autoimmune hemolytic anemia in early childhood. *J Pediatr* 1981; **99**: 704-711 [PMID: 7299542 DOI: 10.1016/S0022-3476(81)80388-5]

56 **Czaja AJ**, Carpenter HA. Autoimmune hepatitis with incidental histologic features of bile duct injury. *Hepatology* 2001; **34**: 659-665 [PMID: 11584360 DOI: 10.1053/jhep.2001.27562]

57 **Vajro P**, Hadchouel P, Hadchouel M, Bernard O, Alagille D. Incidence of cirrhosis in children with chronic hepatitis. *J Pediatr* 1990; **117**: 392-396 [PMID: 2391593 DOI: 10.1016/S0022-3476(05)81078-9]

58 **Floreani A**, Liberal R, Vergani D, Mieli-Vergani G. Autoimmune hepatitis: Contrasts and comparisons in children and adults - a comprehensive review. *J Autoimmun* 2013; **46**: 7-16 [PMID: 24035197 DOI: 10.1016/j.jaut.2013.08.004]

59 **Hennes EM**, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, Bittencourt PL, Porta G, Boberg KM, Hofer H, Bianchi FB, Shibata M, Schramm C, Eisenmann de Torres B, Galle PR, McFarlane I, Dienes HP, Lohse AW. Simplified criteria for the diagnosis of autoimmune hepatitis. *Hepatology* 2008; **48**: 169-176 [PMID: 18537184 DOI: 10.1002/hep.22322]

60 **Czaja AJ**. Performance parameters of the diagnostic scoring systems for autoimmune hepatitis. *Hepatology* 2008; **48**: 1540-1548 [PMID: 18924244 DOI: 10.1002/hep.22513]

61 **Ebbeson RL**, Schreiber RA. Diagnosing autoimmune hepatitis in children: is the International Autoimmune Hepatitis Group scoring system useful? *Clin Gastroenterol Hepatol* 2004; **2**: 935-940 [PMID: 15476158 DOI: 10.1016/S1542-3565(04)00396-9]

62 **Hiejima E**, Komatsu H, Sogo T, Inui A, Fujisawa T. Utility of simplified criteria for the diagnosis of autoimmune hepatitis in children. *J Pediatr Gastroenterol Nutr* 2011; **52**: 470-473 [PMID: 21407112 DOI: 10.1097/MPG.0b013e3181fc1e0b]

63 **Ferri PM**, Ferreira AR, Miranda DM, Simões E Silva AC. Diagnostic criteria for autoimmune hepatitis in children: a challenge for pediatric hepatologists. *World J Gastroenterol* 2012; **18**: 4470-4473 [PMID: 22969217 DOI: 10.3748/wjg.v18.i33.4470]

64 **Vitfell-Pedersen J**, Jørgensen MH, Müller K, Heilmann C. Autoimmune hepatitis in children in Eastern Denmark. *J Pediatr Gastroenterol Nutr* 2012; **55**: 376-379 [PMID: 22644464 DOI: 10.1097/MPG.0b013e3182602b20]

65 **Ferreira AR**, Roquete ML, Toppa NH, de Castro LP, Fagundes ED, Penna FJ. Effect of treatment of hepatic histopathology in children and adolescents with autoimmune hepatitis. *J Pediatr Gastroenterol Nutr* 2008; **46**: 65-70 [PMID: 18162836 DOI: 10.1097/01.mpg.0000304456.84552.13]

66 **Clark JH**, Fitzgerald JF. Effect of exogenous corticosteroid therapy on growth in children with HBsAg-negative chronic aggressive hepatitis. *J Pediatr Gastroenterol Nutr* 1984; **3**: 72-76 [PMID: 6694051 DOI: 10.1097/00005176-198401000-00016]

67 **Johnson PJ**, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. *N Engl J Med* 1995; **333**: 958-963 [PMID: 7666914 DOI: 10.1056/NEJM199510123331502]

68 **Nastasio S**, Sciveres M, Riva S, Filippeschi IP, Vajro P, Maggiore G. Celiac disease-associated autoimmune hepatitis in childhood: long-term response to treatment. *J Pediatr Gastroenterol Nutr* 2013; **56**: 671-674 [PMID: 23403438 DOI: 10.1097/MPG.0b013e31828b1dfa]

69 **Terrabuio DR**, Abrantes-Lemos CP, Carrilho FJ, Cançado EL. Follow-up of pregnant women with autoimmune hepatitis: the disease behavior along with maternal and fetal outcomes. *J Clin Gastroenterol* 2009; **43**: 350-356 [PMID: 19077726 DOI: 10.1097/MCG.0b013e318176b8c5]

70 **Heneghan MA**, Norris SM, O'Grady JG, Harrison PM, McFarlane IG. Management and outcome of pregnancy in autoimmune hepatitis. *Gut* 2001; **48**: 97-102 [PMID: 11115829 DOI: 10.1136/gut.48.1.97]

71 **Alvarez F**, Ciocca M, Cañero-Velasco C, Ramonet M, de Davila MT, Cuarterolo M, Gonzalez T, Jara-Vega P, Camarena C, Brochu P, Drut R, Alvarez E. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. *J Hepatol* 1999; **30**: 222-227 [PMID: 10068099 DOI: 10.1016/S0168-8278(99)80065-8]

72 **Debray D**, Maggiore G, Girardet JP, Mallet E, Bernard O. Efficacy of cyclosporin A in children with type 2 autoimmune hepatitis. *J Pediatr* 1999; **135**: 111-114 [PMID: 10393616 DOI: 10.1016/S0022-3476(99)70339-2]

73 **Sciveres M**, Caprai S, Palla G, Ughi C, Maggiore G. Effectiveness and safety of ciclosporin as therapy for autoimmune diseases of the liver in children and adolescents. *Aliment Pharmacol Ther* 2004; **19**: 209-217 [PMID: 14723612 DOI: 10.1046/j.1365-2036.2003.01754.x]

74 **Aw MM**, Dhawan A, Samyn M, Bargiota A, Mieli-Vergani G. Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. *J Hepatol* 2009; **51**: 156-160 [PMID: 19446911 DOI: 10.1016/j.jhep.2009.02.024]

75 **Woynarowski M**, Nemeth A, Baruch Y, Koletzko S, Melter M, Rodeck B, Strassburg CP, Pröls M, Woźniak M, Manns MP. Budesonide versus prednisone with azathioprine for the treatment of autoimmune hepatitis in children and adolescents. *J Pediatr* 2013; **163**: 1347-1353.e1 [PMID: 23810723 DOI: 10.1016/j.jpeds.2013.05.042]

76 **Mieli-Vergani G**, Vergani D. Budesonide for juvenile autoimmune hepatitis? Not yet. *J Pediatr* 2013; **163**: 1246-1248 [PMID: 23932214 DOI: 10.1016/j.jpeds.2013.06.064]

77 **D'Agostino D**, Costaguta A, Álvarez F. Successful treatment of refractory autoimmune hepatitis with rituximab. *Pediatrics* 2013; **132**: e526-e530 [PMID: 23821693 DOI: 10.1542/peds.2011-1900]

78 **Martin SR**, Alvarez F, Anand R, Song C, Yin W. Outcomes in children who underwent transplantation for autoimmune hepatitis. *Liver Transpl* 2011; **17**: 393-401 [PMID: 21445922 DOI: 10.1002/lt.22244]

79 **Murray-Lyon IM**, Stern RB, Williams R. Controlled trial of prednisone and azathioprine in active chronic hepatitis. *Lancet* 1973; **1**: 735-737 [PMID: 4121073 DOI: 10.1016/S0140-6736(73)92125-9]

80 **Stellon AJ**, Hegarty JE, Portmann B, Williams R. Randomised controlled trial of azathioprine withdrawal in autoimmune chronic active hepatitis. *Lancet* 1985; **1**: 668-670 [PMID: 2858619 DOI: 10.1016/S0140-6736(85)91329-7]

81 **Cook GC**, Mulligan R, Sherlock S. Controlled prospective trial of corticosteroid therapy in active chronic hepatitis. *Q J Med* 1971; **40**: 159-185 [PMID: 4933363]

82 **Maggiore G**, Bernard O, Hadchouel M, Alagille D. Life-saving immunosuppressive treatment in severe autoimmune chronic active hepatitis. *J Pediatr Gastroenterol Nutr* 1985; **4**: 655-658 [PMID: 4032180 DOI: 10.1097/00005176-198508000-00028]

83 **Soloway RD**, Summerskill WH, Baggenstoss AH, Geall MG, Gitnick GL, Elveback IR, Schoenfield LJ. Clinical, biochemical, and histological remission of severe chronic active liver disease: a controlled study of treatments and early prognosis. *Gastroenterology* 1972; **63**: 820-833 [PMID: 4538724]

84 **Mistilis SP**, Skyring AP, Blackburn CR. Natural history of active chronic hepatitis. I. Clinical features, course, diagnostic criteria, morbidity, mortality and survival. *Australas Ann*

Med 1968; **17**: 214-223 [PMID: 5684607]
85 **Krawitt EL**. Autoimmune hepatitis. *N Engl J Med* 2006; **354**: 54-66 [PMID: 16394302 DOI: 10.1056/NEJMra050408]
86 **Feld JJ**, Dinh H, Arenovich T, Marcus VA, Wanless IR, Heathcote EJ. Autoimmune hepatitis: effect of symptoms and cirrhosis on natural history and outcome. *Hepatology* 2005; **42**: 53-62 [PMID: 15954109 DOI: 10.1002/hep.20732]
87 **Saadah OI**, Smith AL, Hardikar W. Long-term outcome of autoimmune hepatitis in children. *J Gastroenterol Hepatol* 2001; **16**: 1297-1302 [PMID: 11903750 DOI: 10.1046/j.1440-1746.2001.02615.x]
88 **Radhakrishnan KR**, Alkhouri N, Worley S, Arrigain S, Hupertz V, Kay M, Yerian L, Wyllie R, Feldstein AE. Autoimmune hepatitis in children--impact of cirrhosis at presentation on natural history and long-term outcome. *Dig Liver Dis* 2010; **42**: 724-728 [PMID: 20163994 DOI: 10.1016/j.dld.2010.01.002]
89 **Deneau M**, Book LS, Guthery SL, Jensen MK. Outcome after Discontinuation of Immunosuppression in Children with Autoimmune Hepatitis: A Population-Based Study. *J Pediatr* 2014; **164**: 714-719.e2 [DOI: 10.1016/j.jpeds.2013.12.008]
90 **Whitington PF**, Vos MB, Bass LM, Melin-Aldana H, Romero R, Roy CC, Alvarez F. Humoral immune mechanism of liver injury in giant cell hepatitis with autoimmune hemolytic anemia. *J Pediatr Gastroenterol Nutr* 2014; **58**: 74-80 [PMID: 23969541 DOI: 10.1097/MPG.0b013e3182a98dbe]
91 **Maggiore G**, Sciveres M, Fabre M, Gori L, Pacifico L, Resti M, Choulot JJ, Jacquemin E, Bernard O. Giant cell hepatitis with autoimmune hemolytic anemia in early childhood: long-term outcome in 16 children. *J Pediatr* 2011; **159**: 127-132.e1 [PMID: 21349541 DOI: 10.1016/j.jpeds.2010.12.050]
92 **Shores D**, Kobak G, Pegram LD, Whitington PF, Shneider BL. Giant cell hepatitis and immune thrombocytopenic purpura: reversal of liver failure with rituximab therapy. *J Pediatr Gastroenterol Nutr* 2012; **55**: e128-e130 [PMID: 21921813 DOI: 10.1097/MPG.0b013e3182359002]
93 **Lega S**, Maschio M, Taddio A, Maggiore G, Ventura A. Giant cell hepatitis with Coombs-positive haemolytic anaemia: steroid sparing with high-dose intravenous immunoglobulin and cyclosporine. *Acta Paediatr* 2013; **102**: e137-e139 [PMID: 23205764 DOI: 10.1111/apa.12114]
94 **Czaja AJ**. Autoantibody-negative autoimmune hepatitis. *Dig Dis Sci* 2012; **57**: 610-624 [PMID: 22187100 DOI: 10.1007/s10620-011-2017-z]
95 **Gassert DJ**, Garcia H, Tanaka K, Reinus JF. Corticosteroid-responsive cryptogenic chronic hepatitis: evidence for seronegative autoimmune hepatitis. *Dig Dis Sci* 2007; **52**: 2433-2437 [PMID: 17429719 DOI: 10.1007/s10620-006-9665-4]
96 **Czaja AJ**, Carpenter HA, Santrach PJ, Moore SB, Homburger HA. The nature and prognosis of severe cryptogenic chronic active hepatitis. *Gastroenterology* 1993; **104**: 1755-1761 [PMID: 8500735]
97 **Czaja AJ**. Comparability of probable and definite autoimmune hepatitis by international diagnostic scoring criteria. *Gastroenterology* 2011; **140**: 1472-1480 [PMID: 21324319 DOI: 10.1053/j.gastro.2011.02.010]
98 **Czaja AJ**, Rakela J, Ludwig J. Features reflective of early prognosis in corticosteroid-treated severe autoimmune chronic active hepatitis. *Gastroenterology* 1988; **95**: 448-453 [PMID: 3391372]
99 **Czaja AJ**. Corticosteroids or not in severe acute or fulminant autoimmune hepatitis: therapeutic brinksmanship and the point beyond salvation. *Liver Transpl* 2007; **13**: 953-955 [PMID: 17600348 DOI: 10.1002/lt.21088]
100 **Quail MA**, Russell RK, Bellamy C, Mieli-Vergani G, Gillett PM. Seronegative autoimmune hepatitis presenting after diagnosis of coeliac disease: a case report. *Eur J Gastroenterol Hepatol* 2009; **21**: 576-579 [PMID: 19318969 DOI: 10.1097/MEG.0b013e3282fa1400]
101 **Tosun MS**, Ertekin V, Selimoğlu MA. Autoimmune hepatitis associated with celiac disease in childhood. *Eur J Gastroenterol Hepatol* 2010; **22**: 898-899 [PMID: 20535074 DOI: 10.1097/MEG.0b013e32832faf09]
102 **El-Shabrawi M**, El-Karaksy H, Mohsen N, Isa M, Al-Biltagi M, El-Ansari M. Celiac disease in children and adolescents with autoimmune hepatitis: a single-centre experience. *J Trop Pediatr* 2011; **57**: 104-108 [PMID: 20571152 DOI: 10.1093/tropej/fmq057]
103 **Di Biase AR**, Colecchia A, Scaioli E, Berri R, Viola L, Vestito A, Balli F, Festi D. Autoimmune liver diseases in a paediatric population with coeliac disease - a 10-year single-centre experience. *Aliment Pharmacol Ther* 2010; **31**: 253-260 [PMID: 19878151 DOI: 10.1111/j.1365-2036.2009.04186.x]
104 **Clemente MG**, Meloni A, Obermayer-Straub P, Frau F, Manns MP, De Virgiliis S. Two cytochromes P450 are major hepatocellular autoantigens in autoimmune polyglandular syndrome type 1. *Gastroenterology* 1998; **114**: 324-328 [PMID: 9453493 DOI: 10.1016/S0016-5085(98)70484-6]
105 **Lankisch TO**, Mourier O, Sokal EM, Habes D, Lacaille F, Bridoux-Henno L, Hermeziu B, Lenaerts C, Strassburg CP, Jacquemin E. AIRE gene analysis in children with autoimmune hepatitis type I or II. *J Pediatr Gastroenterol Nutr* 2009; **48**: 498-500 [PMID: 19322061 DOI: 10.1097/MPG.0b013e31818550de]
106 **Kerkar N**, Hadzić N, Davies ET, Portmann B, Donaldson PT, Rela M, Heaton ND, Vergani D, Mieli-Vergani G. De-novo autoimmune hepatitis after liver transplantation. *Lancet* 1998; **351**: 409-413 [PMID: 9482295 DOI: 10.1016/S0140-6736(97)06478-7]
107 **Mieli-Vergani G**, Vergani D. De novo autoimmune hepatitis after liver transplantation. *J Hepatol* 2004; **40**: 3-7 [PMID: 14672607 DOI: 10.1016/j.jhep.2003.10.022]
108 **Andries S**, Casamayou L, Sempoux C, Burlet M, Reding R, Bernard Otte J, Buts JP, Sokal E. Posttransplant immune hepatitis in pediatric liver transplant recipients: incidence and maintenance therapy with azathioprine. *Transplantation* 2001; **72**: 267-272 [PMID: 11477351 DOI: 10.1097/00007890-200107270-00018]
109 **Hernandez HM**, Kovarik P, Whitington PF, Alonso EM. Autoimmune hepatitis as a late complication of liver transplantation. *J Pediatr Gastroenterol Nutr* 2001; **32**: 131-136 [PMID: 11321380 DOI: 10.1097/00005176-200102000-00007]
110 **Heneghan MA**, Portmann BC, Norris SM, Williams R, Muiesan P, Rela M, Heaton ND, O'Grady JG. Graft dysfunction mimicking autoimmune hepatitis following liver transplantation in adults. *Hepatology* 2001; **34**: 464-470 [PMID: 11526530 DOI: 10.1053/jhep.2001.26756]
111 **Gupta P**, Hart J, Millis JM, Cronin D, Brady L. De novo hepatitis with autoimmune antibodies and atypical histology: a rare cause of late graft dysfunction after pediatric liver transplantation. *Transplantation* 2001; **71**: 664-668 [PMID: 11292299 DOI: 10.1097/00007890-200103150-00016]
112 **Spada M**, Bertani A, Sonzogni A, Petz W, Riva S, Torre G, Melzi ML, Alberti D, Colledan M, Segalin A, Lucianetti A, Gridelli B. A cause of late graft dysfunction after liver transplantation in children: de-novo autoimmune hepatitis. *Transplant Proc* 2001; **33**: 1747-1748 [PMID: 11267495 DOI: 10.1016/S0041-1345(00)02826-8]
113 **Salcedo M**, Vaquero J, Bañares R, Rodríguez-Mahou M, Alvarez E, Vicario JL, Hernández-Albújar A, Tíscar JL, Rincón D, Alonso S, De Diego A, Clemente G. Response to steroids in de novo autoimmune hepatitis after liver transplantation. *Hepatology* 2002; **35**: 349-356 [PMID: 11826408 DOI: 10.1053/jhep.2002.31167]
114 **Clemente MG**, Vajro P, Musu MP, Cicotto L, Ciccimarra E, Mandato C, De Virgiliis S. Autoimmune manifestations in children transplanted for non- autoimmune liver diseases. *J Hepatol* 2001; **34**: 45 (abstract) [DOI: 10.1016/S0168-8278(01)81023-0]
115 **Petz W**, Sonzogni A, Bertani A, Spada M, Lucianetti A,

Colledan M, Gridelli B. A cause of late graft dysfunction after pediatric liver transplantation: de novo autoimmune hepatitis. *Transplant Proc* 2002; **34**: 1958-1959 [PMID: 12176643 DOI: 10.1016/S0041-1345(02)03137-8]

116 **Gibelli NE**, Tannuri U, Mello ES, Cançado ER, Santos MM, Ayoub AA, Maksoud-Filho JG, Velhote MC, Silva MM, Pinho-Apezzato ML, Maksoud JG. Successful treatment of de novo autoimmune hepatitis and cirrhosis after pediatric liver transplantation. *Pediatr Transplant* 2006; **10**: 371-376 [PMID: 16677364 DOI: 10.1111/j.1399-3046.2005.00470.x]

P- Reviewer: Tannuri U **S- Editor**: Song XX
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.477

World J Hepatol 2014 July 27; 6(7): 477-485
ISSN 1948-5182 (online)

REVIEW

Focal liver lesions detection and characterization: The advantages of gadoxetic acid-enhanced liver MRI

Stefano Palmucci

Stefano Palmucci, Radiodiagnostic and Radiotherapy Unit, University Hospital "Policlinico-Vittorio Emanuele", 95123 Catania, Italy
Author contributions: Palmucci S designed and wrote the manuscript.
Correspondence to: Stefano Palmucci, MD, Radiodiagnostic and Radiotherapy Unit, Universiy Hospital "Policlinico-Vittorio Emanuele", Via Santa Sofia 78, 95123 Catania, Italy. spalmucci@sirm.org
Telephone: +39-95-3781769 Fax: +39-95-3782368
Received: March 28, 2014 Revised: May 14, 2014
Accepted: June 10, 2014
Published online: July 27, 2014

Abstract

Since its clinical introduction, several studies in literature have investigated gadolinium ethoxybenzhyl diethylenetriaminepentaacetic acid or gadoxetic acid (Gd-EOB-DTPA) properties. Following contrast injection, it provides dynamic vascular phases (arterial, portal and equilibrium phases) and hepatobiliary phase, the latter due to its uptake by functional hepatocytes. The main advantages of Gd-EOB-DTPA of focal liver lesion detection and characterization are discussed in this paper. Namely, we focus on the possibility of distinguishing focal nodular hyperplasia (FNH) from hepatic adenoma (HA), the identification of early hepatocellular carcinoma (HCC) and the pre-operative assessment of metastasis in liver parenchyma. Regarding the differentiation between FNH and HA, adenoma typically appears hypointense in hepatobiliary phase, whereas FNH is isointense or hyperintense to the surrounding hepatic parenchyma. As for the identification of early HCCs, many papers recently published in literature have emphasized the contribution of hepatobiliary phase in the characterization of nodules without a typical hallmark of HCC. Atypical nodules (no hypervascularizaton observed on arterial phase and/or no hypovascular appearance on portal phase) with low signal intensity in the hepatobiliary phase, have a high probability of malignancy. Finally, regarding the evaluation of focal hepatic metastases, magnetic resonance pre-operative assessment using gadoxetic acid allows for more accurate diagnosis.

Key words: Magnetic resonance imaging; Liver; Image enhancement; Gadolinium diethylenetriaminepentaacetic acid; Carcinoma; Hepatocellular

Core tip: This study highlights the added value of gadoxetic acid-enhanced liver magnetic resonance imaging (MRI) in the detection and characterization of focal liver lesions. Three main topics are summarized: the role of gadoxetic acid in the evaluation of solid benign hepatic lesions, represented by hepatocellular adenoma and focal nodular hyperplasia; the diagnostic capability of hepatobiliary phase of gadoxetic acid-enhanced liver magnetic resonance imaging in the early identification of small hepatocellular carcinoma; the high diagnostic accuracy powered by gadoxetic enhanced-liver MRI in the detection of hepatic metastasis.

Palmucci S. Focal liver lesions detection and characterization: The advantages of gadoxetic acid-enhanced liver MRI. *World J Hepatol* 2014; 6(7): 477-485 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/477.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.477

INTRODUCTION

Since the first studies were reported in literature in 1991-1992, several authors have investigated the potentialities of gadolinium ethoxybenzhyl diethylenetriaminepentaacetic acid or gadoxetic acid (Gd-EOB-DTPA) enhanced magnetic resonance imaging (MRI) liver[1-5]. In a previous article published by Mühler *et al*[5], spin-echo

Table 1 Imaging features of focal liver lesions in the dynamic vascular phases (after contrast administration) and in the hepatobiliary phase

	Phases			
	Arterial	Portal	Delayed	Hepato-biliary
FNH	Hyperintense	Isointense	Isointense	Hyperintense/isointense (hypointense[1])
Adenoma	Hyperintense	Isotense/slightly hypointense	Isotense/slightly hypointense	Hypointense (hyperintense or mixed hypo/hyperintense[1])
Typical HCC	Hyperintense	Hypointense	Hypointense	Hypointense
Pre/early HCC (decreased portal supply)	Isointense	Hypointense	Hypointense	Hypointense
Pre/early HCC (increased arterial supply)	Hyperintense	Isointense	Isointense	Hypointense
Metastasis (hypovascular)	Irregularly hypointense	Irregularly hypointense	Irregularly hypointense	Hypointense
Metastasis (hypervascular)	Irregularly hyperintense	Isointense or hypointense	Inhomogeneously hypointense	Hypointense

[1]Atypical behaviours of focal liver lesions. FNH: Focal nodular hyperplasia; HCC: Hepatocellular carcinoma.

(SE) sequences and short tau inversion recovery (STIR) sequences were compared in the detection of experimental liver metastases[5]. Relative enhancement and lesion-to-liver contrast were also analysed in the mentioned study. After contrast administration, the authors reported lesion-to-liver contrast increased by approximately 500% with both SE and STIR sequences. Therefore, we can see that the role of Gd-EOB-DTPA in focal liver lesion (FLLs) detection has been studied from the beginning.

Subsequently, the usefulness of hepatospecific contrast in liver MRI has been confirmed by other studies. In fact, detection and characterization of focal liver tumours have been compared in the same patient using Gd-EOB-DTPA and Gd-DTPA enhanced MRI[6]. In the assessment of FLLs, Gd-EOB-DTPA has also been compared with intra-operative findings in a multicenter analysis[7].

Although research on focal lesions is the most common, some authors have observed that, because of its properties, Gd-EOB-DTPA could be potentially used as a tracer of liver functionality[8-10].

The mechanisms of contrast uptake and excretion have been documented[11-14]. The uptake of Gd-Eob-DTPA is achieved by functional hepatocytes, which have the cloned organic anion transporting polypeptides (OATPs). In humans the contrast is introduced through OATP1 and OATP3 transporters, located at the apical membrane of hepatocytes[15]. Then, the contrast has urinary and biliary excretion rates (the latter up to 50%, much higher than other hepatospecific contrasts). Regarding biliary excretion, the contrast is excreted through Multidrug Resistance-associated Proteins (MRPs) to bile canaliculi (MRP2 = apical transporter) or sinusoidal spaces (MRP3, MRP4 = basolateral transporters)[11-15].

Thus, in normal liver parenchyma starting during dynamic vascular phases, hepatocytes increase the uptake of gadoxetic acid. The uptake process is gradually followed by contrast discharging through the bile canaliculi. Generally, the hepatobiliary phase, where hepatocytes reach maximum signal intensity, is obtained 20 min after contrast administration. The variable contrast uptake by FLLs represents an additional diagnostic tool in liver imaging.

The aim of this topic highlight is to discuss the advantages of gadoxetic acid-enhanced liver MRI in the study of FLLs, focusing on: (1) Evaluation of hepatic adenoma and focal nodular hyperplasia; (2) Identification of early hepatocellular carcinoma (HCC); and (3) Detection of hepatic metastases detection in oncology patients. Typical and atypical behaviours of FLLs using gadoxetic acid-enhanced MRI are summarized in Table 1, which shows imaging features observed also in the hepatobiliary phase.

EVALUATION OF HEPATIC ADENOMA AND FOCAL NODULAR HYPERPLASIA

The use of Gd-Eob-DTPA allows for characterization of hepatic adenoma (HA) and focal nodular hyperplasia (FNH). In some cases, diagnosis between these solid lesions cannot be reliably achieved using only dynamic vascular phases, and hepatobiliary contrast agents are very useful in their differentiation. In fact, in a previous study, although using gadobenate dimeglumine-a different liver specific contrast from gadoxetic acid-Grazioli *et al*[16] reported an overall accuracy of 98.3% in the differentiation of FNH from HA and liver adenomatosis, with positive predictive value of 100% and negative predictive value of 96.4%.

FNH was described for the first time by Edmondson in 1956[17]. The lesion is considered a non-neoplastic and hyperplastic response of the liver parenchyma to "a pre-existing local arterial spiderlike malformation"[18]. It occurs in asymptomatic women. The relationship between FNH and contraceptives is still unclear as several authors have demonstrated that contraceptives may favour FNH progression[19]. The lesion is generally represented by a solid circumscribed mass, sometimes with lobulated contour (Figure 1), with a central scar surrounded by nodules of hyperplastic hepatocytes and small bile ductuli[20]. FNHs may show a certain degree of histological heterogeneity, due to the variable degree of intra-lesional inflammation, fibrosis or fat content (the latter has been described as steatotic FNH).

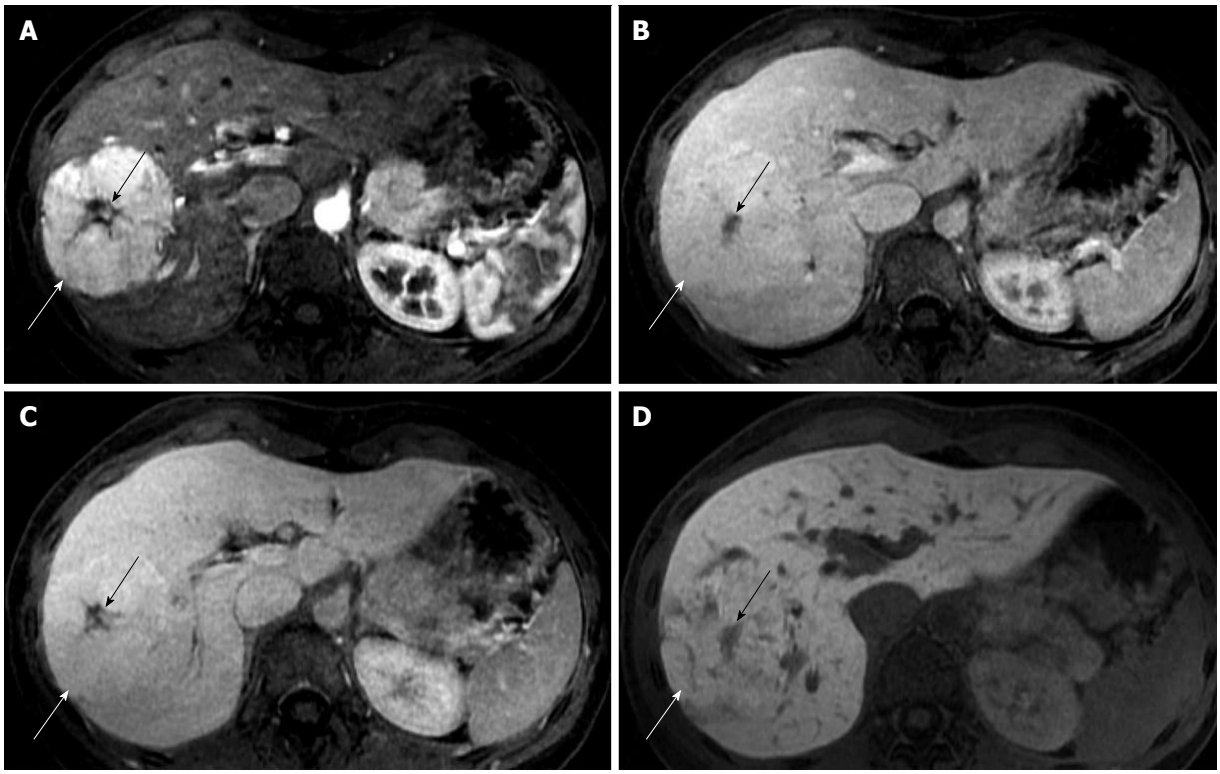

Figure 1 Typical imaging features of focal nodular hyperplasia in a 29-year-old woman. Gadoxetic acid-enhanced magnetic resonance imaging; axial images (A-D) were obtained in dynamic phases and hepatobiliary phase. A shows a solid circumscribed mass (white arrow), lobulated in contour, with a central scar (black arrow); the lesion is hyperintense on the arterial phase (A) and persists slightly hyperintense in the portal and venous phases (B and C respectively). In hepatobiliary phase (D) the mass is slightly hyperintense or isointense to the surrounding liver. The presence of biliary canaliculi, even if not functioning, leads to retention of gadoxetic acid in comparison to the surrounding parenchyma.

Hepatic adenoma is a rare monoclonal benign liver tumour, predominantly found in young females and associated with the use of contraceptives[21]. It generally appears as an uncapsulated mass, formed by large plats or cord cells very similar to hepatocytes. In a work by Grazioli *et al*[22], they are defined as "these plats are separated by sinusoids, which consist of small capillaries perfused through the arterial pressure". This histological architecture explains the morphological behaviour of adenomas during the dynamic phases after contrast administration. In fact, lesions often appear hypervascular in the arterial phase, and are generally isointense or hypointense to the surrounding liver in the portal phase. The vascular supply in the portal phase is not observed because of the adenomas lack of a portal vascularization[22]. Adenomas have a poor number of Kupfer cells, and this histological feature could explain the absence of technetium (Tc)-99m sulfur colloid uptake. In addition, HAs do not have bile canaliculi[23,24].

The significant capability of Gd-Eob-DTPA in distinguishing FNH from adenomas depends on histological features and cellular expression of molecular transporters. Bile ductuli are present in FNHs, whereas they are missing in HAs. The molecular transporter Organic Anion Transporting Polypeptide 8 (OATP 8) is usually absent or minimally expressed in cellular adenomas. This transporter is instead expressed in FNH, explaining the uptake of Gd-Eob-DTPA[25].

Thus, typically HAs appear hypointense, whereas FNHs are isointense or hyperintense to the surrounding hepatic parenchyma (Figures 1 and 2, Table 1). Several studies have described the mentioned imaging features.

In a work published in 2001, all three adenomas studied in the hepatobiliary phase by Grazioli *et al*[22] showed hypointense appearance following liver contrast agent administration. Zech *et al*[25] reported enhancement in the hepatobiliary phase in the 90% of FNH examined in their series where only a minority of lesions showed no enhancement or peripheral enhancement. The presence of biliary canaliculi, even if not functioning, leads to a "slower excretion in comparison to the surrounding parenchyma", and this gadoxetic acid retention explains the hyperintense appearance of FNH[26] (Figures 1 and 2).

Nevertheless, atypical lesions are very difficult to diagnose, even using Gd-EOB-DTPA. In fact, the heterogeneity of FNH could also explain the atypical imaging presentation that has recently been well described in many articles[27,28]. In another case series published in literature, Grazioli *et al*[29] found that 62 out of 68 FNHs (91.2%) were hyperintense or isointense to the surrounding liver, with only 6 lesions showing an atypical pattern[29]. One atypical enhancement pattern explanation was the presence of a large central scar. These lesions appeared hypointense in hepatobiliary phase, showing only a little marginal enhancement. Two atypical lesions, in the series reported by Grazioli *et al*[29], were hypointense for

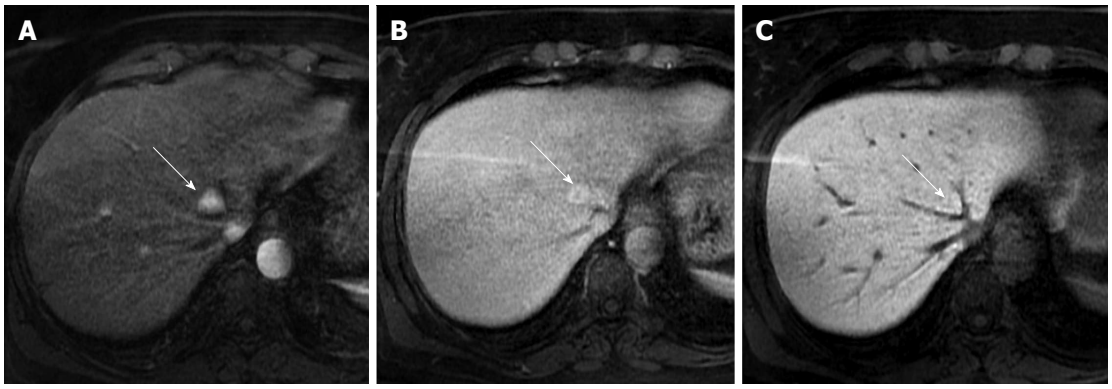

Figure 2 Magnetic resonance imaging of a small focal nodular hyperplasia. Arterial, venous and hepatobiliary phases (A, B and C), acquired in a 44-year-old woman shows the typical enhancement of a small focal nodular hyperplasia (white arrows). The lesion is located in the fourth liver segment, between medium and left sovrahepatic vein. In hepatobiliary phase (C) the lesion is slightly hyperintense to the surrounding liver parenchyma, due to uptake of hepatospecific contrast.

the presence of large fibrous components and abundant fat contents (steatotic FNH).

On the other hand, atypical HAs may not appear hypointense in the hepatospecific phase. Atypical behaviours, appearing as hyperintense lesions, have been reported in literature[15]. In fact, inflammatory adenomas could enhance in the hepatospecific phase. Hyperintense HAs in the hepatobiliary phase have been observed in the series by Denecke *et al*[15]. They reported one hepatic adenoma homogeneously hyperintense and two HAs with a mixed pattern (hypo-/hyperintense). In the subgroup of fatty hepatic adenomas, 14 adenomas were hypointense and 1 was mixed hyper-/hypointense. Also, Huppertz *et al*[30] describe in their FLLs series two out of three adenomas with hyperintense appearance in comparison to the surrounding liver. However, based on a quantitative analysis, all HAs, with hypointense signal to the surrounding liver on hepatobiliary phase, showed a certain degree of increase in signal intensity[15]. This could probably be explained by contrast retention in the interstitium or fibrotic tissue.

In addition, in the series reported by Denecke *et al*[15], the proportion between hyperintense and hypointense adenomas in hepatobiliary phase was approximately equal both in the non-steatotic group and in the steatotic of fatty adenomas[29]. The mechanism of Gd-EOB-DTPA uptake in these minority HAs is still unclear and further studies with histological correlation are needed.

IDENTIFICATION OF EARLY HCC

The progressive differentiation of a regenerative nodule to a dysplastic nodule, and then to an early-HCC has been well investigated[31-34]. In this differentiation, the nodule increases its arteriolar supply progressively and reduces the portal vascularization[32,33]. This vascular change is a crucial step in the carcinogenesis. In view of this consideration, HCC diagnosis with imaging techniques is based on a "vascular analysis" of enhancing pattern, with an increased signal intensity or "wash-in" during the arterial phase and a "wash-out" pattern in the portal or equilibrium phase[35] (Table 1).

In 2012 the European Association for the Study of the Liver (EASL) and European Organization for Research and Treatment of Cancer (EORTC) provided common guidelines for the management of the liver[36]. The joint committee established that non-invasive assessment for HCC could be made only by applying a 4-phase multidetector computed tomography (CT) scan or dynamic contrast-enhanced MRI. In addition, the guidelines postulated that diagnosis is based on a typical morphological hallmark of HCC (Figure 3), with hypervascular pattern in the arterial phase and wash-out in the portal venous or delayed phases[36]. It has to be remarked that while only one technique is required for nodules greater than 1 cm in diameter (evidence 2D, recommendation 2B), a more conservative approach using 2 techniques is recommended in suboptimal settings[36].

Similarly, in 2010 an update of The American Association for the Study of Liver Disease (AASLD) recommended that nodules greater than 1 cm should be investigated with either 4-phase multidetector CT scan or dynamic contrast enhanced MRI[37]. In case of atypical nodules, a second contrast methodical is required (level Ⅱ), or alternatively a biopsy.

Nevertheless, the characterization of a nodule, based on these approaches, is not possible if both mentioned imaging features, "wash-in" and "wash-out", are not observed. Nodules may have hypervascular appearance in arterial phase, without evident wash-out in the portal or equilibrium phase (Figure 4). They could also have the same attenuation or signal intensity to the surrounding liver parenchyma during the dynamic arterial phase on CT and MRI images respectively, and may manifest a wash-out only in the portal phase. In this case the diagnosis is difficult and so, a further analysis is usually required in order to evaluate other important features such as a change in size or a tumour marker. A more invasive approach could be also adopted by choosing a biopsy.

In addition, small nodules (< 2 cm) very often lack the typical behaviour of HCC. Arterial neovascularization or reduced portal supply cannot be identified on imaging techniques, probably because these vascular changes are not significant. Adopting only hypervascularity criteria in

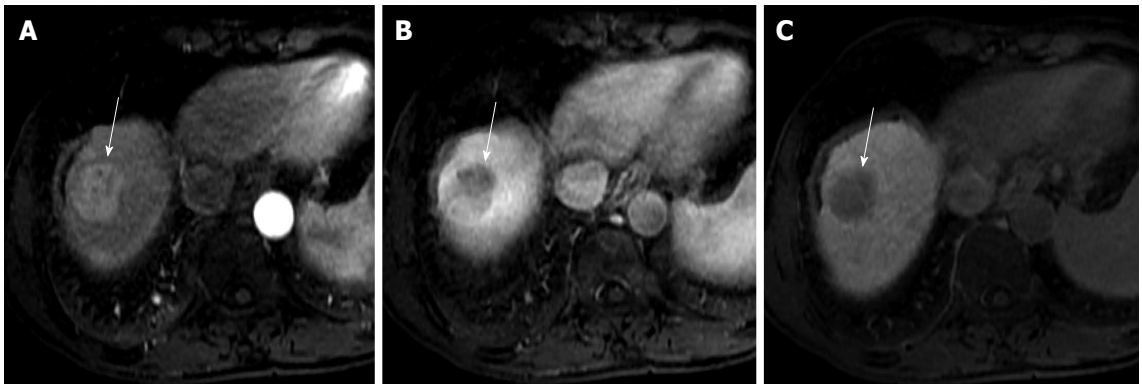

Figure 3 Imaging features of a typical hepatocellular carcinoma. Axial magnetic resonance images show a hypervascular lesion in the arterial phase (A, white arrow), located in the top of the liver, with wash-out clearly in the portal venous phase (B, white arrow). This enhancement pattern represents the typical morphological hallmark of hepatocellular carcinoma. The nodule has an increased arteriolar supply and reduced portal vascularization. In hepatobiliary phase, the lesion appears hypointense to the surrounding liver parenchyma.

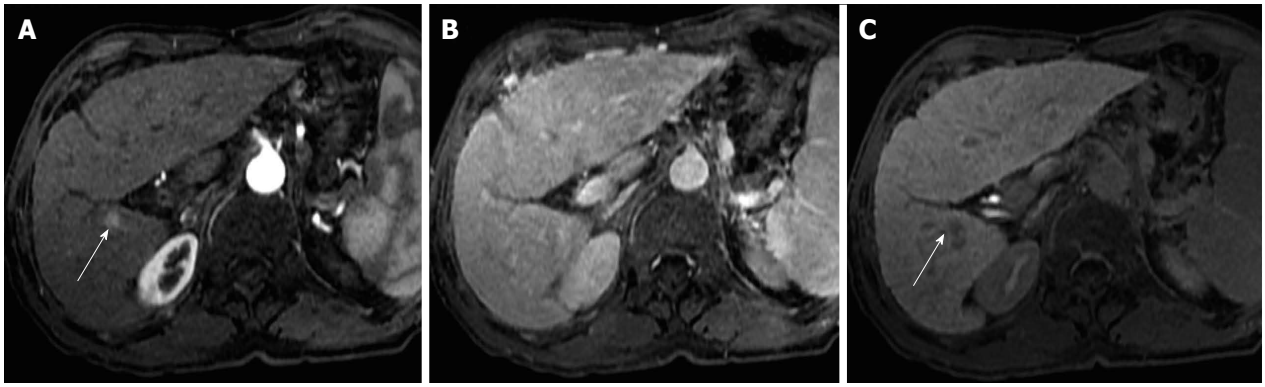

Figure 4 Imaging features of a small hepatocellular carcinoma. The lesion (white arrow), located in the fifth segment of right hepatic lobe, is detectable in the arterial and hepatobiliary phase. It has hypervascular appearance in arterial phase (A), without evident wash-out in the portal phase (B). The lesion is hypointense in the hepatobiliary phase (C). As reported in literature, the low or absence of gadolinium ethoxybenzhyl diethylenetriaminepentaacetic acid or gadoxetic acid uptake could precede the decrease of portal vascularization in malignant differentiation.

the diagnosis of HCC, MR sensitivity for nodules < 20 mm is about 63%[38,39].

An important diagnostic tool for the evaluation of lesions in the hepatospecific phase has now been added. In fact, papers have recently emphasized the contribution of hepatobiliary phase in the characterization of nodules without a typical hallmark of HCC. In a recent paper by Iannicelli *et al*[40], a total of 120 nodules were retrospectively evaluated using gadoxetic acid-enhanced liver MRI. In this study, 92 out of 120 nodules (76.6%) reported typical vascular behaviour of HCC, with hypervascularization appearance in the arterial phase. In the hepatobiliary phase, 90/92 nodules showed low signal intensity, whereas two nodules were hyperintense. The other 28 cases, with non-hypervascular behaviour in the arterial phase, were hypointense in hepatobiliary phase. Among these non-hypervascular nodules, only 15 cases had hypointense signal in the equilibrium phase. In the follow-up study, 50% of non-hypervascular nodules with low signal intensity in the hepatobiliary phase acquired the typical vascular behaviour of HCC.

The high accuracy in the identification of early HCCs will probably change the diagnostic algorithm in hepatocellular carcinoma[41]. It facilitates the diagnosis of hypervascular advanced HCC and the differentiation of early HCC and dysplastic nodules from pseudovascular lesions.

The hypointense appearance in hepatobiliary phase will probably be considered a "radiological marker of nodule differentiation". In the study by Golfieri *et al*[42], 62 out of 215 nodules were atypical for radiological behaviour. Their histological analysis showed 20 high-grade dysplastic nodules (HGDN)/early HCC, 21 low-grade nodules dysplasia, 17 regenerative nodules and 4 nodular regenerative hyperplasia. Nineteen out of 20 HGDN/early HCC nodules were hypointense in hepatobiliary phase. In another work, Kogita *et al*[43] found that low or absence of Gd-EOB-DTPA uptake precedes the decrease of portal vascularization in malignant differentiation (Figure 4).

In conclusion, gadoxetic acid-enhanced liver MRI could be very helpful in the early identification of HCC. However, differentiation between HCC and dysplastic nodule remain very difficult. Atypical nodules require better investigation, studying their behaviour in the hepatospecific phase.

LIVER METASTASES DETECTION IN ONCOLOGY PATIENTS

Detection of liver metastases in oncology patients is essential in order to choose the best possible management and treatment. In this regard, many studies have demonstrated the high diagnostic accuracy of liver MRI[44]. Nevertheless, routine liver MRI is generally not performed for the staging of extra-hepatic oncology diseases. For example, the American College of Radiology Appropriateness Criteria for pre-treatment staging of colorectal cancer recommended CT of the chest, abdomen and pelvis for the initial evaluation of disease[45]. In the majority of the cases, staging liver MRI is required to evaluate doubtful FLLs.

The identification of liver involvement by metastases disease is essential because surgical resection has improved patient survival, especially in cases of colorectal cancer[46,47].

Gadoxetic acid-enhanced liver MRI allows for a vascular dynamic study of the hepatic parenchyma and adds hepatospecific phase for characterization of FLLs[46,48-50]. Lee *et al*[46] evaluated Gd-EOB-DTPA liver MRI and triple-phase multidetector computed tomography (MDCT) in the detection of suspected hepatic metastases, reporting that dynamic MR images with or without hepatospecific phase show better diagnostic performance than MDCT images. The sensitivity increased significantly with the addition of hepatobiliary phase in gadoxetic acid-enhanced MRI ($P < 0.0001$). In particular, the diagnostic accuracy was greater for small lesions (< 1 cm)[46]. Gadoxetic acid-enhanced liver MRI showed higher capability than enhanced MDCT in detection liver metastases from pancreatic carcinoma. In fact, in a recent work by Motosugi *et al*[48], higher values of sensitivity for detection of metastases were reported, with values of 85% for MRI and 69% for MDCT.

Acquisition of hepatospecific phase takes some time in a liver MRI protocol because it is generally performed 20 min after contrast administration. Less time would be important, in order to reduce the length of a liver MRI protocol. Diagnostic accuracy for metastases detection and lesion conspicuity was evaluated in hepatospecific images obtained 10 min and 20 min after gadoxetic acid administration[51]. In the study performed by Jeong *et al*[51], the hepatobiliary phase images obtained at 10 and 20 min after Gd-EOB-DTPA administration improve detection of metastases in comparison with pre-contrast images and dynamic acquisitions only. It has been demonstrated that sensitivity in the detection of metastases does not differ significantly using delay images acquired at 10 min and 20 min after contrast injection. However, in our opinion, the interval time between dynamic acquisitions and 10-min hepatobiliary phase, and between the 10-min and 20-min hepatobiliary phases, could be maintained in a standard liver MRI protocol. In fact, these intervals offer the possibility to acquire other sequences, thus acquiring a more complete liver MRI protocol. Diffusion weighted imaging (DWI) using multiple *b* values could require more time for its acquisition. In line with what has previously been reported in literature[52], morphological T2-weighted sequences, including axial breath-hold steady-state free-precession, axial breath-hold single shot spin-echo and axial breath-hold fast spin-echo sequences are acquired after dynamic imaging in our protocol. After these T2-weighted sequences, radiologists may acquire the first hepatospecific phase (10 min after contrast administration). Then, between 10-min and 20-min hepatobiliary phases, DWI could be placed without any considerable influence on imaging quality[52].

Recently in the field of FLL detection and characterization, it has been evaluated whether diagnostic performance of gadoxetic acid-enhanced liver MRI could be enriched by DWI. The contribution of DWI has been widely applied in different radiology fields[53-58]. In detection and characterization of FLLs, diffusion imaging reported higher scores in comparison with conventional T2-weighted sequences. In view of these results, several studies have compared the diagnostic capability of DWI and gadoxetic acid-enhanced liver MRI in detection FLLs. Donati *et al*[59] found that adding DWI to Gd-EOB-DTPA did not significantly increase diagnostic accuracy compared to Gd-EOB-DTPA imaging alone. Considering the detection of small metastases, Shimada *et al*[60] reported higher diagnostic accuracy of Gd-EOB-DTPA in comparison to DWI. Probably, both imaging modalities represent very important diagnostic tools in the evaluation of FLLs, as recently described in a study by Macera *et al*[61]. They found that the combination of DWI with Gd-EOB-DTPA-enhanced MRI imaging significantly increases the diagnostic accuracy sensitivity in patients with colorectal liver metastases treated with pre-operative chemotherapy[61].

CONCLUSION

The topics discussed clearly demonstrate the importance of gadoxetic acid-enhanced liver MRI in the evaluation of FLLs. In fact, it significantly increases diagnostic accuracy in the detection and characterization of FLLs. Furthermore, it allows for the diagnosis of benign solid hepatic lesions such as FNH and HA, thanks to the different contrast uptake observed in hepatobiliary phase.

Some atypical nodules in vascular behaviours could be diagnosed as HCC if they lack Gd-EOB-DTPA retention in the hepatobiliary phase. The HCC guidelines need to underline the recent use of a liver hepatospecific agent. Finally, MR pre-operative assessment using gadoxetic acid allows for higher diagnostic accuracy in the detection of hepatic metastases.

REFERENCES

1 **Weinmann HJ**, Schuhmann-Giampieri G, Schmitt-Willich H, Vogler H, Frenzel T, Gries H. A new lipophilic gadolinium chelate as a tissue-specific contrast medium for MRI. *Magn Reson Med* 1991; **22**: 233-237; discussion 242 [PMID: 1812351]

2 **Schuhmann-Giampieri G**, Schmitt-Willich H, Press WR, Negishi C, Weinmann HJ, Speck U. Preclinical evaluation

of Gd-EOB-DTPA as a contrast agent in MR imaging of the hepatobiliary system. *Radiology* 1992; **183**: 59-64 [PMID: 1549695 DOI: 10.1148/radiology.183.1.1549695]

3 **Clément O**, Mühler A, Vexler V, Berthezène Y, Brasch RC. Gadolinium-ethoxybenzyl-DTPA, a new liver-specific magnetic resonance contrast agent. Kinetic and enhancement patterns in normal and cholestatic rats. *Invest Radiol* 1992; **27**: 612-619 [PMID: 1428739]

4 **Clément O**, Mühler A, Vexler VS, Rosenau W, Berthezène Y, Kuwatsuru R, Brasch RC. Evaluation of radiation-induced liver injury with MR imaging: comparison of hepatocellular and reticuloendothelial contrast agents. *Radiology* 1992; **185**: 163-168 [PMID: 1523301 DOI: 10.1148/radiology.185.1.1523301]

5 **Mühler A**, Clément O, Vexler V, Berthezène Y, Rosenau W, Brasch RC. Hepatobiliary enhancement with Gd-EOB-DTPA: comparison of spin-echo and STIR imaging for detection of experimental liver metastases. *Radiology* 1992; **184**: 207-213 [PMID: 1609081 DOI: 10.1148/radiology.184.1.1609081]

6 **Hammerstingl R**, Zangos S, Schwarz W, Rosen T, Bechstein WO, Balzer T, Vogl TJ. Contrast-enhanced MRI of focal liver tumors using a hepatobiliary MR contrast agent: detection and differential diagnosis using Gd-EOB-DTPA-enhanced versus Gd-DTPA-enhanced MRI in the same patient. *Acad Radiol* 2002; **9** Suppl 1: S119-S120 [PMID: 12019845]

7 **Huppertz A**, Balzer T, Blakeborough A, Breuer J, Giovagnoni A, Heinz-Peer G, Laniado M, Manfredi RM, Mathieu DG, Mueller D, Reimer P, Robinson PJ, Strotzer M, Taupitz M, Vogl TJ. Improved detection of focal liver lesions at MR imaging: multicenter comparison of gadoxetic acid-enhanced MR images with intraoperative findings. *Radiology* 2004; **230**: 266-275 [PMID: 14695400 DOI: 10.1148/radiol.2301020269]

8 **Ryeom HK**, Kim SH, Kim JY, Kim HJ, Lee JM, Chang YM, Kim YS, Kang DS. Quantitative evaluation of liver function with MRI Using Gd-EOB-DTPA. *Korean J Radiol* 2004; **5**: 231-239 [PMID: 15637473]

9 **Tamada T**, Ito K, Higaki A, Yoshida K, Kanki A, Sato T, Higashi H, Sone T. Gd-EOB-DTPA-enhanced MR imaging: evaluation of hepatic enhancement effects in normal and cirrhotic livers. *Eur J Radiol* 2011; **80**: e311-e316 [PMID: 21315529 DOI: 10.1016/j.ejrad.2011.01.020]

10 **Tajima T**, Takao H, Akai H, Kiryu S, Imamura H, Watanabe Y, Shibahara J, Kokudo N, Akahane M, Ohtomo K. Relationship between liver function and liver signal intensity in hepatobiliary phase of gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging. *J Comput Assist Tomogr* 2010; **34**: 362-366 [PMID: 20498536 DOI: 10.1097/RCT.0b013e3181cd3304]

11 **Jeong WK**, Kim YK, Song KD, Choi D, Lim HK. The MR imaging diagnosis of liver diseases using gadoxetic acid: emphasis on hepatobiliary phase. *Clin Mol Hepatol* 2013; **19**: 360-366 [PMID: 24459639 DOI: 10.3350/cmh.2013.19.4.360]

12 **Kim SH**, Jeong WK, Kim Y, Kim MY, Kim J, Pyo JY, Oh YH. Hepatocellular carcinoma composed of two different histologic types: imaging features on gadoxetic acid-enhanced liver MRI. *Clin Mol Hepatol* 2013; **19**: 92-96 [PMID: 23593616 DOI: 10.3350/cmh.2013.19.1.92]

13 **Pastor CM**, Planchamp C, Pochon S, Lorusso V, Montet X, Mayer J, Terrier F, Vallee JP. Kinetics of gadobenate dimeglumine in isolated perfused rat liver: MR imaging evaluation. *Radiology* 2003; **229**: 119-125 [PMID: 12944603 DOI: 10.1148/radiol.2291020726]

14 **Tsuboyama T**, Onishi H, Kim T, Akita H, Hori M, Tatsumi M, Nakamoto A, Nagano H, Matsuura N, Wakasa K, Tomoda K. Hepatocellular carcinoma: hepatocyte-selective enhancement at gadoxetic acid-enhanced MR imaging--correlation with expression of sinusoidal and canalicular transporters and bile accumulation. *Radiology* 2010; **255**: 824-833 [PMID: 20501720 DOI: 10.1148/radiol.10091557]

15 **Denecke T**, Steffen IG, Agarwal S, Seehofer D, Kröncke T, Hänninen EL, Kramme IB, Neuhaus P, Saini S, Hamm B, Grieser C. Appearance of hepatocellular adenomas on gadoxetic acid-enhanced MRI. *Eur Radiol* 2012; **22**: 1769-1775 [PMID: 22437921 DOI: 10.1007/s00330-012-2422-5]

16 **Grazioli L**, Morana G, Kirchin MA, Schneider G. Accurate differentiation of focal nodular hyperplasia from hepatic adenoma at gadobenate dimeglumine-enhanced MR imaging: prospective study. *Radiology* 2005; **236**: 166-177 [PMID: 15955857 DOI: 10.1148/radiol.2361040338]

17 **Edmondson HA**. Tumors of the liver and intrahepatic bile ducts. Washington: Armed Forces Institute of Pathology, 1958

18 **Wanless IR**, Mawdsley C, Adams R. On the pathogenesis of focal nodular hyperplasia of the liver. *Hepatology* 1985; **5**: 1194-1200 [PMID: 4065824]

19 **Shen YH**, Fan J, Wu ZQ, Ma ZC, Zhou XD, Zhou J, Qiu SJ, Qin LX, Ye QH, Sun HC, Huang XW, Tang ZY. Focal nodular hyperplasia of the liver in 86 patients. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 52-57 [PMID: 17287167]

20 **Bartolozzi C**, Cioni D, Donati F, Lencioni R. Focal liver lesions: MR imaging-pathologic correlation. *Eur Radiol* 2001; **11**: 1374-1388 [PMID: 11519546]

21 **Maillette de Buy Wenniger L**, Terpstra V, Beuers U. Focal nodular hyperplasia and hepatic adenoma: epidemiology and pathology. *Dig Surg* 2010; **27**: 24-31 [PMID: 20357448 DOI: 10.1159/000268404]

22 **Grazioli L**, Federle MP, Brancatelli G, Ichikawa T, Olivetti L, Blachar A. Hepatic adenomas: imaging and pathologic findings. *Radiographics* 2001; **21**: 877-892; discussion 892-894 [PMID: 11452062 DOI: 10.1148/radiographics.21.4.g01jl04877]

23 **Rubin RA**, Lichtenstein GR. Hepatic scintigraphy in the evaluation of solitary solid liver masses. *J Nucl Med* 1993; **34**: 697-705 [PMID: 8384256]

24 **Boulahdour H**, Cherqui D, Charlotte F, Rahmouni A, Dhumeaux D, Zafrani ES, Meignan M. The hot spot hepatobiliary scan in focal nodular hyperplasia. *J Nucl Med* 1993; **34**: 2105-2110 [PMID: 8254396]

25 **Zech CJ**, Grazioli L, Breuer J, Reiser MF, Schoenberg SO. Diagnostic performance and description of morphological features of focal nodular hyperplasia in Gd-EOB-DTPA-enhanced liver magnetic resonance imaging: results of a multicenter trial. *Invest Radiol* 2008; **43**: 504-511 [PMID: 18580333 DOI: 10.1097/RLI.0b013e3181705cd1]

26 **Mohajer K**, Frydrychowicz A, Robbins JB, Loeffler AG, Reed TD, Reeder SB. Characterization of hepatic adenoma and focal nodular hyperplasia with gadoxetic acid. *J Magn Reson Imaging* 2012; **36**: 686-696 [PMID: 22674623 DOI: 10.1002/jmri.23701]

27 **Baranes L**, Chiaradia M, Pigneur F, Decaens T, Djabbari M, Zegaï B, Costentin C, Laurent A, Calderaro J, Rahmouni A, Luciani A. Imaging benign hepatocellular tumors: atypical forms and diagnostic traps. *Diagn Interv Imaging* 2013; **94**: 677-695 [PMID: 23830777 DOI: 10.1016/j.diii.2013.05.002]

28 **Ronot M**, Paradis V, Duran R, Kerbaol A, Vullierme MP, Belghiti J, Valla DC, Vilgrain V. MR findings of steatotic focal nodular hyperplasia and comparison with other fatty tumours. *Eur Radiol* 2013; **23**: 914-923 [PMID: 23064717 DOI: 10.1007/s00330-012-2676-y]

29 **Grazioli L**, Bondioni MP, Haradome H, Motosugi U, Tinti R, Frittoli B, Gambarini S, Donato F, Colagrande S. Hepatocellular adenoma and focal nodular hyperplasia: value of gadoxetic acid-enhanced MR imaging in differential diagnosis. *Radiology* 2012; **262**: 520-529 [PMID: 22282184 DOI: 10.1148/radiol.11101742]

30 **Huppertz A**, Haraida S, Kraus A, Zech CJ, Scheidler J, Breuer J, Helmberger TK, Reiser MF. Enhancement of focal liver lesions at gadoxetic acid-enhanced MR imaging: correlation with histopathologic findings and spiral CT--initial observations. *Radiology* 2005; **234**: 468-478 [PMID: 15591431]

31 **Lee JM**, Zech CJ, Bolondi L, Jonas E, Kim MJ, Matsui O, Merkle EM, Sakamoto M, Choi BI. Consensus report of the

4th International Forum for Gadolinium-Ethoxybenzyl-Diethylenetriamine Pentaacetic Acid Magnetic Resonance Imaging. *Korean J Radiol* 2011; **12**: 403-415 [PMID: 21852900 DOI: 10.3348/kjr.2011.12.4.403]

32 **Haradome H**, Grazioli L, Tinti R, Morone M, Motosugi U, Sano K, Ichikawa T, Kwee TC, Colagrande S. Additional value of gadoxetic acid-DTPA-enhanced hepatobiliary phase MR imaging in the diagnosis of early-stage hepatocellular carcinoma: comparison with dynamic triple-phase multidetector CT imaging. *J Magn Reson Imaging* 2011; **34**: 69-78 [PMID: 21598343 DOI: 10.1002/jmri.22588]

33 **Kudo M**. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. *J Gastroenterol* 2009; **44** Suppl 19: 112-118 [PMID: 19148804 DOI: 10.1007/s00535-008-2274-6]

34 **Chou CT**, Chou JM, Chang TA, Huang SF, Chen CB, Chen YL, Chen RC. Differentiation between dysplastic nodule and early-stage hepatocellular carcinoma: the utility of conventional MR imaging. *World J Gastroenterol* 2013; **19**: 7433-7439 [PMID: 24259975 DOI: 10.3748/wjg.v19.i42.7433]

35 **Joo I**, Choi BI. New paradigm for management of hepatocellular carcinoma by imaging. *Liver Cancer* 2012; **1**: 94-109 [PMID: 24159577 DOI: 10.1159/000342404]

36 **European Association For The Study Of The Liver1**; European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

37 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

38 **Forner A**, Vilana R, Ayuso C, Bianchi L, Solé M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Brú C, Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; **47**: 97-104 [PMID: 18069697 DOI: 10.1002/hep.21966]

39 **International Consensus Group for Hepatocellular NeoplasiaThe International Consensus Group for Hepatocellular Neoplasia**. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009; **49**: 658-664 [PMID: 19177576 DOI: 10.1002/hep.22709]

40 **Iannicelli E**, Di Pietropaolo M, Marignani M, Briani C, Federici GF, Delle Fave G, David V. Gadoxetic acid-enhanced MRI for hepatocellular carcinoma and hypointense nodule observed in the hepatobiliary phase. *Radiol Med* 2014; **119**: 367-376 [PMID: 24297598 DOI: 10.1007/s11547-013-0364-x]

41 **Kudo M**. Will Gd-EOB-MRI change the diagnostic algorithm in hepatocellular carcinoma? *Oncology* 2010; **78** Suppl 1: 87-93 [PMID: 20616589 DOI: 10.1159/000315235]

42 **Golfieri R**, Renzulli M, Lucidi V, Corcioni B, Trevisani F, Bolondi L. Contribution of the hepatobiliary phase of Gd-EOB-DTPA-enhanced MRI to Dynamic MRI in the detection of hypovascular small (≤ 2 cm) HCC in cirrhosis. *Eur Radiol* 2011; **21**: 1233-1242 [PMID: 21293864 DOI: 10.1007/s00330-010-2030-1]

43 **Kogita S**, Imai Y, Okada M, Kim T, Onishi H, Takamura M, Fukuda K, Igura T, Sawai Y, Morimoto O, Hori M, Nagano H, Wakasa K, Hayashi N, Murakami T. Gd-EOB-DTPA-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading and portal blood flow. *Eur Radiol* 2010; **20**: 2405-2413 [PMID: 20490505 DOI: 10.1007/s00330-010-1812-9]

44 **Imam K**, Bluemke DA. MR imaging in the evaluation of hepatic metastases. *Magn Reson Imaging Clin N Am* 2000; **8**: 741-756 [PMID: 11149677]

45 **Dewhurst C**, Rosen MP, Blake MA, Baker ME, Cash BD, Fidler JL, Greene FL, Hindman NM, Jones B, Katz DS, Lalani T, Miller FH, Small WC, Sudakoff GS, Tulchinsky M, Yaghmai V, Yee J. ACR Appropriateness Criteria pretreatment staging of colorectal cancer. *J Am Coll Radiol* 2012; **9**: 775-781 [PMID: 23122343 DOI: 10.1016/j.jacr.2012.07.025]

46 **Lee KH**, Lee JM, Park JH, Kim JH, Park HS, Yu MH, Yoon JH, Han JK, Choi BI. MR imaging in patients with suspected liver metastases: value of liver-specific contrast agent gadoxetic acid. *Korean J Radiol* 2013; **14**: 894-904 [PMID: 24265564 DOI: 10.3348/kjr.2013.14.6.894]

47 **Baker ME**, Pelley R. Hepatic metastases: basic principles and implications for radiologists. *Radiology* 1995; **197**: 329-337 [PMID: 7480672 DOI: 10.1148/radiology.197.2.7480672]

48 **Motosugi U**, Ichikawa T, Morisaka H, Sou H, Muhi A, Kimura K, Sano K, Araki T. Detection of pancreatic carcinoma and liver metastases with gadoxetic acid-enhanced MR imaging: comparison with contrast-enhanced multi-detector row CT. *Radiology* 2011; **260**: 446-453 [PMID: 21693662 DOI: 10.1148/radiol.11103548]

49 **Böttcher J**, Hansch A, Pfeil A, Schmidt P, Malich A, Schneeweiss A, Maurer MH, Streitparth F, Teichgräber UK, Renz DM. Detection and classification of different liver lesions: comparison of Gd-EOB-DTPA-enhanced MRI versus multiphasic spiral CT in a clinical single centre investigation. *Eur J Radiol* 2013; **82**: 1860-1869 [PMID: 23932636 DOI: 10.1016/j.ejrad.2013.06.013]

50 **Muhi A**, Ichikawa T, Motosugi U, Sou H, Nakajima H, Sano K, Sano M, Kato S, Kitamura T, Fatima Z, Fukushima K, Iino H, Mori Y, Fujii H, Araki T. Diagnosis of colorectal hepatic metastases: comparison of contrast-enhanced CT, contrast-enhanced US, superparamagnetic iron oxide-enhanced MRI, and gadoxetic acid-enhanced MRI. *J Magn Reson Imaging* 2011; **34**: 326-335 [PMID: 21780227 DOI: 10.1002/jmri.22613]

51 **Jeong HT**, Kim MJ, Park MS, Choi JY, Choi JS, Kim KS, Choi GH, Shin SJ. Detection of liver metastases using gadoxetic-enhanced dynamic and 10- and 20-minute delayed phase MR imaging. *J Magn Reson Imaging* 2012; **35**: 635-643 [PMID: 22095933 DOI: 10.1002/jmri.22880]

52 **Saito K**, Araki Y, Park J, Metoki R, Katsuyama H, Nishio R, Kakizaki D, Moriyasu F, Tokuuye K. Effect of Gd-EOB-DTPA on T2-weighted and diffusion-weighted images for the diagnosis of hepatocellular carcinoma. *J Magn Reson Imaging* 2010; **32**: 229-234 [PMID: 20578029 DOI: 10.1002/jmri.22219]

53 **Morani AC**, Elsayes KM, Liu PS, Weadock WJ, Szklaruk J, Dillman JR, Khan A, Chenevert TL, Hussain HK. Abdominal applications of diffusion-weighted magnetic resonance imaging: Where do we stand. *World J Radiol* 2013; **5**: 68-80 [PMID: 23671743 DOI: 10.4329/wjr.v5.i3.68]

54 **Lee NK**, Kim S, Kim GH, Kim DU, Seo HI, Kim TU, Kang DH, Jang HJ. Diffusion-weighted imaging of biliopancreatic disorders: correlation with conventional magnetic resonance imaging. *World J Gastroenterol* 2012; **18**: 4102-4117 [PMID: 22919242 DOI: 10.3748/wjg.v18.i31.4102]

55 **Palmucci S**, Mauro LA, Messina M, Russo B, Failla G, Milone P, Berretta M, Ettorre GC. Diffusion-weighted MRI in a liver protocol: its role in focal lesion detection. *World J Radiol* 2012; **4**: 302-310 [PMID: 22900131 DOI: 10.4329/wjr.v4.i7.302]

56 **Ichikawa T**, Haradome H, Hachiya J, Nitatori T, Araki T. Diffusion-weighted MR imaging with a single-shot echo-planar sequence: detection and characterization of focal hepatic lesions. *AJR Am J Roentgenol* 1998; **170**: 397-402 [PMID: 9456953 DOI: 10.2214/ajr.170.2.9456953]

57 **Bruegel M**, Rummeny EJ. Hepatic metastases: use of diffusion-weighted echo-planar imaging. *Abdom Imaging* 2010; **35**: 454-461 [PMID: 19471997 DOI: 10.1007/s00261-009-9541-8]

58 **Palmucci S**, Mauro LA, Failla G, Foti PV, Milone P, Sinagra N, Zerbo D, Veroux P, Ettorre GC, Veroux M. Magnetic resonance with diffusion-weighted imaging in the evaluation of transplanted kidneys: updating results in 35 patients. *Transplant Proc* 2012; **44**: 1884-1888 [PMID: 22974862 DOI: 10.1016/j.transproceed.2012.06.045]

59 **Donati OF**, Fischer MA, Chuck N, Hunziker R, Weishaupt D, Reiner CS. Accuracy and confidence of Gd-EOB-DTPA

enhanced MRI and diffusion-weighted imaging alone and in combination for the diagnosis of liver metastases. *Eur J Radiol* 2013; **82**: 822-828 [PMID: 23287713 DOI: 10.1016/j.ejrad.2012.12.005]

60 **Shimada K**, Isoda H, Hirokawa Y, Arizono S, Shibata T, Togashi K. Comparison of gadolinium-EOB-DTPA-enhanced and diffusion-weighted liver MRI for detection of small hepatic metastases. *Eur Radiol* 2010; **20**: 2690-2698 [PMID: 20563726 DOI: 10.1007/s00330-010-1842-3]

61 **Macera A**, Lario C, Petracchini M, Gallo T, Regge D, Floriani I, Ribero D, Capussotti L, Cirillo S. Staging of colorectal liver metastases after preoperative chemotherapy. Diffusion-weighted imaging in combination with Gd-EOB-DTPA MRI sequences increases sensitivity and diagnostic accuracy. *Eur Radiol* 2013; **23**: 739-747 [PMID: 22976920 DOI: 10.1007/s00330-012-2658-0]

P- Reviewer: Borzio M, Penkova-Radicheva MP **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.486

World J Hepatol 2014 July 27; 6(7): 486-495
ISSN 1948-5182 (online)

REVIEW

Differential diagnosis and management of liver tumors in infants

Israel Fernandez-Pineda, Rosa Cabello-Laureano

Israel Fernandez-Pineda, Rosa Cabello-Laureano, Department of Pediatric Surgery, Virgen del Rocio Children's Hospital, Sevilla 41013, Spain
Author contributions: Fernandez-Pineda I and Cabello-Laureano R designed the review article and wrote the manuscript.
Correspondence to: Israel Fernandez-Pineda, MD, Department of Pediatric Surgery, Virgen del Rocio Children's Hospital, Av. Manuel Siurot s/n, t. 0034 955012924, Sevilla 41013, Spain. israfdez@hotmail.com
Telephone: +34-955-012924 Fax: +34-955-012805
Received: January 21, 2014 Revised: March 21, 2014
Accepted: May 31, 2014
Published online: July 27, 2014

Abstract

During the first year of life, most of the liver neoplasms are benign in origin, but some of these histologically benign lesions may be challenging in their management. Although most hepatic hemangiomas can be safely observed until involution is documented, some patients will need treatment due to progressive hepatomegaly, hypothyroidism and/or cardiac failure. Large mesenchymal hamartomas may require extensive hepatic resection and an appropriate surgical plan is critical to obtain good results. For malignant neoplasms such as hepatoblastoma, complete surgical resection is the mainstay of curative therapy. The decision about whether to perform an upfront or delayed resection of a primary liver malignant tumor is based on many considerations, including the ease of resection, surgical expertise, tumor histology and stage, and the likely chemosensitivity of the tumor. This article reviews the initial management of the more common hepatic tumors of infancy, focusing on the differential diagnosis and treatment options.

Key words: Hepatoblastoma; Hepatic hemangioma; Mesenchymal hamartoma; Undifferentiated embryonal sarcoma of the liver; Focal nodular hyperplasia

Core tip: Management of liver neoplasms during the first year of life may be challenging. Some of these tumors may be observed but others require extensive surgical resection and adjuvant therapies. Differential diagnosis and treatment options are discussed in our article.

Fernandez-Pineda I, Cabello-Laureano R. Differential diagnosis and management of liver tumors in infants. *World J Hepatol* 2014; 6(7): 486-495 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/486.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.486

INTRODUCTION

The management of infants with liver tumors may be challenging and it may require a complete work-up because of symptoms or concern about malignancy. Initial evaluation should be focused on patient history, pregnancy evaluation, gestational age at birth, weight and findings on physical exam. Diagnostic imaging modalities may facilitate the identification of benign and malignant liver tumors, however biopsy or resection for histological diagnosis sometimes becomes necessary. Some of these infantile hepatic neoplasms are highly vascularized and surgical interventions are at high risk of bleeding. Certain tumor markers may be helpful in the initial work-up and evaluation of response to therapy. Alpha-fetoprotein (AFP) level may be elevated in children with malignant lesions such as hepatoblastoma and hepatocellular carcinoma, but cautious interpretation is warranted as AFP level is frequently elevated in infants up to 6 mo of age and may be slightly elevated with benign tumors and with hepatic insult or regeneration. Therapy must be tailored according to the nature of the lesion. Observation is recommended for asymptomatic hepatic hemangioma,

Table 1 Hepatic tumors characterisctics

	Clinical findings	Laboratory findings	Biopsy findings	Therapy	Outcome
Hepatic hemangioma	Cutaneous hemangiomas	Decreased T3, T4	Glut-1 positive/negative	Observation Propranolol Embolization	Favourable
Focal nodular hyperplasia	Bleeding Torsion	-	Glutamine synthetase	Observation Surgery	Favourable
Mesenchymal hamartoma	Hepatomegaly	-	Vimentin, desmin, a-1 antitrypsin, actin, cytokeratins	Surgery	Favourable
Hepatoblastoma	Hepatomegaly	Elevated AFP	Small cells Embryonal epithelial cells	Chemotherapy Surgery	EFS 30%-90%
Billiary tract rhabdomyosarcoma	Jaundice Hilum of the liver	Cholestasis	Embryonal or botryoid subtype	Chemotherapy Radiation therapy Surgery	EFS 60%-90%
Angiosarcoma	Metastatic disease	-	Glut-1 negative	Chemotherapy Radiation therapy Surgery	Unfavourable
Malignant rhabdoid tumor	Metastatic disease	-	INI1/BAF 47	Chemotherapy Surgery	Unfavourable
Undifferentiated embryonal sarcoma	Right lobe of the liver	-	SMA, a-ACT, desmin, vimentin	Chemotherapy Surgery	Unfavourable
Metastatic hepatic disease from NB	Hepatomegaly	Elevated catecholamines	MYC-N	Chemotherapy Radiation therapy Surgery	EFS 50%-90%

AFP: Alpha-fetoprotein; MYC-N: MYC-N proto-oncogene protein; EFS: Event free survival; SMA: Smooth muscle actin; ACT: Actin; INI1/BAF: INI1/BAF protein; NB: Neuroblastoma.

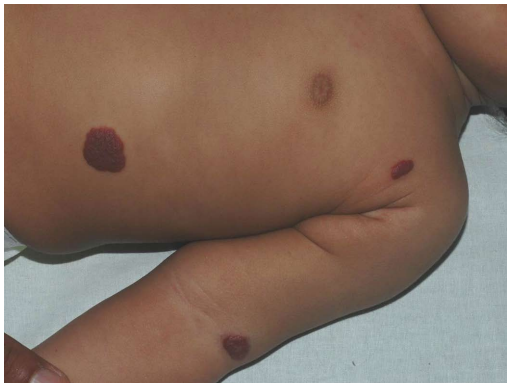

Figure 1 Cutaneous hemangiomatosis.

whereas complete surgical resection in the mainstay of treatment in hepatoblastoma. Benign primary liver tumors described in infants include hemangioma, focal nodular hyperplasia and mesenchymal hamartomas. Hepatic adenoma is almost exclusively a disease of older children. Malignant lesions include hepatoblastoma, biliary tract rhabdomyosarcoma, angiosarcoma, rhabdoid tumor, undifferentiated embryonal sarcoma and metastatic neuroblastoma (Table 1). The aim of this article is to review the clinical features and management of infants diagnosed with a liver tumor.

BENIGN LIVER TUMORS IN INFANTS

Hepatic hemangioma

Hepatic hemangioma (HH) is the most common benign liver tumor of infancy and it must be differentiated from misnamed hepatic hemangiomas seen in adults, which correspond actually to hepatic venous malformations[1,2]. These adult cases are histologically described as cavernous hemangiomas with large, dilated, blood-filled vessels lined by flattened endothelium, whereas HH are true vascular tumors composed of proliferating endothelial cells. A great variety of pediatric vascular lesions is incorrectly referred to as "hemangiomas" in the medical literature and a significant number of patients receive ineffective and potentially harmful treatment based on misclassification. In 2007, Christison-Lagay *et al*[3] from Vascular Anomalies Center in Boston Children's Hospital postulated three principal categories of HH (focal, multifocal, and diffuse) and a clinical practice algorithm. These lesions share the same patterns of growth, histological findings and involution as their cutaneous counterparts, the infantile hemangioma (IH) and the Rapidly Involuting Congenital Hemangioma (RICH)[4-6]. Focal hemangioma seems to correspond with a RICH, a vascular tumor completed formed at birth with no postnatal growth in which involution is normally observed in the first 12-18 mo after birth. Multifocal and diffuse HH correspond with IH, the most common vascular tumor in children that shows a rapid postnatal growth (0-12 mo) followed by slow involution (1-5 years). It is probable that most HH remain undiagnosed since they are asymptomatic self-limiting lesions, although they often come to clinical attention while screening for visceral hemangioma based on the presence of multiple cutaneous IH (Figure 1), since the liver is the most commonly involved organ[3,7,8]. Some patients may develop a congestive heart failure associated with high-volume vascular shunting and treatment is warranted. Unresponsive patients to therapy may develop a severe cardiac failure with hypothyroidism (IH express type 3

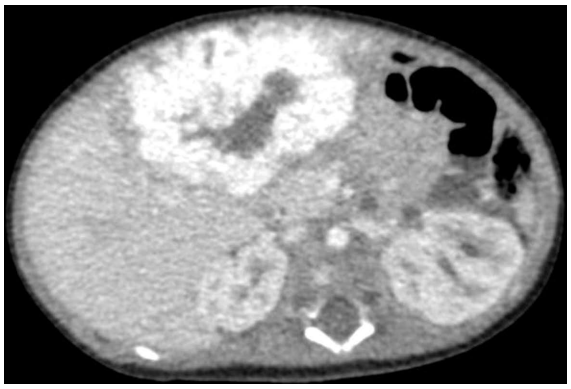

Figure 2 Abdominal computed tomography-contrast. Focal hepatic hemangioma that shows centripetal enhancement and central sparing because of thrombosis, necrosis and/or intralesional hemorrhage.

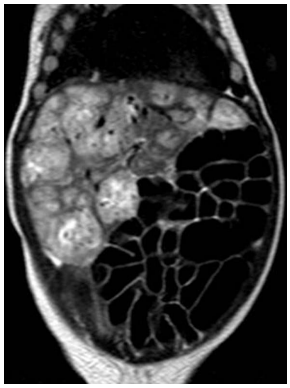

Figure 3 Abdominal magnetic resonance imaging-contrast. Diffuse hepatic hemangioma that nearly totally replaces the liver.

iodothyronine deiodinase that converts thyroid hormone to its inactive form, resulting in an acquired hypothyroidism), abdominal compartment syndrome, and death[9-12].

Differential diagnosis with malignant liver tumors should be performed and AFP should be included in the initial lab work. Focal HH (Figure 2) shows centripetal enhancement and central sparing because of thrombosis, necrosis, or intralesional hemorrhage on computed tomography (CT) or gadolinium magnetic resonance imaging (MRI). Multifocal HH shows multiple well-defined, spherical lesions with intervening areas of normal hepatic parenchyma, whereas diffuse lesions (Figure 3) nearly totally replace the liver. On CT, lesions are hypodense relative to liver without contrast but enhance centripetally with contrast. Central sparing, thrombosis, or necrosis is not seen in multifocal and diffuse HH. Radiologists who are very specialized in looking at vascular lesions feel comfortable in many cases saying that something is an hemangioma *vs* another tumor based upon its radiographic presentation. Hepatoblastomas tend to be heterogeneous on T2-weighted imaging and angiosarcomas seem to have central enhancement rather than centrifugal enhancement, but if there is any question about the diagnosis, a biopsy is recommended, although this procedure is at high risk of bleeding[13-16].

Most of the diagnosed HH may be observed closely with serial abdominal ultrasonography until involution is documented. If the lesions become symptomatic (hemodynamically significant shunting), medical therapy is firstly recommended. Recently, propranolol has been introduced as an effective treatment for cutaneous IH and several recent cases have been reported showing excellent response of diffuse HH to propranolol, even in patients with associated hypothyroidism. Corticosteroids have been first line treatment of infantile hemangioma, but the use of propranolol is emerging as the treatment of choice for high-risk infantile hemangiomas[17,18]. Other therapeutic options include arterial embolization, hepatic artery ligation, resection, or liver transplantation[3].

Focal nodular hyperplasia

Focal nodular hyperplasia (FNH) of the liver is a rare benign lesion, usually seen in older children rather than infants. Girls are more affected than boys. An asymptomatic incidental finding on a diagnostic study is commonly observed[19,20]. A cumulative incidence is reported in oncologic pediatric patients after completion of therapy and differential diagnosis to other focal hepatic lesions, such as metastasis, is often challenging. Infants with neuroblastoma and metastatic hepatic disease seem to be a specific risk-group for FNH development, especially if they underwent chemotherapy and/or radiation therapy to the liver during treatment[21-23]. Gutweiler *et al*[24] reported a hepatoblastoma case presenting with FNH after treatment of neuroblastoma. FNH should be considered in patients with persistent late imaging changes. Classical CT-contrast picture is a lesion enhanced when compared with normal liver and a central scar that becomes hyperintense owing to concentration of the contrast. Currently, liver ultrasound (US) and MRI are the recommended diagnostic imaging tools for characterizing the lesion and subsequent follow-up. Glutamine synthetase is a nitrogen metabolism enzyme with a distribution in the human liver characterized by its strict pericentrolobular localization[25]. It has emerged as a good marker for identification of resected FNH and for differentiating FNH from all other types of hepatocellular nodules developed on normal liver[26]. Acute abdominal pain may develop owing to torsion or rupture of the lesion with bleeding. Although FNH is a benign lesion that is typically managed conservatively in adults, most children with FNH undergo biopsy or resection because of increasing size, concerning symptoms or inability to rule out malignancy, especially in pediatric cancer survivors[27].

Mesenchymal hamartoma

After hemangiomas, mesenchymal hamartoma of the liver (MHL) is the second commonest benign hepatic tumor in childhood, but these tumors are relatively rare. Most MHLs are large benign multicystic masses that present in the first 2 years of life[28]. Prenatal diagnosis of MHL has been reported, most often in the last trimester of pregnancy and it may be a cause of severe hydrops. An early prenatal diagnosis and a subsequent follow-up could help to establish the best time for delivery. Fetal intervention

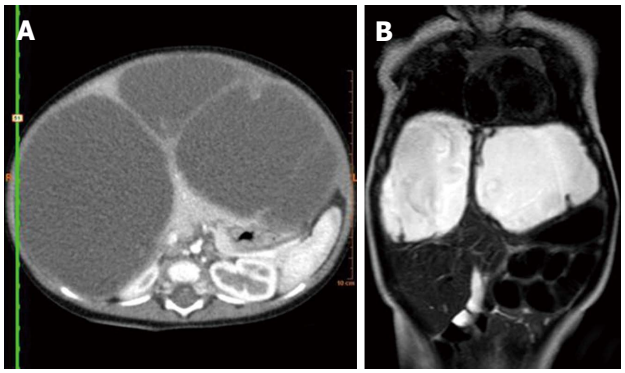

Figure 4 Abdominal computed tomography and magnetic resonance imaging. A: Abdominal computed tomography-contrast shows enhancement of the solid component, septate, and the peripheral rim; B: Abdominal magnetic resonance imaging-contrast shows a high signal intensity on T2-weighted magnetic resonance sequences.

may be beneficial in selected cases. If the fetus is becoming hydropic, early delivery or fetal treatment (particularly if the tumor is composed of a few large cysts) should be considered. Most affected fetuses have been successfully delivered vaginally[29].

Postnatal presentation is more common with abdominal distension and/or an upper abdominal mass. Liver function tests are usually normal. AFP is occasionally elevated though not to the degree that occurs in hepatoblastoma. About 75% of MHL occur in the right lobe of the liver. In the newborn, the tumor may expand rapidly and cause life-threatening abdominal distension with respiratory distress[30]. Diagnostic imaging studies demonstrate a multiloculated cystic tumor with a variable amount of solid tissue[31]. This may be seen in undifferentiated embryonal sarcoma of the liver (UESL), but rarely in hepatoblastoma. Intratumor calcification, which can be frequently detected in hepatoblastoma or hepatic hemangioma, has been reported very rarely for a MHL.

Ultrasound demonstrates the presence of thin mobile septate and/or round hyperechoic parietal nodules within the cysts, but rarely containing debris. The hepatic architecture is normal beyond the outer rim of compressed liver. On CT-contrast the solid component, septate, and the peripheral rim may enhance. On MRI, MHL has a low signal intensity on T1-weighted magnetic resonance sequences and a variable signal intensity on T2-weighted sequences (Figure 4)[32]. In most patients, the diagnosis of MHL is suggested by imaging and confirmed by histological examination of the resected specimen. If radiological diagnosis is not clear, a percutaneous or open tumor biopsy can be performed[33].

Although a laparoscopic or open surgical biopsy is considered by some authors, SIOPEL (International Childhood Liver Tumor Study Group of the International Society of Paediatric Oncology) currently recommends image-guided coaxial plugged needle biopsy for liver tumors (obtaining numerous cores)[34]. Fine needle aspiration cytology is of limited value because hepatoblastoma or a malignant mesenchymal tumor is difficult to exclude. MHL has been considered a focal tumor, but small satellite lesions at the tumor margin have been described, which could explain tumor recurrence after apparent complete resection. Clinical and histological evidence suggest that UESL can develop within a preexisting MHL[28,30]. Both tumors share similar features on gross pathology (cystic and solid components, sometimes pedunculated), histology (mesenchymal elements with benign bile duct epithelial structures), and immunohistochemistry (positive staining for vimentin, desmin, a-1-antitrypsin, actin, cytokeratins). Flow cytometry studies have shown that although most MHLs are diploid, some are aneuploid and cytogenetic studies have demonstrated a balanced translocation involving the same breakpoint on chromosome 19 (band 19q13.4) and chromosome 11. These abnormalities have been found in both, UESL and MHL[28].

The management of MHL remains still controversial. MHL has the potential to involute spontaneously, especially for those tumors with a prominent angiomatous component. Nonoperative management may be appropriate in selected cases (*e.g.*, infants with a biopsy-proven MHL and a prominent vascular component). Percutaneous aspiration or drainage of larger cysts may temporarily control tumor size in life-threatening lesions and it may helpful for the definitive surgical resection. The standard of care is complete resection with the goal of achieving negative margins to avoid the risks of local recurrence and long-term malignant transformation. Enucleation may be adequate in case of very large tumors that replace most of the liver parenchyma. Liver infiltration by MHL is rarely seen and a surgical plain is normally found for resection (Figure 5). Pedunculated lesions are amenable to laparoscopic resection. Marsupialization or partial resection are suboptimal because of the risk of tumor recurrence. Liver transplantation can be considered for unresectable tumors[28,30].

MALIGNANT LIVER TUMORS IN INFANTS

Hepatoblastoma

Hepatoblastoma (HB) is the most common malignant liver tumor in infancy and early childhood, accounting for over 65% of all liver cancer diagnosed in children under 15 years of age. Recent publications indicate that the incidence rates for HB have increased in the last decades[34,35]. Maternal smoking, parental occupation and genetic susceptibility (gene *MPO*, *NQ01*, *SULT*, *IGF-2* and so on) have been associated with HB and recent studies provide support for an increased risk of HB in low (1500-2500 g) and very low (< 1500 g), birth weight infants, in which HB is diagnosed at older ages and in more advanced stages than HB cases of normal birth weight. Neonatal therapies including supplemental oxygen, phototherapy, administration of numerous drugs, total parenteral nutrition and blood transfusions may play a role in the development of HB[36]. An infant with HB usually presents with an abdominal mass often detected by a parent.

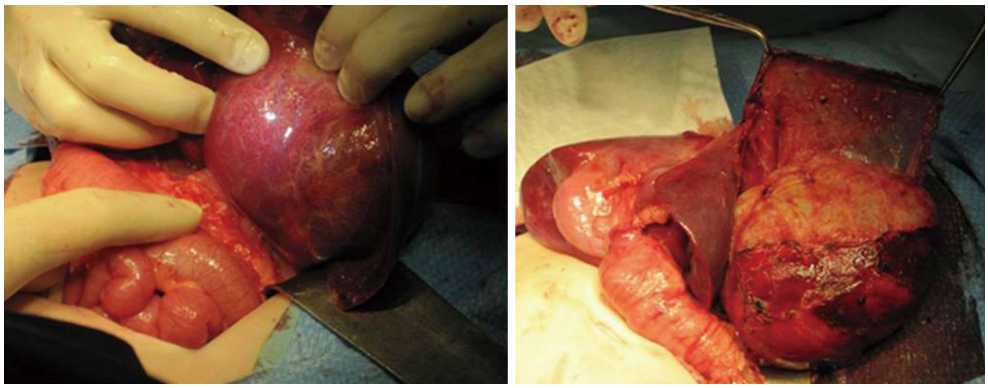

Figure 5 Surgical resection of mesenchymal hamartoma of the liver.

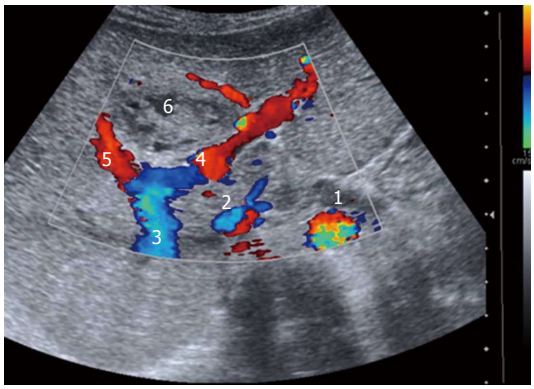

Figure 6 Doppler-US of the liver allows to investigate the relation between the tumor and the hepatic vessels. 1: Aorta; 2: Inferior vena cava; 3: Hepatic portal vein; 4: Left portal vein; 5: Right portal vein; 6 Tumor.

Table 2 Pre-treatment evaluation system of tumor extension staging system

PRETEXT Ⅰ	One section is involved and three adjoining sections are free
PRETEXT Ⅱ	One or two sections are involved, but two adjoining sections are free
PRETEXT Ⅲ	Two or three sections are involved, and no two adjoining sections are free
PRETEXT Ⅳ	All four sectors involved, any involvement of caudate lobe indicates a minimum of PRETEXT Ⅱ

PRETEXT: Pre-treatment evaluation system of tumor extension.

Other frequent presenting findings include anorexia, failure to thrive, abdominal pain, and abdominal distension. Jaundice is rarely seen since the liver function is otherwise normal. The presence of jaundice in a pediatric patient with a liver mass is most commonly seen in biliary rhabdomyosarcoma and undifferentiated sarcoma of the liver. Marked thrombocytosis is a typical finding in the laboratory work of a HB patient due to a paraneoplastic effect related to the tumor production of interleukin-6, a potent growth factor for megakaryocytes. The measurement of the serum AFP level is an useful test in infants with a liver mass and is elevated in at least 70% of children with HB. Moreover, patients with low AFP level at diagnosis (< 100 ng/mL) tend to have a more aggressive biological tumor behaviour and ultimately an unfavourable clinical outcome[37]. AFP is also an extremely useful marker of the tumor response to therapy and in the early detection of tumor recurrence. Attention must be paid to the correction of residual fetal.

AFP in infants under 6 mo of age. Elevation of AFP may also be seen in infants with yolk-sac tumors, sarcomas and hamartomas.

Abdominal ultrasonography-Doppler should be the first imaging modality in an infant with suspicion of a liver tumor and provides information about the origin of the mass, the extention of the lesion, and discerns whether the lesion is solid or cystic and whether it is a solitary or a multifocal tumor. It represents a valuable tool of resectability assessment as it allows to investigate the relation between the tumor and the hepatic vessels (Figure 6). It can be also used intraoperatively. On CT, HB shows heterogenous, low attenuation mass which enhance during arterial phase and hypoattenuates during portal phase. MRI shows HB as hypointense in comparison to normal liver in T1-weighted sequences and hyperintense in T2-weighted sequences, while dynamic imaging with gadolinium shows early enhancement with rapid washout. Chest CT should be performed to investigate pulmonary metastatic disease[34].

The pre-treatment evaluation system of tumor extension was developed by the SIOPEL and aims to define tumor extension before any therapeutic intervention. This system divides the liver into four sectors, an anterior and a posterior sector on the right and a medial and a lateral sector on the left (Table 2). The Children's Oncology Group (COG) adopted a different system based mostly on surgical findings[38-40].

In SIOPEL protocol, a tumor biopsy is required to confirm diagnosis before starting chemotherapy and this does not upstage a patient if a subsequent complete resection is performed. Biopsy can be done with open or laparoscopic surgical technique, but a percutaneous approach ultrasound-guided is preferred. Tumor seeding should be prevented by advancing the needle through a short depth of normal liver tissue (a portion that will be resected at future surgery)[41,42]. COG protocol allows

a primary tumor resection without a biopsy if it seems feasible. Patients with negative margin and microscopic positive margin will receive a less intensive chemotherapy regimen compared with those patients with gross residual disease or initial biopsy only. Patients with negative margin and pure fetal histology are observed and will receive no adjuvant chemotherapy in the COG protocol[43].

For both protocols, surgical resection is the mainstay of curative therapy, but only one-third to one-half of newly diagnosed patients with HB will have resectable disease at diagnosis. The combination of cisplatin-based chemotherapy and surgery has improved survival in patients with unresectable HB by increasing the number of patients whose tumors can be resected. Patients whose tumor may not be resectable even after neoadjuvant chemotherapy should be referred to a liver transplant center[44-48].

Biliary tract rhabdomyosarcoma

Rhabdomyosarcoma (RMS) of the biliary tree is a rare mesenchymal neoplasm that arises as an intraluminal biliary mass or cluster of grape-like masses and it typically presents with features of obstructive jaundice[49]. Median age at presentation is 3 years, but it should be included in the differential diagnosis of an infant presenting with jaundice and a mass in the porta hepatis. Other diagnostic possibilities include undifferentiated sarcoma of the liver, pancreateoblastoma, papillary cystic tumor of the pancreas, metastatic lesions and more rarely, hepatoblastoma[50,51]. Additionally, a RMS in this location can mimic the radiological appearance of a choledochal cyst because of its combined cystic and solid component[52]. Once the radiological diagnosis is performed by US, CT or MRI, an endoscopic retrograde cholangio-pancreatography can be performed to relieve biliary obstruction, visualize the biliary tree and obtain a biopsy[53,54]. This procedure may be challenging in an infant and an open, laparoscopic or needle biopsy may be required to confirm the diagnosis. Outcome in RMS appears to have improved over the last several decades secondary to the tumor chemosensitivity. Multiagent neoadjuvant chemotherapy following the biopsy may avoid important complications associated to a massive primary resection. Even after chemotherapy, gross total resection is rarely possible but outcome is good despite residual disease after second-look surgery[48,51].

Angiosarcoma

Most of the vascular liver neoplasms in infants are benign and correspond to infantile hemangioma (multifocal and diffuse hepatic hemangioma) and rapidly involuting congentinal hemangioma (focal hepatic hemangioma). If an hepatic hemangioma shows an unusual progression, malignancy should be suspected and a tumor biopsy is warranted. Hepatic angiosarcoma is a rare and high-grade malignant neoplasm that accounts for 2% of liver tumors in children[55-57]. Early metastatic disease to the lungs is commonly seen. Diagnosis may be challenging and an open wedge biopsy may be a good choice to avoid potential bleeding and obtain an accurate histological diagnosis. Prognosis is poor, even after multiagent chemotherapy, surgical resection, radiation, and liver transplantation[58,59].

Malignant rhabdoid tumor

Malignant rhabdoid tumor of the liver (MRTL) is a rare and aggressive neoplasm that share clinical features with HB such as male predominance, thrombocytosis, anemia, and only moderate derangement of overall liver function at presentation[60,61]. However, patients at diagnosis are younger compared with HB patients and LDH is typically elevated. Accurate diagnosis of MRTL may be challenging due to extensive tumor necrosis and immunohistochemistry studies for INI1/BAF 47 protein (which is abnormally lost in all rhabdoid tumors) has emerged as an useful tool for diagnosis[62]. For infants with liver tumors and normal AFP level at diagnosis, detailed cytogenetic, immunohistochemical and/ or molecular analysis of INI1/BAF 47 protein may be helpful in distinguishing MRTL from HB[63]. Hepatoblastomas of small cell undifferentiated histology can mimic MRTL but do not have *INI1* mutations[63,64]. Outcomes for patients with MRTL are very poor. Multiagent chemotherapy including vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide in combination with complete surgical resection is the mainstay of treatment[65].

Undifferentiated embryonal sarcoma

Undifferentiated embryonal sarcoma of the liver (UESL) is an uncommon malignant hepatic neoplasm that occurs more frequently in older children but has also been described in infancy[66,67]. Malignant transformation from mesenchymal hamartoma or a solitary liver cyst to UESL has been reported. It is generally considered to be a highly invasive malignant tumor with lung, peritoneum and pleura as the typical sites for distant metastasis. The diagnosis may be challenging and relies on postoperative pathology and immunostaining analysis (positive expression of SMA, a-ACT, desmin, vimentin). If the tumor is not suitable to primary resection, a biopsy should be obtained followed by chemoradiation. Survival rates have significantly improved in the last decades and long-term survival cases have been reported[68,69]. In an Italian-German soft tissue sarcoma study[70], 12 of 17 children with UESL achieved remission following treatment with chemoradiation and surgery. Patients whose tumor is not able to be resected or who have postoperative local recurrence of the tumor without distant metastasis may be candidates for liver transplantation.

Metastatic neuroblastoma

Neuroblastoma (NB) is the most common extracranial solid tumor in the pediatric population, accounting for 6%-10% of all childhood cancers and 15% of all cancer related mortalities in children. Common sites for metastasis are bone marrow, bone and liver. Stage 4S or MS represents 5% of NB cases and it is defined as disease with a localized primary adrenal or extra-adrenal tumor

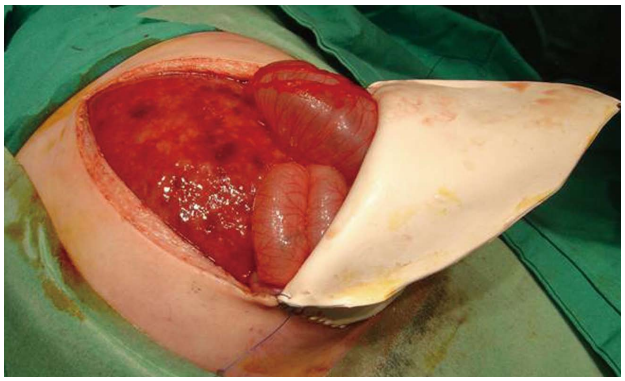

Figure 7 Extensive hepatic infiltration by neuroblastoma. Surgical abdominal decompression by patch placement.

and metastasis restricted to the liver, skin, and bone marrow involvement less than 10%[71,72]. Although, it is associated with survival rates of 70%-97% due to the possibility of regression and spontaneous tumor maturation, some of these young patients may present with extensive and diffuse liver involvement (Figure 7) that can cause respiratory compromise and symptoms of abdominal compartment syndrome with decreased venous return, renal impairment and coagulation disorders secondary to extensive hepatic infiltration. Chemotherapy and liver radiation have been advocated as therapeutical options for those infants who present with progressive disease and life-threatening symptoms[73,74]. Surgical management by abdominal decompression may be necessary in case an abdominal compartment syndrome is present[75,76]. However, the decision of when and how to treat remains controversial.

Our experience

Benign hepatic tumors: we have evolved from corticosteroids treatment to oral propranolol in the last 5 years for the management of symptomatic hepatic hemangiomas. We have observed a more rapid response to propranolol on ultrasound follow-up compared with steroids. Oral propranolol is discontinued until lesion involution is documented which it normally occurs after the first year of age. Patients with asymptomatic lesions have been observed with good results.

Most of our FNH patients underwent incisional biopsy to rule out malignancy. We have observed FNH as a residual lesion of primary vascular anomalies.

In our experience, a surgical plain may lead the resection of MHL with good residual liver parenchyma. All our patients have a normal liver function on follow-up.

Malignant hepatic tumors: at our institution, we follow the SIOP protocol for the management of hepatoblastoma with an initial incisional biopsy at presentation followed by cisplatin-based chemotherapy and surgical resection. Our overall survival is 70% and it does not differ from the results published by other groups.

As for RMS in other locations, our experience in the management of biliary tract RMS has evolved from primary tumor resection in the last decades to initial biopsy followed by neoadjuvant chemotherapy and non-massive second look surgery for tumor resection and evaluation of tumor response.

We have anecdotal cases of infants with tumors other than hepatoblastoma (angiosarcoma, malignant rhabdoid tumor, undifferentiated embryonal sarcoma) and conclusions are difficult to be drawn.

We have successfully managed liver infiltration by neuroblastoma with standard protocols based on chemotherapy and radiation therapy. We have only performed one surgical abdominal decompression by patch placement in a patient with an abdominal compartment syndrome (Figure 7) who finally died.

REFERENCES

1 **MullikenJB**, Glowacki J. Hemangiomas and vascular malformations in infants and children: a classification based on endothelial characteristics. *Plast Reconstr Surg* 1982; **69**: 412-422 [PMID:7063565]

2 **Mulliken JB**, Fishman SJ, Burrows PE. Vascular anomalies. *Curr Probl Surg* 2000; **37**: 17-84 [DOI: 10.1016/S0011-3840(00)80013-1]

3 **Christison-Lagay ER**, Burrows PE, Alomari A, Dubois J, Kozakewich HP, Lane TS, Paltiel HJ, Klement G, Mulliken JB, Fishman SJ. Hepatic hemangiomas: subtype classification and development of a clinical practice algorithm and registry. *J Pediatr Surg* 2007; **42**: 62-67; discussion 67-68 [PMID: 17208542 DOI: 10.1016/j.jpedsurg.2006.09.041]

4 **Drut RM**, Drut R. Extracutaneous infantile haemangioma is also Glut1 positive. *J Clin Pathol* 2004; **57**: 1197-1200 [PMID: 15509684 DOI: 10.1136/jcp.2003.012682]

5 **Mo JQ**, Dimashkieh HH, Bove KE. GLUT1 endothelial reactivity distinguishes hepatic infantile hemangioma from congenital hepatic vascular malformation with associated capillary proliferation. *Hum Pathol* 2004; **35**: 200-209 [DOI: 10.1016/j.humpath.2003.09.017]

6 **Hernández F**, Navarro M, Encinas JL, López Gutiérrez JC, López Santamaría M, Leal N, Martínez L, Patrón M, Tovar JA. The role of GLUT1 immunostaining in the diagnosis and classification of liver vascular tumors in children. *J Pediatr Surg* 2005; **40**: 801-804 [PMID: 15937818 DOI: 10.1016/j.jpedsurg.2005.01.046]

7 **Metry DW**, Hawrot A, Altman C, Frieden IJ. Association of solitary, segmental hemangiomas of the skin with visceral hemangiomatosis. *Arch Dermatol* 2004; **140**: 591-596 [PMID: 15148105 DOI: 10.1001/archderm.140.5.591]

8 **Mulliken JB**, Enjolras O. Congenital hemangiomas and infantile hemangioma: missing links. *J Am Acad Dermatol* 2004; **50**: 875-882 [PMID: 15153887 DOI: 10.1016/j.jaad.2003.10.670]

9 **Güven A**, Aygun C, Ince H, Aydin M, Pinarli FG, Baysal K, Küçüködük S. Severe hypothyroidism caused by hepatic hemangioendothelioma in an infant of a diabetic mother. *Horm Res* 2005; **63**: 86-89 [PMID: 15711094 DOI: 10.1159/000083879]

10 **Konrad D**, Ellis G, Perlman K. Spontaneous regression of severe acquired infantile hypothyroidism associated with multiple liver hemangiomas. *Pediatrics* 2003; **112**: 1424-1426 [PMID: 14654623 DOI: 10.1542/peds.112.6.1424]

11 **Ho J**, Kendrick V, Dewey D, Pacaud D. New insight into the pathophysiology of severe hypothyroidism in an infant with multiple hepatic hemangiomas. *J Pediatr Endocrinol Metab* 2005; **18**: 511-514 [PMID: 15921182 DOI: 10.1515/-JPEM.2005.18.5.511]

12 **Huang SA**, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, Fishman SJ, Larsen PR. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hem-

angiomas. *N Engl J Med* 2000; **343**: 185-189 [PMID: 10900278 DOI: 10.1056/NEJM200007203430305]
13 **Kassarjian A**, Dubois J, Burrows PE. Angiographic classification of hepatic hemangiomas in infants. *Radiology* 2002; **222**: 693-698 [PMID: 11867787]
14 **Kassarjian A**, Zurakowski D, Dubois J, Paltiel HJ, Fishman SJ, Burrows PE. Infantile hepatic hemangiomas: clinical and imaging findings and their correlation with therapy. *AJR Am J Roentgenol* 2004; **182**: 785-795 [PMID: 14975986 DOI: 10.2214/ajr.182.3.1820785]
15 **Regier TS**, Ramji FG. Pediatric hepatic hemangioma. *Radiographics* 2004; **24**: 1719-1724 [PMID: 15537980 DOI: 10.1148/rg.246035188]
16 **Hughes JA**, Hill V, Patel K, Syed S, Harper J, De Bruyn R. Cutaneous haemangioma: prevalence and sonographic characteristics of associated hepatic haemangioma. *Clin Radiol* 2004; **59**: 273-80 [DOI: 10.1016/S0009-9260(03)00267-8]
17 **Léauté-Labrèze C**, Dumas de la Roque E, Hubiche T, Boralevi F, Thambo JB, Taïeb A. Propranolol for severe hemangiomas of infancy. *N Engl J Med* 2008; **358**: 2649-2651 [PMID: 18550886 DOI: 10.1056/NEJMc0708819]
18 **Marsciani A**, Pericoli R, Alaggio R, Brisigotti M, Vergine G. Massive response of severe infantile hepatic hemangioma to propanolol. *Pediatr Blood Cancer* 2010; **54**: 176 [PMID: 19743301 DOI: 10.1002/pbc.22262]
19 **Stocker JT**, Ishak KG. Focal nodular hyperplasia of the liver: A study of 21 pediatric cases. *Cancer* 1981; **48**: 336
20 **Whelan TJ**, Baugh JH, Chandor S. Focal nodular hyperplasia of the liver. *Ann Surg* 1973; **177**: 150-158 [PMID: 4698535 DOI: 10.1097/00000658-197302000-00004]
21 **Sugito K**, Uekusa S, Kawashima H, Furuya T, Ohashi K, Inoue M, Ikeda T, Koshinaga T, Tomita R, Mugishima H, Maebayashi T. The clinical course in pediatric solid tumor patients with focal nodular hyperplasia of the liver. *Int J Clin Oncol* 2011; **16**: 482-487 [PMID: 21455626 DOI: 10.1007/s10147-011-0210-x]
22 **French AE**, Irwin MS, Navarro OM, Greenberg M, Nathan PC. Long-term hepatic outcomes in survivors of stage 4S and 4 neuroblastoma in infancy. *Pediatr Blood Cancer* 2012; **58**: 283-288 [PMID: 21370436 DOI: 10.1002/pbc.23077]
23 **Benz-Bohm G**, Hero B, Gossmann A, Simon T, Körber F, Berthold F. Focal nodular hyperplasia of the liver in long-term survivors of neuroblastoma: how much diagnostic imaging is necessary? *Eur J Radiol* 2010; **74**: e1-e5 [PMID: 19369017 DOI: 10.1016/j.ejrad.2009.05.002]
24 **Gutweiler JR**, Yu DC, Kim HB, Kozakewich HP, Marcus KJ, Shamberger RC, Weldon CB. Hepatoblastoma presenting with focal nodular hyperplasia after treatment of neuroblastoma. *J Pediatr Surg* 2008; **43**: 2297-2300 [PMID: 19040959 DOI: 10.1016/j.jpedsurg.2008.08.069]
25 **Bioulac-Sage P**, Cubel G, Taouji S, Scoazec JY, Leteurtre E, Paradis V, Sturm N, Nhieu JT, Wendum D, Bancel B, Ramos J, Paraf F, Saint Paul MC, Michalak S, Fabre M, Guettier C, Le Bail B, Zucman-Rossi J, Balabaud C. Immunohistochemical markers on needle biopsies are helpful for the diagnosis of focal nodular hyperplasia and hepatocellular adenoma subtypes. *Am J Surg Pathol* 2012; **36**: 1691-1699 [PMID: 23060349 DOI: 10.1097/PAS.0b013e3182653ece]
26 **Shafizadeh N**, Kakar S. Diagnosis of well-differentiated hepatocellular lesions: role of immunohistochemistry and other ancillary techniques. *Adv Anat Pathol* 2011; **18**: 438-445 [PMID: 21993269 DOI: 10.1097/PAP.0b013e318234abb4]
27 **Meyers RL**, Scaife ER. Benign liver and biliary tract masses in infants and toddlers. *Semin Pediatr Surg* 2000; **9**: 146-155 [PMID: 10949425 DOI: 10.1053/spsu.2000.7562]
28 **Stringer MD**, Alizai NK. Mesenchymal hamartoma of the liver: a systematic review. *J Pediatr Surg* 2005; **40**: 1681-1690 [PMID: 16291152 DOI: 10.1016/j.jpedsurg.2005.07.052]
29 **Cignini P**, Coco C, Giorlandino M, Bagolan P, Morini F, Giorlandino C. Fetal hepatic mesenchymal hamartoma: a case report. *J Prenat Med* 2007; **1**: 45-46 [PMID: 22470828]
30 **Thompson PA**, Chintagumpala M. Renal and hepatic tumors in the neonatal period. *Semin Fetal Neonatal Med* 2012; **17**: 216-221 [PMID: 22595862 DOI: 10.1016/j.siny.2012.04.002]
31 **Kim SH**, Kim WS, Cheon JE, Yoon HK, Kang GH, Kim IO, Yeon KM. Radiological spectrum of hepatic mesenchymal hamartoma in children. *Korean J Radiol* 2007; **8**: 498-505 [PMID: 18071280 DOI: 10.3348/kjr.2007.8.6.498]
32 **Anil G**, Fortier M, Low Y. Cystic hepatic mesenchymal hamartoma: the role of radiology in diagnosis and perioperative management. *Br J Radiol* 2011; **84**: e91-e94 [PMID: 21511744 DOI: 10.1259/bjr/41579091]
33 **Millard J**, Fraser N, Stewart RJ. Mesenchymal hamartoma of the liver: is biopsy always necessary? *Pediatr Surg Int* 2006; **22**: 622-625 [PMID: 16807720 DOI: 10.1007/s00383-006-1702-z]
34 **Roebuck DJ**. Assessment of malignant liver tumors in children. *Cancer Imaging* 2009; **9**: S98-S103 [PMID: 19965302 DOI: 10.1102/1470-7330.2009.9041]
35 **Siegel MJ**. Pediatric liver imaging. *Semin Liver Dis* 2001; **21**: 251-269 [PMID: 11436576 DOI: 10.1055/s-2001-15339]
36 **Mann JR**, Kasthuri N, Raafat F, Pincott JR, Parkes SE, Muir KR, Ingram LC, Cameron AH. Malignant hepatic tumours in children: incidence, clinical features and aetiology. *Paediatr Perinat Epidemiol* 1990; **4**: 276-289 [PMID: 2374747 DOI: 10.1111/j.1365-3016.1990.tb00651.x]
37 **Maruyama K**, Ikeda H, Koizumi T, Tsuchida Y. Prenatal and postnatal histories of very low birthweight infants who developed hepatoblastoma. *Pediatr Int* 1999; **41**: 82-89 [PMID: 10200142]
38 **De Ioris M**, Brugieres L, Zimmermann A, Keeling J, Brock P, Maibach R, Pritchard J, Shafford L, Zsiros J, Czaudzerna P, Perilongo G. Hepatoblastoma with a low serum alpha-fetoprotein level at diagnosis: the SIOPEL group experience. *Eur J Cancer* 2008; **44**: 545-550 [PMID: 18166449 DOI: 10.1016/j.ejca.2007.11.022]
39 **Aronson DC**, Schnater JM, Staalman CR, Weverling GJ, Plaschkes J, Perilongo G, Brown J, Phillips A, Otte JB, Czauderna P, MacKinlay G, Vos A. Predictive value of the pretreatment extent of disease system in hepatoblastoma: results from the International Society of Pediatric Oncology Liver Tumor Study Group SIOPEL-1 study. *J Clin Oncol* 2005; **23**: 1245-1252 [PMID: 15718322 DOI: 10.1200/JCO.2005.07.145]
40 **Roebuck DJ**, Aronson D, Clapuyt P, Czauderna P, de Ville de Goyet J, Gauthier F, Mackinlay G, Maibach R, McHugh K, Olsen OE, Otte JB, Pariente D, Plaschkes J, Childs M, Perilongo G. 2005 PRETEXT: a revised staging system for primary malignant liver tumours of childhood developed by the SIOPEL group. *Pediatr Radiol* 2007; **37**: 123-132; quiz 249-250 [PMID: 17186233 DOI: 10.1007/s00247-006-0361-5]
41 **Czauderna P**, Otte JB, Aronson DC, Gauthier F, Mackinlay G, Roebuck D, Plaschkes J, Perilongo G. Guidelines for surgical treatment of hepatoblastoma in the modern era--recommendations from the Childhood Liver Tumour Strategy Group of the International Society of Paediatric Oncology (SIOPEL). *Eur J Cancer* 2005; **41**: 1031-1036 [PMID: 15862752 DOI: 10.1016/j.ejca.2005.02.004]
42 **Hoffer FA**. Liver biopsy methods for pediatric oncology patients. *Pediatr Radiol* 2000; **30**: 481-488 [PMID: 10929368 DOI: 10.1007/s002470000244]
43 **Maturen KE**, Nghiem HV, Marrero JA, Hussain HK, Higgins EG, Fox GA, Francis IR. Lack of tumor seeding after percutaneous biopsy of hepatocellular carcinoma using coaxial cutting needle technique. *AJR Am J Roentgenol* 2005; **184**: 37
44 **Davidoff AM**, Fernandez-Pineda I, Santana VM, Shochat SJ. The role of neoadjuvant chemotherapy in children with malignant solid tumors. *Semin Pediatr Surg* 2012; **21**: 88-99 [PMID: 22248974 DOI: 10.1053/j.sempedsurg.2011.10.010]
45 **Brown J**, Perilongo G, Shafford E, Keeling J, Pritchard J, Brock P, Dicks-Mireaux C, Philips A, Vos A, Plaschkes J.

Pretreatment prognostic factors for children with hepatoblastoma - results from the International Society of Paediatric Oncology (SIOP) study SIOPEL 1. *Eur J Cancer* 2000; **36**: 1418-1425 [DOI: 10.1016/S0959-8049(00)00074-5]

46 **Perilongo G**, Shafford E, Maibach R, Aronson D, Brugières L, Brock P, Childs M, Czauderna P, MacKinlay G, Otte JB, Pritchard J, Rondelli R, Scopinaro M, Staalman C, Plaschkes J. Risk-adapted treatment for childhood hepatoblastoma. final report of the second study of the International Society of Paediatric Oncology--SIOPEL 2. *Eur J Cancer* 2004; **40**: 411-421 [PMID: 14746860 DOI: 10.1016/j.ejca.2003.06.003]

47 **Katzenstein HM**, London WB, Douglass EC, Reynolds M, Plaschkes J, Finegold MJ, Bowman LC. Treatment of unresectable and metastatic hepatoblastoma: a pediatric oncology group phase II study. *J Clin Oncol* 2002; **20**: 3438-3444 [PMID: 12177104 DOI: 10.1200/JCO.2002.07.400]

48 **Pritchard J**, Brown J, Shafford E, Perilongo G, Brock P, Dicks-Mireaux C, Keeling J, Phillips A, Vos A, Plaschkes J. Cisplatin, doxorubicin, and delayed surgery for childhood hepatoblastoma: a successful approach--results of the first prospective study of the International Society of Pediatric Oncology. *J Clin Oncol* 2000; **18**: 3819-3828 [PMID: 11078495]

49 **Spunt S**, Lobe T, Pappo A, Parham DS, Wharam MD Jr, Arndt C, Andersen JR, Crist WM, Paidas C, Wiener E, Andrassy RJ, Schwartz CL. Aggressive surgery is unwarranted for biliary tract rhabdomyosarcoma. *J Pediatr Surg* 2000; **35**: 309-316 [DOI: 10.1016/S0022-3468(00)90030-7]

50 **Zampieri N**, Camoglio F, Corroppolo M, Cecchetto M, Ornis S, Ottolenghi A. Botryoid rhabdomyosarcoma of the biliary tract in children: a unique case report. *Eur J Cancer Care* (Engl) 2006; **15**: 463-466 [PMID: 17177904 DOI: 10.1111/j.1365-2354.2006.00683.x]

51 **Perera MT**, McKiernan PJ, Brundler MA, Hobin DA, Mayer DA, Mirza DF, Sharif K. Embryonal rhabdomyosarcoma of the ampulla of Vater in early childhood: report of a case and review of literature. *J Pediatr Surg* 2009; **44**: e9-e11 [PMID: 19231522 DOI: 10.1016/j.jpedsurg.2008.10.113]

52 **Pollono DG**, Tomarchio S, Berghoff R, Drut R, Urrutia A, Cedola J. Rhabdomyosarcoma of extrahepatic biliary tree: initial treatment with chemotherapy and conservative surgery. *Med Pediatr Oncol* 1998; **30**: 290-293

53 **Tireli GA**, Sander S, Dervisoglu S, Demirali O, Unal M. Embryonal rhabdomyosarcoma of the common bile duct mimicking choledochal cyst. *J Hepatobiliary Pancreat Surg* 2005; **12**: 263-265 [PMID: 15995817 DOI: 10.1007/s00534-004-0959-7]

54 **Himes RW**, Raijman I, Finegold MJ, Russell HV, Fishman DS. Diagnostic and therapeutic role of endoscopic retrograde cholangiopancreatography in biliary rhabdomyosarcoma. *World J Gastroenterol* 2008; **14**: 4823-4825 [PMID: 18720547 DOI: 10.3748/wjg.14.4823]

55 **Awan S**, Davenport M, Portmann B, Howard ER. Angiosarcoma of liver in children. *J Pediatr Surg* 1996; **31**: 1729-32 [DOI: 10.1016/S0022-3468(96)90065-2]

56 **Noronha R**, Gonzalez-Crussi F. Hepatic angiosarcoma in childhood. A case report and review of the literature. *Am J Surg Pathol* 1984; **8**: 863-871 [PMID: 6542321 DOI: 10.1097/00000478-198411000-00007]

57 **Dimashkieh HH**, Mo JQ, Wyatt-Ashmead J, Collins MH. Pediatric hepatic angiosarcoma: case report and review of the literature. *Pediatr Dev Pathol* 2004; **7**: 527-532 [PMID: 15547777 DOI: 10.1007/s10024-004-4041-x]

58 **Ferrari A**, Casanova M, Bisogno G, Cecchetto G, Meazza C, Gandola L, Garaventa A, Mattke A, Treuner J, Carli M. Malignant vascular tumors in children and adolescents: a report from the Italian and German Soft Tissue Sarcoma Cooperative Group. *Med Pediatr Oncol* 2002; **39**: 109-114 [PMID: 12116058 DOI: 10.1002/mpo.10078]

59 **Lezama-del Valle P**, Gerald WL, Tsai J, Meyers P, La Quaglia MP. Malignant vascular tumors in young patients. *Cancer* 1998; **83**: 1634-1639

60 **Trobaugh-Lotrario AD**, Finegold MJ, Feusner JH. Rhabdoid tumors of the liver: rare, aggressive, and poorly responsive to standard cytotoxic chemotherapy. *Pediatr Blood Cancer* 2011; **57**: 423-428 [PMID: 21744471 DOI: 10.1002/pbc.22857]

61 **Wagner LM**, Garrett JK, Ballard ET, Hill DA, Perry A, Biegel JA, Collins MH. Malignant rhabdoid tumor mimicking hepatoblastoma: a case report and literature review. *Pediatr Dev Pathol* 2007; **10**: 409-415 [PMID: 17929989 DOI: 10.2350/06-08-0155.1]

62 **Perry A**, Fuller CE, Judkins AR, Dehner LP, Biegel JA. INI1 expression is retained in composite rhabdoid tumors, including rhabdoid meningiomas. *Mod Pathol* 2005; **18**: 951-958 [PMID: 15761491 DOI: 10.1038/modpathol.3800375]

63 **Al Nassan A**, Sughayer M, Matalka I, Ghandour K, Masarweh M, Zimmermann A, Sultan I. INI1 (BAF 47) immunohistochemistry is an essential diagnostic tool for children with hepatic tumors and low alpha fetoprotein. *J Pediatr Hematol Oncol* 2010; **32**: e79-e81 [PMID: 20048688 DOI: 10.1097/MPH.0b013e3181b79613]

64 **Trobaugh-Lotrario AD**, Tomlinson GE, Finegold MJ, Gore L, Feusner JH. Small cell undifferentiated variant of hepatoblastoma: adverse molecular and clinical features similar to rhabdoid tumors. *Pediatric Blood and Cancer* 2009; **52**: 328-334

65 **Jayaram A**, Finegold MJ, Parham DM, Jasty R. Successful management of rhabdoid tumor of the liver. *J Pediatr Hematol Oncol* 2007; **29**: 406-408 [PMID: 17551403 DOI: 10.1097/MPH.0b013e3180601011]

66 **Ismail H**, Dembowska-Bagińska B, Broniszczak D, Kaliciński P, Maruszewski P, Kluge P, Swięszkowska E, Kościesza A, Lembas A, Perek D. Treatment of undifferentiated embryonal sarcoma of the liver in children--single center experience. *J Pediatr Surg* 2013; **48**: 2202-2206 [DOI: 10.1016/j.jpedsurg.2013.05.020]

67 **Pachera S**, Nishio H, Takahashi Y, Yokoyama Y, Oda K, Ebata T, Igami T, Nagino M. Undifferentiated embryonal sarcoma of the liver: case report and literature survey. *J Hepatobiliary Pancreat Surg* 2008; **15**: 536-544 [PMID: 18836810 DOI: 10.1007/s00534-007-1265-y]

68 **Webber EM**, Morrison KB, Pritchard SL, Sorensen PH. Undifferentiated embryonal sarcoma of the liver: results of clinical management in one center. *J Pediatr Surg* 1999; **34**: 1641-1644 [DOI: 10.1016/S0022-3468(99)90634-6]

69 **Gao J**, Fei L, Li S, Cui K, Zhang J, Yu F, Zhang B. Undifferentiated embryonal sarcoma of the liver in a child: A case report and review of the literature. *Oncol Lett* 2013; **5**: 739-742 [PMID: 23426588]

70 **Bisogno G**, Pilz T, Perilongo G, Ferrari A, Harms D, Ninfo V, Treuner J, Carli M. Undifferentiated sarcoma of the liver in childhood: a curable disease. *Cancer* 2002; **94**: 252-257 [PMID: 11815984 DOI: 10.1002/cncr.10191]

71 **Stephenson SR**, Cook BA, Mease AD, Ruymann FV. The prognostic significance of age and patterns of metastasis in stage IV-S neuroblastoma. *Cancer* 1986; **58**: 372-375

72 **Evans AE**, Chatten J, D'Angio GJ, Gerson JM, Robinson J, Schnaufer L. A review of 17 IV-S neuroblastoma patients at the Children's Hospital of Philadelphia. *Cancer* 1980; **45**: 833-839

73 **Bishop MW**, Yin H, Shimada H, Towbin AJ, Miethke A, Weiss B. Management of stage 4S composite neuroblastoma with a MYCN-amplified nodule. *J Pediatr Hematol Oncol* 2014; **36**: e31-e35 [PMID: 23528904 DOI: 10.1097/MPH.0b013e3182847376]

74 **Steele M**, Jones NL, Ng V, Kamath B, Avitzur Y, Chami R, Cutz E, Fecteau A, Baruchel S. Successful liver transplantation in an infant with stage 4S(M) neuroblastoma. *Pediatr Blood Cancer* 2013; **60**: 515-517 [PMID: 23152322 DOI: 10.1002/pbc.24391]

75 **Nuchtern JG**, London WB, Barnewolt CE, Naranjo A, McGrady PW, Geiger JD, Diller L, Schmidt ML, Maris JM, Cohn SL, Shamberger RC. A prospective study of expectant

observation as primary therapy for neuroblastoma in young infants: a Children's Oncology Group study. *Ann Surg* 2012; **256**: 573-580 [PMID: 22964741]

76 **Mabrut JY**, Dubois R, Pelizzo G, Floret D, Frappaz D, Chapuis JP. Abdominal expansion using a polytetrafluoroethylene prosthesis in the treatment of Pepper syndrome. *Pediatr Surg Int* 2000; **16**: 219-221 [PMID: 10786988 DOI: 10.1007/s003830050729]

P- Reviewer: Wakiyama S **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.496

World J Hepatol 2014 July 27; 6(7): 496-503
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

CEUS and Fibroscan in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

Sila Cocciolillo, Giustino Parruti, Leonardo Marzio

Sila Cocciolillo, Leonardo Marzio, Digestive Physiopathology Unit, Gabriele d'Annunzio University, Pescara Civic Hospital, 65124 Pescara, Italy
Giustino Parruti, Infectious and Tropical Diseases Unit, Pescara Civic Hospital, 65124 Pescara, Italy
Author contributions: Cocciolillo S contributed to the recruitment of patients, acquisition of data, design the study, analysis and interpretation of the data and writing of the draft manuscript; Parruti G supervised and analyzed data from fibroscan; Marzio L contributed to the conceptual design, provided administrative support and overall supervision of the study, analyzed the data and critically revised the manuscript.
Correspondence to: Sila Cocciolillo, MD, Digestive Physiopathology Unit, Gabriele d'Annunzio University, Pescara Civic Hospital, Via Fonte Romana 8, 65124 Pescara, Italy. s_sila2000@yahoo.it
Telephone: +39-329-2066508 Fax: +39-85-4295547
Received: April 18, 2014 Revised: June 12, 2014
Accepted: June 27, 2014
Published online: July 27, 2014

Abstract

AIM: To determine intra-hepatic blood flow and liver stiffness in patients with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) using contrast-enhanced ultrasound and fibroscan.

METHODS: This prospective study included 15 patients with NAFLD, 17 patients with NASH and 16 healthy controls. In each patient, real-time ultrasound was used to locate the portal vein (PV) and the right liver lobe, and 5 mL of SonoVue® was then injected intravenous in a peripheral vein of the left arm over a 4-s span. Digital recording was performed for 3 min thereafter. The recording was subsequently retrieved to identify an area of interest in the PV area and in the right liver parenchyma (LP) to assess the blood flow by processing the data using dedicated software (Qontrast®, Bracco, Italy). The following parameters were evaluated: percentage of maximal contrast activity (Peak%), time to peak (TTP, s), regional blood volume (RBV, cm^3), regional blood flow (RBF, cm^3/s) and mean transit time (MTT, s). At 24-48 h post-injection, liver stiffness was evaluated using Fibroscan and measured in kPa. The statistical evaluation was performed using Student's *t* test.

RESULTS: In the PV, the Peak%, RBV and RBF were significantly reduced in the NAFLD and NASH patients compared with the controls (Peak%: NAFLD 26.3 ± 6.6, NASH 28.1 ± 7.3 *vs* controls 55.8 ± 9.9, $P < 0.001$; RBV: NAFLD 4202.3 ± 3519.7, NASH 3929.8 ± 1941.3 *vs* controls 7473 ± 3281, $P < 0.01$; RBF: NAFLD 32.5 ± 10.8, NASH 32.7 ± 12.1 *vs* controls 73.1 ± 13.9, $P < 0.001$). The TTP in the PV was longer in both patient groups but reached statistical significance only in the NASH patients compared with the controls (NASH 79.5 ± 37.8 *vs* controls 43.2 ± 30, $P < 0.01$). In the LP, the Peak%, RBV and RBF were significantly reduced in the NAFLD and NASH patients compared with the controls (Peak%: NAFLD 43.2 ± 7.3, NASH 41.7 ± 7.7 *vs* controls 56.6 ± 6.3, $P < 0.001$; RBV: NAFLD 4851.5 ± 2009, NASH 5069.4 ± 2292.5 *vs* controls 6922.9 ± 2461.5, $P < 0.05$; RBF: NAFLD 55.7 ± 10.1, NASH 54.5 ± 12.1 *vs* controls 75.9 ± 10.5, $P < 0.001$). The TTP was longer in both patient groups but did not reach statistical significance. The MTT in both the PV and LP in the NAFLD and NASH patients was not different from that in the controls. Liver stiffness was significantly increased relative to the controls only in the NASH patients (NASH: 6.4 ± 2.2 *vs* controls 4.6 ± 1.5, $P < 0.05$).

CONCLUSION: Blood flow derangement within the liver present not only in NASH but also in NAFLD suggests that a vascular flow alteration precedes liver fibrosis development.

Key words: Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Contrast-enhanced ultrasound; Fibroscan; Hepatic blood flow; Liver stiffness

Core tip: The use of contrast-enhanced ultrasound (CEUS) assisted by dedicated software (Qontrast®) in combination with Fibroscan examination could provide a non-invasive tool to evaluate the level of fatty-liver disease. In this study, we found that there were reductions in portal and intra-parenchymal blood flow in patients affected by non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH), whereas liver stiffness was increased only in NASH patients. Qontrast®-assisted CEUS could be used to quantify early changes in intra-parenchymal liver flow before the onset of fibrosis.

Cocciolillo S, Parruti G, Marzio L. CEUS and Fibroscan in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *World J Hepatol* 2014; 6(7): 496-503 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/496.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.496

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide[1,2]. Liver biopsy, which is the gold standard for diagnosing NAFLD is an invasive procedure with potential adverse effects and large inter- and intra-observer variability[3]. NAFLD cannot be diagnosed reliably without clear imaging or biopsy evidence of hepatic steatosis and without excluding excessive alcohol consumption, viral hepatitis and medications. NAFLD is further divided into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). NAFL is simple steatosis with no evidence of hepatocellular injury, whereas NASH is steatosis with inflammation, hepatocellular injury and possible fibrosis. NASH can lead to cirrhosis and hepatocellular carcinoma, whereas NAFLD has a very slow, if any, progression to NASH. NAFL and NASH, therefore, can be considered different steps in the same histological disease spectrum[3,4]. The pathogenesis of NAFLD is not completely known[5,6]. The fat accumulation occurring in NAFLD is key to the onset of vascular impairment[7]. The fat accumulation is responsible for liver structural and functional changes, leading to increased hepatic vascular resistance and finally to portal hypertension.

To study blood flow in the liver, pulsed continuous Doppler ultrasound (US) is used as the first-line imaging investigation. Doppler US can evaluate the blood flow in large and small vessels but fails to analyze the flow in the capillaries or sinusoids, where the velocity of the red blood cells is too slow to produce a Doppler signal[8]. Hence, changes in the hepatic microcirculation may be assessed using contrast-enhanced ultrasonography (CEUS) that consists of an intravenously administered suspension of gas-filled microbubbles that remain entirely within the intravascular space, thus acting as a blood pool tracer[9,10]. The obtained data can be processed using a post-processing computational tool (Qontrast®, Esaote, Florence, Italy) that includes a suite of software applications for image analysis designed to use alternative representations to extract and present brightness information that is already present in the image.

Table 1 Study populations

Population characteristics	Controls	NAFLD	NASH
Number	16	15	17
Male/female	8/8	12/3	16/1
Mean age (range)	37 yr (26-69 yr)	48 yr (26-75 yr)	45 yr (20-74 yr)
AST (mean ± SD)	20.6 ± 4.5	19.3 ± 5[b]	45.2 ± 22.1[d]
ALT (mean ± SD)	24.4 ± 7.0	27.4 ± 8.1[b]	86.4 ± 55.7[d]
GGT (mean ± SD)	18.3 ± 10.1	25.6 ± 20[b]	73.1 ± 43[d]
ALP (mean ± SD)	144.4 ± 45.4	154.1 ± 38.3	176.5 ± 57.4

[b]$P < 0.001$, NAFLD *vs* NASH; [d]$P < 0.001$, NASH *vs* controls. AST: Aspartate aminotransferase: reference range 0-37 (IU/L); ALT: Alanine aminotransferase: reference range 0-40 (IU/L); GGT: Gamma-glutamyl transferase: reference range 7-50 (IU/L); ALP: Alkaline phosphatase: reference range 98-279 (IU/L); NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease.

Liver fibrosis directly affects the mechanical properties of the liver parenchyma, such as stiffness, which indicates tissue resistance to deformation under mechanical stress. A greater stiffness corresponds to a higher tissue resistance to deformation. Liver stiffness can be studied using three physical measurements: two measures based on sonographic techniques, such as Fibroscan[11] and acoustic radiation force impulse[12,13], and one that is MR-based, such as magnetic resonance elastography[14]. Regardless of the specific technique, the measured parameter is correlated with the histological fibrosis stage, and the results can be used to accurately predict moderate to severe fibrosis[10,11,15].

In NASH and NAFLD, it remains unclear whether early changes in intrahepatic blood flow are associated with an early production of fibrous tissue. Therefore, the aim of this study was to evaluate the liver blood flow in the large and small intra-parenchymal vessels and fibrosis using CEUS and Fibroscan in patients with NAFLD and NASH compared with healthy controls.

MATERIALS AND METHODS

Populations

The study population was enrolled from August 2010 to December 2013. All of the participants were Caucasian and underwent physical examinations, laboratory tests for liver function, upper and lower abdominal real-time ultrasonography (RUS) and computed tomography (CT) scan when necessary.

Sixteen healthy controls and 32 patients with US-documented steatosis were recruited (Table 1). Fifteen patients affected by NAFLD as defined according the latest guidelines established by the American Association for the study of liver diseases[3] and 17 patients with NASH defined as having fatty liver on abdominal ultrasound examination and either aspartate aminotransferase or alanine aminotransferase more than 1.5 times the upper normal limit on two occasions during the six months before en-

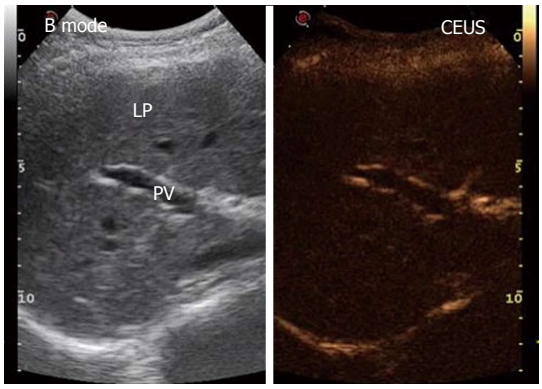

Figure 1 Example in a healthy control of the split-screen display during the contrast-enhanced ultrasound procedure upon the injection of SonoVue® using a low mechanical index. Left: B-mode frame; Right: Contrast-enhanced ultrasound frame. PV: Portal vein; LP: Liver parenchyma. The B-mode frame shows more detail because of the higher gain.

rollment were included. Exclusion criteria were laboratory data and image studies as assessed with ultrasound or CT scan when necessary, compatible with hepatitis B and C, autoimmune hepatitis, sclerosing cholangitis, Wilson's disease, alpha-1 anti-trypsin deficiency, hemochromatosis and hepatic cirrhosis[16]. Additional exclusion criteria were patients with medical histories of malignancy, previous abdominal or thoracic surgery and history of heart and pulmonary disease that may impair the flow of the contrast bubbles to the liver as well as severe concomitant diseases. Finally, patients with pregnancy and breastfeeding as well as pediatric patients were also excluded.

Contrast-enhanced ultrasound and dedicated software

The patients were examined after fasting for 8 h and after having obtained written informed consent. The CEUS examination was always performed by the same expert operator using RUS with a 3.5-MHz convex probe (MyLab70 XVision, Esaote, Ansaldo, Italy) through a longitudinal intercostal scan, in which the portal vein (PV) and right liver parenchyma (LP) could be easily identified while keeping the patient or subject in the supine position. The US contrast medium (SonoVue®, Bracco Spa, Milan, Italy) consisted of 2.5 μm sulfur hexafluoride-filled microbubbles (hence, they are smaller than red blood cells, which have a diameter of 7 μm) stabilized by a lipid monolayer membrane[9]. The microbubbles can generate a nonlinear harmonic response to a low mechanical index (MI), thus permitting continuous real-time imaging. In our study, we used a signal-processing algorithm installed on the ultrasonographic machine (Contrast-Tuned Imaging™, CnTI™, Esaote, Genoa, Italy) that automatically sets a low MI of 0.06 and holds this value constant during the entire CEUS procedure. These features allow the contrast medium microbubbles to travel through the smallest blood vessels without bursting. SonoVue, a blood-pool contrast agent, has no cellular uptake; thus, it enhances only the US image generated by the blood vessels[9]. In CEUS studies, there are 3 overlapping vascular phases: the arterial phase, which starts within 20 s after the injection and lasts 30-45 s; the portal venous phase, which usually lasts until 2 min after the injection; and the late phase, which corresponds to the clearance of the US contrast agent from the circulation. The CEUS screen, because of the low gain**,** shows signals only from intensely reflective structures, which limits the ability to identify the proper scan area. To overcome this problem, a split-screen display was used on the ultrasound machine to show the conventional B-mode image beside the CEUS image (Figure 1). Using contrast-processed data, the blood flow through the small capillaries of the liver interstitial tissue could be measured in terms of the volume and flow.

The procedure started with a 5-mL contrast medium injection (always performed by the same expert nurse) using a 20-gauge (G) needle cannula over a 4-s span into the antecubital vein of the left arm with the patient in the supine position. The line was then flushed with a 5-mL bolus of saline solution, also injected over a 4-s span. During the contrast medium injection, digital recording was started and performed for 3 min; during this operation, the patients were asked to breathe slowly to minimize respiration-related movements. The video recordings were then analyzed by the same trained operator using Qontrast® software (Esaote, Florence, Italy), which performs a parametric analysis of perfusion within a selected set of higher signal intensity frames in the region of interest (ROI). In each patient, we evaluated two ROIs: one in the PV and one in the right LP. To correct for translational movements in the ROI, a Gamma variate (bolus)-corrected parametric curve model was selected. The Qontrast® software was then allowed to process the perfusion in each of the previously determined ROIs, calculate the parameters automatically and plot the measured and calculated curves. The following parameters were generated (Figure 2): Peak%, the maximum signal intensity (SI) reached during SonoVue® bolus transit at time T, where T was the time to peak (TTP, s), the time to reach the maximum SI; regional blood volume (RBV, cm^3), the blood volume in the ROI, proportional to the area under the time intensity curve; mean transit time (MTT, s), the contrast medium mean transit time in the ROI; and regional blood flow (RBF, cm^3/s), the RBV to MTT ratio. The reproducibility of the data obtained by Qontrast® analysis of CEUS was tested according to the method of Ridolfi *et al*[17].

Transient elastography

Transient elastography was performed 24-48 h after CEUS using a Fibroscan device (Echosens, Paris, France). Fibroscan consists of a 5-MHz US transducer probe installed on the axis of a vibrator that generates a 50-Hz vibration (completely painless to the patient) that causes an elastic shear wave to propagate through the skin and subcutaneous tissue and finally to the LP, the stiffness of which is directly related to the velocity of the wave. Fibroscan measures the stiffness of a cylindrical volume 1 cm in diameter, 4 cm in length and 25 to 45 cm from

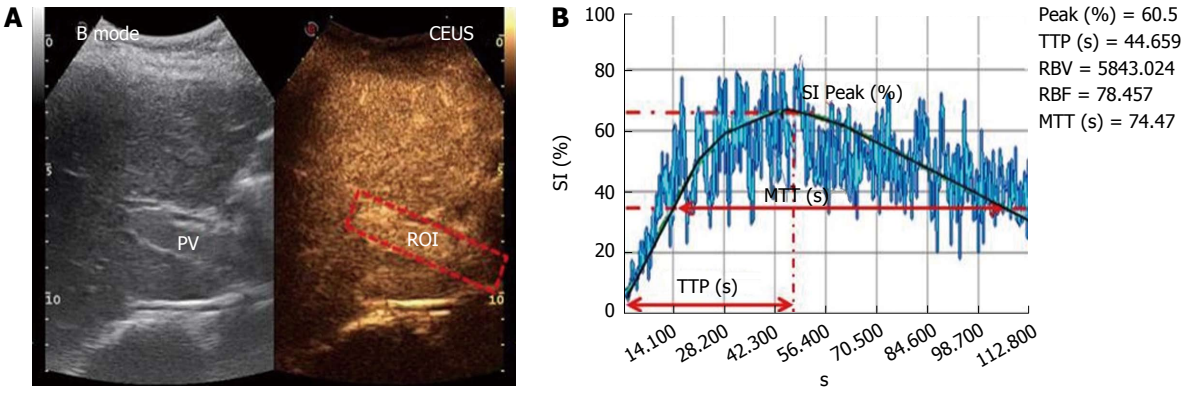

Figure 2 Example of region of interest selection in the portal vein and Qontrast®-assisted contrast-enhanced ultrasound analysis of portal vein parameters in healthy control. A: Region of interest (ROI) drawn in a region of the portal vein (PV) in a selected set of higher signal intensity frames 1 min and 10 s after SonoVue® injection; B: A gamma variate (bolus)-corrected parametric curve for the translational movement caused by breathing activity. SI (%): Signal intensity; PEAK (%): Maximum signal intensity reached during SonoVue® bolus injection; TTP (s): Time to peak; RBV (cm^3): Regional blood volume; RBF (cm^3/s): Regional blood flow; MTT (s): Mean transit time.

the skin. Acquisition was performed by the same expert operator through an intercostal scan, in which the probe was placed perpendicular to an area free of large vascular structures. During acquisition, the patient lay in the supine position with the right arm in abduction. Liver stiffness was determined by computing the median value of 10 successful acquisitions in kPa.

Statistical analysis

Continuous variables (laboratory values, SonoVue® data processed by Qontrast® software and elastosonography data) are expressed as group means ± SDs. Age was analyzed as a mean. Comparisons of all gathered data among the groups were tested by a Welch-corrected unpaired *t* test. *P* values were two-tailed, and all *P* values less than 0.05 were considered statistically significant. All statistical relationships were assessed using correlation analysis. The statistical analyses were performed using the GraphPad Prism software, version 3.00 (GraphPad Software, San Diego, California, Unites States).

RESULTS

Qontrast

We could not analyze two PV ROIs in NAFLD patients and three in NASH patients because of poor video recording due to liver steatosis that interfered with the returning echoes to the US probe.

The PV analysis showed a significantly shorter Peak% (Figure 3A) and decreased RBV and RBF (Figure 3C and D) in both the NASH and NAFLD patients compared with the controls. The TTP in the PV was longer in both patient groups but reached significance only in the NASH patients (Figure 3B).

The LP analysis yielded similar results, with Peak% (Figure 4A), RBV and RBF (Figure 4C and D) significantly reduced in both the NASH and NAFLD groups compared with the normal controls. The TTP was longer in both NASH and NAFLD patients compared with the controls but did not reach significance (Figure 4B). The MTT in both the PV and LP in the NAFLD and NASH patients was similar to that in the controls (Figure 5).

Fibroscan

The values of liver stiffness measured in kPa were found to be significantly greater in the NASH patients compared with the control group (Figure 6).

Adverse effects

CEUS studies were performed successfully in all of the patients and were well tolerated, with no side or adverse effects reported.

DISCUSSION

Our study showed that blood flow, as assessed by Qontrast®-assisted CEUS analysis of the PV and LP, was decreased in patients affected by NAFLD and NASH. We also found that liver stiffness, as assessed by Fibroscan, was increased only in NASH patients.

Based on the data obtained by the Qontrast® analysis of ROIs in the PV and LP, significant reductions in the Peak%, RBV and RBF were found in both groups of patients, whereas a delayed TTP was found only in the PV of the NASH group. Our results suggest the hypothesis that in patients with NAFLD, there is a reduced vascular compliance in the liver due to augmented hepatic vascular resistance to portal blood flow and an increased hepatic vascular tone that starts before the onset of fibrosis. This change was previously demonstrated by Francque *et al*[18] in an experimental animal model; in their study, Wistar rats fed with a methionine- and choline-deficient diet for four weeks developed severe steatosis associated with a significant increase in intrahepatic resistance before the onset of fibrosis and inflammation. These changes involved functional (liver endothelial dysfunction and vasoconstrictor overproduction) and structural (sinusoidal altered microvascular architecture) factors. Another study by Pasarín *et al*[19], performed on rats fed with a cafeteria diet for one month, showed that the impaired response

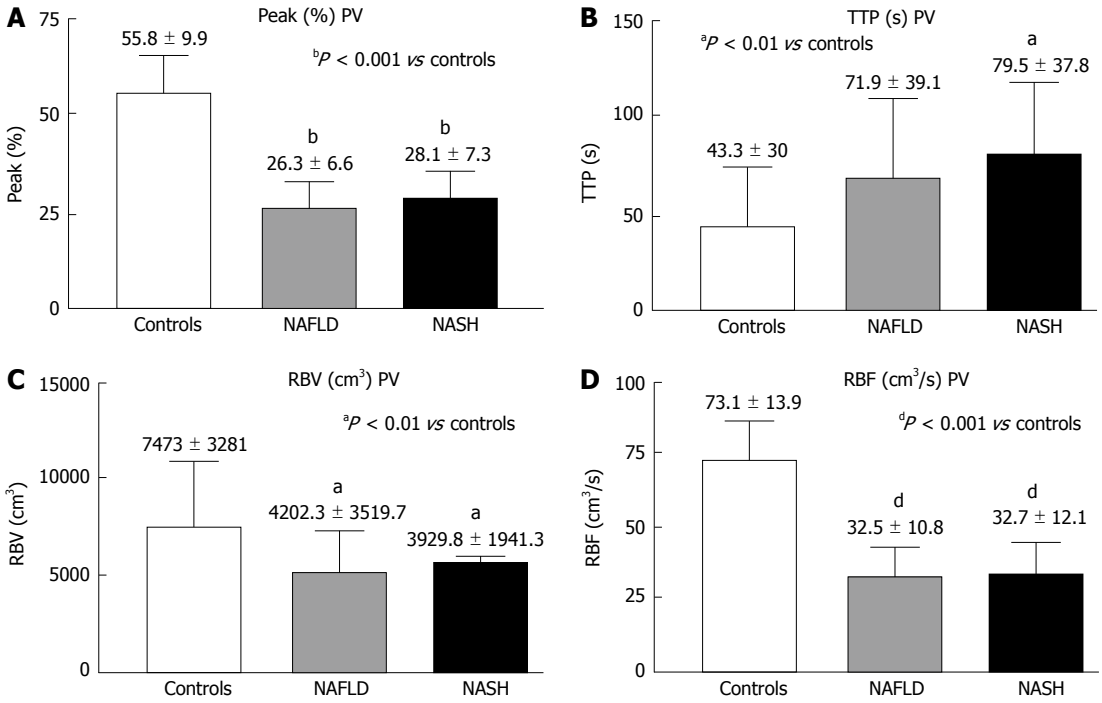

Figure 3 Contrast-enhanced ultrasound of the portal vein in controls, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. A: Peak (%); B: TTP (s); C: RBV (cm^3); D: RBF (cm^3/s); Peak (%): Maximum signal intensity (SI) reached during SonoVue® bolus injection; TTP (s): Time to peak; RBV (cm^3): Regional blood volume; RBF (cm^3/s): Regional blood flow; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

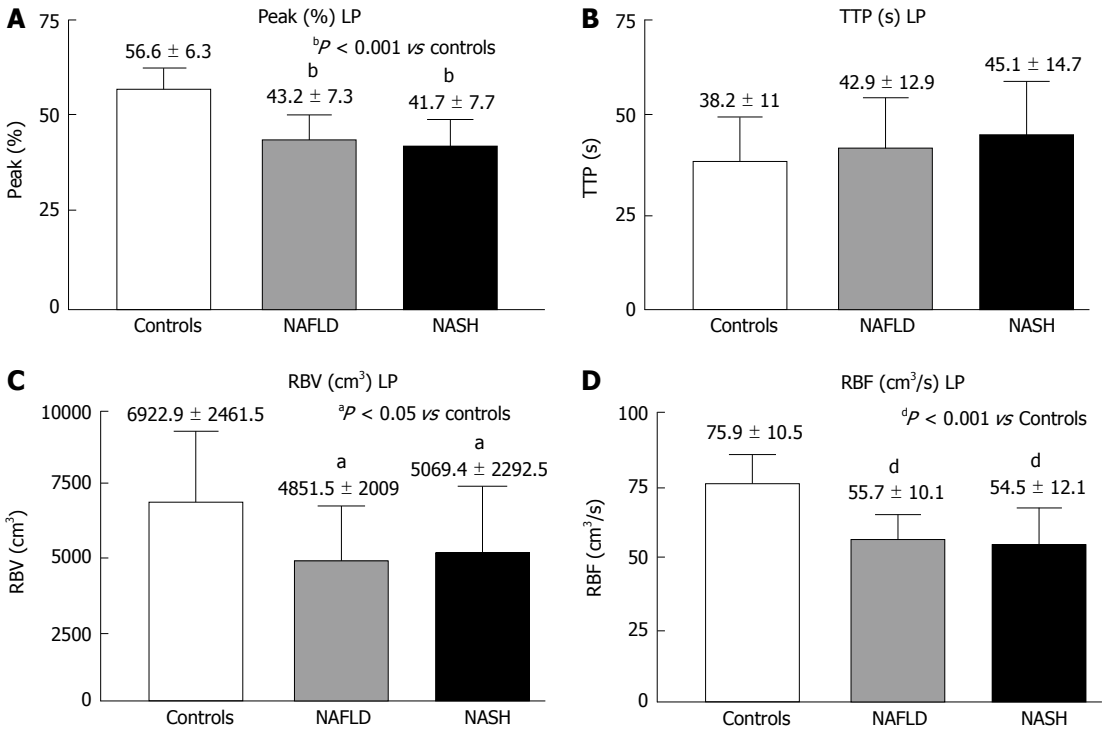

Figure 4 Contrast-enhanced ultrasound of liver parenchyma in controls, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. A: Peak (%); B: TTP (s); C: RBV (cm^3); D: RBF (cm^3/s); Peak (%): Maximum signal intensity (SI) reached during SonoVue® bolus injection; TTP (s): Time to peak; RBV (cm^3): Regional blood volume; RBF (cm^3/s): Regional blood flow; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

to endothelial-dependent vasodilation caused endothelial dysfunction, leading to augmented intrahepatic resistance and reduced portal flow. Even in this study, the functional features of intrahepatic vascular changes preceded the onset of fibrosis and inflammation[19].

Another interesting observation is that our data obtained by Qontrast®-assisted CEUS were similar to those from other studies that analyzed liver blood flow with

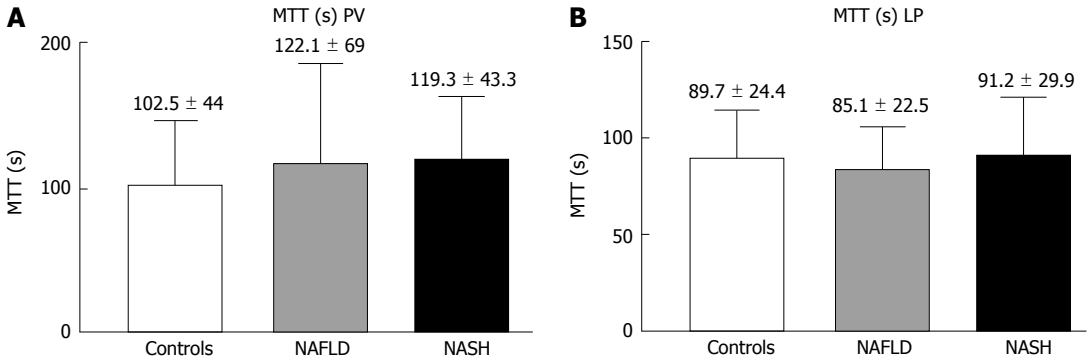

Figure 5 Contrast-enhanced ultrasound of the portal vein (A) and liver parenchyma (B) in controls, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Mean transit time (s). NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

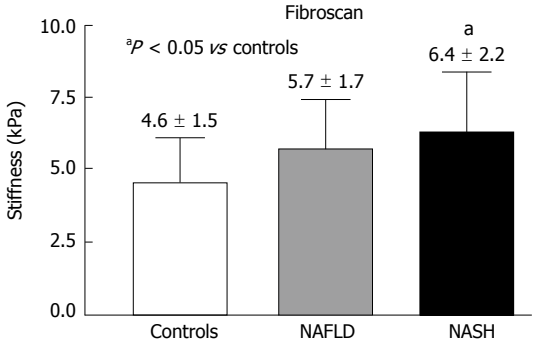

Figure 6 Fibroscan in controls, non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

CEUS using SonoVue® as the contrast medium in cirrhotic patients. Lin *et al*[20] studied the flow in the right PV by means of color Doppler and CEUS, and they found that the arrival time of SonoVue® in the right PV was prolonged, whereas the velocity and flow volume were decreased. Similar results were found in another study by Ridolfi *et al*[17], who evaluated liver blood flow in the PV and in the parenchyma by means of CEUS and subsequent analysis by Qontrast® in cirrhotic patients and healthy subjects. They found a reduced Peak% and prolonged TTP and MTT in cirrhotic patients compared with controls. These data suggest that NASH and, more interestingly, NAFLD might be considered precursors of liver cirrhosis due to the presence of similar hemodynamic changes in liver blood flow.

In our patients affected by NASH, the delay in reaching the maximum signal intensity (TTP), only present in the PV, together with the reduction in blood flow in either the PV or LP, could be the consequence of not only intraparenchymal microcirculation variations but also increased liver stiffness. Liver stiffness data in our patients with NAFLD and NASH could be included among those with no fibrosis (NAFLD) or mild fibrosis (NASH) as classified by Wong *et al*[21]. These authors studied a large cohort of patients with hepatic steatosis using Fibroscan and liver histology. They found that patients with no fibrosis or mild fibrosis showed liver stiffness values (kPa) that were consistent with those of our patients with NAFLD (no fibrosis) and NASH (mild fibrosis), respectively. They also identified some patients with steatosis with liver stiffness values that were much higher than those found in this present study. These patients were classified at histology as having a fibrosis pattern compatible with early cirrhosis, which was an exclusion criterion in our study.

The major limitation of this present study was the small number of patients that were examined; further studies in a much larger population are required to draw definitive conclusions regarding the value of the digital data generated by Qontrast®. However, the differences between the control, NAFLD and NASH groups for the main measure of the analysis (Peak% in the PV and LP) were so large that the statistical power of the study could be considered satisfactory. The absence of liver biopsy data in our study was another limitation, although other authors have found a significant correlation between US and histopathologic data in the evaluation of steatosis[22-24]. The CEUS procedure may be incorrectly applied when the US machine does not meet the criteria of good sensitivity, good tissue suppression and good temporal and spatial resolution as reported in the Guidelines of European Federation of Societies for Ultrasound in Medicine and Biology[25]. In our study, we obtained a good tissue suppression by means of CnTI™, which maintains a low MI throughout the study and avoids microbubble bursting as well as bioeffects in the target organs.

In conclusion, CEUS evaluated by Qontrast® might be able to quantify functional vascular liver changes not otherwise detectable with any other non-invasive procedure and before the development of fibrosis. The combined use of Fibroscan and Qontrast®-assisted CEUS could be helpful in assessing the level of disease and could be potentially useful for monitoring the effects of therapeutic interventions.

COMMENTS

Background

Non-alcoholic fatty liver disease (NAFLD) is among the most common cause of chronic liver disease worldwide. NAFLD is further subdivided into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). Whereas NASH is represented by steatosis with inflammation, hepatocellular injury and possible fibrosis, NAFL is a simple steatosis with no evidence of hepatocellular injury.

Fat accumulation within the hepatocytes leads to narrowing and distortion of the sinusoidal lumen, leading to increased hepatic vascular resistance and finally to portal hypertension and fibrosis that are among the stigmata of hepatic cirrhosis. Whether these vascular hemodynamic changes are present in NAFLD and NASH remains unclear. Pulsed continuous Doppler ultrasound (US) is the first-line imaging tool for studying blood flow in the liver and allows for the evaluation of flow in the great hepatic vessels but fails to analyze the flow in the capillaries or sinusoids, where the velocity of the red blood cells is too slow to produce a Doppler signal. Hence, to assess changes in hepatic microcirculation, the authors used US to analyze a Doppler signal generated by an intravenously administered suspension of gas-filled microbubbles (each bubble is one-third the diameter of a red blood cell) stabilized by a lipid monolayer membrane; these features allow these bubbles to remain entirely within the intravascular space, thus acting as a blood pool tracer. The obtained data can be processed with a post-processing computational tool (Qontrast®, Esaote, Firenze, Italy), which allowed them to extrapolate objective and quantitative parameters of microvascular damage in the liver. Liver fibrosis directly affects the mechanical properties of the liver parenchyma and may also contribute to portal hypertension. Liver stiffness can be studied with Fibroscan that consists of measuring the resistance of the liver tissue to the propagation of a US beam within the tissue.

Research frontiers

Considering the increasing prevalence of NAFLD with potentially severe outcomes and the limitations of the actual gold standard (liver biopsy) as a diagnostic procedure, the development of a non-invasive technique that allows for an early assessment of liver damage in terms of the derangement of intrahepatic microcirculation and the development of fibrosis appears to be a stimulating research field. This approach also has therapeutic implications in terms of the development of new drugs and monitoring of their therapeutic effects.

Innovations and breakthroughs

The US contrast medium (SonoVue®, Bracco Spa, Milan, Italy) consisted of 2.5 μm sulfur hexafluoride-filled microbubbles, which are smaller than red blood cells. The microbubbles have no cellular uptake, unlike the contrast media used for computed tomography scan or magnetic resonance, and can travel through the smallest liver blood vessels without bursting. A Doppler signal not otherwise detectable with the standard US machine is therefore generated, and the flow in microvessels can be measured. In addition, the use of a computer program that analyzes the signal intensity within the US image allows for the standardization of data in term of the blood flow and volume. By means of contrast-enhanced ultrasound (CEUS) and computer-assisted determination of flow and volume, it has been possible for the first time to detect a derangement in the microcirculation within the liver parenchyma not only in NASH but also in NAFLD. Fibrosis otherwise appears to be limited only to NASH.

Applications

To non-invasively monitor the development of liver disease and to study the effect of drugs on hepatic micro-circulation and fibrosis.

Terminology

CEUS: contrast-enhanced ultrasound, SonoVue®: microbubbles of 2.5 μm in diameter filled with sulfur hexafluoride that are stabilized by a lipid monolayer membrane. QONTRAST® is a suite of software applications for image analysis designed to extract and present, in alternative representation, brightness information that is already contained within the images. FibroScan® is a sonography-based non-invasive and rapid bedside method for the diagnosis and quantification of hepatic fibrosis (by measuring liver stiffness).

Peer review

This is a clinical study which evaluated the findings of contrast-enhanced US and Fibroscan in patients with NAFLD and control. The authors found some differences of the hepatic hemodynamics and liver stiffness among control NAFL and NASH.

REFERENCES

1 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]

2 **Torres DM**, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology* 2008; **134**: 1682-1698 [PMID: 18471547 DOI: 10.1053/j.gastro.2008.02.077]

3 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

4 **Yilmaz Y**. Review article: is non-alcoholic fatty liver disease a spectrum, or are steatosis and non-alcoholic steatohepatitis distinct conditions? *Aliment Pharmacol Ther* 2012; **36**: 815-823 [PMID: 22966992]

5 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]

6 **Vernon G**, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Aliment Pharmacol Ther* 2011; **34**: 274-285 [PMID: 21623852 DOI: 10.1111/j.1365-2036.2011.04724.x]

7 **Farrell GC**, Teoh NC, McCuskey RS. Hepatic microcirculation in fatty liver disease. *Anat Rec* (Hoboken) 2008; **291**: 684-692 [PMID: 18484615 DOI: 10.1002/ar.20715]

8 **Bernatik T**, Strobel D, Hahn EG, Becker D. Doppler measurements: a surrogate marker of liver fibrosis? *Eur J Gastroenterol Hepatol* 2002; **14**: 383-387 [PMID: 11943950 DOI: 10.1097/00042737-200204000-00008]

9 **Greis C**. Technology overview: SonoVue (Bracco, Milan). *Eur Radiol* 2004; **14** Suppl 8: P11-P15 [PMID: 15700328 DOI: 10.1007/s10406-004-0076-3]

10 **Quaia E**. Assessment of tissue perfusion by contrast-enhanced ultrasound. *Eur Radiol* 2011; **21**: 604-615 [PMID: 20927527 DOI: 10.1007/s00330-010-1965-6]

11 **Yoneda M**, Yoneda M, Mawatari H, Fujita K, Endo H, Iida H, Nozaki Y, Yonemitsu K, Higurashi T, Takahashi H, Kobayashi N, Kirikoshi H, Abe Y, Inamori M, Kubota K, Saito S, Tamano M, Hiraishi H, Maeyama S, Yamaguchi N, Togo S, Nakajima A. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with nonalcoholic fatty liver disease (NAFLD). *Dig Liver Dis* 2008; **40**: 371-378 [PMID: 18083083 DOI: 10.1016/j.dld.2007.10.019]

12 **Friedrich-Rust M**, Nierhoff J, Lupsor M, Sporea I, Fierbinteanu-Braticevici C, Strobel D, Takahashi H, Yoneda M, Suda T, Zeuzem S, Herrmann E. Performance of Acoustic Radiation Force Impulse imaging for the staging of liver fibrosis: a pooled meta-analysis. *J Viral Hepat* 2012; **19**: e212-e219 [PMID: 22239521 DOI: 10.1111/j.1365-2893.2011.01537.x]

13 **Yoneda M**, Suzuki K, Kato S, Fujita K, Nozaki Y, Hosono K, Saito S, Nakajima A. Nonalcoholic fatty liver disease: US-based acoustic radiation force impulse elastography. *Radiology* 2010; **256**: 640-647 [PMID: 20529989 DOI: 10.1148/radiol.10091662]

14 **Yin M**, Talwalkar JA, Glaser KJ, Manduca A, Grimm RC, Rossman PJ, Fidler JL, Ehman RL. Assessment of hepatic fibrosis with magnetic resonance elastography. *Clin Gastroenterol Hepatol* 2007; **5**: 1207-1213.e2 [PMID: 17916548 DOI: 10.1016/j.cgh.2007.06.012]

15 **Mori M**, Fujii H, Ogawa T, Kobayashi S, Iwai S, Morikawa H, Enomoto M, Tamori A, Sawada A, Takeda S, Kawada N. Close correlation of liver stiffness with collagen deposition and presence of myofibroblasts in non-alcoholic fatty liver disease. *Hepatol Res* 2011; **41**: 897-903 [PMID: 21682831 DOI: 10.1111/j.1872-034X.2011.00842.x]

16 **Barzin G**, Merat S, Nokhbeh-Zaeem H, Saniee P, Pedramnia S, Mostashfi Habibabadi A, Nasseri-Moghaddam S. Oral Nitrate Reductase Activity Is Not Associated with Development of Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH): A Pilot Study. *Middle East J Dig Dis* 2014; **6**: 23-27 [PMID: 24829701]

17 **Ridolfi F**, Abbattista T, Busilacchi P, Brunelli E. Contrast-enhanced ultrasound evaluation of hepatic microvascular

changes in liver diseases. *World J Gastroenterol* 2012; **18**: 5225-5230 [PMID: 23066317 DOI: 10.3748/wjg.v18.i37.5225]

18 **Francque S**, Laleman W, Verbeke L, Van Steenkiste C, Casteleyn C, Kwanten W, Van Dyck C, D'Hondt M, Ramon A, Vermeulen W, De Winter B, Van Marck E, Van Marck V, Pelckmans P, Michielsen P. Increased intrahepatic resistance in severe steatosis: endothelial dysfunction, vasoconstrictor overproduction and altered microvascular architecture. *Lab Invest* 2012; **92**: 1428-1439 [PMID: 22890552 DOI: 10.1038/labinvest.2012.103]

19 **Pasarín M**, La Mura V, Gracia-Sancho J, García-Calderó H, Rodríguez-Vilarrupla A, García-Pagán JC, Bosch J, Abraldes JG. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. *PLoS One* 2012; **7**: e32785 [PMID: 22509248 DOI: 10.1371/journal.pone.0032785]

20 **Lin LW**, Duan XJ, Wang XY, Xue ES, He YM, Gao SD, Yu LY. Color Doppler velocity profile and contrast-enhanced ultrasonography in assessment of liver cirrhosis. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 34-39 [PMID: 18234636]

21 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]

22 **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 [PMID: 1999069 DOI: 10.1016/S0009-9260(05)80350-2]

23 **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 [PMID: 16000931 DOI: 10.1097/00004836-200508000-00012]

24 **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54**: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452]

25 **Piscaglia F**, Nolsøe C, Dietrich CF, Cosgrove DO, Gilja OH, Bachmann Nielsen M, Albrecht T, Barozzi L, Bertolotto M, Catalano O, Claudon M, Clevert DA, Correas JM, D'Onofrio M, Drudi FM, Eyding J, Giovannini M, Hocke M, Ignee A, Jung EM, Klauser AS, Lassau N, Leen E, Mathis G, Saftoiu A, Seidel G, Sidhu PS, ter Haar G, Timmerman D, Weskott HP. The EFSUMB Guidelines and Recommendations on the Clinical Practice of Contrast Enhanced Ultrasound (CEUS): update 2011 on non-hepatic applications. *Ultraschall Med* 2012; **33**: 33-59 [PMID: 21874631 DOI: 10.1055/s-0031-1281676]

P- Reviewer: Maruyama H, Nagarajan P, Videla LA
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.504

World J Hepatol 2014 July 27; 6(7): 504-512
ISSN 1948-5182 (online)

RETROSPECTIVE STUDY

Clinical outcomes of compensated and decompensated cirrhosis: A long term study

Dimitrios N Samonakis, Mairi Koulentaki, Constantina Coucoutsi, Aikaterini Augoustaki, Chryssavgi Baritaki, Emmanuel Digenakis, Nikolaos Papiamonis, Maria Fragaki, Erminia Matrella, Maria Tzardi, Elias A Kouroumalis

Dimitrios N Samonakis, Mairi Koulentaki, Constantina Coucoutsi, Aikaterini Augoustaki, Chryssavgi Baritaki, Emmanuel Digenakis, Nikolaos Papiamonis, Maria Fragaki, Erminia Matrella, Elias A Kouroumalis, Department of Gastroenterology and Hepatology, University Hospital of Heraklion, Heraklion 71110, Crete, Greece

Maria Tzardi, Department of Histopathology, University Hospital of Heraklion, Heraklion 71110, Crete, Greece

Author contributions: Samonakis DN wrote the manuscript, did the statistics and was responsible for patient management; Koulentaki M contributed to data base design, patient management and paper corrections; Coucoutsi C, Augustaki A and Baritaki C kept data base and patient management; Digenakis E, Papiamonis N and Fragaki M contributed to patient management and data base update, patient survival follow up; Matrella E contributed to long term patient management and outpatient clinic details; Tzardi M diagnosed all liver biopsies; Kouroumalis EA contributed to the study design, patient managment, contributed to the manuscript, had overall responsibility for the study.

Correspondence to: Dr. Dimitrios N Samonakis, Department of Gastroenterology and Hepatology, University Hospital of Heraklion, Buidling A-4th Floor, Voutes, Herklion 71110, Crete, Greece. dsamonakis@gmail.com
Telephone: +30-2810-392356 Fax: +30-2810-542085

Received: December 27, 2013 Revised: May 16, 2014
Accepted: June 10, 2014
Published online: July 27, 2014

Abstract

AIM: To study these characteristics and prognostic patterns in a Greek patient population.

METHODS: We analyzed a large cohort of cirrhotic patients referred to the department of Gastroenterology and Hepatology and the outpatient clinics of this tertiary hospital, between 1991 and 2008. We included patients with established cirrhosis, either compensated or decompensated, and further decompensation episodes were registered. A data base was maintained and updated prospectively throughout the study period. We analyzed differences in cirrhosis aetiology, time to and mode of decompensation, hepatocellular carcinoma (HCC) occurrence and ultimately patient survival.

RESULTS: Five hundreds and twenty-two patients with median age 67 (range, 29-91) years and average follow up 9 years-10 mo (range, 1-206 mo) were studied. Commonest aetiology was hepatitis C virus (HCV, 41%) followed by alcohol (31%). The median survival time in compensated cirrhotics was 115 mo (95%CI: 95-133), whereas in decompensated patients was 55 mo (95%CI: 36-75). HCV patients survived longer while HBV patients had over twice the risk of death of HCV patients. The median time to decompensation was 65 mo (95%CI: 51-79), with alcoholics having the highest risk (RR = 2.1 *vs* HCV patients). Hepatitis B virus (HBV) patients had the highest risk of HCC, alcoholics the lowest. Leading causes of death: liver failure, hepatorenal syndrome, sepsis and HCC progression.

CONCLUSION: Cirrhosis aetiology and decompensation at presentation were predictors of survival. Alcoholics had the highest decompensation risk, HBV cirrhotics the highest risk of HCC and HCV cirrhotics the highest decompensation-free time.

Key words: Survival; Decompensation; Hepatocellular carcinoma; Bleeding; Ascites

Core tip: Hepatitis C was the most common cause in our cirrhotics and many hepatitis C virus patients were aged and demonstrated a long, mild course. Alcoholic and non alcoholic steatohepatitis cirrhosis is becoming a significant problem. Ascites was the commonest type of decompensation. Survival in compensated cirrhotics was at least double that of decompensated patients. Variceal bleeding was more frequent in alcoholics; nevertheless it was unexpectedly related to better survival

than decompensation with ascites or encephalopathy. This was attributed to the improvements in the management of variceal bleeding together with the importance of abstinence from alcohol after the episode was successfully treated. Hepatocellular carcinoma patients with a history of hepatitis B virus had the highest risk of mortality.

Samonakis DN, Koulentaki M, Coucoutsi C, Augoustaki A, Baritaki C, Digenakis E, Papiamonis N, Fragaki M, Matrella E, Tzardi M, Kouroumalis EA. Clinical outcomes of compensated and decompensated cirrhosis: A long term study. *World J Hepatol* 2014; 6(7): 504-512 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/504.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.504

INTRODUCTION

Cirrhosis and its complications represent the end in the spectrum of chronic liver diseases, irrespective of aetiology. The natural history of cirrhosis is classically characterised by an asymptomatic phase termed compensated cirrhosis, followed by the development of complications from portal hypertension and/or liver dysfunction, termed decompensated cirrhosis. The transition has been estimated to occur at a rate of 5%-7% per year. In recent years this process has been proposed as a series of critical steps that if unchecked, culminate in hepatic decompensation[1].

Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV), represents the commonest cause of cirrhosis worldwide[2]. Nevertheless, hepatitis C often has indolent course for a long period of time[3,4] with a median time from infection to cirrhosis of 30 years; several confounding factors have been associated with disease progression[4]. Despite higher risk for decompensation in HCV infection, cirrhosis presents earlier in HBV patients[5]. Longitudinal studies of patients with chronic hepatitis B have shown a 5 year cumulative incidence of developing cirrhosis 8%-20% with a 5 year survival in compensated cirrhosis 85% and 15%-35% in decompensated cirrhosis[6]. Various factors are related with progression in HBV infection, but clearly its course is modified by antiviral therapy and HBV DNA suppression[7,8]. Sustained response to anti-HCV treatment also significantly determines patients' outcome regarding decompensation, liver failure, death or orthotopic liver transplantation and decreases does not completely eliminate the HCC risk[9].

Hepatocellular carcinoma (HCC) is a major complication of viral cirrhosis, both compensated and decompensated, and a major cause of death[2]. HCC incidence appears to be increasing worldwide[10] and several clinicopathological variables have been identified as predictors for outcome[11,12]. The annual incidence of HBV related HCC ranges from 2%-5%[6]. Two other very common causes of chronic liver disease and subsequent complications are non alcoholic steatohepatitis (NASH) and alcoholic liver disease, which especially in western countries are becoming significant public health issues[13,14].

The purpose of this study was to evaluate-in a region of southern Greece-a cohort of patients with either compensated or decompensated cirrhosis at presentation; to identify the time to and mode of decompensation, investigate the occurrence of HCC and assess the impact of all the aforementioned on patient survival.

MATERIALS AND METHODS

Study design

The study was performed in a tertiary hospital which is the reference centre for the island of Crete, in the south of Greece. The population of 800000 is largely homogenous. The few non-Greek patients are mostly from Eastern Europe and the Balkans. The study conformed to the principles of declaration of Helsinki and was approved by the ethics committee of the University Hospital of Heraklion. The participants in the study provided verbal consent; for those patients with hepatic encephalopathy, consent was given by relatives. Written informed consent was provided from those undergoing interventional procedures (*i.e.*, liver biopsies, endoscopies and abdominal paracentesis).

We included patients with a diagnosis of cirrhosis who were seen as outpatients in the liver clinic or were hospitalized, mostly for chronic liver disease complications. Starting date of the study was January 1991 and their data were registered in a data base until June 2008. During the long period of this study, many patients that fulfilled the criteria were included and were therefore followed up prospectively. In that sense the study is both prospective and retrospective.

All patients with established cirrhosis were included. Diagnosis was based on liver biopsy (all patients with compensated cirrhosis) and/or clinical evidence of decompensation combined with endoscopic and radiological findings. We excluded from the study (1) paediatric liver disease; (2) patients with primary biliary cirrhosis (PBC); (3) autoimmune hepatitis (AIH) cirrhosis; and (4) 12 patients who did not wish to participate in the study. PBC patients have a discrete clinical course and our experience has been reported elsewhere[15]. AIH patients are few in Crete (less than 20 patients have been diagnosed during the study period) and all but one run a good course under treatment. Thus AIH as a separate group for cirrhosis aetiology was excluded due to small numbers.

The diagnosis of decompensated cirrhosis was based on the presence of any of the following: ascites, variceal bleeding or encephalopathy. The classification as compensated cirrhosis precluded any past history of the above criteria. The diagnosis of liver failure was made when one or more of the following were observed in decompensated cirrhotics: Hepato-renal syndrome type 2/type 1, progressively worsening liver biochemistry with prolongation of international normalized ratio and/or deepening jaundice (frequently due to sepsis), severely worsening encephalopathy, or liver failure in the context

of massive infiltration from tumour.

All patients with HBV related cirrhosis had received standard antivirals, initially lamivudine/adefovir and later either entecavir or tenofovir. Antiviral treatment started at the time of initial diagnosis of chronic HBV infection and continued after the diagnosis of cirrhosis until death or end of follow up.

No patient with decompensated hepatitis C related cirrhosis received antiviral treatment with the standard regimen. The number of compensated HCV cirrhotics in this population on treatment with interferon and ribavirin was too small to draw conclusions. HCV decompensated cirrhotics received only supportive treatment as indicated (diuretics, antibiotics, varices ligation, repeated paracentesis and terlipressin). Approximately half of HCV cirrhotics had no antiviral treatment prior to their cirrhosis diagnosis due to either old age, side effects or unavailability of treatment. In any case antivirals were discontinued on diagnosis of cirrhosis according to the guidelines at a certain time period. A 30% of alcoholics discontinued alcohol consumption.

A careful evaluation was performed to document any episode of decompensation at scheduled outpatient Hepatology clinic visits or at hospitalization for any reason. For patients who had not attended the outpatient clinic for three months after their previous visit, information regarding the outcome was obtained by telephone interviews with patients or relatives.

Liver biopsies were taken using ultrasound guidance and were initially performed with Menghini needles, later substituted by Tru-cut needles; few biopsies were done intraoperatively or transjugularly. All patients with bleeding were scoped within 24 h to diagnose and treat portal hypertensive bleeding. Ascites and encephalopathy were diagnosed according to standard criteria; all patients with ascites on presentation and according to clinical suspicion underwent abdominal paracentesis to check for spontaneous bacterial peritonitis ever since this was internationally accepted practice. Screening for HCC was performed every 6 mo with ultrasound (US) and a-fetoprotein, and during the last 3 years of the study contrast-enhanced US was used. HCC was diagnosed either histologically or according to European association of the study of the liver/American association of the study if the liver criteria ever since these were published[16,17].

Viral hepatitis markers were detected by Abbott Elisa immunoassays and viral load was measured quantitatively using polymerase chain reaction test wherever appropriate since the method was available. Alcohol misuse (defined as exceeding 40 g of ethanol daily in male-20 g daily in female patients) was identified after interviewing the patient during hospitalisation or in the outpatient alcoholic clinic, as well from information provided by social services. The study included patients with a diagnosis of alcoholic cirrhosis who were either active drinkers or were abstainers at evaluation. Three distinct end points were considered: decompensation, death (or liver transplantation) and HCC. Few patients received a transplant due to the late development of liver transplantation services in the country. NASH related cirrhosis and cryptogenic cirrhosis were evaluated as a single group.

Statistical analysis

Univariate comparisons of patient characteristics between the aetiological groups were undertaken using the chi-squared test and one-way ANOVA according to the type of characteristic. Bonferroni post-hoc comparisons were made when the ANOVA comparison was found to be statistically significant.

The median follow-up time was calculated using the reverse Kaplan Meier estimator[18]. Kaplan-Meier estimates of survival curves were constructed for both overall survival and decompensation-free survival. Median survival times were compared using the log-rank test. Both univariate and multivariate Cox's proportional hazards models were used to estimate hazard ratios (relative risks). A significance level of 5% was chosen for all hypothesis tests. SPSS version 17 was used throughout.

RESULTS

A total of 522 patients were included in the study. The majority of these patients had compensated cirrhosis on presentation (n = 360, 69%). One hundred and eighty five patients developed decompensation during follow up (35.4% of the entire cohort and 51.3% of the initially compensated cirrhotics) and there were 231 deaths (44%) over the follow-up period. Median follow-up was 9 years 10 mo, and ranged from 1 mo to just over 17 years. There were 183 patients with a minimum follow up of 5 years in the entire cohort.

Seventy eight patients (15%) were lost to follow up. The distribution of cirrhosis causes in those lost to follow up was found to be similar to those remaining in the study (n = 444). Leading causes of death were: liver failure which resulted in 55 deaths (23.8%) hepatorenal syndrome (n = 50, 21.6%), sepsis (n = 25, 10.8%), massive portal hypertensive bleeding (n = 15, 6.5%). These were followed by HCC progression, extrahepatic cancer, cardiovascular events, and other causes. In 21 patients (9%) who died the cause was not verified.

Characteristics of the patient cohort are presented in Table 1. Mean patient age was 67 (range 29 to 91) years. The most common cause of cirrhosis was hepatitis C (41%, 215 patients), followed by alcoholic liver disease (31%, 162 patients). The age distribution within each etiologic group is summarized in Figure 1.

The mean age of the alcoholic liver disease (ALD) patients was 62 (SD ± 12) years, of HBV patients 67 (SD ± 10) years, of HBV + ALD patients 56 (SD ± 15) years, of HCV patients 71 (SD ± 9) years, HCV + ALD patients 65 (SD ± 11) years, of NASH/cryptogenic patients 70 (SD ± 13) years. Average patient age differed to a statistically significant extent between groups (P < 0.0001). Post-hoc pairwise comparisons indicated that HCV and NASH/cryptogenic patients were older on average than ALD or

Table 1 Patients' cohort characteristics

	n (%)
Number of patients	522
Male	342 (66)
Female	180 (34)
Cirrhosis aetiology	
HCV	180 (34)
HCV/ALD	35 (7)
HBV	67 (13)
HBV/ALD	15 (3)
ALD	162 (31)
NASH/other	63 (12)
HCC	88 (17)
Patients alive	213 (41)
Patients died	231 (44)
Lost to follow up	78 (15)
Initially compensated	358 (69)
Initially decompensated	164 (31)
Decompensated during Follow Up	185 (35)
Initial episode of decompensation	
Ascites	256 (73)
Variceal bleed	37 (11)
Encephalopathy	10 (3)
More than 1	22 (6)
Not known	24 (7)

HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBV: Hepatitis B virus; ALD: Alcoholic liver disease; NASH: Non alcoholic steatohepatitis.

HBV/ALD patients ($P < 0.0001$ in all cases). HBV/ALD patients were also younger, on average, than patients with HBV alone ($P = 0.035$). There have been only 5 patients with HDV co-infection, three of them with HBV/HDV/HCV with a history of intravenous drug use. Six patients had co infection HBV/HCV. Due to the small number no separate analysis for the viral co infections was performed.

The median survival time of those presenting with compensated cirrhosis was 115 (95%CI: 95-135) mo whereas decompensated patients had a median survival of 55 (95%CI: 36-75) mo. Kaplan-Meier survival curves also indicated a worse overall prognosis for patients presenting with decompensated cirrhosis (Figure 2) ($P < 0.0001$).

Survival was also strongly influenced by cirrhosis aetiology: Kaplan-Meier survival curves are presented according to etiologic group in Figure 3, in which HBV patients (90% e-antigen negative) appear to have the worst overall survival ($P = 0.004$). Using univariate Cox regression analysis, HBV patients were found to have just over twice the risk of death of HCV patients (RR = 2.1, $P < 0.0001$) whilst the NASH/cryptogenic group had a RR of 1.6 ($P = 0.042$) compared to the HCV group.

At presentation, both cirrhosis aetiology and decompensation remained significant predictors of survival (P values 0.007 and < 0.0001 respectively) after adjusting for age ($P = 0.633$) and sex ($P = 0.505$) in a multivariable model. The RR was 2.6 for patients that were decompensated at diagnosis (95%CI: 1.9-3.6) compared to compensated patients. Patients with HBV had RR = 1.8 (95%CI: 1.2-2.7, $P = 0.005$) compared to HCV patients but none of the other groups had a statistically significantly elevated risk compared to HCV patients (all P values > 0.1).

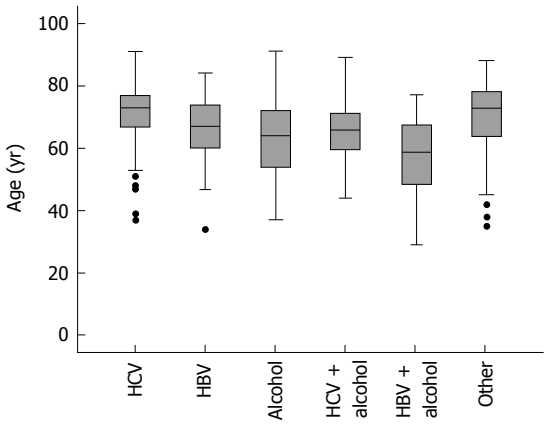

Figure 1 Patients' age in the different etiologies of cirrhosis. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

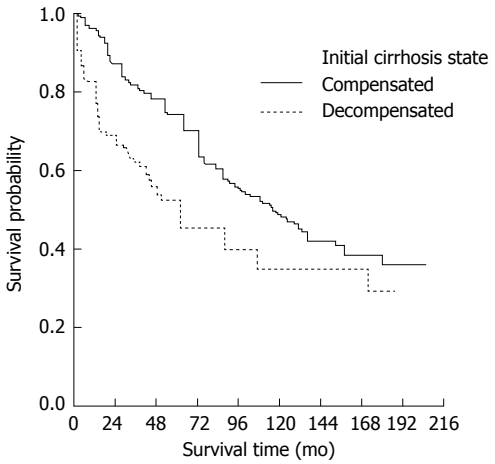

Figure 2 Survival curves in compensated and decompensated cirrhosis.

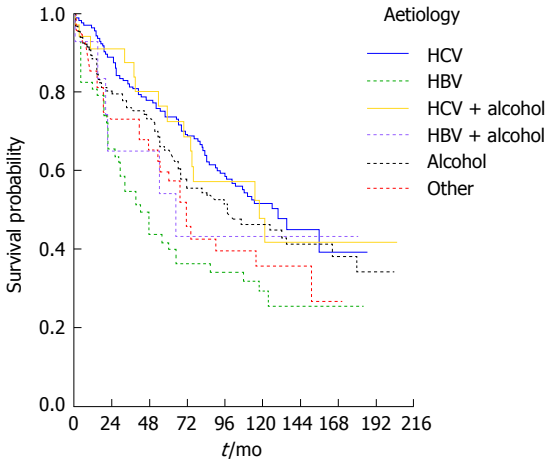

Figure 3 Survival curves according to the etiology of cirrhosis. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

The median time to decompensation was 65 mo (95%CI: 51-79 mo) and varied according to aetiology. Kaplan-Meier curves are presented by etiologic group in Figure 4 ($P = 0.003$). The highest median decompensation-free time was seen in the HCV patient group (median 105, 95%CI: 60-150 mo), the lowest in

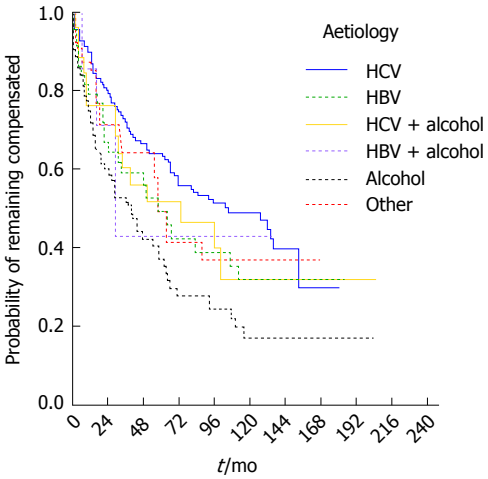

Figure 4 Risk for decompensation according to the etiology of cirrhosis. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

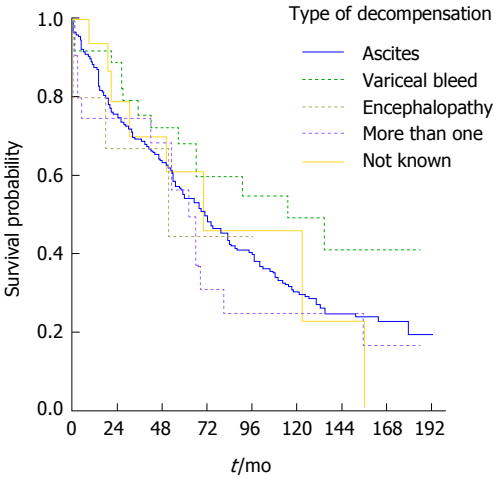

Figure 5 Survival in relation to the type of decompensation.

the NASH/cryptogenic (median 58, 95%CI: 48-68) and HBV groups (median 57, 95%CI: 35-79 mo). Aetiology remained a statistically significant predictor of risk of decompensation in a multivariable Cox model (P = 0.026), adjusting for age (P = 0.611) and sex (P = 0.878). Patients with alcoholic aetiology had the highest risk of decompensation compared to those with HCV (RR = 2.1, 95%CI: 1.3-3.2).

The most frequent type of decompensation was presentation of ascites (73%, 256 patients) while 6% (22 patients) had more than one complication on the same date, 15 having both ascites and encephalopathy. The latter group of patient had high mortality (64%, 14 patients out of 22, died during follow-up). Variceal bleeding was diagnosed in 37 patients. The leading aetiology in patients with variceal bleeding was ALD (51%, 19 patients), followed by NASH/crypto (27%, 10 patients) and then HCV (19%, 7 patients).

From the Kaplan-Meier curve it appears that patients who decompensated with variceal bleeding had the best overall survival, followed by those decompensating with ascites whilst the worst outcomes were evident in the group presenting with more than one complication (Figure 5). The corresponding log rank test indicated, however that the differences in survival were not statistically significant (P = 0.354).

HCC was diagnosed in 10 patients at the time of first presentation, whilst 78 patients developed HCC over the follow-up period. The mean time to the development of HCC in the entire cohort was 164 mo (95%CI: 156-172 mo). The incidence of HCC during the follow-up period was associated with cirrhosis aetiology (P = 0.003), even after adjusting for age and sex in a multivariable model (P = 0.027); the only pairwise statistically significant comparison was ALD compared to HBV, with ALD patients having an HCC risk of 0.3 times that of those with HBV aetiology (95%CI: 0.15-0.60, P = 0.001). In addition, female cirrhotics had an HCC risk 0.38 times that of men (95%CI: 0.20-0.71). Age was not statistically significant (P = 0.205). Cirrhosis aetiology was a borderline statistically significant predictor of survival after the diagnosis of HCC (P = 0.064) after adjusting for age (P = 0.494) and sex (P = 0.159).

DISCUSSION

In this homogenous cohort of patients with extended follow up from a single centre we studied the clinical course of cirrhosis and analyzed it according to the aetiology. The most common aetiology in this southern region of Greece was hepatitis C, in keeping with previous publications from the island and mainland[19,20]. In our cohort alcoholic was the second most common cause of cirrhosis and this cause has displayed an increasing trend over recent years.

Hepatitis C patients were, on average, older followed by the NASH/cryptogenic group, with HBV cirrhotics tending to be diagnosed at a younger age in this study. The older age in the HCV cohort could be expected as HCV infection is asymptomatic in the majority of patients with slow progression over decades, until cirrhosis is established[5]. Alcohol abuse in HCV infected patients is a well recognised negative prognostic factor and explains the younger average age of this group compared to the HCV group. Even in the younger HBV cirrhotics, however, alcohol abuse significantly lowered the age of cirrhosis diagnosis. In our study, age appeared to influence the survival after decompensation while comorbidities like cardiovascular diseases or diabetes had significant effect in those with NASH related cirrhosis.

NASH is an increasingly recognised cause of cirrhosis, delineating a significant percentage of cryptogenic cases[13]. Although the adult obesity epidemic has not been yet evident in Greece, most of the NASH cirrhotics were identified recently and many of these cases were linked to diabetes mellitus. Their relatively small number in our study is obviously due to the usually long period until cirrhosis develops. This picture is expected to change over the next decade. In our department in a sur-

vey of 2000 liver biopsies, NAFLD/NASH comprised 22.5% of the biopsies between the years 2003-2006, as compared to 5% between the years 1990-1995[21].

The majority of patients were diagnosed with compensated cirrhosis, but a considerable number decompensated during follow up. Patients who presented with compensated cirrhosis had a significantly better survival than those presenting with decompensated cirrhosis. A recent interesting study from the United Kingdom showed that survival of cirrhosis is significantly higher in patients diagnosed and followed in an ambulatory setting than those with first diagnosis in the occasion of a hospital admission[22]. In this study aetiology affected prognosis in young patients to a greater extent than in older ones.

In our cohort, those with HCV aetiology remained compensated for a longer period of time on average, with alcoholics having the highest risk for decompensation. These data are similar to those of other studies of Greek cirrhotic patients (as reported by Giannousis *et al*[20]) as well as to those from a cohort of 4537 cirrhotics from a general practice data base in the United Kingdom. In the later study, alcoholic aetiology had higher rate of decompensation compared to others during the first year after diagnosis; nevertheless this difference was not evident following the first year[23].

Ascites was the most common type of presentation in decompensated cirrhosis while patients with multiple presentations (*i.e.*, combination of ascites, variceal bleeding, and encephalopathy) had the worst prognosis. In a study on acute-on-chronic liver failure (ACLF) by Moreau *et al*[24], ascites was a risk factor for development ACLF because it is an independent predictor of kidney failure following bacterial infections. Benvegnù *et al*[2] reported (using a large cohort of viral cirrhosis, mainly HCV related, cirrhosis patients) that the most frequent complication was HCC, followed by ascites which is also the experience published by Sangiovanni *et al*[25], in an elegant natural history study of 214 HCV patients. A recent paper[26] showed that the HCC incidence was significantly higher among HCV patients with varices compared to those without.

In the present study, alcoholics had significantly more episodes of variceal bleeding. Unexpectedly patients who decompensated with variceal bleeding displayed a better survival compared to other presentations of decompensation. This may be attributed to the large number of alcoholics in this group of decompensated patients, in whom abstinence may have effectively influenced the prognosis. Moreover the established approach in variceal bleeding which includes a combination of pharmacologic and early endoscopic therapy may also be responsible for improved survival displayed in these patients[27]. Primary and secondary prophylaxis might also account for the decreased incidence of variceal bleeding observed in the recent years as compared to episodes seen in the first years of the study.

Our cohort's average survival was almost similar in compensated cirrhotics (10 years) and slightly better in decompensated (4.5 years) to the survival reported in the seminal natural history paper by D'Amico *et al*[28]. The somewhat better survival in our decompensated group could be due to our study being more recent (with documented improvements in the medical and endoscopic management of these patients), and also due to the development of alcohol services in our department and the course of HCV patients with the longest survival. Fattovich *et al*[3] in a previous classical study also reported a long survival in a cohort of 384 HCV cirrhotics[25,29]. It should be stressed that survival in HCV cirrhosis was better compared to HBV cirrhotics despite the fact that HCV cirrhotics received only symptomatic and supportive treatment while practically the vast majority HBV patients received antiviral treatment.

The lowest survival rates were found in the HBV group. This might be related to the increased incidence of HCC in this group and to the fact that more than 90% of our HBV patients had HBeAg negative chronic hepatitis. Indeed the incidence of cirrhosis and its subsequent complications are much more frequent in HBeAg negative than in HBeAg positive HBV infected patients, both in Europe and in Asia[7]. Moreover, we have included patients from the first era of the antiviral therapy when treatment for HBV aetiology was not as effective as treatments now available. This, together with the development of lamivudine resistance in a percentage of the HBV patients (data under preparation) as well as with the correlation with HCC all contributed to the uneven outcome of these patients. The poor outcomes for the combined aetiology, HBV plus alcohol group, is no surprise as alcohol can worsen the natural course of viral hepatitis at any time[4,7].

Alcoholic cirrhotics despite their higher decompensation risk had a relatively high overall survival rates and this can again be explained by the fact that a proportion of these patients successfully discontinued or reduced their alcohol intake. Thirty percent of the whole cohort of patients with alcoholic aetiology became abstinent, mostly by attending the alcohol services at the hospital. Similar to our findings, the study by Toshikuni *et al*[30] reported that survival of HCV cirrhotics was similar to survival of alcoholic cirrhotics, with the same risk for decompensation and mortality. A study in Danish patients[31] showed that alcoholic cirrhotics had high prevalence of complications at the time of diagnosis and these were predictors of 1-year mortality. In this series ascites was also the most frequent type of decompensation, while there was also high risk of variceal bleeding or encephalopathy. As in our series, more than one complication was associated with worse prognosis.

HCC development was observed mostly in HCV and HBV cirrhosis, and NASH had the smallest incidence. The risk was highest in HBV cirrhosis and lowest in those with alcoholic aetiology. Similarly, Fattovich *et al*[11] reported that in the absence of HBV or HCV infection, HCC incidence is lower in alcoholic cirrhotics and these data were confirmed by a retrospective study from Japan.

However recent data confirm that heavy alcohol consumption significantly increases the risk of HCC in HBV-related cirrhotics[32].

Survival after HCC development was marginally related to the aetiology in our group of patients in keeping with the data by Trevisani *et al*[12]. However, the development of HCC was a catastrophic event in the natural course of the disease[2,25]. The poor survival of the HCC group was also influenced by the fact that many of these patients were referred from district hospitals after the diagnosis of large tumours, not amenable to radical treatments (resection or transplantation). This, together with a heterogeneous approach to HCC screening amongst the referring hospitals obviously affected both the actual incidence and the outcome. Treatment of these patients has been reported in the randomized trial with sc octreotide[33] or im long acting somatostatin analogues[34]. The remaining few patients underwent chemoembolization and have been reported elsewhere (Samonakis *et al*[34] submitted). Only 3 patients were transplanted due to the recent development of transplant services in the country, where even today there is only one liver transplant centre with a rather limited activity.

The causes of death in this cohort of cirrhotic patients were mostly related to complications of liver disease and/or HCC rather than the presence of comorbidities. This in keeping with most published experience in natural history studies[35]. An exception to this was the NASH-cryptogenic group where death from cardiovascular complications was frequent (data not shown). It is increasingly recognised that cardiovascular diseases may seriously contribute to the mortality of cirrhosis, contrary to previously thoughts that liver cirrhosis is protective for coronary artery disease[36].

This study has several limitations. Due to the original design it has a retrospective and a prospective arm. Moreover some patients were lost to follow up after a successful management of an acute episode, so data on survival or cause of death are missing for this population. We could not provide an analysis in relation to the model end-stage liver disease (MELD) score as it was introduced after 2002. A recent publication[37] showed that aetiology of cirrhosis has an impact on 1-year survival predicted by the MELD score. The study findings are further limited by a long accrual period. Standard survival analysis methods, such as those applied in the present study, are valid under the assumption that the probabilities of death are stable with respect to absolute time.

In conclusion, in this cohort of patients with a long follow up we found that cirrhosis aetiology and decompensation were predictors of survival at presentation. Alcoholics had the highest risk of decompensation and HBV cirrhotics were at the highest risk of developing HCC. On average HCV cirrhotics had the highest decompensation-free time. The improvement in the management of cirrhosis complications, recent advances in the treatment of viral hepatitis and the development of specialized services for alcoholic liver disease could affect the development of complications and ultimately patient survival.

ACKNOWLEDGMENTS

We are grateful to Dr. Moschandreas for her advice regarding data analysis and for English language corrections. We are also grateful to Dr. Economaki, Dr. Leontidis, Dr. Chatzicostas for their contribution in data selection and information for patient update.

COMMENTS

Background

Cirrhosis and its complications represent the end in the spectrum of chronic liver diseases, irrespective of aetiology. Its natural history is typically characterised by an asymptomatic phase termed compensated cirrhosis, followed by the development of complications from portal hypertension and/or liver dysfunction, termed decompensated cirrhosis. Cirrhotics have diverse presentation and prognosis according to stage, aetiology and geographic region.

Research frontiers

The research objective was to study disease progression in the authors' cohort of compensated and decompensated cirrhotics; to identify the time to and mode of decompensation, to assess the occurrence of hepatocellular carcinoma and the ultimate impact of cirrhosis on patient survival.

Innovations and breakthroughs

Previous studies from various parts of the world have presented local experience. This is the largest study in their country, both with respect to the study population and the follow up period. It reflects various critical issues on the epidemiology, natural history and survival of this large cohort of cirrhotics.

Applications

The study provides insight on the natural course of common causes of liver cirrhosis, denotes the increasing problem of alcoholic liver disease, whereas provides useful information on the importance of aetiology in prognosis.

Terminology

Cirrhosis arises as a result of different mechanisms of liver injury and represents a common denominator to various aetiologies; it represents an increasing cause of liver morbidity and mortality. Chronic infection with hepatitis B and C virus, represent the commonest cause of cirrhosis. Hepatocellular carcinoma is a primary neoplasm that frequently develops on a cirrhotic liver.

Peer review

The article of Samonakis *et al* entitled "Natural history study of compensated and decompensated cirrhosis: a long term single centre study" is a retrospective study aimed to reporting the characteristics and evolution of a wide cohort of cirrhotic patients in Greece. The article is of interest in clinical practice, the methodology is appropriate and the size of the population is big enough to support the conclusions that authors have drawn in this comprehensive work.

REFERENCES

1 **Garcia-Tsao G**, Friedman S, Iredale J, Pinzani M. Now there are many (stages) where before there was one: In search of a pathophysiological classification of cirrhosis. *Hepatology* 2010; **51**: 1445-1449 [PMID: 20077563 DOI: 10.1002/hep.23478]

2 **Benvegnù L**, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut* 2004; **53**: 744-749 [PMID: 15082595 DOI: 10.1136/gut.2003.020263]

3 **Fattovich G**, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, Nevens F, Solinas A, Mura D, Brouwer JT, Thomas H, Njapoum C, Casarin C, Bonetti P, Fuschi P, Basho J, Tocco A, Bhalla A, Galassini R, Noventa F, Schalm SW, Realdi G. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. *Gastroenterology* 1997; **112**: 463-472 [PMID: 9024300 DOI: 10.1053/gast.1997.v112.pm9024300]

4 **Massard J**, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, Poynard T. Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 2006; **44**: S19-S24 [PMID: 16356583 DOI: 10.1016/j.jhep.2005.11.009]

5 **Fattovich G**, Pantalena M, Zagni I, Realdi G, Schalm SW, Christensen E. Effect of hepatitis B and C virus infections on the natural history of compensated cirrhosis: a cohort study of 297 patients. *Am J Gastroenterol* 2002; **97**: 2886-2895 [PMID: 12425564 DOI: 10.1111/j.1572-0241.2002.07057.x]

6 **McMahon BJ**. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; **49**: S45-S55 [PMID: 19399792 DOI: 10.1002/hep.22898]

7 **Fattovich G**, Bortolotti F, Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol* 2008; **48**: 335-352 [PMID: 18096267 DOI: 10.1016/j.jhep.2007.11.011]

8 **Manno M**, Cammà C, Schepis F, Bassi F, Gelmini R, Giannini F, Miselli F, Grottola A, Ferretti I, Vecchi C, De Palma M, Villa E. Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; **127**: 756-763 [PMID: 15362032 DOI: 10.1053/j.gastro.2004.06.021]

9 **Manesis EK**, Papatheodoridis GV, Touloumi G, Karafoulidou A, Ketikoglou J, Kitis GE, Antoniou A, Kanatakis S, Koutsounas SJ, Vafiadis I. Natural course of treated and untreated chronic HCV infection: results of the nationwide Hepnet.Greece cohort study. *Aliment Pharmacol Ther* 2009; **29**: 1121-1130 [PMID: 19222410 DOI: 10.1111/j.1365-2036.2009.03974.x]

10 **Shariff MI**, Cox IJ, Gomaa AI, Khan SA, Gedroyc W, Taylor-Robinson SD. Hepatocellular carcinoma: current trends in worldwide epidemiology, risk factors, diagnosis and therapeutics. *Expert Rev Gastroenterol Hepatol* 2009; **3**: 353-367 [PMID: 19673623 DOI: 10.1586/egh.09.35]

11 **Fattovich G**, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology* 2004; **127**: S35-S50 [PMID: 15508101 DOI: 10.1053/j.gastro.2004.09.014]

12 **Trevisani F**, Magini G, Santi V, Morselli-Labate AM, Cantarini MC, Di Nolfo MA, Del Poggio P, Benvegnù L, Rapaccini G, Farinati F, Zoli M, Borzio F, Giannini EG, Caturelli E, Bernardi M. Impact of etiology of cirrhosis on the survival of patients diagnosed with hepatocellular carcinoma during surveillance. *Am J Gastroenterol* 2007; **102**: 1022-1031 [PMID: 17313497 DOI: 10.1111/j.1572-0241.2007.01100.x]

13 **Liou I**, Kowdley KV. Natural history of nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2006; **40** Suppl 1: S11-S16 [PMID: 16540761]

14 **Tome S**, Lucey MR. Review article: current management of alcoholic liver disease. *Aliment Pharmacol Ther* 2004; **19**: 707-714 [PMID: 15043511 DOI: 10.1111/j.1365-2036.2004.01881.x]

15 **Koulentaki M**, Moscandrea J, Dimoulios P, Chatzicostas C, Kouroumalis EA. Survival of anti-mitochondrial antibody-positive and -negative primary biliary cirrhosis patients on ursodeoxycholic acid treatment. *Dig Dis Sci* 2004; **49**: 1190-1195 [PMID: 15387345 DOI: 10.1023/B:DDAS.0000037811.48575.da]

16 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. *J Hepatol* 2001; **35**: 421-430 [DOI: 10.1016/S0168-8278(01)00130-1]

17 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

18 **Schemper M**, Smith TL. A note on quantifying follow-up in studies of failure time. *Control Clin Trials* 1996; **17**: 343-346 [DOI: 10.1016/0197-2456(96)00075-X]

19 **Kouroumalis EA**, Skordilis PG, Moschandrea J, Alexandrakis G, Charoulakis N, Tzardi M, Manousos ON. Natural history of advanced hepatocellular carcinoma in Crete. Association with hepatitis C virus. *Eur J Gastroenterol Hepatol* 1997; **9**: 981-988 [PMID: 9391788 DOI: 10.1097/00042737-199710000-00011]

20 **Giannousis IP**, Papatheodoridis GV, Deutsch MJ, Manolakopoulos SG, Manesis EK, Koskinas JS, Archimandritis AJ. The burden and recent epidemiological changes of the main chronic liver diseases in a Greek referral tertiary centre. *Eur J Gastroenterol Hepatol* 2010; **22**: 172-179 [PMID: 19738477]

21 **Avgerinos A**, Koulentaki M, Tzardi M, Samonakis D, Matrella E, Kouroumalis EA. Increased incidence of steatohepatitis during the last sixteen years in Crete. EASL special conference on NASH/NAFLD. Bologna, Italy, 2009

22 **Ratib S**, Fleming KM, Crooks CJ, Aithal GP, West J. 1 and 5 year survival estimates for people with cirrhosis of the liver in England, 1998-2009: a large population study. *J Hepatol* 2014; **60**: 282-289 [PMID: 24128415]

23 **Fleming KM**, Aithal GP, Card TR, West J. The rate of decompensation and clinical progression of disease in people with cirrhosis: a cohort study. *Aliment Pharmacol Ther* 2010; **32**: 1343-1350 [PMID: 21050236 DOI: 10.1111/j.1365-2036.2010.04473.x]

24 **Moreau R**, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, Durand F, Gustot T, Saliba F, Domenicali M, Gerbes A, Wendon J, Alessandria C, Laleman W, Zeuzem S, Trebicka J, Bernardi M, Arroyo V. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; **144**: 1426-1437, 1437.e1-e9 [PMID: 23474284 DOI: 10.1053/j.gastro.2013.02.042]

25 **Sangiovanni A**, Prati GM, Fasani P, Ronchi G, Romeo R, Manini M, Del Ninno E, Morabito A, Colombo M. The natural history of compensated cirrhosis due to hepatitis C virus: A 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303-1310 [PMID: 16729298 DOI: 10.1002/hep.21176]

26 **Gomez EV**, Rodriguez YS, Bertot LC, Gonzalez AT, Perez YM, Soler EA, Garcia AY, Blanco LP. The natural history of compensated HCV-related cirrhosis: a prospective long-term study. *J Hepatol* 2013; **58**: 434-444 [PMID: 23111008 DOI: 10.1016/j.jhep.2012.10.023]

27 **Samonakis DN**, Triantos CK, Thalheimer U, Patch DW, Burroughs AK. Management of portal hypertension. *Postgrad Med J* 2004; **80**: 634-641 [PMID: 15537846 DOI: 10.1136/pgmj.2004.020446]

28 **D'Amico G**, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; **44**: 217-231 [PMID: 16298014 DOI: 10.1016/j.jhep.2005.10.013]

29 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [DOI: 10.1016/S0140-6736(96)07642-8]

30 **Toshikuni N**, Izumi A, Nishino K, Inada N, Sakanoue R, Yamato R, Suehiro M, Kawanaka M, Yamada G. Comparison of outcomes between patients with alcoholic cirrhosis and those with hepatitis C virus-related cirrhosis. *J Gastroenterol Hepatol* 2009; **24**: 1276-1283 [PMID: 19486451 DOI: 10.1111/j.1440-1746.2009.05851.x]

31 **Jepsen P**, Ott P, Andersen PK, Sørensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology* 2010; **51**: 1675-1682 [PMID: 20186844]

32 **Lin CW**, Lin CC, Mo LR, Chang CY, Perng DS, Hsu CC, Lo GH, Chen YS, Yen YC, Hu JT, Yu ML, Lee PH, Lin JT, Yang SS. Heavy alcohol consumption increases the incidence of hepatocellular carcinoma in hepatitis B virus-related cirrhosis. *J Hepatol* 2013; **58**: 730-735 [PMID: 23220252 DOI: 10.1016/j.jhep.2012.11.045]

33 **Kouroumalis E**, Skordilis P, Thermos K, Vasilaki A, Moschandrea J, Manousos ON. Treatment of hepatocellular carci-

noma with octreotide: a randomised controlled study. *Gut* 1998; **42**: 442-447 [PMID: 9577356 DOI: 10.1136/gut.42.3.442]

34 **Samonakis DN**, Moschandreas J, Arnaoutis T, Skordilis P, Leontidis C, Vafiades I, Kouroumalis E. Treatment of hepatocellular carcinoma with long acting somatostatin analogues. *Oncol Rep* 2002; **9**: 903-907 [PMID: 12066229]

35 **Asrani SK**, Kamath PS. Natural history of cirrhosis. *Curr Gastroenterol Rep* 2013; **15**: 308 [PMID: 23314828 DOI: 10.1007/s11894-012-0308-y]

36 **Kalaitzakis E**, Rosengren A, Skommevik T, Björnsson E. Coronary artery disease in patients with liver cirrhosis. *Dig Dis Sci* 2010; **55**: 467-475 [PMID: 19242795]

37 **Angermayr B**, Luca A, König F, Bertolini G, Ploner M, Gridelli B, Ulbrich G, Reiberger T, Bosch J, Peck-Radosavljevic M. Aetiology of cirrhosis of the liver has an impact on survival predicted by the Model of End-stage Liver Disease score. *Eur J Clin Invest* 2009; **39**: 65-71 [PMID: 19087131 DOI: 10.1111/j.1365-2362.2008.02063.x]

P- Reviewer: Castiella A, Koch TR, Romero MR, Squadrito G
S- Editor: Song XX **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.513

World J Hepatol 2014 July 27; 6(7): 513-519
ISSN 1948-5182 (online)

CLINICAL TRIALS STUDY

Patients with multiple synchronous colonic cancer hepatic metastases benefit from enrolment in a "liver first" approach protocol

Dimitrios Kardassis, Achilleas Ntinas, Dimosthenis Miliaras, Alexandros Kofokotsios, Konstantinos Papazisis, Dionisios Vrochides

Dimitrios Kardassis, Achilleas Ntinas, Dionisios Vrochides, Centre for Hepato-Pancreato-Biliary Surgery, "Euromedica Geniki Kliniki" General Hospital, 54645 Thessaloniki, Greece
Dimosthenis Miliaras, Department of Pathology, "Euromedica Geniki Kliniki" General Hospital, 54645 Thessaloniki, Greece
Alexandros Kofokotsios, Department of Interventional Gastroenterology, "Euromedica Geniki Kliniki" General Hospital, 54645 Thessaloniki, Greece
Konstantinos Papazisis, Department of Medical Oncology, "Euromedica Geniki Kliniki" General Hospital, 54645 Thessaloniki, Greece
Author contributions: Kardassis D, Ntinas A and Vrochides D conceived and designed the study, performed surgical procedures, analysed and interpreted the data, and wrote the manuscript; Kofokotsios A, Papazisis K and Miliaras D provided multi-disciplinary treatment of the enlisted patients based on their respective specialties and were also involved in editing the manuscript; all authors approved the manuscript.
Correspondence to: Dimitrios Kardassis, MD, Centre for Hepato-Pancreato-Biliary Surgery, "Euromedica Geniki Kliniki" General Hospital, R.: 701, 11 Maria Callas Street, 54645 Thessaloniki, Greece. dimitrios.kardassis@gmx.net
Telephone: +30-231-0895469 Fax: +30-23-10895196
Received: November 4, 2013 Revised: May 15, 2014
Accepted: May 28, 2014
Published online: July 27, 2014

Abstract

AIM: To assess a protocol for treating patients with multiple synchronous colonic cancer liver metastases, which are unresectable in one stage.

METHODS: Patients enrolled in the "liver first" protocol presented with colon-only (not rectal) cancer and multiple synchronous hepatic metastases (type Ⅱ or Ⅲ). All patients showed good performance status (ECOG PS 0-1) and were treated with curative intent. Complete oncologic staging including positron emission tomography-computed tomography was performed in order to rule out extrahepatic disease. If bowel obstruction was imminent, an intraluminal colonic stent was placed endoscopically. Subsequently, all patients received standardised neo-adjuvant chemotherapy, that is, FOLFOX or XELOX regimens combined with an antiangiogenic agent (bevacizumab or cetuximab). Provided that a response to chemotherapy was observed, patients underwent either one or two hepatectomies with or without portal vein embolization followed by the indicated colectomy. Further chemotherapy was administered after each procedure. Re-staging was performed after each chemotherapeutic treatment. Disease progression at any stage resulted in discontinuation of the protocol and conversion to palliative disease management.

RESULTS: Prospectively recorded data from 11 consecutive patients (8 men) were analysed for this study. Their mean age at the time of their first assessment was 65.7 (SD ± 15.3) years. Six (54.6%) patients presented with type Ⅲ metastatic disease. The minimum and maximum follow-up periods were 7.3 and 39.6 mo, respectively. The mean overall survival of all patients was 16.5 (95%CI: 10.0-23.2) mo. A colonic stent had to be placed in 5 (45.5%) patients due to the onset of an intraluminal obstruction. Four (36.4%) patients succeeded in completing all planned surgical operations. Their mean overall survival was 27.2 (95%CI: 15.1-39.3) mo and the mean disease-free survival was 7.7 (95%CI: 3.0-12.5) mo. Patients, who were obliged to shift to palliative treatment due to disease progression, had a mean overall survival of 10.5 (95%CI: 8.6-12.4) mo. None of these patients underwent palliative colectomy. No postoperative mortality was recorded.

CONCLUSION: The implementation of a structured "liver first" approach protocol for the treatment of pa-

tients with extensive, liver-limited colon cancer metastatic disease may be beneficial.

Key words: Clinical protocols; Colectomy; Colon cancer; Hepatectomy; Liver neoplasm

Core tip: Complete tumour burden resection remains the only possible curative therapy for liver-limited colon cancer metastatic disease. However, there are different approaches regarding treatment of the primary tumour and its hepatic metastases, if the latter are synchronous and unresectable with one surgical procedure. For this subgroup of patients, a "liver first" approach protocol is introduced in order to assess standardised treatment as well as to prevent overtreatment in cases of undetected extra-hepatic metastatic dissemination or disease progression.

Kardassis D, Ntinas A, Miliaras D, Kofokotsios A, Papazisis K, Vrochides D. Patients with multiple synchronous colonic cancer hepatic metastases benefit from enrolment in a "liver first" approach protocol. *World J Hepatol* 2014; 6(7): 513-519 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/513.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.513

INTRODUCTION

Approximately every second patient who suffers from colorectal cancer (CRC) will at some point be diagnosed with either synchronous or metachronous metastatic disease[1,2]. Liver is the most frequently affected organ. Resection of the complete tumour load has long been accepted as the only therapeutic option that results in improved long-term survival or even cure[3]. During the past decade a significant prolongation of overall survival and an increase in survival rates has been reported. This development is based on the improvement of systemic chemotherapy and introduction of antiangiogenic agents, but also on the utilisation of advanced surgical strategies and equipment[4-6].

Whereas in metachronous resectable disease, the timing of necessary operative procedures seems obvious, various approaches are currently being implemented if resectable (or potentially resectable) hepatic metastases, with no evidence of extrahepatic disease, are detected at the time of the primary tumour diagnosis[7]. The "classic" approach consists of targeting the primary tumour first, followed by chemotherapy and resection of the hepatic metastases[8]. This strategy remains essential, if diagnosis of the disease coincides with an existing acute lower gastrointestinal bleeding or significant bowel obstruction. The "simultaneous" approach includes resection of the primary tumour as well as any hepatic metastases in one stage. This option is often preferred, especially in experienced centres, when a minor hepatectomy is sufficient in clearing the existing tumour load[9]. Finally, the "reverse" strategy has been introduced in recent years[10,11]. In this approach, liver specific procedures such as portal vein embolization and hepatectomies come first, followed by colectomy. All operative procedures take place either after chemotherapy alone or after combination with radiotherapy, when the diagnosis is rectal cancer. The rationale behind this strategy is that patients with multiple hepatic metastases are more likely to become incurable by not timely confronting the extensive liver metastatic disease.

Important criteria for choosing the appropriate therapeutic plan are patient's performance status, primary tumour location, disease extent, available diagnostic and therapeutic tools and methods, as well as the centre's medical and surgical team experience. Due to the complexity of the disease, the patient population is heterogenic. In addition, conclusions regarding best possible management are based on retrospective series of patients suffering from CRC and liver metastases[12]. Therefore, treatment of those patients is routinely based on patient and centre specific ("individually tailored") approaches rather than generally accepted guidelines.

For this study, a subgroup of CRC patients was defined, that is, patients who had been diagnosed with stage Ⅳ colonic (not rectal) cancer and presented with multiple, bilobar, synchronous, liver-only metastases, that were either potentially resectable after more than one procedure (type Ⅱ) or initially unresectable, but possibly resectable after tumour downsizing (type Ⅲ)[13,14]. These patients were enrolled in a prospective "liver first" approach protocol which included staging, certain oncologic therapy and surgical therapeutic steps. The aim of the study was to assess the implementation of this algorithm, especially in terms of applicability and safety.

MATERIALS AND METHODS

Ethics

This study was conducted in a tertiary care private hospital according to the guidelines of the Declaration of Helsinki of the World Medical Association[15]. The hospital's ethics committee approved the study protocol. Written informed consent was obtained from all patients. Their enrolment was discussed during and approved by the hospital's weekly tumour board. All patients were treated with curative intent.

Definitions

Nomenclature regarding the extent of hepatic resections is that endorsed by the International Hepato-Pancreato-Biliary Association[16]. Decisions on resectability were taken by the hepato-pancreato-biliary surgeons of our centre based on the recommendations made on the Consensus Conferences on the Multidisciplinary Treatment of Colorectal Cancer Metastases[17,18]. Postoperative complications are reported according to the Dindo-Clavien

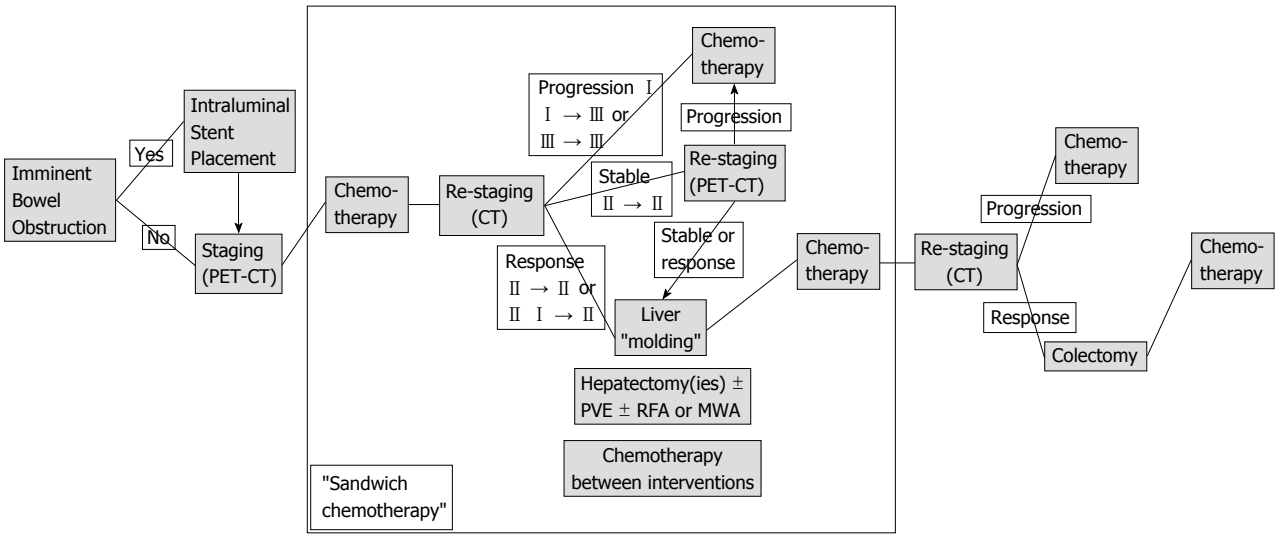

Figure 1 Algorithm of the "liver first" protocol. PET-CT: Positron emission tomography–computed tomography; PVE: Portal vein embolization; RFA: Radiofrequency ablation; MWA: Microwave ablation.

classification[19].

Patients

Inclusion criteria for patients enrolled in the "liver first" protocol included the diagnosis of colon-only (not rectal) cancer and synchronous, multiple, bilobar, liver metastases (type Ⅱ or Ⅲ), age ⩾ 18 years, no previous disease-specific therapeutic management and Eastern Cooperative Oncology Group (ECOG) performance status grade 0 or 1. Patients who were diagnosed with extrahepatic disease were excluded.

Study protocol

The protocol was performed within the scope of an intent-to-treat study. Initially, a complete oncologic staging, that is clinical examination, blood tests, liver function tests, tumour marker determination, coloscopy, primary tumour histology, abdominal and thoracic cross-sectional imaging, positron emission tomography-computed tomography (PET-CT), was performed. In the case of an imminent bowel obstruction, an intraluminal colonic stent was placed by endoscopy (Figure 1). All patients then received standardised neo-adjuvant chemotherapy including an antiangiogenic agent. In the case of post-chemotherapy disease response, patients underwent either portal vein embolization, in order to achieve an increase in the future liver remnant, or/and one or two hepatectomies. If indicated, radiofrequency ablation or microwave ablation was performed intraoperatively. In between, (sandwich) chemotherapy was administered. This particular protocol phase was called "liver molding". If the disease remained stable, a PET-CT scan was performed in order to assess the neoplasm's response to chemotherapy. Following the "liver molding" phase, chemotherapy and re-staging was repeated. Only in the case of absence of extrahepatic disease at this stage, patients underwent the indicated colectomy. Adjuvant chemotherapy regimens were administered. On the other hand, disease progression at any stage of the protocol resulted in its discontinuation and conversion to palliative disease management.

Chemotherapy

First-line chemotherapy comprised of 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX4), or capecitabine and oxaliplatin (XELOX) combined with a vascular endothelial growth factor inhibitor (bevacizumab). In second-line chemotherapy, oxaliplatin was replaced by irinotecan and/or bevacizumab was replaced by an epidermal growth factor receptor inhibitor (panitumumab), the latter was administered if patients had non-mutated disease (KRAS wild-type).

Statistical analysis

Continuous and categorical variables were recorded and analysed with descriptive statistics. Survival analysis was performed by the use of Kaplan-Meier curves. Statistical analysis was performed by means of the IBM SPSS Statistics Package, version 19.9 (SPSS Inc., Chicago, IL, United States).

RESULTS

For this study, prospectively collected data were analysed. Between July, 2010 and October, 2011 eleven consecutive patients (eight men) who met the inclusion criteria were enrolled in the "liver first" protocol. Demographic and clinical characteristics at the time of their first assessment are displayed in Table 1. Patients' mean age was 65.7 (SD ± 15.3) years. Seven patients (63.6%) presented with the primary tumour located in the sigmoid colon. Five patients (45.5%) presented with type Ⅱ metastatic disease. Six patients (54.6%) presented with type Ⅲ metastatic disease. The number of hepatic metastases

Table 1 Patients' first assessment demographic and clinical characteristics

Patients	Gender	Age (yr)	Primary colonic tumour location	Metastatic type (liver-limited)	Colonic obstruction > stent placement
1	Male	67	Sigmoid	Ⅱ	-
2	Male	75	Sigmoid	Ⅱ	-
3	Female	37	Sigmoid	Ⅲ	-
4	Male	79	Sigmoid	Ⅲ	√
5	Male	79	Descending	Ⅲ	√
6	Male	40	Sigmoid	Ⅱ	√
7	Female	75	Sigmoid	Ⅱ	-
8	Male	59	Descending	Ⅲ	-
9	Female	78	Descending	Ⅲ	√
10	Male	59	Sigmoid	Ⅲ	√
11	Male	75	Ascending	Ⅱ	-

ranged between seven and more than thirty, while their size ranged between 2 cm and 16 cm. A colonic stent was placed in five patients (45.5%) before the start of neo-adjuvant therapy due to an imminent intraluminal obstruction. Four patients (36.4%), all presenting with type Ⅱ metastatic disease at the time of first assessment, completed all scheduled surgical procedures and correspondingly the entire protocol. They underwent two or three operations (mean: 2.75), including the indicated colectomy as the last operative step. Pathology confirmed negative margins (R0) of all resected specimens. One out of five "type Ⅱ" patients (20.0%) suffered disease progression before reaching the time point of the planned hepatectomy. In only one out of six "type Ⅲ" patients (16.7%) the neoplasm was able to be converted to "type Ⅱ" following neo-adjuvant chemotherapy. No palliative colectomy was necessary for the seven patients who had to be allocated to palliative therapy due to disease progression (Table 2).

The minimum and maximum follow-up periods were 7.3 mo and 39.6 mo, respectively. The mean overall survival of all patients was 16.5 (95%CI: 10.0-23.2) mo. Patients who were able to complete the "liver first" protocol had a mean disease-free survival of 7.7 (95%CI: 3.0-12.5) mo and a mean overall survival of 27.2 (95%CI: 15.1-39.3) mo. On the contrary, patients, who were obliged to shift to palliative treatment due to disease progression during the period of their enrolment did not became free of disease at any time point and had a mean overall survival of 10.5 (95%CI: 8.6-12.4) mo (Table 2).

With regard to severe complications associated with chemotherapy, one patient suffered from upper gastrointestinal bleeding after receiving the FOLFOX and bevacizumab regimen. Two severe postoperative complications (Grade Ⅲ) were documented. One patient suffered an anastomotic site bleeding following sigmoidectomy, which was confirmed and treated by endoscopy and blood transfusions, and one patient suffered a bile leakage following hepatectomy, requiring percutaneous drainage. Furthermore, no postoperative (90-d) mortality was recorded.

DISCUSSION

Patients presenting with metastatic CRC represent a large, but significantly heterogeneous population as distinctions can be made based on primary tumour location, extension of metastatic spread and diagnosis time point of metastases (synchronous *vs* metachronous). Currently, complete neoplasm resection is regarded as the only curative therapeutic option for those patients[20]. Despite broadening resectability criteria in recent years, only a selected group (20%-30%) will be candidates for curative resection[21]. Historically, the first step of implementing therapeutic treatment was to resect the primary colorectal tumour and subsequently target hepatic metastases ("classic" approach). Due to improvements in both chemotherapy and surgical techniques, simultaneous resection of primary and liver-limited secondary disease ("combined" approach) or the prioritised resection of liver metastases ("reverse" approach) are being performed in experienced centres[22,23].

For this study, we selected a patient cohort as homogenous as possible. To be more specific, we included patients with synchronous liver-only metastatic disease that was diagnosed at the same time as the primary tumour and was either resectable in more than one stage or potentially resectable after successful downsizing. We excluded patients with rectal cancer because of the "interference" of radiotherapy treatment phases with the specific protocol steps. We also excluded patients who had to be treated with the "classic" approach, for example patients with ileus secondary to complete bowel obstruction. In addition, patients who could be treated with the "combined" approach, for example due to the presence of a solitary liver metastasis, were also excluded. Finally, we excluded patients with potentially resectable extrahepatic neoplasm dissemination.

In theory, the proposed "liver first" protocol may take advantage of the fact that neo-adjuvant chemotherapy in CRC patients provides an assessment of tumour biology[24]. Its effectiveness influences future therapeutic strategies because it may downsize the existing tumour load, so that initially unresectable metastases may become resectable[25]. Adding biological agents reportedly increases oncologic response and resectability rate[26]. On the other hand, this approach helps to avoid unnecessary operative procedures, and thus potential complications and delay in chemotherapy administration in patients whose neoplasm's biology is not favourable.

Upfront colectomy in the treatment of CRC with synchronous hepatic metastases in the context of the curative or even palliative setting became controversial the last few years. Even though some authors conclude that upfront colectomy is beneficial in terms of overall survival, this standpoint has been challenged because the rate of primary-related complications seems low, even when using modern antiangiogenic therapy[27-30]. In our small cohort of patients, we did not encounter any primary-related complications. Whenever a bowel obstruction

Table 2 Patients' operative treatment and oncologic characteristics

Patients	Metastatic type (liver-limited)	Hepatectomy 1	Hepatectomy 2	Colectomy	Disease-free Survival period (mo)	Overall Survival period (mo)
1	II	RE and wedge and RFA-MWA	-	√	8.23	39.57
2	II	Right	Left lateral and RFA-MWA	√	2.20	14.17
3	III	-	-	-	-	13.97
4	III	-	-	-	-	7.33
5	III	-	-	-	-	13.37
6	II	Laparoscopic left lateral	Right	√	15.27	39.17
7	II	Left lateral	Right	√	5.27	15.57
8	III	-	-	-	-	9.43
9	III	Laparoscopic left lateral	-	-	-	7.80
10	III	-	-	-	-	11.5
11	II	-	-	-	-	10.1

RE: Right extended; RFA: Radiofrequency ablation; MWA: Microwave ablation.

was imminent, a stent placement prevented acute surgery and enabled the protocol enrolment for each patient. In fact, one of five patients who received a colonic stent completed all planned operations and thus, the stent was resected with the colectomy specimen.

In spite of meticulous and repeated staging, three out of four patients (75.0%), who completed the "liver first" protocol and became disease-free, were finally diagnosed with recurrence (mean disease-free survival of 7.7 mo). This trend coincides with several large retrospective series[31,32]. A recent study suggests that pathologic characteristics of the primary colorectal tumour are more prognostic than relevant metastatic features[33].

A significant limitation of this study is the absence of a control group with matched diagnosis for comparing the "reverse" with the "classic" approach. Another important limitation is that the number of patients enrolled in the applied protocol is small.

The main goal of this work was to examine the feasibility and safety of realising a prospective "liver first" approach protocol-to our knowledge, it is the first one - for patients with liver-limited metastatic colon cancer. It focuses on a specific subgroup, namely patients with synchronous, multiple, bilobar hepatic metastases that are resectable after several interventions or disease downsizing. Treatment for these patients is usually "individually tailored" since the criterion of metastatic load resectability and the availability of therapeutic options may differ significantly among medical teams. Even though the number of patients is low, a noticeable trend can be observed, that is, patients who showed disease progression during the various steps of this algorithm had a worse outcome than those patients who succeeded in completing the protocol and became disease free, even for a short period of time. Furthermore, patients with disease progression avoided at least one operation (colectomy) without developing primary-related complications that needed surgical intervention.

In conclusion, the implementation of a structured "liver first" approach protocol for the treatment of patients with extensive, liver-limited colon cancer metastatic disease is feasible, safe, and may be beneficial. The application of such a protocol requires strict multidisciplinary decision-making process and therapeutic management.

COMMENTS

Background

Liver-limited colon cancer metastatic disease is a common entity in oncological and surgical practice. Complete tumour burden resection combined with systemic chemotherapy currently constitutes the only possible curative therapy.

Research frontiers

No consensus has yet been reached concerning both the timing and the sequence of primary tumour and synchronous, multiple hepatic metastases resection in case this cannot be achieved in one stage ("simultaneous" approach). Depending on the patient's clinical situation and the existing medical expertise, the primary tumour is either targeted upfront ("classic" approach) or subsequent to one or more liver resections ("reverse" or "liver first" approach).

Research frontiers

For this subgroup of patients, a structured "liver first" approach protocol has been introduced and implemented in order to assess standardised treatment as well as to prevent overtreatment in cases of undetected extra-hepatic metastatic dissemination or disease progression.

Applications

This study suggests that, regarding the treatment of patients with multiple synchronous colonic cancer liver metastases, which are unresectable in one stage, the application of a "liver first" approach protocol, which is based on a strict multidisciplinary decision-making process and therapeutic management is feasible, safe and potentially beneficial.

Terminology

A synchronous colorectal cancer metastasis is usually defined as metastatic neoplasmatic tissue that is detected either concurrently with diagnosis of the primary tumour or three to twelve months after the diagnosis. With respect to the described treatment protocol, a synchronous colorectal cancer metastasis was defined as metastatic neoplasmatic tissue which was diagnosed at the same time as the primary tumour. In contrast, metachronous metastases were identified at a later stage.

Peer review

The present manuscript deals with a novel and very interesting approach protocol to treat patients with colon cancer and hepatic metastasis.

REFERENCES

1 **Abdalla EK**, Adam R, Bilchik AJ, Jaeck D, Vauthey JN, Mahvi D. Improving resectability of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1271-1280 [PMID: 16955381 DOI: 10.1245/s10434-006-9045-5]

2 **Bova R**, Kamphues C, Neuhaus P, Puhl G. [Impact of time of occurrence of liver metastases (synchronous vs. metachronous) on early postoperative outcome and long-term sur-

vival of colorectal cancer patients]. *Zentralbl Chir* 2014; **139**: 220-225 [PMID: 23846535]
3 **Scheele J**, Stangl R, Altendorf-Hofmann A. Hepatic metastases from colorectal carcinoma: impact of surgical resection on the natural history. *Br J Surg* 1990; **77**: 1241-1246 [PMID: 2253003 DOI: 10.1002/bjs.1800771115]
4 **Wang CC**, Li J. An update on chemotherapy of colorectal liver metastases. *World J Gastroenterol* 2012; **18**: 25-33 [PMID: 22228967 DOI: 10.3748/wjg.v18.i1.25]
5 **Padman S**, Padbury R, Beeke C, Karapetis CS, Bishnoi S, Townsend AR, Maddern G, Price TJ. Liver only metastatic disease in patients with metastatic colorectal cancer: impact of surgery and chemotherapy. *Acta Oncol* 2013; **52**: 1699-1706 [PMID: 24102180 DOI: 10.3109/0284186X.2013.831473]
6 **Donati M**, Stavrou GA, Oldhafer KJ. Current position of ALPPS in the surgical landscape of CRLM treatment proposals. *World J Gastroenterol* 2013; **19**: 6548-6554 [PMID: 24151380 DOI: 10.3748/wjg.v19.i39.6548]
7 **Tzeng CW**, Aloia TA. Colorectal liver metastases. *J Gastrointest Surg* 2013; **17**: 195-201; quiz p.201-202 [PMID: 23054896 DOI: 10.1007/s11605-012-2022-3]
8 **Gennari L**, Doci R, Bignami P, Bozzetti F. Surgical treatment of hepatic metastases from colorectal cancer. *Ann Surg* 1986; **203**: 49-54 [PMID: 3942421 DOI: 10.1097/00000658-198601000-00009]
9 **Mayo SC**, Pulitano C, Marques H, Lamelas J, Wolfgang CL, de Saussure W, Choti MA, Gindrat I, Aldrighetti L, Barrosso E, Mentha G, Pawlik TM. Surgical management of patients with synchronous colorectal liver metastasis: a multicenter international analysis. *J Am Coll Surg* 2013; **216**: 707-716; discussion 716-718 [PMID: 23433970 DOI: 10.1016/j.jamcollsurg.2012.12.029]
10 **Mentha G**, Majno PE, Andres A, Rubbia-Brandt L, Morel P, Roth AD. Neoadjuvant chemotherapy and resection of advanced synchronous liver metastases before treatment of the colorectal primary. *Br J Surg* 2006; **93**: 872-878 [PMID: 16671066 DOI: 10.1002/bjs.5346]
11 **Mentha G**, Roth AD, Terraz S, Giostra E, Gervaz P, Andres A, Morel P, Rubbia-Brandt L, Majno PE. 'Liver first' approach in the treatment of colorectal cancer with synchronous liver metastases. *Dig Surg* 2008; **25**: 430-435 [PMID: 19212115 DOI: 10.1159/000184734]
12 **Andres A**, Toso C, Adam R, Barroso E, Hubert C, Capussotti L, Gerstel E, Roth A, Majno PE, Mentha G. A survival analysis of the liver-first reversed management of advanced simultaneous colorectal liver metastases: a LiverMetSurvey-based study. *Ann Surg* 2012; **256**: 772-778; discussion 778-779 [PMID: 23095621 DOI: 10.1097/SLA.0b013e3182734423]
13 **Adam R**, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, Giacchetti S, Paule B, Kunstlinger F, Ghémard O, Levi F, Bismuth H. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: a model to predict long-term survival. *Ann Surg* 2004; **240**: 644-657; discussion 657-658 [PMID: 15383792]
14 **Choti MA**. Defining resectable metastatic CRC: indications, outcomes, and controversies. In: Marshall JA, Choti MA. Managing CRC: the resectable and potentially resectable patient-A multidisciplinary approach. New Jersey: CMP-Medica-United Business Media, 2008: 9-15
15 Adopted by the 18th World Medical Association General Assembly (Helsinki, Finland, June 1964) and amended by the 64th World Medical Association General Assembly. Fortaleza, Brazil, 2013
16 The Brisbane 2000 terminology of liver anatomy and resections. HPB 2000; 2: 333-339. *HPB* (Oxford) 2002; **4**: 99-100 [DOI: 10.1080/136518202760378489]
17 **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]
18 **Adams RB**, Aloia TA, Loyer E, Pawlik TM, Taouli B, Vauthey JN. Selection for hepatic resection of colorectal liver metastases: expert consensus statement. *HPB* (Oxford) 2013; **15**: 91-103 [PMID: 23297719 DOI: 10.1111/j.1477-2574.2012.00557.x]
19 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
20 **Reuter NP**, Woodall CE, Scoggins CR, McMasters KM, Martin RC. Radiofrequency ablation vs. resection for hepatic colorectal metastasis: therapeutically equivalent? *J Gastrointest Surg* 2009; **13**: 486-491 [PMID: 18972167 DOI: 10.1007/s11605-008-0727-0]
21 **Poultsides GA**, Paty PB. Reassessing the need for primary tumor surgery in unresectable metastatic colorectal cancer: overview and perspective. *Ther Adv Med Oncol* 2011; **3**: 35-42 [PMID: 21789154 DOI: 10.1177/1758834010386283]
22 **Brouquet A**, Mortenson MM, Vauthey JN, Rodriguez-Bigas MA, Overman MJ, Chang GJ, Kopetz S, Garrett C, Curley SA, Abdalla EK. Surgical strategies for synchronous colorectal liver metastases in 156 consecutive patients: classic, combined or reverse strategy? *J Am Coll Surg* 2010; **210**: 934-941 [PMID: 20510802 DOI: 10.1016/j.jamcollsurg.2010.02.039]
23 **Jamal MH**, Hassanain M, Chaudhury P, Tran TT, Wong S, Yousef Y, Jozaghi Y, Salman A, Jabbour S, Simoneau E, Al-Abbad S, Al-Jiffry M, Arena G, Kavan P, Metrakos P. Staged hepatectomy for bilobar colorectal hepatic metastases. *HPB* (Oxford) 2012; **14**: 782-789 [PMID: 23043668 DOI: 10.1111/j.1477-2574.2012.00543.x]
24 **Mayo SC**, Pawlik TM. Colorectal hepatic metastasis - current therapeutic approach. *EGHR* 2011; **7**: 54-60
25 **Adam R**, Avisar E, Ariche A, Giachetti S, Azoulay D, Castaing D, Kunstlinger F, Levi F, Bismuth F. Five-year survival following hepatic resection after neoadjuvant therapy for nonresectable colorectal. *Ann Surg Oncol* 2001; **8**: 347-353 [PMID: 11352309]
26 **Nordlinger B**, Adam R, Arnold D, Zalcberg JR, Gruenberger T. The role of biological agents in the resection of colorectal liver metastases. *Clin Oncol* (R Coll Radiol) 2012; **24**: 432-442 [PMID: 22794325 DOI: 10.1016/j.clon.2012.01.002]
27 **Ferrand F**, Malka D, Bourredjem A, Allonier C, Bouché O, Louafi S, Boige V, Mousseau M, Raoul JL, Bedenne L, Leduc B, Deguiral P, Faron M, Pignon JP, Ducreux M. Impact of primary tumour resection on survival of patients with colorectal cancer and synchronous metastases treated by chemotherapy: results from the multicenter, randomised trial Fédération Francophone de Cancérologie Digestive 9601. *Eur J Cancer* 2013; **49**: 90-97 [PMID: 22926014 DOI: 10.1016/j.ejca.2012.07.006]
28 **Scheer MG**, Sloots CE, van der Wilt GJ, Ruers TJ. Management of patients with asymptomatic colorectal cancer and synchronous irresectable metastases. *Ann Oncol* 2008; **19**: 1829-1835 [PMID: 18662955 DOI: 10.1093/annonc/mdn398]
29 **Poultsides GA**, Servais EL, Saltz LB, Patil S, Kemeny NE, Guillem JG, Weiser M, Temple LK, Wong WD, Paty PB. Outcome of primary tumor in patients with synchronous stage IV colorectal cancer receiving combination chemotherapy without surgery as initial treatment. *J Clin Oncol* 2009; **27**: 3379-3384 [PMID: 19487380 DOI: 10.1200/JCO.2008.20.9817]
30 **Cirocchi R**, Trastulli S, Abraha I, Vettoretto N, Boselli C, Montedori A, Parisi A, Noya G, Platell C. Non-resection versus resection for an asymptomatic primary tumour in patients with unresectable stage IV colorectal cancer. *Cochrane Database Syst Rev* 2012; **8**: CD008997 [PMID: 22895981 DOI: 10.1002/14651858.CD008997.pub2]
31 **Topal B**, Kaufman L, Aerts R, Penninckx F. Patterns of failure following curative resection of colorectal liver metasta-

ses. *Eur J Surg Oncol* 2003; **29**: 248-253 [PMID: 12657235]

32 **Tan MC**, Butte JM, Gonen M, Kemeny N, Fong Y, Allen PJ, Kingham TP, Dematteo RP, Jarnagin WR, D'Angelica MI. Prognostic significance of early recurrence: a conditional survival analysis in patients with resected colorectal liver metastasis. *HPB* (Oxford) 2013; **15**: 803-813 [PMID: 23782400 DOI: 10.1111/hpb.12136]

33 **Cardona K**, Mastrodomenico P, D'Amico F, Shia J, Gönen M, Weiser MR, Paty PB, Kingham TP, Allen PJ, De Matteo RP, Fong Y, Jarnagin WR, D'Angelica MI. Detailed pathologic characteristics of the primary colorectal tumor independently predict outcome after hepatectomy for metastases. *Ann Surg Oncol* 2013; **20**: 148-154 [PMID: 22847127 DOI: 10.1245/s10434-012-2540-y]

P- Reviewer: Galvao FHF, Ramos S **S- Editor**: Song XX
L- Editor: Webster JR **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.520

World J Hepatol 2014 July 27; 6(7): 520-526
ISSN 1948-5182 (online)

CLINICAL TRIALS STUDY

Pegylated interferon alfa-2b plus ribavirin for treatment of chronic hepatitis C

PN Rao, Abraham Koshy, Jacob Philip, Narayanan Premaletha, Joy Varghese, Krishnasamy Narayanasamy, Samir Mohindra, Nitin Vikas Pai, Manoj Kumar Agarwal, Ashoknanda Konar, Hasmukh B Vora

PN Rao, Department of Gastroenterology, Asian Institute of Gastroenterology, Hyderabad 500082, India
Abraham Koshy, Department of Hepatology, Lakeshore Hospital, Kochi 682040, India
Jacob Philip, Department of Gastroenterology, Cosmopolitan Hospital, Thiruvananthapuram 695004, India
Narayanan Premaletha, Department of Gastroenterology, Medical College, Thiruvananthapuram 695011, India
Joy Varghese, Department of Hepatology, Global Hospitals and Health City, Chennai 600100, India
Krishnasamy Narayanasamy, Department of Hepatology, GGH and MMC, Chennai 600003, India
Samir Mohindra, Department of Gastroenterology, SGPGIMS, Lucknow 226014, India
Nitin Vikas Pai, Department of Gastroenterology, Pai Clinic and Diagnostic Center, Pune 411005, India
Manoj Kumar Agarwal, Department of Gastroenterology, Belle Vue Clinic, Kolkata 700017, India
Ashoknanda Konar, Department of Gastroenterology, Peerless Hospital and B.K. Roy Research Center, Kolkata 700094, India
Hasmukh B Vora, Department of Gastroenterology, Alka Hospital, Ahmedabad 380019, India
Author contributions: All eleven authors of the manuscript carried out the field research for the study in addition to contributing to the conception and design of the study; Rao PN was also instrumental in writing the paper.
Supported by Virchow Biotech Private Limited, Hyderabad, India
Correspondence to: PN Rao, MD, DM, Department of Gastroenterology, Asian Institute of Gastroenterology, 6-3-661, Somajiguda, Hyderabad 500082, India. pnrcraig@gmail.com
Telephone: +91-40-23378888 Fax: +91-40-23324255
Received: December 19, 2013 Revised: April 11, 2014
Accepted: May 28, 2014
Published online: July 27, 2014

Abstract

AIM: To study the safety and efficacy of pegylated interferon alfa-2b, indigenously developed in India, plus ribavirin in treatment of hepatitis C virus (HCV).

METHODS: One-hundred HCV patients were enrolled in an open-label, multicenter trial. Patients were treated with pegylated interferon alfa-2b 1.5 μg/kg per week subcutaneously plus oral ribavirin 800 mg/d for patients with genotypes 2 and 3 for 24 wk. The same dose of peginterferon plus weight-based ribavirin (800 mg/d for ≤ 65 kg; 1000 mg/d for > 65-85 kg; 1200 mg/d for > 85-105 kg; 1400 mg/d for > 105 kg body weight) was administered for 48 wk for patients with genotypes 1 and 4. Serological and biochemical responses of patients were assessed.

RESULTS: Eighty-two patients (35 in genotypes 1 and 4 and 47 in 2 and 3), completed the study. In genotype 1, 25.9% of patients achieved rapid virologic response (RVR): while the figures were 74.1% for early virologic response (EVR) and 44.4% for sustained virologic response (SVR). For genotypes 2 and 3, all patients bar one belonged to genotype 3, and of those, 71.4%, 87.5%, and 64.3% achieved RVR, EVR, and SVR, respectively. In genotype 4, 58.8%, 88.2%, and 52.9% of patients achieved RVR, EVR, and SVR, respectively. The majority of patients attained normal levels of alanine aminotransferase by 4-12 wk of therapy. Most patients showed a good tolerance for the treatment, although mild-to-moderate adverse events were exhibited; only two patients discontinued the study medication due to serious adverse events (SAEs). Eleven SAEs were observed in nine patients; however, only four SAEs were related to study medication.

CONCLUSION: Peginterferon alfa-2b, which was developed in India, in combination with ribavirin, is a safe and effective drug in the treatment of HCV.

Key words: Hepatitis C virus; Genotype; Peginterferon alfa-2b; Ribavirin; Treatment

Core tip: In a multicenter study, the safety and efficacy of pegylated interferon alfa-2b, indigenously developed

in India, plus ribavirin was evaluated on 100 hepatitis C virus (HCV) patients with genotypes 1, 2, 3, and 4. Eighty-two patients completed the study. Most patients had mild-to-moderate adverse events, although 11 serious adverse events were reported in 9 patients. However, only 4 of these were related to study medication. The percentage of serologic response (rapid virologic response, early virologic response, and sustained virologic response rates) of patients was similar to that reported in published studies. In conclusion, peginterferon alfa-2b, developed in India, is a safe and cost-effective drug in the treatment of Indian patients with HCV infection.

Rao PN, Koshy A, Philip J, Premaletha N, Varghese J, Narayanasamy K, Mohindra S, Pai NV, Agarwal MK, Konar A, Vora HB. Pegylated interferon alfa-2b plus ribavirin for treatment of chronic hepatitis C. *World J Hepatol* 2014; 6(7): 520-526 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/520.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.520

INTRODUCTION

According to the World Health Organization's estimates, over 170 million people (3% of the world's population) are infected with chronic hepatitis C virus (HCV) worldwide[1]. Each year, about five million people are newly infected, and more than 350000 people, despite availability of treatment, die from HCV-related complications[2]. Hepatitis is an emerging infection in India, with a paucity of large scale prevalence studies on hepatitis C in the general population. The reported prevalence rates also vary widely (range 0.09% to 7.89%)[3]. However, regardless of prevalence rates, the burden of HCV infection in India is expected to be high with a population over 1.2 billion; as a result, its treatment modalities, as well as success rates, demand attention.

The HCV genotype plays a significant role in therapeutic guidelines, since HCV genotypes 1 and 4 are more resistant to treatment compared to HCV genotypes 2 and 3. Yet, irrespective of genotype, pegylated interferon, in combination with ribavirin, is considered the gold standard in the treatment of chronic HCV infection[4-7]. Currently, both pegylated interferon alfa-2a and pegylated alfa-2b are available in India. These drugs are exorbitantly priced and are not easily accessible to the majority of Indian patients. In view of this, Virchow Biotech developed pegylated interferon alfa-2b from *Escherichia coli* by using recombinant DNA technology, and priced it competitively. The aim of the present study is to evaluate the safety and efficacy of pegylated interferon alfa-2b in chronic hepatitis C patients.

MATERIALS AND METHODS

Patient selection

Male and female patients aged 18-65 years-old (both years inclusive) that attended the outpatient department of 12 hospitals were screened. 100 consecutive patients were enrolled if they had chronic hepatitis C infection as per the following criteria: presence of HCV RNA and persistent elevation of serum alanine aminotransferase (ALT) levels 1.5 times greater than normal (N < 40 IU/L); compensated liver disease at the time of baseline visit as defined by Child-Pugh class A; hemoglobin $\geqslant$ 9 g/dL (females), $\geqslant$ 10 g/dL (males); platelet count $\geqslant 75 \times 10^9$/L; neutrophil count $\geqslant 1.5 \times 10^9$/L; and thyroid stimulating hormone within normal limits (0.35-5.50 mIU/mL). Only treatment näive patients were included in the study. Patients were excluded if they had evidence of other liver diseases such as hepatitis A virus, hepatitis B virus, alfa-2 antitrypsin deficiency, Wilson's disease, primary biliary cirrhosis, autoimmune liver disease, or hemochromatosis. Other criteria for exclusion were: chronic alcoholism; history of drug abuse; immune suppression associated with organ transplantation; history of hypersensitivity to interferon or its diluents; significant psychiatric disease, especially depression; severe cardiovascular disease; patients with co-infection of human immunodeficiency virus infection; and pregnant and lactating women. Study procedures were explained to each participant and written informed consent was obtained before enrolment into the study.

Study design

This is an open-label, multicenter study that was conducted, with the approval of the Drugs Controller General of India, at 12 centers across eight Indian cities between March 2010 and March 2013. The study, conducted in accordance with principles under the 1964 Declaration of Helsinki and later revisions, was initiated after obtaining approval of the study protocol from the institutional ethical committee at respective centers. This trial was registered in Clinical Trial Registry India (CTRI/2011/000028).

Treatment regimen

Treatment consisted of the administration of peginterferon alfa-2b (manufactured by Virchow Biotech Private Ltd, Hyderabad, India) 1.5 μg/kg per week subcutaneously, in combination with ribavirin 800 mg/d orally, for patients with genotypes 2 and 3 for 24 wk. The same dose of peginterferon was administered in combination with weight-based ribavirin (800 mg/d for $\leqslant$ 65 kg; 1000 mg/d for > 65-85 kg; 1200 mg/d for > 85-105 kg; 1400 mg/d for > 105 kg body weight) for 48 wk for patients with genotypes 1 and 4.

Dose modification/discontinuation

Ribavirin dose was reduced to half if hemoglobin level was < 10 g/dL; treatment was discontinued if hemoglobin level was < 8.5 g/dL. Peginterferon dose was reduced to half in patients with white blood cells (WBC) < 1.5×10^9/L, neutrophils < 0.75×10^9/L, or platelet count < 50×10^9/L. Peginterferon treatment was discontinued in

Table 1 Baseline characteristics of patients with genotypes 1, 3 and 4

Parameter	Genotype 1 (n = 27)	Genotype 3 (n = 56)[1]	Genotype 4 (n = 17)
Age (yr)	41.9 ± 13.2	41.7 ± 10.9	46.3 ± 9.3
Weight (kg)	60.5 ± 12.0	63.3 ± 11.5	63.7 ± 10.8
Male number (%)[2]	19 (70.3%)	11 (19.7%)	11 (64.7%)
Hemoglobin (g/dL)	14.1 ± 1.6	13.8 ± 1.9	14.2 ± 1.2
White blood cell count (10^9/L)	6682 ± 1682	7086 ± 1886	7201 ± 1886
Neutrophils (%)	58.4 ± 8.4	56.0 ± 11.8	53.3 ± 8.0
Platelet count (10^3/L)	200 ± 80	199 ± 78	170 ± 50
Alanine Aminotransferase (U/L)	88.1 ± 41	127.7 ± 87.4	104.9 ± 61.1
HCV RNA $\log_{10}$ IU/mL	5.5 ± 1.2	5.4 ± 1.1	5.5 ± 0.9

[1]Includes one patient with genotype 2; [2]Value in percentage. HCV: Hepatitis C virus.

patients with WBC < 1.0 × 10^9/L, neutrophils < 0.5 × 10^9/L, or platelet count < 25 × 10^9/L.

Assessment of efficacy

The primary efficacy endpoint was the percentage of patients with sustained virologic response (SVR), defined as undetectable serum HCV RNA 24 wk after cessation of therapy. Secondary efficacy endpoints were: rapid virologic response (RVR), defined as undetectable serum HCV RNA at week 4; early virologic response (EVR), defined as undetectable serum HCV RNA or 2-$\log_{10}$ reduction in HCV RNA from the baseline at week 12; end of treatment virologic response (ETVR), defined as undetectable serum HCV RNA at weeks 24 and 48[1]; with normalization of ALT at weeks 12, 24, 48[1], and 24 after cessation of therapy ([1]only for patients with genotypes 1 and 4). Data on non-responders, relapse, and breakthrough were also collected[4]. Non-responders were defined as those who failed to clear HCV RNA from serum after 24 wk of therapy. Relapse was defined as undetectable HCV RNA at the end of treatment, followed by the reappearance of HCV RNA during follow-up. Breakthrough was defined as undetectable HCV RNA during treatment, followed by the appearance of HCV RNA, despite continued treatment.

Blood samples were obtained for serologic tests for quantitative HCV RNA by polymerase chain reaction (PCR) at baseline and at weeks 4, 12, 24, and 48 for genotypes 2 and 3; while for genotypes 1 and 4 this was at baseline and at weeks 4, 12, 24, 48, and 72. Cobas Taqman HCV test (Roche), using the real-time PCR method with a lower detection limit of < 25 IU/mL, was employed for quantification of HCV RNA in serum. A linear array detection kit from Roche was used in HCV genotyping.

Assessment of safety

Vitals (respiratory rate, pulse rate, body temperature, and blood pressure), hematology (complete blood picture, hemoglobin, and platelet count), and ALT levels were measured at each visit. Biochemical parameters (serum lactate dehydrogenase, creatinine, potassium, and phosphorus) were also measured at specified screening visits; weeks 4, 12, 24, and 48 for genotypes 2 and 3, and weeks 4, 12, 24, 48, and 72 for genotypes 1 and 4. Patients were monitored for adverse events (AE) and medication compliance throughout the duration of study. Adverse events were graded as mild, moderate, or severe. Treatment was suspended or modified according to the severity of adverse events. The dosage of peginterferon alfa-2b, ribavirin, or combination of the two was again increased to the original level after the resolution of adverse events. Serious adverse events (SAEs) were documented and communicated to the institutional ethics committee and Drugs Controller General of India.

Sample size

Various trials conducted on patients with genotypes 1 and 4 or 2 and 3 have reported around 40%-80% SVR, which reflects the efficacy of peginterferon alfa-2b in the treatment of HCV[6,7]. In our earlier pilot study conducted on 25 patients with HCV infection, a SVR of 60% was observed. Considering the 60% efficacy, 95%CI, 80% power, and 15% error with a 15% dropout rate with two tailed t-test, the calculated sample size was 100 patients.

Statistical analysis

Values were expressed as mean (SD). Since an open-label study design was adopted, efficacy assessment basically relied upon descriptive statistics rather than inferential analysis. Intention-to-treat (ITT) analysis was carried out on the population that included all patients who met the eligibility criteria and had received at least one dose of the investigational drug during the study period. Per protocol analysis was also carried out, which included patients who completed the stipulated study period.

Safety parameters, such as vital signs and laboratory findings including hematology and biochemical parameters, were analyzed by repeated measure analysis of variance. Two-sided P-values were reported, with those less than 0.05 being considered statistically significant. All analyses were performed using IBM SPSS version 19.0 for Windows.

RESULTS

Patients' characteristics

A total of 100 consecutive patients with chronic hepatitis C who met the inclusion/exclusion criteria were enrolled into the study. Among them, 27 pertained to genotype 1, 17 for genotype 4, only one for genotype 2, and 55 for genotype 3. Since there was only one patient with genotype 2, the results presented on genotypes 2 and 3 basically represent only those of genotype 3. The demographic and baseline characteristics of the 100 enrolled patients are presented in Table 1. At baseline, values of hematological and biochemical investigations were within normal limits except for liver function tests such as serum ALT, aspartate aminotransferase, and alkaline

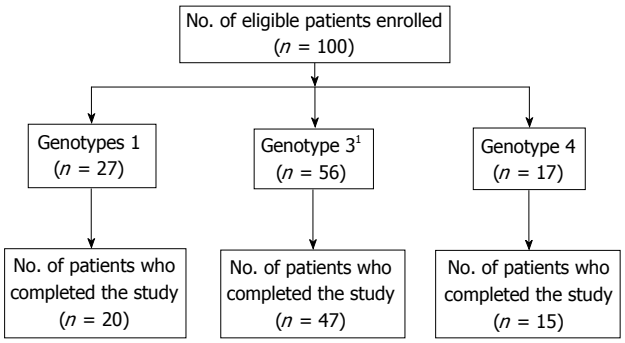

Figure 1 Disposition of patients. [1]Includes one patient with genotype 2.

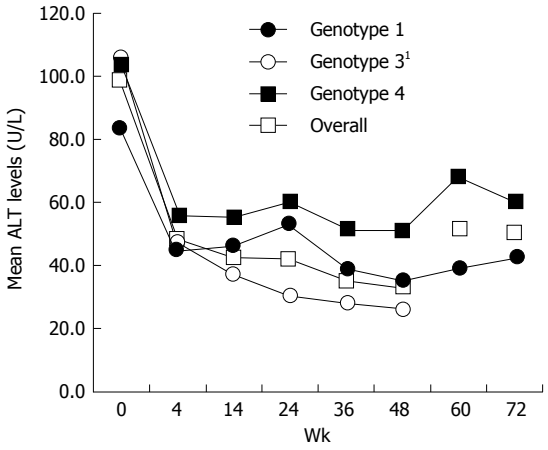

Figure 2 Mean alanine aminotransferase levels during the study period in patients with different genotypes. [1]Includes one patient with genotype 2. ALT: Alanine aminotransferase.

phosphatase. Barring serum ALT levels, other demographic, hematological, and biochemical parameters, including HCV RNA levels, were not significantly different between genotypes 1, 3, and 4. The mean ALT levels in genotype 3 patients were significantly higher ($P < 0.02$) than those in genotype 1; but these were similar to those of genotype 4.

Figure 1 shows the flow of patients through the study. Among the 100 patients, 82 completed the study. Eighteen patients did not complete the study for the following reasons: lost to follow-up (8), withdrew (6), discontinued due to SAE (2), and discontinued therapy due to non-response by the investigator (2). Treatment compliance was monitored by maintaining a patient dairy. During the study period, the mean daily intake of ribavirin was 14.3 ± 1.84 mg/kg body weight in genotypes 1 and 4 and 12.84 ± 2.29 mg/kg body weight in genotype 3.

Treatment response

Overall, 57%, 84%, 72%, and 57% of enrolled patients achieved RVR, EVR, ETVR, and SVR, respectively. Results on virologic response of genotypes 1, 3, and 4, evaluated by ITT and per protocol analysis, are presented in Tables 2 and 3, respectively.

Data on the percentage of patients with normalization of ALT at weeks 4, 12, and 24 of treatment, at the end of treatment (week 48 in genotypes 1 and 4, and week 24 in genotypes 2 and 3), and at 24 wk after cessation of therapy are presented in Table 4. In general, the majority of patients, irrespective of their genotype, attained normal levels of ALT by 4 to 12 wk of therapy and the effect was sustained even during follow-up. Mean ALT levels during different study periods are presented in Figure 2.

Table 2 Percentage of patients who responded in terms of rapid virologic response, early virologic response, end of the treatment virologic response, and sustained virologic response in genotypes 1, 3 and 4 by intention-to-treat analysis

Parameter	Genotype 1 (*n* = 27)	Genotype 3[1] (*n* = 56)	Genotype 4 (*n* = 17)
RVR	25.9%	71.4%	58.8%
EVR	74.1%	87.5%	88.2%
ETVR	59.2%	78.6%	70.5%
SVR	44.4%	64.3%	52.9%

[1]Includes one patient with genotype 2. RVR: Rapid virologic response; EVR: Early virologic response; SVR: Sustained virologic response; ETVR: End of treatment virologic response.

Table 3 Percentage of patients who responded in terms of rapid virologic response, early virologic response, end of treatment virologic response, and sustained virologic response in genotypes 1, 3 and 4 by per protocol analysis

Parameter	Genotype 1 (*n*/N)	Genotype 3[1] (*n*/N)	Genotype 4 (*n*/N)
RVR	25.9% (7/27)	74.1% (40/54)	58.8% (10/17)
EVR	74.1% (20/27)	100% (49/49)	88.2% (15/17)
ETVR	84.2% (16/19)	89.8% (44/49)	75.0% (12/16)
SVR	60.0% (12/20)	76.6% (36/47)	60.0% (9/15)

[1]Includes one patient with genotype 2; *n*: Number of responding patients; N: Total number of patients studied; RVR: Rapid virologic response; EVR: Early virologic response; SVR: Sustained virologic response; ETVR: End of treatment virologic response.

Table 4 Percentage of patients with normalization of alanine aminotransferase levels during different study periods *n* (%)

Weeks	Genotype 1 (*n* = 27)	Genotype 3[1] (*n* = 56)	Genotype 4 (*n* = 17)
4	16 (59.2)	27 (48.2)	8 (47.0)
12	17 (62.9)	29 (51.7)	8 (47.0)
24	17 (62.9)	35 (62.5)	9 (52.9)
48	17 (62.9)	40 (71.4)	11 (64.7)
72	17 (62.9)	-	12 (70.6)

[1]Includes one patient with genotype 2.

Side-effects

The majority of patients tolerated the scheduled treatment with peginterferon and ribavirin, though with the usual known adverse events with these drugs. Adverse events were analyzed for safety of peginterferon alfa-2b and presented in Table 5. Ninety-one patients reported 328 adverse events; 95 events by genotype 1 patients, 68 events by genotype 4 patients, and 165 events in geno-

Table 5 Patients with adverse events

Adverse event	*n* (%) of patients		
	Genotype 1 (*n* = 27)	Genotype 3[1] (*n* = 56)	Genotype 4 (*n* = 17)
Injection-site reactions	9 (33.3)	16 (28.6)	7 (41.2)
Flu-like symptoms	24 (88.8)	49 (87.5)	14 (82.3)
Tiredness	4 (14.8)	5 (8.9)	2 (11.7)
Weight loss	1 (3.7)	3 (5.4)	1 (5.8)
Chest discomfort	1 (3.7)	2 (3.6)	2 (11.7)
Arthralgia	3 (11.1)	0 (0)	1 (5.8)
Alopecia	2 (7.4)	10 (17.9)	3 (17.6)
Anorexia	2 (7.4)	7 (12.5)	3 (17.6)
Nausea	3 (11.1)	8 (14.3)	3 (17.6)
Vomiting	2 (7.4)	3 (5.4)	0
Dyspepsia	1 (3.7)	3 (5.4)	2 (11.7)
Gastritis	1 (3.7)	0 (0)	0
Mucous stool	1 (3.7)	0 (0)	0
Diarrhea	1 (3.7)	4 (7.1)	1 (5.8)
Melena	1 (3.7)	6 (10.7)	0
Ascites	1 (3.7)	0 (0)	0
Thrombocytopenia	2 (7.4)	5 (8.9)	1 (5.8)
Anemia	9 (33.3)	13 (23.2)	6 (35.3)
Neutropenia	12 (44.4)	15 (26.8)	8 (47.0)
Anxiety	1 (3.7)	3 (5.4)	1 (5.8)
Depression	2 (7.4)	4 (7.1)	3 (17.6)
Insomnia	1 (3.7)	1 (1.8)	2 (11.7)
Hypothyroidism	2 (7.4)	2 (3.6)	2 (11.7)
Giddiness	1 (3.7)	1 (1.8)	2 (11.7)
Dry throat	1 (3.7)	1 (1.8)	0 (0)
Cough	2 (7.4)	2 (3.6)	2 (11.7)
Sinusitis	1 (3.7)	0 (0)	0 (0)
Bleeding gums	2 (7.4)	1 (1.8)	0 (0)
Palpitation	1 (3.7)	0 (0)	0 (0)
Pruritus	0 (0)	0 (0)	1 (5.8)
Yellow-colored sputum	0 (0)	0 (0)	1 (5.8)
Urinary tract infection	1 (3.7)	0 (0)	0 (0)
Death	0 (0)	1 (1.8)	0 (0)
No. of patients reporting AEs	24 (88.8)	49 (87.5)	14 (82.3)
Discontinued due to SAEs	0 (0)	2 (3.6)	0 (0)
Temporary discontinuation of therapy	4 (14.8)	3 (5.4)	3 (17.6)
Temporary dose reduction	11 (40.7)	16 (29.6)	5 (29.4)

[1]Includes one patient with genotype 2. SAE: Serious adverse event.

type 3 patients. Administration of peginterferon alfa-2b resulted in common mild-to-moderate AEs, which included flu-like symptoms, nausea, and loss of appetite. None of the patients permanently stopped treatment due to adverse events, with the exception of two patients who discontinued due to SAEs. Ribavirin was temporarily discontinued due to anemia in ten patients. Twenty-four patients required ribavirin dose reduction, four needed peginterferon alfa-2b dose reduction, and four required both ribavirin and peginterferon alfa-2b dose reduction for management of anemia and thrombocytopenia. Nine patients reported 11 SAEs, which were all relieved with relevant therapy aside from one patient who died. Among the 11 SAEs, four were related to the study medication and the remaining seven, including the case of death, were unrelated to it.

DISCUSSION

Infection with HCV is one of the most important medical and public health problems worldwide in view of its life-threatening complications, including hepatocellular carcinoma, cirrhosis, and liver failure[8-10]. The goal of therapy in chronic HCV infection is to achieve SVR and thereby prevent long-term complications. Despite the promising role of new antiviral therapies[11], the use of pegylated-interferon alfa combined with ribavirin continues, to date, to be the standard care of treatment in HCV infection.

Since genotype constitutes one of the important determinants of the course and outcome of therapy, 24 or 48 wk combination therapy with peginterferon alfa and ribavirin has been recommended for genotypes 2 and 3 and genotypes 1 and 4 patients, respectively[4-7]. The present open-label, multicenter study using standard-of-care therapy was undertaken to establish that the safety and efficacy of peginterferon alfa-2b is comparable to the results of historical controls in the treatment of chronic HCV infection.

One-hundred eligible patients with chronic HCV infection were enrolled, with the majority (55%) having HCV genotype 3 which is in accordance with the published prevalence studies conducted in India[12,13]. There was only one patient with genotype 2, which is rare among Indians, and thus it should be noted that the reported combined results of patients with genotypes 2 and 3, in fact, only reflects those of genotype 3. Anticipating 15% attrition, 100 patients were enrolled. However, there was instead an 18% dropout, and as a result, 82 patients completed the specified study period of therapy.

Since the dose and duration of therapy were different, the data on outcome measurements were analyzed separately for genotypes 1 and 4 and for genotypes 2 and 3. The SVR (44.4%) observed in the present study for genotype 1 is comparable with those of reported studies[14-16]. In genotypes 2 and 3, 64.3% of patients achieved SVR, which fits with the conformity figure results reported by Manns *et al*[17]. The rates of SVR in treatment naïve genotype 2 patients were reported to be 86.5%[18], which is higher than that of genotype 3. Since our genotype 2 and 3 patients, except for one, belonged to genotype 3, a lower SVR (64.3%) was observed in the present study. In genotype 4, 52.9% patients achieved SVR, which is comparable with values from published studies[19,20]. Apart from genotype, baseline viral load has been shown to be one of the determinants of SVR[21]. However, perhaps due to the small number of patients covered in the present study, our stratified statistical analysis showed that baseline viral load had no impact on SVR.

In view of the cost factor and incidence of adverse events with peginterferon use during long-duration treatment, individualized treatment, based on the results of RVR and EVR, has been emphasized. In this respect, the presence of RVR is highly predictive of ultimate SVR with a full treatment course of 48 wk in genotype 1 patients[22]. In the current study, all genotype 1 patients (*n* = 7) who achieved RVR also attained SVR; while a study reported a SVR rate of 86.8% in patients with RVR[15]. In genotype 4 patients, 80% with RVR attained SVR,

whereas the published study reported 86%[23]. Similarly, among the genotype 3 patients who had RVR, 83.3% attained SVR, which is similar (83.7%) to that reported in the literature[24]. This further confirms the utility of RVR in predicting SVR.

Among the patients who attained EVR, 10 (76.9%) in genotype 1 and 9 (75%) in genotype 4 achieved SVR. In patients with genotypes 2 and 3, the percentage with EVR attaining SVR was 100%. This is in line with the literature[25], which shows that patients with genotype 3 who fail to achieve EVR also fail to achieve SVR. Since the duration of treatment for genotypes 2 and 3 is only 24 wk, it has been reported that EVR testing is not cost-effective in these patients[25]. This indicates that utility of RVR is higher than EVR in the prediction of SVR.

Overall, 16 patients had relapse; 5 (31.2%) patients in genotype 1, 8 (18.2%) patients in genotypes 2 and 3, and 3 (25%) patients in genotype 4. Among the 100 patients, 5 were non-responders to the study treatment; 1 (3.7%) patient in genotype 1; 2 (3.6%) patients in genotypes 2 and 3, and 2 (11.7%) in genotype 4. In addition 4 patients had breakthrough during the treatment; 2 (7.4%) patients in genotype 1; 1 (1.8%) patient in genotype 3, and 1 (5.8%) patient in genotype 4.

Biochemical response of peginterferon alfa-2b was assessed by the percentage of patients attaining normalization of ALT levels. Overall, the majority of patients (51%) had normalization of ALT levels as early as week 4. This denotes that peginterferon is very effective in producing a biochemical response in patients with chronic hepatitis C.

The treatment was well-tolerated in the majority of patients, though with the common side-effects usually attributed with interferon or ribavirin. In 32% of patients, temporary dose modifications in peginterferon (4%), ribavirin (24%), or both (4%), and temporary discontinuation of therapy in 10% of patients, were required. Though 11 SAEs were observed in 9 patients, only 4 were related to study medication, with such SAEs also being reported in earlier studies[15,17,26,27].

The limitations of the study are that it is a single arm study and the results on the outcome measures were compared with those of historical controls. Earlier studies on Indian patients with HCV infection were conducted using peginterferon alfa-2b in two studies-one study was carried out on 103 patients, but only on genotype 3 patients[25]; the other study, despite covering all four genotypes, had only 16 patients[28]. We are not aware of any study conducted with an adequately powered sample of Indian patients with HCV infection following the global guidelines on peginterferon plus ribavirin[4-7,29].

Therefore, despite the limitation of a lack of comparator, our results on serological responses such as RVR, EVR, ETVR, and SVR provide valuable information on the safety and efficacy of peginterferon alfa-2b, in combination with ribavirin, in the treatment of Indian patients with chronic HCV infection. Currently, Virchow Biotech-developed peginterferon alfa-2b is marketed in India and other emerging countries at a very competitive rate. In view of the relatively low incidence of the adverse events and improved virologic and biochemical response, the results of the study show that peginterferon alfa-2b, in combination with ribavirin, is a safe and cost-effective drug in the treatment of chronic hepatitis C.

COMMENTS

Background

Hepatitis C virus (HCV) infection is a major cause of chronic liver disease in India, with a high morbidity and mortality due to its complications. Pegylated interferon, in combination with ribavirin, is the standard recommended treatment for chronic hepatitis C. One of the reasons for this could be due to its cost factor, with another being that studies evaluating the safety and efficacy of these drugs in India are limited. Therefore, an attempt is being made to evaluate the efficacy of peginterferon alfa-2b, a drug locally developed in India, in combination with ribavirin.

Research frontiers

This prospective study presents results on the efficacy, in terms of virologic response, of indigenously-developed peginterferon alfa-2b plus ribavirin in Indian patients with different genotypes of chronic hepatitis C. Adverse events observed with this combination are also reported.

Innovations and breakthroughs

There have been a few prior studies on Indian patients with HCV infection using peginterferon alfa-2b. However, these were limited to a small number of patients or confined to one genotype.

Applications

This study demonstrates that virologic response of peginterferon alfa-2b and ribavirin, when given as per global guidelines in Indian patients with different types of chronic hepatitis C, is similar to that of historical controls.

Terminology

Success rate of treatment is assessed based on sustained virologic response, which is defined as undetectable HCV RNA in blood 24 wk after cessation of therapy.

Peer review

This is a straightforward clinical control study.

REFERENCES

1 Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat* 1999; **6**: 35-47 [PMID: 10847128 DOI: 10.1046/j.1365-2893.1999.6120139.x]

2 **WHO**. Hepatitis C. WHO Fact sheet No 164. Available from: URL: http: //www.who.int/mediacentre/factsheets/fs164/en/

3 **Mukhopadhyaya A**. Hepatitis C in India. *J Biosci* 2008; **33**: 465-473 [PMID: 19208972 DOI: 10.4103/1947-2714.103325]

4 **Ghany MG**, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49**: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

5 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

6 **Omata M**, Kanda T, Yu ML, Yokosuka O, Lim SG, Jafri W, Tateishi R, Hamid SS, Chuang WL, Chutaputti A, Wei L, Sollano J, Sarin SK, Kao JH, McCaughan GW. APASL consensus statements and management algorithms for hepatitis C virus infection. *Hepatol Int* 2012; **6**: 409-435 [DOI: 10.1007/s12072-012-9342-y]

7 **Ghany MG**, Nelson DR, Strader DB, Thomas DL, Seeff LB. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011; **54**: 1433-1444 [PMID: 21898493 DOI: 10.1002/hep.24641]

8 **Kenny-Walsh E**. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med* 1999; **340**: 1228-1233 [PMID: 10210705 DOI: 10.1056/NEJM199904223401602]

9 **Di Bisceglie AM**. Natural history of hepatitis C: its impact on clinical management. *Hepatology* 2000; **31**: 1014-1018 [PMID: 10733560 DOI: 10/S0270913900298170]

10 **Barrera JM**, Bruguera M, Ercilla MG, Gil C, Celis R, Gil MP, del Valle Onorato M, Rodés J, Ordinas A. Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. *Hepatology* 1995; **21**: 639-644 [PMID: 7533121]

11 **Kanda T**, Imazeki F, Yokosuka O. New antiviral therapies for chronic hepatitis C. *Hepatol Int* 2010; **4**: 548-561 [PMID: 21063477 DOI: 10.1007/s12072-010-9193-3]

12 **Hissar SS**, Goyal A, Kumar M, Pandey C, Suneetha PV, Sood A, Midha V, Sakhuja P, Malhotra V, Sarin SK. Hepatitis C virus genotype 3 predominates in North and Central India and is associated with significant histopathologic liver disease. *J Med Virol* 2006; **78**: 452-458 [PMID: 16482560 DOI: 10.1002/jmv.20561]

13 **Narahari S**, Juwle A, Basak S, Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort. *Infect Genet Evol* 2009; **9**: 643-645 [PMID: 19460332 DOI: 10.1016/j.meegid.2009.04.001]

14 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965 [PMID: 11583749 DOI: 10.1016/S0140-6736(01)06102-5]

15 **Mangia A**, Minerva N, Bacca D, Cozzolongo R, Ricci GL, Carretta V, Vinelli F, Scotto G, Montalto G, Romano M, Cristofaro G, Mottola L, Spirito F, Andriulli A. Individualized treatment duration for hepatitis C genotype 1 patients: A randomized controlled trial. *Hepatology* 2008; **47**: 43-50 [PMID: 18069698 DOI: 10.1002/hep.22061]

16 **Kainuma M**, Furusyo N, Kajiwara E, Takahashi K, Nomura H, Tanabe Y, Satoh T, Maruyama T, Nakamuta M, Kotoh K, Azuma K, Shimono J, Shimoda S, Hayashi J. Pegylated interferon α-2b plus ribavirin for older patients with chronic hepatitis C. *World J Gastroenterol* 2010; **16**: 4400-4409 [PMID: 20845506 DOI: 10.3748/wjg.v16.i35.4400]

17 **Manns M**, Zeuzem S, Sood A, Lurie Y, Cornberg M, Klinker H, Buggisch P, Rössle M, Hinrichsen H, Merican I, Ilan Y, Mauss S, Abu-Mouch S, Horban A, Müller TH, Welsch C, Chen R, Faruqi R, Pedicone LD, Wedemeyer H. Reduced dose and duration of peginterferon alfa-2b and weight-based ribavirin in patients with genotype 2 and 3 chronic hepatitis C. *J Hepatol* 2011; **55**: 554-563 [PMID: 21237227 DOI: 10.1016/j.jhep.2010.12.024]

18 **Kanda T**, Imazeki F, Azemoto R, Yonemitsu Y, Mikami S, Kita K, Takashi M, Sunaga M, Wu S, Nakamoto S, Tawada A, Arai M, Kato K, Yoshida Y, Koma Y, Fujiwara K, Fukai K, Suzuki N, Yokosuka O. Response to peginterferon-alfa 2b and ribavirin in Japanese patients with chronic hepatitis C genotype 2. *Dig Dis Sci* 2011; **56**: 3335-3342 [PMID: 21604145 DOI: 10.1007/s10620-011-1750-7]

19 **Al-Ali J**, Siddique I, Varghese R, Hasan F. Pegylated interferon-alpha2b plus ribavirin for the treatment of chronic hepatitis C virus genotype 4 infection in patients with normal serum ALT. *Ann Hepatol* 2012; **11**: 186-193 [PMID: 22345335]

20 **Khuroo MS**, Khuroo MS, Dahab ST. Meta-analysis: a randomized trial of peginterferon plus ribavirin for the initial treatment of chronic hepatitis C genotype 4. *Aliment Pharmacol Ther* 2004; **20**: 931-938 [PMID: 15521839 DOI: 10.1111/j.1365-2036.2004.02208.x]

21 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676]

22 **Poordad F**, Landaverde C. Rapid virological response to peginterferon alfa and ribavirin treatment of chronic hepatitis C predicts sustained virological response and relapse in genotype 1 patients. *Therap Adv Gastroenterol* 2009; **2**: 91-97 [PMID: 21180537 DOI: 10.1177/1756283X08101217]

23 **Kamal SM**, El Kamary SS, Shardell MD, Hashem M, Ahmed IN, Muhammadi M, Sayed K, Moustafa A, Hakem SA, Ibrahiem A, Moniem M, Mansour H, Abdelaziz M. Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: The role of rapid and early virologic response. *Hepatology* 2007; **46**: 1732-1740 [PMID: 17943989]

24 **Poordad FF**. Review article: the role of rapid virological response in determining treatment duration for chronic hepatitis C. *Aliment Pharmacol Ther* 2010; **31**: 1251-1267 [PMID: 20236258 DOI: 10.1111/j.1365-2036.2010.04300.x]

25 **Gralewicz S**, Eckersdorf B, Gołebiewski H. Hippocampal rhythmic slow activity (RSA) in the cat after intraseptal injections of muscarinic cholinolytics. *Acta Neurobiol Exp* (Wars) 1992; **52**: 211-221 [PMID: 1293959 DOI: 10.1053/jhep.2003.50364]

26 **Jacobson IM**, Brown RS, Freilich B, Afdhal N, Kwo PY, Santoro J, Becker S, Wakil AE, Pound D, Godofsky E, Strauss R, Bernstein D, Flamm S, Pauly MP, Mukhopadhyay P, Griffel LH, Brass CA. Peginterferon alfa-2b and weight-based or flat-dose ribavirin in chronic hepatitis C patients: a randomized trial. *Hepatology* 2007; **46**: 971-981 [PMID: 17894303 DOI: 10.1002/hep.21932]

27 **Sood A**, Midha V, Hissar S, Kumar M, Suneetha PV, Bansal M, Sood N, Sakhuja P, Sarin SK. Comparison of low-dose pegylated interferon versus standard high-dose pegylated interferon in combination with ribavirin in patients with chronic hepatitis C with genotype 3: an Indian experience. *J Gastroenterol Hepatol* 2008; **23**: 203-207 [PMID: 17645472 DOI: 10.1111/j.1440-1746.2007.05057.x]

28 **Ray G**, Pal S, Nayyar I, Dey S. Efficacy and tolerability of pegylated interferon alpha 2b and ribavirin in chronic hepatitis C--a report from eastern India. *Trop Gastroenterol* 2007; **28**: 109-112 [PMID: 18383998]

29 **WHO**. Guidelines for the screening, care and treatment of persons with hepatitis c infection. Available from: URL: http: //apps.who.int/iris/bitstream/10665/111747/1/9789-241548755_eng.pdf?ua=1

P- Reviewer: Ford N, Kanda T, Liu CJ, Shi Z **S- Editor**: Ji FF
L- Editor: Rutherford A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.527

World J Hepatol 2014 July 27; 6(7): 527-531
ISSN 1948-5182 (online)

CASE REPORT

Acute fatty liver of pregnancy associated with severe acute pancreatitis: A case report

Cássio Vieira de Oliveira, Alecsandro Moreira, Julio P Baima, Leticia de C Franzoni, Talles B Lima, Fabio da S Yamashiro, Kunie Yabuki Rabelo Coelho, Ligia Y Sassaki, Carlos Antonio Caramori, Fernando G Romeiro, Giovanni F Silva

Cássio Vieira de Oliveira, Alecsandro Moreira, Julio P Baima, Leticia de C Franzoni, Talles B Lima, Fabio da S Yamashiro, Ligia Y Sassaki, Carlos Antonio Caramori, Fernando G Romeiro, Giovanni F Silva, Department of Internal Medicine, Gastroenterology Division, Botucatu Medical School, São Paulo State University-UNESP, Botucatu, São Paulo 01049-010, Brazil
Kunie Yabuki Rabelo Coelho, Department of Pathology, Botucatu Medical School, São Paulo State University-UNESP, Botucatu, São Paulo 01049-010, Brazil
Author contributions: de Oliveira CV conceived and coordinated the study and participated in the data acquisition and manuscript writing; Lima TB, Baima J, Coelho KYR, Sassaki LY, Caramori CA, Moreira A and Silva GF participated in the data acquisition and manuscript writing; Romeiro FG coordinated the study and participated in the data acquisition and manuscript writing; Coelho KYR coordinated the histopathological analysis.
Supported by The Department of Internal Medicine, Gastroenterology Division, Botucatu Medical School, São Paulo State University-UNESP, Botucatu/SP, Brazil
Correspondence to: Cássio Vieira de Oliveira, PhD, Department of Internal Medicine, Gastroenterology Division, Botucatu Medical School, São Paulo State University-UNESP, Rua Quirino de Andrade, 215, Botucatu, São Paulo 01049-010, Brazil. cassiovieira01@hotmail.com
Telephone: +55-14-997624920 Fax: +55-14-38116213
Received: March 12, 2014 Revised: May 20, 2014
Accepted: June 10, 2014
Published online: July 27, 2014

Abstract

Acute fatty liver of pregnancy is a rare disease that affects women in the third trimester of pregnancy. Although infrequent, the disease can cause maternal mortality. The diagnosis is not always clear until the pregnancy is terminated, and significant complications, such as acute pancreatitis, can occur. Pancreatic involvement typically only occurs in severe cases after the development of hepatic and renal impairment. To date, little knowledge is available regarding how the disease causes pancreatitis. Treatment involves supportive measures and pregnancy interruption. In this report, we describe a case of a previously healthy 26-year-old woman at a gestational age of 27 wk and 6 d who was admitted with severe abdominal pain and vomiting. This case illustrates the clinical and laboratory overlap between acute fatty liver of pregnancy and pancreatitis, highlighting the difficulties in differentiating each disease. Furthermore, the hypothesis for this overlapping is presented, and the therapeutic options are discussed.

Key words: Acute fatty liver of pregnancy; Severe acute pancreatitis; Fulminant hepatic failure; Liver disease in pregnancy

Core tip: A previously healthy 26-year-old woman at 27 wk and 6 d of pregnancy was referred for investigation of abdominal pain. She presented with complaints of diffuse abdominal pain with nausea and vomiting associated with hepatic and renal dysfunction. Acute fatty liver of pregnancy and severe acute pancreatitis were diagnosed. Acute fatty liver of pregnancy is rarely associated with severe acute pancreatitis, which can complicate the diagnosis. The possible mechanisms involved in this association and the current therapies are discussed, focusing on the relevant aspects to improve the management of similar cases.

de Oliveira CV, Moreira A, Baima JP, Franzoni LC, Lima TB, Yamashiro FS, Coelho KYR, Sassaki LY, Caramori CA, Romeiro FG, Silva GF. Acute fatty liver of pregnancy associated with severe acute pancreatitis: A case report. *World J Hepatol* 2014; 6(7): 527-531 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/527.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.527

INTRODUCTION

Acute fatty liver of pregnancy (AFLP) is a disorder unique to pregnancy that is characterized by microvesicular fatty infiltration of hepatocytes[1]. AFLP was first described in 1940 and was initially considered fatal[2]. However, early diagnosis has dramatically improved the prognosis and maternal mortality; therefore, maternal mortality is currently the exception rather than the rule[1]. AFLP typically occurs in the third quarter of pregnancy, but it is not always diagnosed prior to delivery, as was the case described herein.

The most common initial symptoms are anorexia, nausea, vomiting, abdominal pain, and jaundice. A condition that must be excluded is hemolytic anemia elevated liver function and low platelet count syndrome (HELLP) syndrome, which is characterized by hemolysis, elevated liver enzymes, and low platelet count. AFLP and HELLP syndrome can occur together in some overlapping cases, making the diagnosis more difficult. However, the signs of liver failure, such as hypoglycemia and hepatic encephalopathy, are suggestive of AFLP. Additionally, HELLP syndrome is likely to occur in patients with hypertension, whereas AFLP often occurs in the absence of hypertension. The differential diagnosis of these two diseases was evaluated in a recent study, which indicated that the incorporation of antithrombin activity less than 65% into the diagnostic criteria for AFLP may facilitate prompt diagnosis of this disease[3].

CASE REPORT

A previously healthy 26-year-old woman at a gestational age of 27 wk and 6 d was referred to our hospital due to a diagnostic hypothesis of acute appendicitis. She was complaining of diffuse abdominal pain, nausea, and vomiting during the week. During her physical exam, she was pale and prostrated with mild tachycardia (108 beats/min) and normal blood pressure (110/70 mmHg). No signs of acute appendicitis were noted, but she displayed a potent and diffuse abdominal pain. Cardiotocography revealed signs of fetal distress, so an emergency cesarean section was performed. During the surgery, the possibility of appendicitis was eliminated. Because the newborn displayed bradycardia and an absence of heartbeats at delivery, he was submitted to initial resuscitation protocols and sent to the intensive care unit (ICU). Laboratory tests on the mother revealed leukocytosis, anemia, and hepatic and renal impairment, but no significant proteinuria was found (Table 1).

Abdominal ultrasonography revealed only pancreatic edema without signs of biliary obstruction. After the delivery, abdominal computed tomography (CT), upper gastrointestinal endoscopy, and biochemical tests were performed. The endoscopy was performed exclusively to investigate the possibility of peptic ulcer or other gastroduodenal diseases, but no pathological findings were found. The CT showed only pancreatic edema without peripancreatic collections (Figure 1). Given that the amylase increase was greater than sixfold higher than the normal upper limit and that pancreatic edema was confirmed by ultrasonography and CT exams, the presence of pancreatitis was conclusive. According to the Ranson criteria, the patient had a severe disease that achieved 4 points at admission based on the leukocyte count, aspartate aminotransferase, glycemia, and lactate dehydrogenase values (Table 1). Additionally, she had acute renal failure and achieved 14 points according to the APACHE Ⅱ criteria, which corresponds to an estimated 18.6% risk of hospital death.

The patient developed somnolence and exhibited a progressive decrease in her level of consciousness. Tracheal intubation and mechanical ventilation were needed, so she was transferred to the ICU. At this time, the blood glucose remained normal, but she had abdominal distension and decreased bowel sounds. Then, the diagnostic hypotheses changed to acute liver failure, severe acute pancreatitis, and renal failure. Suddenly, she presented recurrent episodes of hypoglycemia, even with continuous

Table 1 Main laboratory tests demonstrating the development of liver and pancreatic

Blood tests	Admission	48 h after admission	Hospital discharge	1 yr after discharge
Hemoglobin (g/dL)	9.8	11.2	-	-
Leukocyte count (mm^3)	24000	21500	-	-
Glucose (mg/dL)	586	71	91	76
Alkaline phosphatase (U/L)	532	392	248	-
g-GTP (U/L)	282	325	234	-
Calcium (mg/dL)	8.4	7.7	-	-
Amylase (U/L)	460	642	-	59
LDH (U/L)	938	977	-	-
ALT (U/L)	202	112	34	15
AST (U/L)	343	179	38	17
TB (mg/dL)	4.0	4.9	0.6	0.5
Creatinine (mg/dL)	2.6	3.3	0.7	0.8
Urea (mg/dL)	78	85	20	28
INR	2.13	2.78	1.16	0.98
Proteinuria	0.06 g/24 h	0.03 g/24 h	-	-

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TB: Total bilirubin; LDH: Lactate dehydrogenase; INR: International normalized ratio; g-GTP: Gamma-glutamyl transpeptidase.

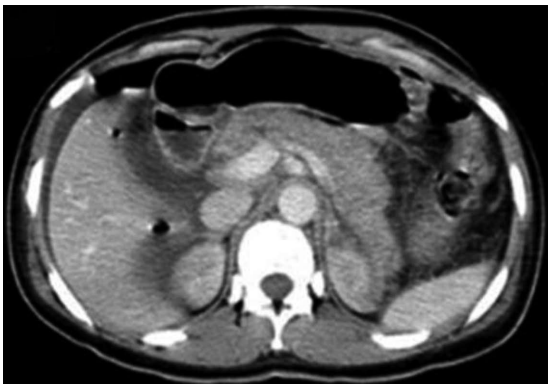

Figure 1 Abdominal computed tomography scan showing diffuse pancreatic edema.

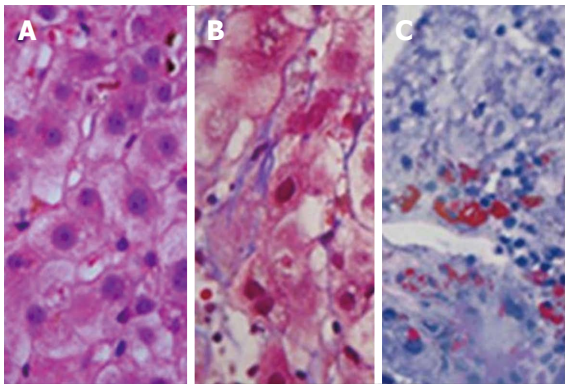

Figure 2 Histopathological analysis of the liver biopsy according to three stains. A: Hematoxylin-eosin staining reveals canalicular cholestasis, hepatocellular ballooning, and microgoticular steatosis; B: Masson staining demonstrates microgoticular steatosis and perivenular and pericellular fibrosis; C: Oil red staining shows the microdroplets of fat clearly stained in red.

dextrose infusion and parenteral nutrition. In response to these new symptoms, AFLP became the major diagnostic hypothesis. Serum factor V was normal, so a percutaneous liver biopsy was performed.

The liver biopsy analysis showed centrilobular microgoticular steatosis, ballooning degeneration, and reticular collapse. The Masson staining showed areas of reticular thickening and intralobular collapse. The "red oil" stain was positive in focal areas, yielding the diagnosis of AFLP (Figure 2).

Seven days following the delivery, she exhibited a clear improvement in consciousness level and liver function tests. She was discharged on postoperative day 24 and returned to the hospital 4 mo later without neurological sequelae. Additionally, laboratory tests and abdominal CT were normal. Despite the problems during the birth, her child exhibited normal development.

DISCUSSION

AFLP is a rare condition that affects approximately 1 in 7000 to 1 in 20000 births[4-8]. AFLP is more common in women with multiple pregnancies and, possibly, in underweight women. However, this case of AFLP occurred in a primiparous, normal-weight woman but not delivering twins.

Approximately half of AFLP patients display signs of preeclampsia at the beginning of or at some time during the course of the disease[9]. Extrahepatic complications may occur, which can be life-threatening[10,11]. The patients rarely develop pancreatitis, which can be severe. Similar to the case described herein, pancreatitis is typically noticed only after the development of hepatic and renal dysfunction[12]. In this case, the patient had AFLP with severe acute pancreatitis, an association that is rarely documented in the literature. The acute renal failure was a complication of the pancreatitis, so it was treated only by supportive measurements and the delivery, thereby confirming that it was a consequence of the underlying disease. No renal replacement therapy was needed. The liver function tests demonstrated severe hepatic impairment, which was the cause of the jaundice. Therefore, even in the presence of severe pancreatitis, liver disease remained the major disease.

Women with AFLP have impaired liver function with increased bilirubin and transaminase levels and leukocyte counts, which are typically higher than those observed in a normal pregnancy. The platelet count can be reduced with or without additional signs of disseminated intravascular coagulation in association with a significant reduction of antithrombin Ⅲ[13]. Severely affected patients also have elevated serum ammonia, prolonged prothrombin times, and hypoglycemia caused by liver failure. Acute renal failure and hyperuricemia are often present[14]. However, in the case presented, the recurrent episodes of hypoglycemia, even with appropriate correction, were the main diagnostic clue.

The association between AFLP and inherited defects in the mitochondrial beta-oxidation of fatty acids, especially the impairment of long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD), suggests that some affected women and fetuses have an inherited enzyme deficiency in beta-oxidation that predisposes the mother to this disorder[15-17]. LCHAD catalyzes the third step of the beta-oxidation of fatty acids in the mitochondrion (the formation of 3-ketoacyl-CoA from 3-hydroxyacyl-CoA). The accumulation of long-chain metabolites of 3-hydroxyacyl produced by the fetus or placenta is toxic to the liver and can serve as the cause of the liver disease. The role of the pathogenesis of LCHAD in AFLP has been illustrated in various studies[18-20].

The mechanism by which pancreatitis may develop as a complication of fatty liver of pregnancy is not well understood because this association is rare. Our hypothesis is that the accumulation of long-chain metabolites of 3-hydroxyacyl is toxic to the liver and the pancreatic tissue. Thus, the pancreas could be affected when an increased concentration of these metabolites is present, as occurs in cases of severe hepatic disease. This hypothesis serves as a reasonable explanation for the pancreatic impairment displayed in this case of hepatic failure.

The diagnosis of LCHAD deficiency in newborns can save lives; therefore, all women with AFLP and their children should be administered a molecular test for LCHAD, which should at least evaluate the most common mutation, namely, G1528C[21,22]. In the present case, it was not possible to perform this type of test because it was not available.

The clinical diagnosis of AFLP is typically performed according to the definition, presentation, and laboratory-compatible image results. The liver imaging is primarily used to exclude other diagnoses, such as hepatic infarction and hematoma[23]. Various authors reported steatosis on ultrasound or CT, but these tests are only useful for performing comparative analyses[24,25]. The AFLP diagnosis can only be made through a liver biopsy showing microvesicular fatty infiltration in hepatocytes. The fat droplets are centrally distributed around the cellular nuclei,

giving the cytoplasm a foamy aspect and typically sparing the cells around the portal tract[26]. A special staining (oil red) must be used to confirm the diagnosis in patients without obvious vacuolation[27,28]. As the liver biopsy is invasive, it is not always performed. Liver biopsy should be performed with caution during pregnancy and be reserved for cases where the diagnosis remains unclear.

A specific treatment is not available for AFLP. The primary treatment is delivery, which typically occurs on an emergency basis after maternal stabilization *via* the infusion of glucose and reversal of the coagulation disturbances. Because hypoglycemia is common and dangerous, glucose levels should be monitored until liver function normalizes[7]. It is typically necessary to treat hypoglycemia with the continuous infusion of a 10% glucose solution. Some patients with severe hypoglycemia may require additional bolus administrations of 50% glucose[7].

The liver function tests typically begin to normalize after delivery; however, after the first few days, a transient worsening of renal and hepatic function may be observed, followed by a definite improvement. In severe cases, particularly when the diagnosis is delayed, more days of illness can be observed, requiring supportive treatment in an ICU. Mechanical ventilation, dialysis, parenteral nutrition, or surgery to treat bleeding during cesarean delivery may be needed. Even the most severely ill patients can recover without liver disease sequelae[7]. However, substantial morbidity and even maternal mortality may occur[8]. Some reports have also described patients who required liver transplantation, which is rarely needed when a diagnosis and pregnancy termination were achieved in sufficient time[29,30]. A case series of five patients indicated good recovery after plasma exchange and renal replacement therapy, showing that the accumulation of long-chain metabolites of 3-hydroxyacyl is most likely the major cause of the liver disease. The authors suggested that these therapies are safe and effective; thus, they should be used immediately at the onset of hepatic encephalopathy and/or renal failure in AFLP patients[31]. None of the patients treated by plasma exchange and renal replacement therapy developed pancreatitis, indicating that our pancreatic toxicity hypothesis may be correct.

Of note, AFLP can recur in subsequent pregnancies despite negative LCHAD mutation screening results[6,15,18,32-35]. However, the exact risk of recurrence is unknown. Affected women should be advised of this possibility, and a maternal-fetal medicine specialist should closely monitor subsequent pregnancies. Given that the initial symptoms of AFLP can be atypical, the diagnosis can be easily misidentified, leading to multiple organ dysfunctions. Once the diagnosis is confirmed, it is important to be vigilant to avoid serious complications, such as pancreatitis, renal failure, and hypoglycemia, as described here.

ACKNOWLEDGMENTS

We acknowledge Professor Maria Rita Piloto for the article corrections.

COMMENTS

Case characteristics

A previously healthy 26-year-old woman with a gestational age of 27 wk and 6 d presented with diffuse abdominal pain associated with hepatic and renal failure.

Clinical diagnosis

Diffuse abdominal pain and vomiting.

Differential diagnosis

Hemolytic anemia elevated liver function and low platelet count syndrome syndrome.

Laboratory diagnosis

Amylase: 642 U/L; glucose: 586 mg/dL; alanine aminotransferase: 202 U/L; aspartate aminotransferase: 343 U/L; total bilirubin: 4.0 mg/dL; creatinine: 2.6 mg/dL; urea: 78 mg/dL; international normalized ratio: 2.13.

Imaging diagnosis

Pancreatic edema was confirmed by ultrasonography and computed tomography.

Pathological diagnosis

The liver biopsy results were compatible with acute fatty liver of pregnancy (AFLP).

Treatment

Pregnancy interruption and supportive measures.

Related reports

AFLP rarely presents with severe acute pancreatitis.

Experiences and lessons

Because the early symptoms of AFLP can be uncharacteristic, the disease can be misdiagnosed. It is important to be vigilant to make the correct diagnosis of AFLP and identify complications, such as pancreatitis.

Peer review

This article describes a rare case with acute fatty liver disease complicated with acute pancreatitis. This is an interesting case report.

REFERENCES

1 **Bacq Y**, Riely CA. Acute fatty liver of pregnancy: the hepatologist's view. *Gastroenterologist* 1993; **1**: 257-264 [PMID: 8055222]

2 **Sheehan H**. The pathology of acute yellow atrophy and delayed chloroform poisoning. *J Obstet Gynaecol* 1940; **47**: 49-53 [DOI: 10.1111/j.1471-0528.1940.tb14731.x]

3 **Minakami H**, Morikawa M, Yamada T, Yamada T, Akaishi R, Nishida R. Differentiation of acute fatty liver of pregnancy from syndrome of hemolysis, elevated liver enzymes and low platelet counts. *J Obstet Gynaecol Res* 2014; **40**: 641-649 [PMID: 24428400 DOI: 10.1111/jog.12282]

4 **Usta IM**, Barton JR, Amon EA, Gonzalez A, Sibai BM. Acute fatty liver of pregnancy: an experience in the diagnosis and management of fourteen cases. *Am J Obstet Gynecol* 1994; **171**: 1342-1347 [PMID: 7977544 DOI: 10.1016/0002-9378(94)90158-9]

5 **Pockros PJ**, Peters RL, Reynolds TB. Idiopathic fatty liver of pregnancy: findings in ten cases. *Medicine* (Baltimore) 1984; **63**: 1-11 [PMID: 6690883 DOI: 10.1097/00005792-198401000-00001]

6 **Reyes H**, Sandoval L, Wainstein A, Ribalta J, Donoso S, Smok G, Rosenberg H, Meneses M. Acute fatty liver of pregnancy: a clinical study of 12 episodes in 11 patients. *Gut* 1994; **35**: 101-106 [PMID: 8307428 DOI: 10.1136/gut.35.1.101]

7 **Castro MA**, Fassett MJ, Reynolds TB, Shaw KJ, Goodwin TM. Reversible peripartum liver failure: a new perspective on the diagnosis, treatment, and cause of acute fatty liver of pregnancy, based on 28 consecutive cases. *Am J Obstet Gynecol* 1999; **181**: 389-395 [PMID: 10454689 DOI: 10.1016/S0002-9378(99)70567-3]

8 **Knight M**, Nelson-Piercy C, Kurinczuk JJ, Spark P, Brocklehurst P. A prospective national study of acute fatty liver of pregnancy in the UK. *Gut* 2008; **57**: 951-956 [PMID: 18332072 DOI: 10.1136/gut.2008.148676]

9 **Riely CA**. Acute fatty liver of pregnancy. *Semin Liver Dis* 1987; **7**: 47-54 [PMID: 3296215 DOI: 10.1055/s-2008-1040563]
10 **Pereira SP**, O'Donohue J, Wendon J, Williams R. Maternal and perinatal outcome in severe pregnancy-related liver disease. *Hepatology* 1997; **26**: 1258-1262 [PMID: 9362370 DOI: 10.1002/hep.510260525]
11 **Kennedy S**, Hall PM, Seymour AE, Hague WM. Transient diabetes insipidus and acute fatty liver of pregnancy. *Br J Obstet Gynaecol* 1994; **101**: 387-391 [PMID: 8018608 DOI: 10.1111/j.1471-0528.1994.tb11909.x]
12 **Moldenhauer JS**, O'brien JM, Barton JR, Sibai B. Acute fatty liver of pregnancy associated with pancreatitis: a life-threatening complication. *Am J Obstet Gynecol* 2004; **190**: 502-505 [PMID: 14981397 DOI: 10.1016/j.ajog.2003.09.022]
13 **Castro MA**, Goodwin TM, Shaw KJ, Ouzounian JG, McGehee WG. Disseminated intravascular coagulation and antithrombin III depression in acute fatty liver of pregnancy. *Am J Obstet Gynecol* 1996; **174**: 211-216 [PMID: 8572009]
14 **Grünfeld JP**, Pertuiset N. Acute renal failure in pregnancy: 1987. *Am J Kidney Dis* 1987; **9**: 359-362 [PMID: 3555010 DOI: 10.1016/S0272-6386(87)80137-3]
15 **Schoeman MN**, Batey RG, Wilcken B. Recurrent acute fatty liver of pregnancy associated with a fatty-acid oxidation defect in the offspring. *Gastroenterology* 1991; **100**: 544-548 [PMID: 1985050]
16 **Wilcken B**, Leung KC, Hammond J, Kamath R, Leonard JV. Pregnancy and fetal long-chain 3-hydroxyacyl coenzyme A dehydrogenase deficiency. *Lancet* 1993; **341**: 407-408 [PMID: 8094173]
17 **Treem WR**, Rinaldo P, Hale DE, Stanley CA, Millington DS, Hyams JS, Jackson S, Turnbull DM. Acute fatty liver of pregnancy and long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Hepatology* 1994; **19**: 339-345 [PMID: 8294091 DOI: 10.1002/hep.1840190211]
18 **Treem WR**, Shoup ME, Hale DE, Bennett MJ, Rinaldo P, Millington DS, Stanley CA, Riely CA, Hyams JS. Acute fatty liver of pregnancy, hemolysis, elevated liver enzymes, and low platelets syndrome, and long chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency. *Am J Gastroenterol* 1996; **91**: 2293-2300 [PMID: 8931405]
19 **Sims HF**, Brackett JC, Powell CK, Treem WR, Hale DE, Bennett MJ, Gibson B, Shapiro S, Strauss AW. The molecular basis of pediatric long chain 3-hydroxyacyl-CoA dehydrogenase deficiency associated with maternal acute fatty liver of pregnancy. *Proc Natl Acad Sci USA* 1995; **92**: 841-845 [PMID: 7846063 DOI: 10.1073/pnas.92.3.841]
20 **Yang Z**, Zhao Y, Bennett MJ, Strauss AW, Ibdah JA. Fetal genotypes and pregnancy outcomes in 35 families with mitochondrial trifunctional protein mutations. *Am J Obstet Gynecol* 2002; **187**: 715-720 [PMID: 12237653 DOI: 10.1067/mob.2002.125893]
21 **Rajasri AG**, Srestha R, Mitchell J. Acute fatty liver of pregnancy (AFLP)--an overview. *J Obstet Gynaecol* 2007; **27**: 237-240 [PMID: 17464801 DOI: 10.1080/01443610701194705]
22 **Ibdah JA**. Acute fatty liver of pregnancy: an update on pathogenesis and clinical implications. *World J Gastroenterol* 2006; **12**: 7397-7404 [PMID: 17167825 DOI: 10.3748/wjg.v12.i46.7397]
23 Strategies for cadaveric organ procurement. Mandated choice and presumed consent. Council on Ethical and Judicial Affairs, American Medical Association. *JAMA* 1994; **272**: 809-812 [PMID: 8078146]
24 **Castro MA**, Ouzounian JG, Colletti PM, Shaw KJ, Stein SM, Goodwin TM. Radiologic studies in acute fatty liver of pregnancy. A review of the literature and 19 new cases. *J Reprod Med* 1996; **41**: 839-843 [PMID: 8951135]
25 **Campillo B**, Bernuau J, Witz MO, Lorphelin JM, Degott C, Rueff B, Benhamou JP. Ultrasonography in acute fatty liver of pregnancy. *Ann Intern Med* 1986; **105**: 383-384 [PMID: 3527013 DOI: 10.7326/0003-4819-105-3-383]
26 **Bacq Y**. Acute fatty liver of pregnancy. *Semin Perinatol* 1998; **22**: 134-140 [PMID: 9638907 DOI: 10.1016/S0146-0005(98)80045-1]
27 **Riely CA**, Latham PS, Romero R, Duffy TP. Acute fatty liver of pregnancy. A reassessment based on observations in nine patients. *Ann Intern Med* 1987; **106**: 703-706 [PMID: 3565968 DOI: 10.7326/0003-4819-106-5-703]
28 **Rolfes DB**, Ishak KG. Acute fatty liver of pregnancy: a clinicopathologic study of 35 cases. *Hepatology* 1985; **5**: 1149-1158 [PMID: 2415437 DOI: 10.1002/hep.1840050615]
29 **Ockner SA**, Brunt EM, Cohn SM, Krul ES, Hanto DW, Peters MG. Fulminant hepatic failure caused by acute fatty liver of pregnancy treated by orthotopic liver transplantation. *Hepatology* 1990; **11**: 59-64 [PMID: 2403963 DOI: 10.1002/hep.1840110112]
30 **Amon E**, Allen SR, Petrie RH, Belew JE. Acute fatty liver of pregnancy associated with preeclampsia: management of hepatic failure with postpartum liver transplantation. *Am J Perinatol* 1991; **8**: 278-279 [PMID: 1741873 DOI: 10.1055/s-2007-999396]
31 **Yu CB**, Chen JJ, Du WB, Chen P, Huang JR, Chen YM, Cao HC, Li LJ. Effects of plasma exchange combined with continuous renal replacement therapy on acute fatty liver of pregnancy. *Hepatobiliary Pancreat Dis Int* 2014; **13**: 179-183 [PMID: 24686545 DOI: 10.1016/S1499-3872(14)60028-X]
32 **Bacq Y**, Assor P, Gendrot C, Perrotin F, Scotto B, Andres C. [Recurrent acute fatty liver of pregnancy]. *Gastroenterol Clin Biol* 2007; **31**: 1135-1138 [PMID: 18176373 DOI: 10.1016/S0399-8320(07)78351-3]
33 **MacLean MA**, Cameron AD, Cumming GP, Murphy K, Mills P, Hilan KJ. Recurrence of acute fatty liver of pregnancy. *Br J Obstet Gynaecol* 1994; **101**: 453-454 [PMID: 8018624 DOI: 10.1111/j.1471-0528.1994.tb11926.x]
34 **Barton JR**, Sibai BM, Mabie WC, Shanklin DR. Recurrent acute fatty liver of pregnancy. *Am J Obstet Gynecol* 1990; **163**: 534-538 [PMID: 2386140]
35 **Visconti M**, Manes G, Giannattasio F, Uomo G. Recurrence of acute fatty liver of pregnancy. *J Clin Gastroenterol* 1995; **21**: 243-245 [PMID: 8648062 DOI: 10.1097/00004836-199510000-00016]

P- Reviewer: Alcazar JL, Cichoz-Lach H, Shimizu Y, Tsikouras P, Wang CC
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v6.i7.532

World J Hepatol 2014 July 27; 6(7): 532-537
ISSN 1948-5182 (online)

CASE REPORT

Portal vein thrombosis with protein C-S deficiency in a non-cirrhotic patient

Gustavo A Rodríguez-Leal, Segundo Morán, Roberto Corona-Cedillo, Rocío Brom-Valladares

Gustavo A Rodríguez-Leal, Segundo Morán, Laboratory of Gastrohepatology Research, Hospital de Pediatría, CMN Siglo XXI, Mexican Institute of Social Security, Delegación Cuauhtémoc, CP 06720, México
Gustavo A Rodríguez-Leal, Gastroenterology Department, Médica Sur Clinic and Foundation, Ciudad de México, DF 14050, México
Roberto Corona-Cedillo, Rocío Brom-Valladares, Radiology Department, Médica Sur Clinic and Foundation, Ciudad de México, DF 14050, México
Author contributions: Rodríguez-Leal GA drafted the manuscript, provided patient details and made the suggested revisions; Morán S assisted in drafting the manuscript, reviewed the manuscript and made suggestions on revisions, and assisted with journal submission; Corona-Cedillo R and Brom-Valladares R reviewed the manuscript and made suggestions on revisions, and provided the radiologic material and comments.
Correspondence to: Segundo Morán, MD, Laboratory of Gastrohepatology Research, Hospital de Pediatría, CMN Siglo XXI, Mexican Institute of Social Security, Av Cuauhtémoc 330, Colonia Doctores, Delegación Cuauhtémoc, CP 06720, México. segundomoran@hotmail.com
Telephone: +52-55-56276900 Fax: +52-55-57610952
Received: December 4, 2013 Revised: March 11, 2014
Accepted: May 28, 2014
Published online: July 27, 2014

Key words: Portal vein thrombosis; Mesenteric venous thrombosis; Protein C and S deficiency; Anticoagulant therapy; Transient elastography

Core tip: Abdominal pain, diarrhea, rectal bleeding, abdominal distention, ascites, anorexia, fever, lactacidosis, sepsis, and splenomegaly are common features of acute portal vein thrombosis (PVT). Etiological factors in non-cirrhotic PVT patients are prothrombotic states and local factors, although more than one factor is often identified. Our patient, a 63-year-old man, without personal or familial history of venous thromboembolism developed portal and mesenteric vein thrombosis after an acute gastrointestinal infection by *Escherichia coli*. Clinicians need to be aware of this potential complication in patients with persistent abdominal pain and ascites after abdominal infections.

Rodríguez-Leal GA, Morán S, Corona-Cedillo R, Brom-Valladares R. Portal vein thrombosis with protein C-S deficiency in a non-cirrhotic patient. *World J Hepatol* 2014; 6(7): 532-537 Available from: URL: http://www.wjgnet.com/1948-5182/full/v6/i7/532.htm DOI: http://dx.doi.org/10.4254/wjh.v6.i7.532

Abstract

There are several conditions that can lead to portal vein thrombosis (PVT), including including infection, malignancies, and coagulation disorders. Anew condition of interest is protein C and S deficiencies, associated with hypercoagulation and recurrent venous thromboembolism. We report the case of a non-cirrhotic 63-year-old male diagnosed with acute superior mesenteric vein thrombosis and PVT and combined deficiencies in proteins C and S, recanalized by short-term low molecular heparin plus oral warfarin therapy.

INTRODUCTION

Portal vein thrombosis (PVT) is defined as complete or partial obstruction of blood flow in the portal vein, associated with a thrombus in the vasal lumen[1]. The first case of PVT was reported in 1868 by Balfour and Stewart, in a patient showing splenomegaly, ascites, and variceal dilatation[2]. PVT is rare in the general population having been reported with mean age-standarized incidence and prevalence rates of 0.7 and 3.7 per 100000 inhabitants, respectively[3]. However among patients with cirrhosis, these rates jump to between 4.4%-15%, and cause about 5%-10% of overall cases of portal hypertension[4]. Some 22%-70% of patients without cirrhosis

demonstrate prothrombotic states and local factors are present in 10%-50%[3-5], although more than one factor is often identified[6]. PVT also shows different clinical presentations in acute *vs* chronic onset patients and collateral circulation, both its development and extent. Intestinal congestion and ischemia, with abdominal pain, diarrhea, rectal bleeding, abdominal distention, nausea, vomiting, anorexia, fever, lactacidosis, sepsis, and splenomegaly are common in acute PVT. More difficult to diagnose, chronic PVT can be completely asymptomatic, or present splenomegaly, pancytopenia, varices, and, on rare occasion, ascites[2].

PVT is classified into four categories: (1) thrombosis confined to the portal vein beyond the confluence of the splenic and superior mesenteric vein (SMV); (2) extension of thrombus into the SMV, but with patent mesenteric vessels; (3) diffuse thrombosis of splanchnic venous system, but with large collaterals; and (4) extensive splanchnic venous thrombosis, but with only fine collaterals. Currently this anatomical classification is mainly used to determine operability, but it may also have etiological and prognostic relevance, since patients with thrombus interference with mesenteric vasculature risk bowel infarction and have a lower risk of variceal bleeding than those with isolated PVT. In all cases, patients with PVT should be tested for an underlying thrombophilic condition[6]. Hereditary thrombophilias known to predispose for PVT include mutations of the prothrombin, or factor V, genes, and deficiency of one of the natural anticoagulant proteins C, S, or antithrombin. Fisher *et al*[7] in a study with twenty-nine adult patients with portal hypertension caused by PVT, found that 18 patients (62%) had deficiencies in one or more of the natural anticoagulant proteins, and six had combined deficiency of all three proteins. Of these, eight cases (28%) had combined C and S protein deficiency, nine (31%) had C protein and antithrombin deficiency, seven (24%) showed protein S and antithrombin deficiency, and six cases (21%),as mentioned, had combined deficiency of all three proteins. Due to increased use and improvement of non-invasive imaging techniques in diagnostic evaluation of abdominal pain, acute portomesenteric venous obstruction is an increasingly recognized disorder[1,2,4,5].

CASE REPORT

The patient was a 63-year-old man with glaucoma treated with timolol and latanoprost. He had undergone a resection of thyrogloid cyst 50 years previously. There was no personal history of venous thromboembolism and familial history was unrevealed. No abdominal trauma was reported. The patient had developed an acute gastrointestinal infection by *Escherichia coli* three months before admission, and received treatment with ciprofloxacin. Since that infection, he had felt intermittent mesogastric abdominal pain after meals, nausea and diarrhea, that increased in frequency 2 wk before admission, when he also noted increased abdominal girdle and peripheral edema. He did not note mucus or blood in feces. On admission, the patient had a fever 39 ℃ and blood pressure of 100/70 mmHg. He was alert and oriented without signs of encephalopathy. His bowel sounds were hypoactive and minimal epimesogastric tenderness was present with no rebound tenderness. He had non-tense ascites and edema in the lower extremities. Heart, lungs, throat and skin were unremarkable. Laboratory studies showed a hematocrit of 42.2%, mean corpuscular volume of 87 fL, and a sedimentation rate of 51%, white cell count of 6.8/mm^3, neutrophils 65.6%, lymphocytes 19.0%, monocytes 15.1%, eosinophils 0.3%, platelet count 271/mm^3, prothrombin time 10.6 s, 97.6%, international normalized ratio (INR) 0.96. Serum chemistry values and urine test were normal. Liver function test showed: albumin 3.3 g/dL; total bilirubin 1.94 mg/dL; alanine aminotransferase 51 U/L; aspartate aminotransferase 40 U/L; alkaline phosphatase was 96 (32-91 U/L); lactic dehydrogenase was 251 U/L (98-192 U/L); g-glutamyl transpeptidase was 139 U/L (7-50 U/L). Amylase 44 U/L, Lipase 23 U/L. Viral B and C antibodies were negative. Tumoral markers CA-19-9, ACE, alkaline phosphatae (AFP) were negative. His antiphospholipid antibodies and cardiolipin antibodies were negative. A thrombophilia workup, not including screening for JAK2V617F mutation, revealed normal homocysteine blood levels; C-reactive protein levels was 216.5 (0-7.4 mg/L); D-dimer was 5770 (0-199 ng/mL); fibrinogen levels was 443 (177-410 mg/dL); low levels and little activity of the protein C antigen [protein C antigen level, 39%; protein C activity, 54% (normal 70%-140%)] and protein S antigen [protein S antigen level, 59%; protein C activity, 30% (normal 65%-140%)] were found; antithrombin Ⅲ levels were 89% (normal 75%-125%). Factor V Leiden mutation was homozygote. His father was dead and his mother and sister neglected screening. Hematological, urine, ascites fluid and pharyngeal cultures were negative. Upper endoscopy revealed mild portal hypertensive gastropathy without gastric and esophageal varices. Ultrasonography of the abdomen showed that the portal vein could not be identified in the porta hepatis, which was occupied by several abnormal tubular structures suggestive of cavernous transformation (Figure 1A). The computed tomography scan of the abdomen showed cavernous transformation following PVT. The portal venous thrombus extended from the superior mesenteric vein (Figure 2). A transient elastography (TE) (Fibroscan) was abnormal with stiffness 7.4 kPa. We treated the patient with low molecular weight heparin (enoxaparine, 1 mg/kg) during the first week and chronic anticoagulation therapy (warfarin 2.5 mg/d, INR 2-3) to date. A new Doppler ultrasound, five months after admission, improved his portal flow with complete recanalization and without ascites (Figure 1B). The patient is asymptomatic three years after hospital discharge.

DISCUSSION

In recent years, PVT has increasingly been diagnosed by

Table 1 Hypercoagulable etiologies

Thrombophilic disorders		Local factors	
Inherited disorders	Acquired disorders	Inflammatory	Related to surgery
Factor V Leyden mutation	Myeloproliferative disorders	Cirrhosis	Post liver transplant
Prothrombin mutation	Malignancy	Sepsis	Splenectomy
Antithrombin Ⅲ	Antiphospholipid syndrome	Pancreatitis/cholecystitis	Colectomy
Protein C deficiency	Anticardiolipin antibody	Diverticulitis	Umbilical vein catheterization
Protein S deficiency	Paroxysmal nocturnal hemoglobinuria	Appendicitis	Portocaval shunting
	Hyperhomocystein-emia	Peptic ulcer disease	
	Oral contraception pills	Inflammatory bowel disease	
	Pregnancy/post-partum	Blunt abdominal trauma	

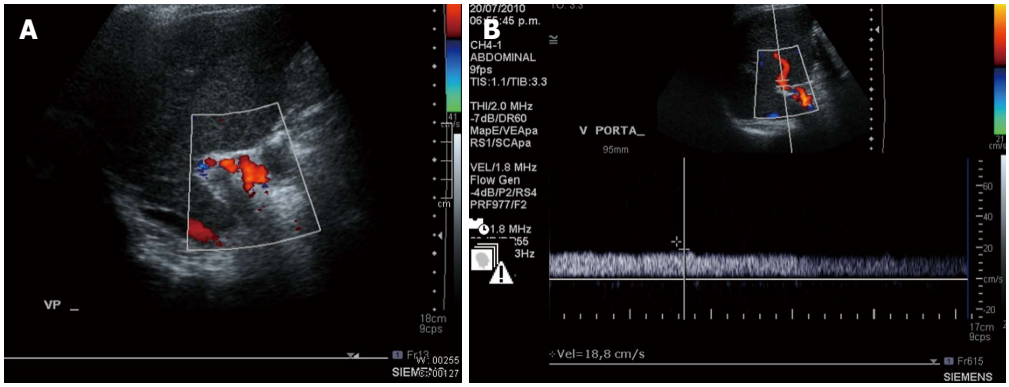

Figure 1 Doppler ultrasound. A: Liver Doppler ultrasound. The image shows the thrombus in the portal vein; B: Doppler ultrasound, performed 4 mo after discharge, revealed that the portal vein thrombi had disappeared and a smooth bloodstream was observed in the portal vein.

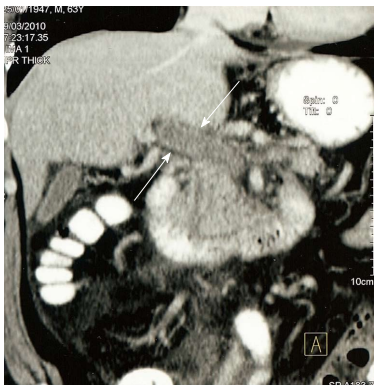

Figure 2 Coronal reconstruction of contrast-enhanced computed tomography image with arrows indicating portal venous thrombosis and evidence of cavernous transformation.

wide use of ultrasound-Doppler equipment. When cirrhosis is not present, the lifetime risk of getting PVT in the general population is reported to be 1%[8,9]. Currently recognized etiologies can be divided into 2 categories: thrombophilic disorders and thromboses thought to be caused from local factors (Table 1).

Protein C is a thrombin-dependent anticoagulant enzyme known to deactivate coagulation cofactors V and VIa and to stimulate fibrinolysis[10]. Protein C deficiency, often inherited as an autosomal dominant trait, is a risk factor for venous thrombosis.

The prevalence of protein C deficiency, as indicated solely by plasma level, is 1 in 200-500 persons in the general population. However, this number is unreliable as many affected individuals remain asymptomatic throughout their lives. However, protein C deficiency is present in approximately 2%-5% patients presenting VTE. Severe homozygous or compound heterozygous protein C deficiency is found in 1 in 500000-750000 live births. Protein S deficiency occurs in 1.35% of the patients with venous thrombosis.

There is evidence to suggest that thrombosis in unusual sites, such as cerebral sinus venous thrombosis, mesenteric vein thrombosis, PVT, and suprahepatic vein thrombosis (Budd-Chiari syndrome), in young individuals is associated with inherited thrombophilia.

Liver function impairment, which can be a result of PVT, cannot account for the low C and S protein levels in our patient, as the levels of other function tests and indirect markers of liver fibrosis (TE) were abnormal.

It is not known whether the unexplained bout of abdominal pain and diarrhea which occurred three months before our patient, was due to thrombosis, to a resolutive episode of intestinal ischaemia secondary to mesenteric vein thrombosis, or to an unrelated illness, although abdominal pain, diarrhea, abdominal distention, nausea, anorexia, and fever are common in acute PVT[4].

In Mexico, Majluf-Cruz *et al*[11], studied 36 patients who had thrombosis-related portal hypertension and found an incidence of 30% of protein C deficiency, whereas 9% had protein S deficiency in patients with primary thrombophilia[12]. Similarly in Mexican patients with non-cirrhotic PVT, 31% had protein C deficiency[13].

Table 2 Proposed mechanism for reduction in concentrations of procoagulant and anticoagulant proteins in patients with portal vein thrombosis

Hereditary or acquired thrombophilia
Reduced hepatic blood flow
Reduced synthesis
Portal hypertension
Portosystemic shunting
Clearance or consumption
Portal pyaemia or other local inflammatory disease
Portal vein thrombosis
Reduced levels of procoagulant and anticoagulant proteins

However, a French study has found a high number of patients with non-cirrhotic PVT showed Protein S deficiency[14] and in a study from United Kingdom, protein S deficiency was found in 38% of patients with PVT[15]. Other cases have also reported C and S protein deficiencies in patients with idiopathic portal hypertension accompanied by PVT[16,17]. Valla *et al*[14], argue that C and S protein deficiencies do not explain the majority of idiopathic portal thrombosis. Nevertheless, we agree with others that measurements of C and S proteins should be performed in patients with portal thrombosis when no overt cause is located. However, since a low number of cases of PVT may be due to underlying hereditary anticoagulant protein deficiency, this can only be confirmed by careful investigation of background of family members, preferably including both parents. When studies of the parents is not feasible, another possibility might be screening siblings, which could be used for both diagnostic and counseling purposes. Lastly, the recent use of gene sequencing in the elucidation of anticoagulant protein gene mutations may now allow determination of whether such anticoagulant deficiencies in PVT are truly primary or not[18]. Some possible mechanisms for reduction in concentrations of procoagulant and anticoagulant proteins in patients with PVT are shown in Table 2.

Visualization of abnormalities associated with PVT is crucial to diagnosis and appropriate intervention. Cavernous transformation of the portal vein occurs in one-third of patients after PVT. An ultrasonographically diagnostic triad would consist of: (1) failure of visualization of the extra-hepatic portal vein; (2) demonstration of high-level echoes in the region of the porta hepatis (the "diamond sign"); and (3) visualization of multiple serpiginous vascular channels around the portal vein[19]. Dynamic contrast-enhanced computed tomography (CT) is the best means of diagnosing PVT and evaluating possible causative diseases. The findings of PVT in a dynamic CT include: filling defect partially or totally occluding the vessel lumen and rim enhancement of the vessel wall[20]. Signs and symptoms of PVT may be subtle or nonspecific and are secondary to the underlying illness. On the other hand, presence of a well-developed cavernoma usually indicates an old thrombosis. A previous PVT, however, can be associated with a recently superimposed thrombus, which is then responsible for the acute manifestations which lead to imaging studies. An abdominal magnetic resonance imaging may prove more useful than Doppler ultrasound in identifying venous collateral development and cavernoma[21]. An important step in PVT is to disclose malignancy. We only performed some tumoral markers (CA-19-9, ACE, AFP), but screening for JAK2V617F in order to discard myeloproliferative neoplasms and positron emission tomography-scan were not performed. TE is a non-invasive technique to assess liver fibrosis, which assesses liver fibrosis by calculating the velocity of a low-frequency transient shear wave produced by a mechanical probe that is placed directly on the skin of the patient. Liver stiffness is expressed in kPa. The method is easy to learn (the procedure can be performed by a technical assistant), and results are immediately available. One meta-analysis evaluating the predictive performance of TE in patients with chronic liver disease suggests the optimal cut-off value for the diagnosis of significant fibrosis is 7.65 kPa and for cirrhosis 13.01 kPa[22]. In our patient, stiffness of 7.4 kPa was highly predictive for significant fibrosis ($F \geqslant 2$). There is no data on the use of TE in PVT, but this method may be useful to determine liver fibrosis in these patients. Complications during follow-up frequently include: esophageal and gastric varices, portal hypertensive gastropathy and bleeding. Portal hypertensive gastropathy is reported to be 44% in patients without cancer and cirrhosis, as was the case with our patient[23]. Therefore, it would be wise to screen all PVT patients endoscopically. Although spontaneous resolution of PVT has been reported in the literature, a specific therapeutic management strategy is necessary. The goal of treatment is similar in acute and chronic PVT, and includes correction of causal factors, prevention of thrombosis extension and achievement of portal vein patency. Currently, anticoagulant therapy is the best way to obtain portal vein recanalization; however, its application is not universally accepted. No controlled trial has been performed on the use of anticoagulants in acute PVT[24]. After 6 mo of therapy, complete recanalization has been reported in about 50% of patients, with good outcomes in mesenteric vein involvement, and very few complications. What is certain is that, in acute PVT onset, the sooner the treatment is given, the better the prognosis; the rate of recanalization is about 69% if anticoagulation is begun within the first week after diagnosis, while it falls to 25% when begun in the second week[25]. Thrombolytic therapy may also be effective, but efficacy is significantly lower and mortality increases compared to conservative treatment[26]. Surgical thrombectomy is usually not recommended. Other approaches, such as transyugular intrahepatic portosystemic shunt, should be reserved for liver transplant patients developing acute PVT or as an alternative when anticoagulation fails[4]. In non-cirrhotic and non-neoplastic patients, PVT has shown promising results with overall survival at 1 year and 5 years of 92% and 76% respectively[3,23,27,28].

In conclusion, our case shows that PVT can be provoked by C and S protein deficiency and that the PVT

can be recanalized by short-term low molecular heparin plus oral warfarin therapy. Although the evidence is not definitive, existing literature supports the idea that the risk-benefit ratio favors anticoagulation in chronic non-cirrhotic PVT.

COMMENTS

Case characteristics

Upon admission the patient felt intermittent colicky abdominal pain and non-bloody diarrhea after meals with increased abdominal girdle and peripheral edema at physical examination.

Clinical diagnosis

The patient presented with non-tense ascites and imaging evidence of portal vein thrombosis (PVT) on a background of non-liver disease.

Differential diagnosis

Differential diagnosis was performed between inherited *vs* acquired disorders of coagulation in PVT using ultrasound Doppler, dynamic computed tomography (CT) and specific laboratory tests.

Laboratory diagnosis

A thrombophilia workup, not including screening for JAK2V617F mutation, revealed normal homocysteine blood levels; C-reactive protein levels were 216.5 (0-7.4 mg/L); D-dimer was 5770 (0-199 ng/mL); fibrinogen levels was 443 (177-410 mg/dL); low levels and little activity of the protein C antigen [protein C antigen level, 39%; protein C activity, 54% (normal 70%-140%)] and protein S antigen [protein S antigen level, 59%; protein C activity, 30% (normal 65%-140%)] and homozygote factor V Leiden mutation was found; abnormal liver function tests (albumin 3.3 g/dL; total bilirubin 1.94 mg/dL; alanine aminotransferase 51 U/L (31-45 U/L); alkaline phosphatase 96 (32-91 U/L); lactic dehydrogenase 251 U/L (98-192 U/L); g-glutamyl transpeptidase 139 U/L (7-50 U/L) were found; antithrombin Ⅲ levels, viral B and C antibodies ,CA-19-9, ACE, alkaline phosphatae, antiphospholipid antibodies and cardiolipin antibodies were normal or negative.

Imaging diagnosis

Liver Doppler ultrasound showed a thrombus in the portal vein that was corroborated by CT image indicating portal venous thrombosis and evidence of cavernous transformation.

Pathologic diagnosis

Histologic examination was not indicated.

Treatment

The patient was treated with low molecular weight heparin (enoxaparine, 1 mg/kg) during the first week and chronic anticoagulation therapy (warfarin 2.5 mg/d, INR 2-3) to date.

Experiences and lessons

Even if Doppler ultrasound or abdominal CT play a key role in the diagnosis of PVT, the protocol to find the etiology of the thrombosis may be complex.

Peer review

This manuscript is interesting and presents a remarkable presentation about diagnosis and management of PVT associated with C and S protein deficiency in a non-cirrhotic patient.

REFERENCES

1 **Kocher G**, Himmelmann A. Portal vein thrombosis (PVT): a study of 20 non-cirrhotic cases. *Swiss Med Wkly* 2005; **135**: 372-376 [PMID: 16106327]

2 **Wang JT**, Zhao HY, Liu YL. Portal vein thrombosis. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 515-518 [PMID: 16286254]

3 **Plessier A**, Darwish-Murad S, Hernandez-Guerra M, Consigny Y, Fabris F, Trebicka J, Heller J, Morard I, Lasser L, Langlet P, Denninger MH, Vidaud D, Condat B, Hadengue A, Primignani M, Garcia-Pagan JC, Janssen HL, Valla D. Acute portal vein thrombosis unrelated to cirrhosis: a prospective multicenter follow-up study. *Hepatology* 2010; **51**: 210-218 [PMID: 19821530 DOI: 10.1002/hep.23259]

4 **Ponziani FR**, Zocco MA, Campanale C, Rinninella E, Tortora A, Di Maurizio L, Bombardieri G, De Cristofaro R, De Gaetano AM, Landolfi R, Gasbarrini A. Portal vein thrombosis: insight into physiopathology, diagnosis, and treatment. *World J Gastroenterol* 2010; **16**: 143-155 [PMID: 20066733 DOI: 10.3748/wjg.v16.i2.143]

5 **Janssen HL**, Wijnhoud A, Haagsma EB, van Uum SH, van Nieuwkerk CM, Adang RP, Chamuleau RA, van Hattum J, Vleggaar FP, Hansen BE, Rosendaal FR, van Hoek B. Extrahepatic portal vein thrombosis: aetiology and determinants of survival. *Gut* 2001; **49**: 720-724 [PMID: 11600478 DOI: 10.1136/gut.49.5.720]

6 **Denninger MH**, Chaït Y, Casadevall N, Hillaire S, Guillin MC, Bezeaud A, Erlinger S, Briere J, Valla D. Cause of portal or hepatic venous thrombosis in adults: the role of multiple concurrent factors. *Hepatology* 2000; **31**: 587-591 [PMID: 10706547 DOI: 10.1002/hep.510310307]

7 **Fisher NC**, Wilde JT, Roper J, Elias E. Deficiency of natural anticoagulant proteins C, S, and antithrombin in portal vein thrombosis: a secondary phenomenon? *Gut* 2000; **46**: 534-539 [PMID: 10716684 DOI: 10.1136/gut.46.4.534]

8 **Webster GJ**, Burroughs AK, Riordan SM. Review article: portal vein thrombosis -- new insights into aetiology and management. *Aliment Pharmacol Ther* 2005; **21**: 1-9 [PMID: 15644039 DOI: 10.1111/j.1365-2036.2004.02301.x]

9 **Ogren M**, Bergqvist D, Björck M, Acosta S, Eriksson H, Sternby NH. Portal vein thrombosis: prevalence, patient characteristics and lifetime risk: a population study based on 23,796 consecutive autopsies. *World J Gastroenterol* 2006; **12**: 2115-2119 [PMID: 16610067]

10 **Clouse LH**, Comp PC. The regulation of hemostasis: the protein C system. *N Engl J Med* 1986; **314**: 1298-1304 [PMID: 3010107 DOI: 10.1056/NEJM198605153142006]

11 **Majluf-Cruz A**, Hurtado Monroy R, Sansores García L, Labardini-Méndez J. The incidence of protein C deficiency in thrombosis-related portal hypertension. *Am J Gastroenterol* 1996; **91**: 976-980 [PMID: 8633591]

12 **Ruiz-Argüelles GJ**, López-Martínez B, Valdés-Tapia P, Gómez-Rangel JD, Reyes-Núñez V, Garcés-Eisele J. Primary thrombophilia in Mexico. V. A comprehensive prospective study indicates that most cases are multifactorial. *Am J Hematol* 2005; **78**: 21-26 [PMID: 15609280 DOI: 10.1002/ajh.20233]

13 **Orozco H**, Guraieb E, Takahashi T, Garcia-Tsao G, Hurtado R, Anaya R, Ruiz-Arguelles G, Hernandez-Ortiz J, Casillas MA, Guevara L. Deficiency of protein C in patients with portal vein thrombosis. *Hepatology* 1988; **8**: 1110-1111 [PMID: 3262079 DOI: 10.1002/hep.1840080522]

14 **Valla D**, Casadevall N, Huisse MG, Tulliez M, Grange JD, Muller O, Binda T, Varet B, Rueff B, Benhamou JP. Etiology of portal vein thrombosis in adults. A prospective evaluation of primary myeloproliferative disorders. *Gastroenterology* 1988; **94**: 1063-1069 [PMID: 3345875]

15 **Aiach M**, Borgel D, Gaussem P, Emmerich J, Alhenc-Gelas M, Gandrille S. Protein C and protein S deficiencies. *Semin Hematol* 1997; **34**: 205-216 [PMID: 9241706]

16 **Hwang S**, Kim do Y, Kim M, Chon YE, Lee HJ, Park YN, Park JY, Ahn SH, Han KH, Chon CY. [Deficiencies in proteins C and S in a patient with idiopathic portal hypertension accompanied by portal vein thrombosis]. *Korean J Hepatol* 2010; **16**: 176-181 [PMID: 20606502 DOI: 10.3350/kjhep.2010.16.2.176]

17 **Das SK**, Ray A, Jana CK, Banerjee N, Khaskil S. Chronic portal vein thrombosis due to combined deficiency of protein C and protein S. *J Indian Med Assoc* 2011; **109**: 753-754 [PMID: 22482325]

18 **Johnson NV**, Khor B, Van Cott EM. Advances in laboratory testing for thrombophilia. *Am J Hematol* 2012; **87** Suppl 1: S108-S112 [PMID: 22473489 DOI: 10.1002/ajh.23186]

19 **Ueno N**, Sasaki A, Tomiyama T, Tano S, Kimura K. Color Doppler ultrasonography in the diagnosis of cavernous transformation of the portal vein. *J Clin Ultrasound* 1997; **25**:

227-233 [PMID: 9314103 DOI: 10.1002/(SICI)1097-0096(199706)25:5<227::AID-JCU2>3.0.CO;2-F]
20 **Lee HK**, Park SJ, Yi BH, Yeon EK, Kim JH, Hong HS. Portal vein thrombosis: CT features. *Abdom Imaging* 2008; **33**: 72-79 [PMID: 17694406 DOI: 10.1007/s00261-007-9200-x]
21 **Smith CS**, Sheehy N, McEniff N, Keogan MT. Magnetic resonance portal venography: use of fast-acquisition true FISP imaging in the detection of portal vein thrombosis. *Clin Radiol* 2007; **62**: 1180-1188 [PMID: 17981166 DOI: 10.1016/j.crad.2007.06.007]
22 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]
23 **Sogaard KK**, Astrup LB, Vilstrup H, Gronbaek H. Portal vein thrombosis; risk factors, clinical presentation and treatment. *BMC Gastroenterol* 2007; **7**: 34 [PMID: 17697371 DOI: 10.1186/1471-230X-7-34]
24 **DeLeve LD**, Valla DC, Garcia-Tsao G. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
25 **Turnes J**, García-Pagán JC, González M, Aracil C, Calleja JL, Ripoll C, Abraldes JG, Bañares R, Villanueva C, Albillos A, Ayuso JR, Gilabert R, Bosch J. Portal hypertension-related complications after acute portal vein thrombosis: impact of early anticoagulation. *Clin Gastroenterol Hepatol* 2008; **6**: 1412-1417 [PMID: 19081529 DOI: 10.1016/j.cgh.2008.07.031]
26 **Malkowski P**, Pawlak J, Michalowicz B, Szczerban J, Wroblewski T, Leowska E, Krawczyk M. Thrombolytic treatment of portal thrombosis. *Hepatogastroenterology* 2003; **50**: 2098-2100 [PMID: 14696472]
27 **Hall TC**, Garcea G, Metcalfe M, Bilku D, Dennison AR. Management of acute non-cirrhotic and non-malignant portal vein thrombosis: a systematic review. *World J Surg* 2011; **35**: 2510-2520 [PMID: 21882035 DOI: 10.1007/s00268-011-1198-0]
28 **Amitrano L**, Guardascione MA, Scaglione M, Pezzullo L, Sangiuliano N, Armellino MF, Manguso F, Margaglione M, Ames PR, Iannaccone L, Grandone E, Romano L, Balzano A. Prognostic factors in noncirrhotic patients with splanchnic vein thromboses. *Am J Gastroenterol* 2007; **102**: 2464-2470 [PMID: 17958760 DOI: 10.1111/j.1572-0241.2007.01477.x]

P- Reviewer: Kobyliak NK, Wong GLH, Wong GLH
S- Editor: Ma YJ **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Hepatology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
www.wjgnet.com

World J Hepatol 2014 July 27; 6(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 peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJH covers topics concerning arrhythmia, heart failure, vascular disease, stroke, hypertension, prevention and epidemiology, dyslipidemia and metabolic disorders, cardiac imaging, pediatrics, nursing, and health promotion. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

WJH is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 43 OA clinical medical journals, including 42 in English, has a total of 15471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJH* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in hepatology; (12) Research Report: To briefly report the novel and innovative findings in hepatology; (13) Meta-Analysis: Covers the systematic review, mixedtreatment comparison, meta-regression, and overview of reviews, in order to summarize a given quantitative effect, *e.g.*, the clinical effectiveness and safety of clinical treatments by combining data from two or more randomized controlled trials, thereby providing more precise and externally valid estimates than those which would stem from each individual dataset if analyzed separately from the others; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJH*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of hepatology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Hepatology

ISSN

ISSN 1948-5182 (online)

Launch date

October 31, 2009

Frequency

Monthly

Editors-in-Chief

Clara Balsano, PhD, Professor, Departement of Biomedicine,

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_20100316080002.htm.

Indexed and Abstracted in
PubMed Central, PubMed, Digital Object Identifier, Directory of Open Access Journals, and Scopus.

SPECIAL STATEMENT

All articles published in journals owned by the BPG represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJH* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of BPG, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible

for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/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 bpgoffice@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

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

Title page

Title: Title should be less than 12 words.

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

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

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

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

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

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

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

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

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

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

Notes in tables and illustrations

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

should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

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

REFERENCES

Coding system

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

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

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.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/index.htm

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

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.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of BPG. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/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 papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJH is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 698 USD per article. All invited articles are published free of charge.

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com