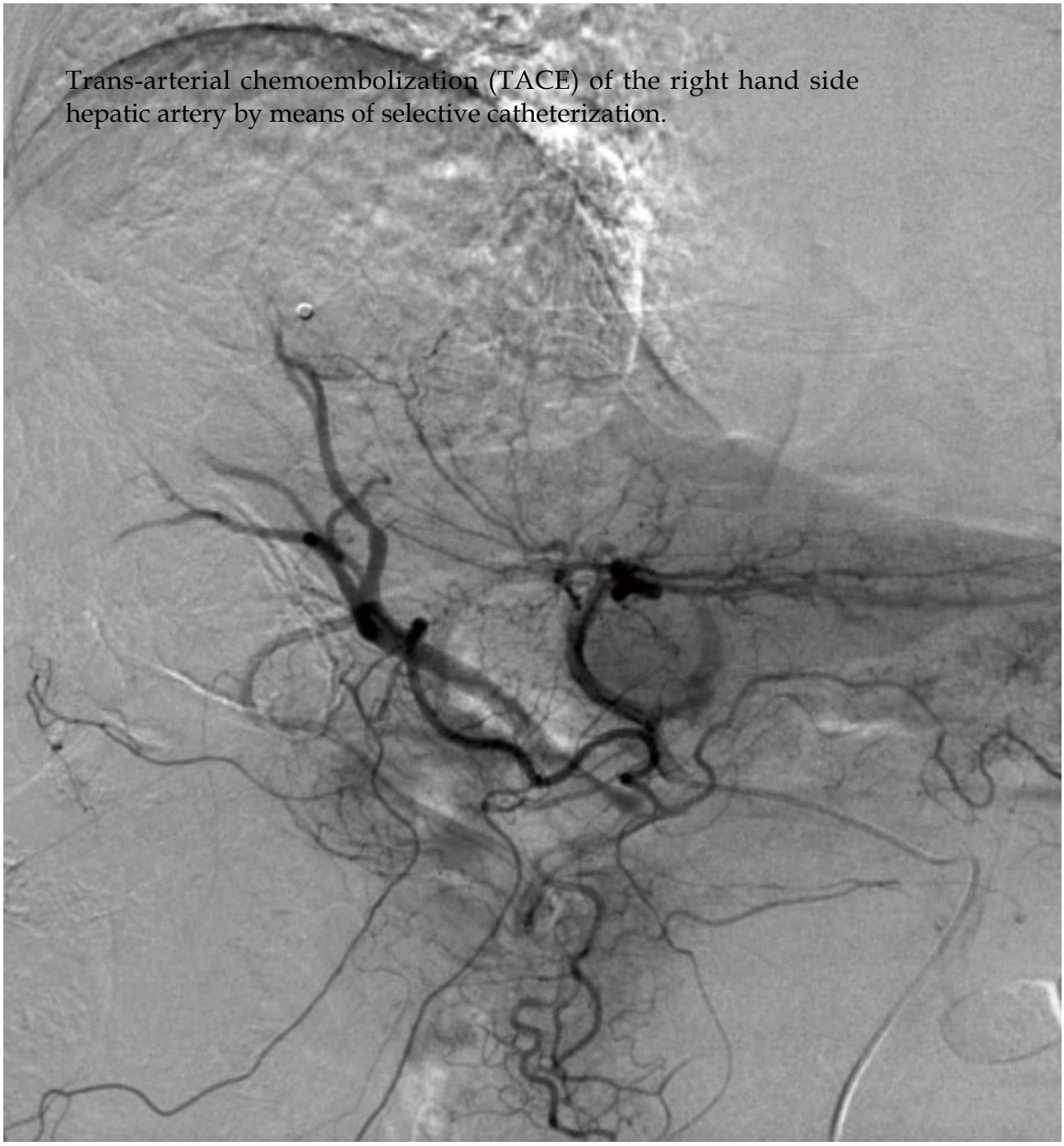
ISSN 1948-5182 (online)

World Journal of Hepatology

World J Hepatol 2010 January 27; 2(1): 1-54

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



Trans-arterial chemoembolization (TACE) of the right hand side hepatic artery by means of selective catheterization.

World Journal of Hepatology

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

Editorial Board

2009-2013

The *World Journal of Hepatology* Editorial Board consists of 575 members, representing a team of worldwide experts in hepatology. They are from 45 countries, including Argentina (4), Australia (7), Austria (2), Bangladesh (1), Belgium (3), Botswana (2), Brazil (8), Brunei Darussalam (1), Bulgaria (1), Canada (10), Chile (1), China (89), Egypt (3), Finland (1), France (15), Germany (28), Greece (8), Hungary (3), India (20), Ireland (1), Israel (7), Italy (65), Japan (45), Malaysia (1), Mexico (4), Pakistan (2), Poland (1), Portugal (1), Philippines (1), Romania (1), Saudi Arabia (1), Singapore (4), South Korea (17), Spain (22), Sri Lanka (1), Sudan (1), Switzerland (3), Thailand (6), The Gambia (1), The Netherland (4), Tunisia (2), Turkey (13), United Kingdom (17), United States (145) and Venezuela (1).

Stephen Lam Chan, *Hong Kong*
George G Chen, *Hong Kong*
Min-Shan Chen, *Guangzhou*
Yang Cheng, *Shanghai*
Siu Tim Cheung, *Hong Kong*
Thomas YC Cheung, *Hong Kong*
Yick-Pang Ching, *Hong Kang*
William Chi-shing Cho, *Hong Kong*
Chui Chung-hin, *Hong Kong*
Shuang-Suo Dang, *Xi'an*
Yi-Tao Ding, *Nanjing*
Jian-Gao Fan, *Shanghai*
Yuen Man Fung, *Hong Kang*
Zuojiong Gong, *Wuhan*
Tian-Quan Han, *Shanghai*
Jin-Yang He, *Guangzhou*
Garrett CL Ho, *Hong Kong*
Ji-Ming Hu, *Wuhan*
Canhua Huang, *Chengdu*
Zhi-Yong Huang, *Wuhan*
Jian-Hui Jiang, *Changsha*
Dong-Yan Jin, *Hong Kong*
Hsiang-Fu Kung, *Hong Kong*
Lai PBS Lai, *Hong Kong*
Wan YJ Lau, *Hong Kong*
Nancy WY Leung, *Hong Kong*
Jin-Qing Li, *Guangzhou*
Li-Ying Li, *Beijing*
Shu-Chen Li, *Harbin*
Xin-Wei Li, *Shanghai*
Yu-Yuan Li, *Guangzhou*
Enqi Liu, *Xi'an*
Yin-Kun Liu, *Shanghai*
Chung-Mau Lo, *Hong Kong*
Lun-Gen Lu, *Shanghai*
Ming-De Lu, *Guangzhou*
John M Luk, *Hong Kong*
Guang-Hua Luo, *Changzhou*
Shuang Mei, *Shanghai*
Kelvin KC Ng, *Hong Kong*
Qin Ning, *Wuhan*
Qin Pan, *Shanghai*
Qi-Jun Qian, *Shanghai*
Jian-Min Qin, *Shanghai*
Xianjun Qu, *Jinan*
Xue-Ying Sun, *Harbin*
Qin Su, *Beijing*
Wu-Yi Sun, *Hefei*
Huiru Tang, *Wuhan*
Peng Tao, *Nanning*
Eric WC Tse, *Hong Kong*
Bin Wang, Weifang
Xiaozhong Wang, *Fuzhou*
Xiu-Jie Wang, *Chengdu*
Zhen Xia Wang, *Huhhot*
Grace LH Wong, *Hong Kong*
Nathalie Wong, *Hong Kong*
Xiong-Zhi Wu, *Tianjin*
De-Xiang Xu, *Hefei*
Rui-An Xu, *Quanzhou*
Xun Di Xu, *Changsha*
Xiao Yang, *Beijing*
Zhen-Fan Yang, *Hong Kong*
Boon Hun Yong, *Hong Kong*
Ting-He Yu, *Chengdu*
Benny CY Zee, *Hong Kong*
Jia-Ning Zhang, *Dalian*
Xiao-Dong Zhang, *Tianjin*
Xiao-Lan Zhang, *Shijiazhuang*
Xiao-Yan Zhang, *Shanghai*
Hong-Chuan Zhao, *Hefei*
Xiao-Ping Zhao, *Beijing*
Jiang Fan Zhu, *Shanghai*
Yi-Ping Zou, *Beijing*

Egypt

Nabil Mohie Abdel-Hamid, *Minia*
Laila AF Eissa, *Mansoura*
Mona Mostafa Fahmy Nosseir, *Giza*

Finland

Thomas Kietzmann, *Oulu*

France

Aramando Abergel, *Clenmont -Ferrant*
Henri Bismuth, *Villejuif Cedex*
Ana CFN Cardoso, *Paris*
Nicolas Chignard, *Paris*
Claude C de Fromentel, *Lyon*
Zdenko Herceg, *Lyon*
Nathalie Janel, *Paris*
Victor de Ledinghen, *Pessac cedex*
Antoinette Lemoine, *Villejuif*
Marcellin Patrick, *Clichy*
Raoul Poupon, *Paris*
Rodrigue Rossignol, *Bordeaux cedex*
Christian Trépo, *Lyon*
Dominique A Vuitton, *Besancon*
Virginie Wautot, *Pierre Benite*

Germany

Thomas Bock, *Tuebingen*
Ali Canbay, *Essen*
Enrico Narciso De Toni, *München*
Joachim Drevs, *Freiburg*
Volker Fendrich, *Marburg*
Peter R Galle, *Mainz*
Erich Gulbins, *Essen*
Roland Kaufmann, *Jena*
Sebastian Hinz, *Kiel*
Philipp Kobbe, *Aachen*
Michael Kremer, *Heidelberg*
Christian Liedtke, *Aachen*
Martin Loss, *Regensburg*
Arun Kumar Mankan, *Munich*
Lars Müller, *MD, Kiel*
Michael D Menger, *Saarbrucken*
Andreas K Nussler, *Munich*
Margarete Odenthal, *Koeln*
Claus Petersen, *Hannover*
Andrej Potthoff, *Hannover*
Thomas Pusl, *München*
Elke Roeb, *Giessen*
Frank Tacke, *Aachen*
Stefan Rose-John, *Kiel*
Andreas Teufel, *Mainz*
Lothar Thomas, *Frankfurt*
Jens JW Tischendorf, *Aachen*
Arndt Vogel, *Hannover*

Greece

Alex P Betrosian, *Athens*
Spiros G Delis, *Athens*
Ioannis Diamantis, *Athens*
Papandreou Dimitrios, *Mela*
Moses S Elisaf, *Ioannina*
Elias A Kouroumalis, *Crete*
George Papatheodoridis, *Athens*
Stamatios E. Theocharis, *Athens*

Hungary

Gábor Bánhegyi, *Budapest*
Subhamay Ghosh, *Pécs*
Peter Nagy, *Budapest*

India

Anjali Deepak Amarapurkar, *Mumbai*
DN Amararpurkar, *Mumbai*
Runu Chakravarty, *Kolkata*
Pronobesh Chattopadhyay, *Moradabad*
Puneet Chopra, *Gurgaon Haryana*
Tanya Das, *Kolkata*
Radha Krishan Dhiman, *Chandigarh*
Ajay Duseja, *Chandigarh*
Devendra K Gupta, *New Delhi*
P Kar, *New Delhi*
Sudhir Kumar, *Lucknow*
Vijay Kumar, *New Delhi*
Anoop Misra, *New Delhi*
Devendra Parmar, *Lucknow*
Rajendra Prasad, *Chandigarh*
K Rajeshwari, *New Delhi*
Pallu Reddanna, *Hyderabad*
Barjesh Chander Sharma, *New Delhi*
Sarman Singh, *New Delhi*
Ajith TA, *Thrissur*

Ireland

Matthew William Lawless, *Dublin*

Israel

Yaron Ilan, *Jerusalem*
Yaakov Maor Kendler, *Tel Hashomer*
Ran Oren, MD, *Tel Aviv*
Amir Shlomai, *Modiin*
Rifaat Safadi, *Jerusalem*
Shira Zelber Sagi, *Tel Aviv*
Yehuda Julius Shoenfeld, *Tel Hahsomer*

Italy

Luca Aasaloni, *Bologna*
Giovanni Addolorato, *Rome*

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

Japan

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

Malaysia

Kamsiah Jaarin, *Kuala Lumpur*

Mexico

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

Pakistan

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

Poland

Maria ES Lotowska, *Bialystok*

Portugal

Felix Dias Carvalho, *Porto*

Philippines

Janus P Ong, *Manila*

Romania

Eugen Georgescu, *Craiova*

Saudi Arabia

Ahmed Helmy, *Riyadh*

Singapore

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

South Korea

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

Spain

Jose AG Agundez, *Badajoz*
Maria Angeles, *Madrid*
Agustin Castiella, *Mendaro*
Ruben Ciria, *Cordoba*
Joan Clari, *Barcelona*
Maria Buti Ferret, *Barcelona*
Puri Fortes, *Pamplona*
Joan Genescà, *Barcelona*
María J Gómez-Lechón, *Valencia*
Arias Jaime, *Madrid*
Ángeles Pajares María, *Madrid*
Jordi Muntane, *Cordoba*
Jose JG Marin, *Salamanca*
Julia P Onsurbe, *Barcelona*
Albert Parés, *Barcelona*
Sonia Ramos, *Madrid*
Cristina Ripoll, *Madrid*
Isabel F Romero, *Barcelona*
Marta R Romero, *Salamanca*
Juan Macias Sanchez, *Sevilla*

Juan Sastre, *Valencia*
Manuel Vázquez-Carrera, *Barcelona*

Sri Lanka

EGD Shaman Rajindrajith, *Ragama*

Sudan

Hatim MY Mudawi, *Khartoum*

Switzerland

Beat Mullhaupt, *Zurich*
Brigitte M Frey-von Matt, *Berne*
Maurer A Christoph, *Liestal*

Thailand

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

The Gambia

Maimuna Ebirunkeh Mendy, *Banjul*

The Netherlands

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

Tunisia

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

Turkey

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

United Kingdom

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

United States

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

WJH World Journal of Hepatology

Contents

Monthly Volume 2 Number 1 January 27, 2010

World Journal of Hepatology
Volume 2 Number 1 January 27, 2010

Contents

NAME OF JOURNAL
World Journal of Hepatology

LAUNCH DATE
October 31, 2009

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

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

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

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

ONLINE SUBSCRIPTION
One-Year Price 216.00 USD

PUBLICATION DATE
January 27, 2010

CSSN
ISSN 1948-5182 (online)

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

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-5182/g_info_17.htm. If you do not have web access please wcontact the editorial office.

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

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.1

World J Hepatol 2010 January 27; 2(1): 1-7
ISSN 1948-5182 (online)

EDITORIAL

Hepatospheres: Three dimensional cell cultures resemble physiological conditions of the liver

Franziska van Zijl, Wolfgang Mikulits

Franziska van Zijl, Wolfgang Mikulits, Department of Medicine I, Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria
Author contributions: van Zijl F prepared and Mikulits W edited the manuscript.
Supported by the Austrian Science Fund, FWF, NO. P19598-B13 and SFB F28, the "Hochschuljubiläumsstiftung der Stadt Wien", the Herzfelder Family Foundation, and the European Union, FP7 Health Research, NO. HEALTH-F4-2008-202047
Correspondence to: Wolfgang Mikulits, PhD, Department of Medicine I, Institute of Cancer Research, Medical University of Vienna, Borschkegasse 8a, A-1090 Vienna, Austria. wolfgang.mikulits@meduniwien.ac.at
Telephone: +43-1-427765250 Fax: +43-1-427765239
Received: July 2, 2009 Revised: November 10, 2009
Accepted: November 17, 2009
Published online: January 27, 2010

Abstract

Studying physiological and pathophysiological mechanisms in the liver on a molecular basis is a challenging task. During two dimensional (2D) culture conditions hepatocytes dedifferentiate rapidly by losing metabolic functions and structural integrity. Hence, inappropriate 2D hepatocellular models hamper studies on the xenobiotic metabolism of the liver which strongly influences drug potency. Also, the lack of effective therapies against hepatocellular carcinoma shows the urgent need for robust models to investigate liver functions in a defined hepatic microenvironment. Here, we summarize and discuss three-dimensional cultures of hepatocytes, herein referred to as hepatospheres, which provide versatile tools to investigate hepatic metabolism, stemness and cancer development.

Key words: Hepatosphere; Hepatocyte; Tissue engineering; 3D culture; Microenvironment

Peer reviewer: Fiona J Warner, Dr, Liver Cell Biology, Centenary Insitute, Locked Bag No. 6, Newtown, NSW, 2042, Australia

van Zijl F, Mikulits M. Hepatospheres: Three dimensional cell cultures resemble physiological conditions of the liver. *World J Hepatol* 2010; 2(1): 1-7 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/1.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.1

INTRODUCTION

The most essential metabolic processes of the human body take place in the liver. The major responsibilities of this multifunctional organ include the regulation of blood glucose levels, the metabolism of lipids and proteins, the detoxification of urea and the production of bile. In addition, the liver has a vast impact on the biotransformation of xenobiotic compounds. Studying the liver in its physiological and pathophysiological situations is therefore crucial for drug development. However, inadequate model systems hinder the investigations of the complex mechanisms underlying those liver functions. The development of experimental tools capable of identifying components in the regulatory circuits of liver functions remains a challenging task.

Hepatocytes, which represent the majority of the liver mass and perform most of the functions of the liver, are mitotically inactive under physiological conditions. Nevertheless, they show tremendous proliferative properties in cases of liver damage[1]. The inability to induce proliferative signals in hepatic cell cultures without losing differentiation is one of the key problems, and is still an unsolved issue. Primary hepatocytes *in vitro* quickly loose their typical characteristics as indicated by the absence of hepatic markers, such as the secretion of albumin (ALB)[2-4]. In particular, hepatocytes show a rapid decrease of liver-specific functions such as

the xenobiotic metabolism, which is of paramount importance for drug development.

Many aspects of liver physiology and liver disease are still open issues and many gaps still need to be filled. The reasons why 3D cultures of hepatocytes, designated as hepatospheres, represent a robust and versatile tool to investigate metabolic functions and mechanisms of hepatocellular carcinoma (HCC) will be summarized and discussed in this review.

3D MODELS: PAST AND PRESENT

A spheroid is the aggregation of cells exhibiting the most energy- and surface-minimized structure[5-7], and remarkably, this self-assembly of cells mimics natural processes in embryogenesis, morphogenesis and organogenesis[8,9]. The overall aim of 3D spheroid cultures is specifically to reflect important aspects of the physiological situation *in vitro*. Cells cultured in 3D spheroids resemble the *in vivo* situation quite efficiently regarding their cell shape and cellular environment, which influences both gene expression and biological behavior.

The attempt to form adequate physiological structures *in vitro* remained a challenge for decades. In 1952, Moscona and Moscona observed that co-cultivation of chondrogenic and myogenic cells of an early chick embryo in suspension spontaneously formed aggregations[9]. Interestingly, the chondrogenic cells formed the inner core, which was surrounded by a layer of myogenic cells. In 1961, A. Moscona described an approach to generate cell interactions *in vitro* by using a rotational technique[10]. Histoformative multicellular aggregates were generated which were quantifiable, controllable and reproducible. The ability of cell types to form aggregates within 24 h was termed 'aggregation pattern' and was described as specific for certain cell types and for a set of conditions, the most important of which was temperature. More than twenty years later, 3D aggregations were first labelled 'spheroid' by Landry[11,12], who observed a spheroid formation by cultivating cells on a non-adherent plastic surface. This early study already showed that this type of cultivation caused cells originating out of a perfused liver to re-aggregate into structures resembling those found *in vivo*[11]. This was accompanied by the production of an extracellular matrix (ECM) consisting of laminin, fibronectin and collagen. Interestingly, this study also showed that hepatocytes cultured in a 3D system display prolonged survival and enhanced metabolic functions, such as ALB secretion and induction of tyrosine aminotransferase, which was maintained for up to two months post isolation.

Nowadays, 3D systems, such as mammospheres, which represent cultures of mammary carcinoma cells, are well developed, and widely used for testing and developing novel drugs. The response to Trastuzumab, for instance, a clinically used antibody against human epidermal growth factor receptor (HER)2/neu, was shown to vary strongly between 2D and 3D cultures. It has been proposed that homodimerization of HER2 is favored in spheres of both mammary carcinoma cells as well as ovarian carcinoma cells, which is the reason for the enhanced activation of the drug in 3D, as compared to 2D, cultures[13]. Furthermore, the tumor-promoting mitogen-activated protein kinase (MAPK) signaling and integrin-β4 phosphorylation are particularly activated in mammospheres. The ability of acini formation in 3D cultures and expression profiling of mammospheres led to defined gene expression patterns which can be used as a prognostic factor for the outcome of estrogen receptor ER+/ER- mammary carcinomas[14,15]. Furthermore, 3D lung organotypic models were employed for studying the mammary tumor outgrowth during the progression to distal metastasis[16]. Interestingly, the authors observed an enhancement of tumor proliferation upon co-cultivation with pulmonary cells.

The spheroid technique has been extended to a variety of other cell types, applications and (patho)physiological situations. For instance, the induction of angiogenesis was studied in oral squamous cells[17] as well as in murine colorectal carcinoma cells[18] where the authors showed that 3D cultures promote angiogenesis during hepatic colon carcinoma metastasis. Furthermore, co-culture models were established to study blood vessel maturation. A human 3D model of mixed spheroids showed that hepatic stellate cells (HSC) caused the quiescence of co-cultured endothelial cells (EC)[19]. However, it is known that HSC infiltrate the tumor tissue and recruit EC to induce angiogenesis, which was shown throughout hepatic metastasis of melanoma cells[20]. Furthermore, rodent hepatospheres were shown to be anchored by co-cultured HSC, whereas hepatic spheres alone detached from the surface[21]. Interestingly, the authors observed that HSC invaded into hepatospheres by forming thin processes which eventually retracted again.

These investigations show that spheroid cultures are nowadays used in a plethora of applications, resembling physiological structures and 3D organotypic models which facilitate the identification of stem cells[22], and the molecular characterization of cell-cell interactions, and allow us to study the invasion of cancer cells[23].

GENERATION OF 3D HCC MODELS

A variety of methods has been developed to form spheroids and to establish physiologically relevant models for reconstituting hepatic tissue. In general, the overall aim is to find robust and reproducible techniques for the generation of spheroids allowing molecular analysis. The easiest approach is plating cells on a non or ultra-low adherent culture dish in a serum-free medium[11] (Figure 1A). Spheroid formation was observed using this technique with the human HCC cell line Huh7 and with embryonal murine primary hepatocytes[24,25]. Interestingly, a combination of hepatocyte growth factor (HGF), basic fibroblast growth

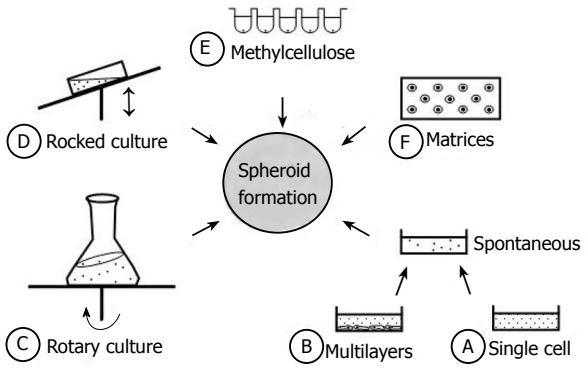


Figure 1 Different approaches to the generation of 3D spheroid cultures.

factor and (EGF) supplemented within the medium led to an 8-fold enhanced spheroid formation[25]. A similar approach was taken with primary rat hepatocytes, by plating 42 000 cells per cm² culture dishes, and culturing them with a special spheroid medium containing Insulin, L-Glutamine, EGF, linoleic acid-albumin, plus traces of copper, selenium and zinc. Hepatocytes initially attached to the culture dishes, forming a monolayer, and subsequently generated spheroids that finally detached from the surface[21]. Another approach observed spheroid formation after seeding murine hepatoma cells into agarose-coated 96-well plates at a density of 2 000 cells per well[26]. In addition, this technique allows spheroids to be fixed in formalin and further processed for immunohistochemistry. Moreover, different co-culturing methods were tested upon induction of hepatic spheroid formation. Qihao *et al*[27] could show that mesenchymal stem cells (MSC) isolated from rat bone marrow differentiated into hepatocytes when overlaid with primary hepatocytes in co-cultures (Figure 1B). Remarkably, MSC gained the ability to form spheroids and expressed hepatic markers after 14 to 21 d.

Apart from stationary cultures, many rotary culture systems were established (Figure 1C). For instance, rat hepatocytes were isolated and either incubated in a rotating 6-well plate[28,29] or a 250 mL spinner vessel which was stirred by a magnetic stir bar at 90-100 rpm[30]. In the latter method, first aggregations were observed after one hour, and compact spheroids after 48 h. Moreover, commercial available rotating wall vessel bioreactors were used, such as the "High Aspect Rotating Vessel" system[2].

Recently, a rocking technique was described (Figure 1D), which in contrast to rotary and stationary techniques uses both non-coated and collagen-coated dishes[3]. Rat primary hepatocytes were plated at a density of 1×10^6 cells/mL and continuously rocked at 0.25 Hz. Spheroid formation was observed after 4 h in both rotational and rocking systems. Due to the enhanced collision in the rocking method, less single cells were observed, along with larger and more compact spheroids. In accordance with the above described studies, spheroids seem to reach their maximum diameter of 125-150 μm on day 2. Thus, a diameter of 100-150 μm is considered the most stable size that spheroids adopt[3]. This is in accordance with the earlier studies in the 1960s[10]. Importantly, spheroid formation in general is strongly dependent on the presence of free divalent Ca^{2+} ions in the medium. Supplementing ethylene glycol tetra acetic acid (EGTA), a chelating agent with high affinity to Ca^{2+}, removes free Ca^{2+} from the medium and results in the rapid disassembly of spheroids and impairment of spheroid nosogenesis[3].

A non-rotating or non-rocking approach is the incubation of cells in methylcellulose-containing medium (Figure 1E), which was, for example, employed for co-cultures of human umbilical vein endothelial cells, human umbilical artery smooth muscle cells, and for rat EC with smooth muscle cells[19]. Using this method, cells are plated in non-adherent round bottom wells to form spheroids of defined cell number and composition. Subsequently, the spheroids can be embedded into collagen gels and are thus suitable for a prolonged observation over a couple of days. As an alternative approach, a variety of different artificial matrices were manufactured to facilitate the formation of 3D cellular aggregations into defined structures (Figure 1F). For instance, a self-designed micromolded non-adhesive agarose hydrogel was used to shape the outgrowth of hepatocytes into rods and honeycombs[31]. In another study, a combination of microfabrication and microcontact printing, which was used to design surfaces with supporting or inhibiting cell adhesion, induced 3D aggregations of hepatocytes of defined size[4,32]. The advantage of this technique is the distinct location and size of the spheroids. However, those spheroids do not show a pronounced polarity, although they express the gap-junction protein Connexin 32 and the adherence junction marker E-cadherin[4]. Another non-rotating and non-rocking technique is the formation of spheres in hanging drops[18,33], where small amounts of cell suspension are incubated upside down until spontaneous spheroid formation occurs.

RELEVANCE OF HEPATOSPHERES

Liver physiology

Whereas 2D culturing of hepatocytes results in rapid loss of differentiation and hepatic gene expression, various studies have shown that hepatospheres closely resemble the situation *in vivo*[29,30]. Self-assembly of hepatocytes results in a structural similarity to native tissue. The surface is smooth and permeated by numerous pore-like openings. These pores have been demonstrated to be the entrances to microvilli-lined channels which are similar to canaliculi. Apically, hepatocytes transport bile acids whereas the basal site conducts trafficking of metabolites from the bloodstream. The polarity of hepatocytes in a sphere was determined by both apical HA4 and basolateral HA321 staining[30], and the network of the numerous channel structures was visualized by FITC-dextran[30]. To test the functional activity of apical secretion, hepatospheres were exposed to a pseudo bile acid, i.e. FITC-glycocholate, and thus active and directed bile secretion of hepatocytes was monitored in 3D

cultures. In order to ensure the integrity of epithelial organization, the adherens junction component E-cadherin was shown as an important mediator for spheroid formation in a variety of cell types, including primary hepatocytes[34], cells of renal carcinoma[35], breast cancer[36] as well as prostate cancer[37]. Moreover, α5β1 integrin and fibronectin were also identified as regulators of spheroid formation[38-40]. Correlating with these findings, self-assembly of hepatocytes and human fibroblasts in micromolded agarose hydrogels could bring about the formation of different shapes, depending on cell type, co-culture conditions and different surface tensions[31].

The dynamic process of spheroid formation is considered a three-step process[33]. In the first phase, single cells rapidly aggregate, depending on ECM-integrin interaction. The second phase is a delay period, involving E-cadherin accumulation, and is followed by the third phase, in which spheroids get their compact features[33]. Interference with integrinβ1 was shown to delay the first phase, whereas interference with E-cadherin blocked the compaction of spheroids. In addition, the expression levels of ECM components and E-cadherin from different human HCC cell lines such as Hep3B, HepG2 or PLC/PRF/5 have been shown to modulate the facility for spheroid formation. Whereas Hep3B and HepG2 rapidly and moderately form spheroids, respectively, PLC/PRF/5 cells fail to aggregate at all, which corresponds to their expression levels of ECM and E-cadherin[33].

Liver-specific metabolism

The metabolic activity of hepatocytes is rapidly lost when cultured in monolayers, but many studies have shown the maintenance of the liver-specific metabolism in hepatospheres. In one such study, 2D and 3D cultures of HCC cells were studied for their differences in gene expression patterns[2]. In hepatospheres, genes involved in xenobiotic metabolism and lipid metabolism, such as cytochrome P450 (CYP)1A1, aldo-keto reductase 1C1, leukotriene B_4 12-hydroxydehydrogenase, epoxidhydrolase X1 and glutathione S-transferase A1, were expressed more highly when compared to their expression in 2D cultures. This was verified by testing the metabolic function of hepatospheres, showing an upregulation of leukotrine, cholesterol metabolism and synthesis of glutathione, ALB[2-4] and ATP. The elevated function of phase I enzymes in hepatospheres was tested by processing 7-ethoxyresurfin to resurfin through CYP1A1 and CYP1A2, and diazepam to oxazepam via CYP3A[3]. In addition, hepatospheres showed an active urea cycle and expression of liver-enriched transcription factors such as hepatocyte nuclear factor (HNF)-4 and CCAAT/enhancer-binding protein (C/EBP-β) both of which are required for the expression of ornithine transcarbamylase[4]. Similarly, a further study compared four different culture conditions on gene expression, namely monolayer cultures in the presence and absence of a collagen coat versus hepatospheres formed either by the rotational or rocked technique[3]. An array of 242 liver-specific genes showed that 85% of these were stably expressed in hepatospheres cultured in the rocked fashion. In particular, ALB synthesis as well as the activities of enzymes involved in the urea cycle, blood clotting, and xenobiotic phase I and II metabolism were significantly enhanced. Interestingly, transfer of hepatospheres onto tissue culture plates, thus culturing them under 2D conditions, revealed a rapid loss of liver-specific functions such as ALB secretion and CYP1A1 activity[2]. These data indicate that metabolic functions of hepatocytes are rapidly lost in 2D cultures whereas liver-specific activities are maintained in hepatospheres.

Further studies have demonstrated, by testing the toxicity of five different compounds[29,41,42], that hepatospheres retain their capability for functional metabolism[29]. All compounds significantly decreased glucose secretion, which is thus suggested as the most sensitive endpoint. Similarly, another investigation established a screening method for hepatotoxicity, where Galactosamine, Propanol, Diclofenac and Paracetamol were analyzed for their negative impact on anchorage dependence, cellular morphology and cell spreading. Interestingly, the xenobiotic influence on those features correlated with elevated lactate dehydrogenase release and gamma-glutamyl transferase levels in both primary liver spheroids as well as on HepG2-derived hepatospheres[28]. Taken together, these studies show that hepatospheres are useful for testing hepatotoxicity, energy metabolism and biotransformation of xenobiotics, as these 3D cultures resemble particular aspects of the physiological situation.

Induction of prolonged survival and stemness

Induction of stemness is a central issue in tissue reconstitution. In mammospheres it has been shown that culturing of mammary epithelial cells in three dimensional spheroids leads to induction of stemness. Some cells derived from mammospheres show repopulating activity capable of regenerating an entire mammary gland after orthotopic transplantation[43,44]. Likewise, many investigators use hepatospheres to identify hepatic stem cells. One experimental approach was able to expand hepatic stem/progenitor cells by using fetal liver tissue of C57BL/6 mice post day 13.5 of gestation[25]. After spontaneous formation of hepatospheres, spheroids were plated onto collagen coated plates, where cells spontaneously formed monolayers. After 21 d in culture, large colonies of bigger cells were visible at the periphery, expressing cytokeratin (CK)-7, a marker characteristic of cholangiocytes, whereas small cell clusters were observed in the center, which were positive for ALB or α-feto protein (AFP). Long-term culturing revealed primary liver cells which could be cultivated for four months by continuously expressing AFP, ALB, α1-antitrypsin, glucose-6 phosphate, CK-19 and biliary glycoprotein[25]. Differentiation of hepatocytes only took place after

Table 1 Characteristics of hepatospheres

Classifications	Ref.
Maintenance of physiological structure	
Reassembling of liver cells in a physiological pattern, formation of ducts	[11,30]
Polarity, apical secretion and function of CD26	[30]
E-cadherin: Induction of spheroid formation	[3,33,34]
Integrin signaling	[33,38-40]
Elevated ECM production	[2,11,33,38-40,45]
Prolonged survival	[11,25]
Physiological expression pattern	[2,3]
Prolonged metabolic functions	
Secretion of albumin	[2-4,11,21,24,25,27]
Tyrosine aminotransferase	[11]
Glutathione S transferase	[2]
Detoxification of urea	[3,4]
Biotransformation of xenobiotics (CYP)	[3,28,41,42]
Induction of stemness	
AFP	[25,27]
CK19	[24,25]
Resembling of HCC	
Angiogenesis	[26,45]

adding Oncostatin M, an inductor of hepatocellular outgrowth. In addition, long-term cultured cells were able to repopulate the liver after orthotopic injection into carbon tetrachloride-treated livers[25].

Another interesting approach showed the differentiation of MSC isolated from Sprangue-Dawley rats into hepatocytes within 21 d, when co-cultured with isolated primary hepatocytes[27]. After differentiation into hepatocytes, the cells produced AFP, CK-18, ALB and glycogen, and spontaneously formed spheroids. This method represents an attempt to generate hepatocytes from MSC which might be a promising approach to cultivate and expand individual hepatocytes for autologous treatment of liver diseases.

Liver cancer

The identification and characterization of hepatic stem cells is not only key for tissue reconstitution, but is also of particular relevance for understanding the molecular mechanisms underlying HCC. In this regard, the human HCC cell line Huh7 was investigated for the presence of a distinct side population by using Pyronin Y and Hoechst 33342 dye. Remarkably, the isolated side population was capable of developing hepatocytes and cholangiocytes which were able to self-renew themselves and may thus contribute to the hepatic cancer stem cell fraction[24]. Almost 80% of the side population was in the G0 phase, and in contrast to the remaining continuously cycling cell fraction, those cells were able to form spheroids in a serum-free medium on non-adherent culture dishes showing an enhanced tumorigenic potential after subcutaneous injection into mice[24]. Interestingly, the cycling cell fraction showed ALB secretion and was negative for CK-19 whereas G0 cells showed only weak expression of ALB but expressed CK-19, the smallest acidic cytokeratin which correlates with poor prognosis in HCC.

Further molecular mechanisms of HCC have been investigated by employing spheroid cultures and focusing on angiogenesis. For example, murine hepatoma cells were tested for the influence of hypoxia-inducible factor (HIF)-1α expression on tumorigenesis[26]. Although the capacity of HIF-1α to induce angiogenesis is well known, the amount of necrosis located in centers of hepatospheres was independent of HIF-1α expression. Yet HIF-1α was found to act as contributing to survival by reducing proliferation but enhancing survival[26].

Hepatospheres also represent a versatile model system to investigate the molecular interaction and communication between different cell types involved in HCC development. Recently, we were able to reveal the promoting role of fibroblasts upon tumor proliferation by subcutaneous co-transplantation of murine HCC cells with activated fibroblasts into mice (van Zijl *et al*; submitted). Moreover, we found an epithelial to mesenchymal transition (EMT) of Ras-transformed hepatocytes at the invasive front of hepatospheres, which has been shown to be dependent on the paracrine secretion of transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF) of co-cultivated myofibroblasts. Interestingly, pharmacological interference with this molecular tumor-stroma crosstalk abrogated cell invasion. In accordance, another study analyzed the tumorigenesis of the human HCC cell lines HepG2, Hep3B and PLC in the presence of HSC[45]. Incubation with conditioned medium of human HSC promoted proliferation and migration. Hepatospheres mixed with HSC grew larger and had fewer necrotic centers when compared with hepatospheres without HSC. This correlated with the finding that co-transplantation of malignant hepatocytes and HSC *in vivo* resulted in increased tumor growth[45,46], elevated ECM production and increased CD31 expression, indicating elevated angiogenesis[45]. Incubation with conditioned medium derived from human HSC cultures and interfering with Erk/MAPK and HGF signaling pathways reduced proliferation of HCC cells, whereas inhibition of TGF-β signaling was not able to modulate tumor proliferation. These data are in contrast to recent findings obtained in mice[46,47].

CONCLUDING REMARKS AND FUTURE PROSPECTS

In summary, the advantages of hepatospheres are numerous (Table 1) and thus, 3D hepatic models are promising for a variety of experimental approaches. In particular, hepatospheres can be used for physiological structure analysis of self-assembled cells, for *in vitro* toxicity testing of different drugs, for investigating the impact of toxic compounds and even for tissue engineering. Furthermore, hepatospheres enhance stem-cell like features and will consequently shed light on stem-cell research, ranging from isolating and expanding stem cells for tissue reconstitution to the benefit of identifying

hepatic cancer stem cells. The ability to study molecular cell-cell interactions in a defined hepatic microenvironment will facilitate the clarification of autocrine and paracrine regulatory loops in it. This might help to answer the question whether fibroblasts co-evolve with tumor cells or cease mutating in HCC development[48]. Drugs targeting the dynamic tumor-stroma interaction can be tested in a well-defined microenvironment and vice versa, and the resistance of hepatic tissues towards drugs can be assessed. Indeed, a variety of recent studies have demonstrated the impact of culture conditions on drug efficiency[11,49-52]. The ultimate task of hepatospheres is the stable and reliable engineering of hepatic tissue for a wide range of applications, including investigations of the molecular mechanisms of liver diseases as well as the development of drugs.

ACKNOWLEDGMENTS

The authors thank Dr. Markus Grubinger for critical comments and helpful discussions.

REFERENCES

1 **Taub R**. Liver regeneration: from myth to mechanism. *Nat Rev Mol Cell Biol* 2004; **5**: 836-847
2 **Chang TT**, Hughes-Fulford M. Monolayer and spheroid culture of human liver hepatocellular carcinoma cell line cells demonstrate distinct global gene expression patterns and functional phenotypes. *Tissue Eng Part A* 2009; **15**: 559-567
3 **Brophy CM**, Luebke-Wheeler JL, Amiot BP, Khan H, Remmel RP, Rinaldo P, Nyberg SL. Rat hepatocyte spheroids formed by rocked technique maintain differentiated hepatocyte gene expression and function. *Hepatology* 2009; **49**: 578-586
4 **Fukuda J**, Sakai Y, Nakazawa K. Novel hepatocyte culture system developed using microfabrication and collagen/polyethylene glycol microcontact printing. *Biomaterials* 2006; **27**: 1061-1070
5 **Jia D**, Dajusta D, Foty RA. Tissue surface tensions guide in vitro self-assembly of rodent pancreatic islet cells. *Dev Dyn* 2007; **236**: 2039-2049
6 **Foty RA**, Pfleger CM, Forgacs G, Steinberg MS. Surface tensions of embryonic tissues predict their mutual envelopment behavior. *Development* 1996; **122**: 1611-1620
7 **Foty RA**, Steinberg MS. The differential adhesion hypothesis: a direct evaluation. *Dev Biol* 2005; **278**: 255-263
8 **Duguay D**, Foty RA, Steinberg MS. Cadherin-mediated cell adhesion and tissue segregation: qualitative and quantitative determinants. *Dev Biol* 2003; **253**: 309-323
9 **Moscona A**, Moscona H. The dissociation and aggregation of cells from organ rudiments of the early chick embryo. *J Anat* 1952; **86**: 287-301
10 **Moscona A**. Rotation-mediated histogenetic aggregation of dissociated cells. A quantifiable approach to cell interactions in vitro. *Exp Cell Res* 1961; **22**: 455-475
11 **Landry J**, Bernier D, Ouellet C, Goyette R, Marceau N. Spheroidal aggregate culture of rat liver cells: histotypic reorganization, biomatrix deposition, and maintenance of functional activities. *J Cell Biol* 1985; **101**: 914-923
12 **Landry J**, Freyer JP. Regulatory mechanisms in spheroidal aggregates of normal and cancerous cells. *Recent Results Cancer Res* 1984; **95**: 50-66
13 **Pickl M**, Ries CH. Comparison of 3D and 2D tumor models reveals enhanced HER2 activation in 3D associated with an increased response to trastuzumab. *Oncogene* 2009; **28**: 461-468
14 **Fournier MV**, Martin KJ, Kenny PA, Xhaja K, Bosch I, Yaswen P, Bissell MJ. Gene expression signature in organized and growth-arrested mammary acini predicts good outcome in breast cancer. *Cancer Res* 2006; **66**: 7095-7102
15 **Martin KJ**, Patrick DR, Bissell MJ, Fournier MV. Prognostic breast cancer signature identified from 3D culture model accurately predicts clinical outcome across independent datasets. *PLoS One* 2008; **3**: e2994
16 **Martin MD**, Fingleton B, Lynch CC, Wells S, McIntyre JO, Piston DW, Matrisian LM. Establishment and quantitative imaging of a 3D lung organotypic model of mammary tumor outgrowth. *Clin Exp Metastasis* 2008; **25**: 877-885
17 **Fischbach C**, Kong HJ, Hsiong SX, Evangelista MB, Yuen W, Mooney DJ. Cancer cell angiogenic capability is regulated by 3D culture and integrin engagement. *Proc Natl Acad Sci USA* 2009; **106**: 399-404
18 **Valcárcel M**, Arteta B, Jaureguibeitia A, Lopategi A, Martínez I, Mendoza L, Muruzabal FJ, Salado C, Vidal-Vanaclocha F. Three-dimensional growth as multicellular spheroid activates the proangiogenic phenotype of colorectal carcinoma cells via LFA-1-dependent VEGF: implications on hepatic micrometastasis. *J Transl Med* 2008; **6**: 57
19 **Wirz W**, Antoine M, Tag CG, Gressner AM, Korff T, Hellerbrand C, Kiefer P. Hepatic stellate cells display a functional vascular smooth muscle cell phenotype in a three-dimensional co-culture model with endothelial cells. *Differentiation* 2008; **76**: 784-794
20 **Olaso E**, Salado C, Egilegor E, Gutierrez V, Santisteban A, Sancho-Bru P, Friedman SL, Vidal-Vanaclocha F. Proangiogenic role of tumor-activated hepatic stellate cells in experimental melanoma metastasis. *Hepatology* 2003; **37**: 674-685
21 **Abu-Absi SF**, Hansen LK, Hu WS. Three-dimensional co-culture of hepatocytes and stellate cells. *Cytotechnology* 2004; **45**: 125-140
22 **Kalabis J**, Oyama K, Okawa T, Nakagawa H, Michaylira CZ, Stairs DB, Figueiredo JL, Mahmood U, Diehl JA, Herlyn M, Rustgi AK. A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. *J Clin Invest* 2008; **118**: 3860-3869
23 **Sodek KL**, Brown TJ, Ringuette MJ. Collagen I but not Matrigel matrices provide an MMP-dependent barrier to ovarian cancer cell penetration. *BMC Cancer* 2008; **8**: 223
24 **Kamohara Y**, Haraguchi N, Mimori K, Tanaka F, Inoue H, Mori M, Kanematsu T. The search for cancer stem cells in hepatocellular carcinoma. *Surgery* 2008; **144**: 119-124
25 **Tsuchiya A**, Heike T, Fujino H, Shiota M, Umeda K, Yoshimoto M, Matsuda Y, Ichida T, Aoyagi Y, Nakahata T. Long-term extensive expansion of mouse hepatic stem/progenitor cells in a novel serum-free culture system. *Gastroenterology* 2005; **128**: 2089-2104
26 **Leek RD**, Stratford I, Harris AL. The role of hypoxia-inducible factor-1 in three-dimensional tumor growth, apoptosis, and regulation by the insulin-signaling pathway. *Cancer Res* 2005; **65**: 4147-4152
27 **Qihao Z**, Xigu C, Guanghui C, Weiwei Z. Spheroid formation and differentiation into hepatocyte-like cells of rat mesenchymal stem cell induced by co-culture with liver cells. *DNA Cell Biol* 2007; **26**: 497-503
28 **Xu J**, Ma M, Purcell WM. Characterisation of some cytotoxic endpoints using rat liver and HepG2 spheroids as in vitro models and their application in hepatotoxicity studies. II. Spheroid cell spreading inhibition as a new cytotoxic marker. *Toxicol Appl Pharmacol* 2003; **189**: 112-119
29 **Xu J**, Purcell WM. Energy metabolism and biotransformation as endpoints to pre-screen hepatotoxicity using a liver spheroid model. *Toxicol Appl Pharmacol* 2006; **216**: 293-302
30 **Abu-Absi SF**, Friend JR, Hansen LK, Hu WS. Structural polarity and functional bile canaliculi in rat hepatocyte spheroids. *Exp Cell Res* 2002; **274**: 56-67

31 **Dean DM**, Napolitano AP, Youssef J, Morgan JR. Rods, tori, and honeycombs: the directed self-assembly of microtissues with prescribed microscale geometries. *FASEB J* 2007; **21**: 4005-4012

32 **Fukuda J**, Nakazawa K. Orderly arrangement of hepatocyte spheroids on a microfabricated chip. *Tissue Eng* 2005; **11**: 1254-1262

33 **Lin RZ**, Chou LF, Chien CC, Chang HY. Dynamic analysis of hepatoma spheroid formation: roles of E-cadherin and beta1-integrin. *Cell Tissue Res* 2006; **324**: 411-422

34 **Nakamura M**, Shinji T, Ujike K, Hirasaki S, Koide N, Tsuji T. Cytoskeletal inhibitors, anti-adhesion molecule antibodies, and lectins inhibit hepatocyte spheroid formation. *Acta Med Okayama* 2002; **56**: 43-50

35 **Shimazui T**, Schalken JA, Kawai K, Kawamoto R, van Bockhoven A, Oosterwijk E, Akaza H. Role of complex cadherins in cell-cell adhesion evaluated by spheroid formation in renal cell carcinoma cell lines. *Oncol Rep* 2004; **11**: 357-360

36 **Alpaugh ML**, Tomlinson JS, Ye Y, Barsky SH. Relationship of sialyl-Lewis(x/a) underexpression and E-cadherin over-expression in the lymphovascular embolus of inflammatory breast carcinoma. *Am J Pathol* 2002; **161**: 619-628

37 **Enmon RM Jr**, O'Connor KC, Song H, Lacks DJ, Schwartz DK. Aggregation kinetics of well and poorly differentiated human prostate cancer cells. *Biotechnol Bioeng* 2002; **80**: 580-588

38 **Casey RC**, Burleson KM, Skubitz KM, Pambuccian SE, Oegema TR Jr, Ruff LE, Skubitz AP. Beta 1-integrins regulate the formation and adhesion of ovarian carcinoma multicellular spheroids. *Am J Pathol* 2001; **159**: 2071-2080

39 **Robinson EE**, Zazzali KM, Corbett SA, Foty RA. Alpha5beta1 integrin mediates strong tissue cohesion. *J Cell Sci* 2003; **116**: 377-386

40 **Robinson EE**, Foty RA, Corbett SA. Fibronectin matrix assembly regulates alpha5beta1-mediated cell cohesion. *Mol Biol Cell* 2004; **15**: 973-981

41 **Xu J**, Ma M, Purcell WM. Optimizing the enzymatic determination of galactose in the culture medium of rat liver and HepG2 cell spheroids. *Anal Biochem* 2002; **311**: 179-181

42 **Ma M**, Xu J, Purcell WM. Biochemical and functional changes of rat liver spheroids during spheroid formation and maintenance in culture: I. morphological maturation and kinetic changes of energy metabolism, albumin synthesis, and activities of some enzymes. *J Cell Biochem* 2003; **90**: 1166-1175

43 **Liao MJ**, Zhang CC, Zhou B, Zimonjic DB, Mani SA, Kaba M, Gifford A, Reinhardt F, Popescu NC, Guo W, Eaton EN, Lodish HF, Weinberg RA. Enrichment of a population of mammary gland cells that form mammospheres and have in vivo repopulating activity. *Cancer Res* 2007; **67**: 8131-8138

44 **Mani SA**, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008; **133**: 704-715

45 **Amann T**, Bataille F, Spruss T, Mühlbauer M, Gäbele E, Schölmerich J, Kiefer P, Bosserhoff AK, Hellerbrand C. Activated hepatic stellate cells promote tumorigenicity of hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 646-653

46 **Gotzmann J**, Mikula M, Eger A, Schulte-Hermann R, Foisner R, Beug H, Mikulits W. Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. *Mutat Res* 2004; **566**: 9-20

47 **Mikula M**, Proell V, Fischer AN, Mikulits W. Activated hepatic stellate cells induce tumor progression of neoplastic hepatocytes in a TGF-beta dependent fashion. *J Cell Physiol* 2006; **209**: 560-567

48 **Weinberg RA**. Coevolution in the tumor microenvironment. *Nat Genet* 2008; **40**: 494-495

49 **Fritz P**, Weber KJ, Frank C, Flentje M. Differential effects of dose rate and superfractionation on survival and cell cycle of V79 cells from spheroid and monolayer culture. *Radiother Oncol* 1996; **39**: 73-79

50 **Graham CH**, Kobayashi H, Stankiewicz KS, Man S, Kapitain SJ, Kerbel RS. Rapid acquisition of multicellular drug resistance after a single exposure of mammary tumor cells to antitumor alkylating agents. *J Natl Cancer Inst* 1994; **86**: 975-982

51 **Kobayashi H**, Man S, Graham CH, Kapitain SJ, Teicher BA, Kerbel RS. Acquired multicellular-mediated resistance to alkylating agents in cancer. *Proc Natl Acad Sci USA* 1993; **90**: 3294-3298

52 **Stuschke M**, Budach V, Stüben G, Streffer C, Sack H. Heterogeneity in the fractionation sensitivities of human tumor cell lines: studies in a three-dimensional model system. *Int J Radiat Oncol Biol Phys* 1995; **32**: 395-408

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

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.8

World J Hepatol 2010 January 27; 2(1): 8-15
ISSN 1948-5182 (online)

TOPIC HIGHLIGHT

Role of contrast enhanced ultrasonography in the assessment of hepatic metastases: A review

Lars Peter Skovgaard Larsen

Lars Peter Skovgaard Larsen, Department of Radiology, Aarhus University Hospital, Noerrebrogade 44, Aarhus 8000 C, Denmark
Author contribution: Larsen LPS contributed solely to this paper.
Correspondence to: Lars Peter Skovgaard Larsen, MD, Department of Radiology, Aarhus University Hospital, Noerrebrogade 44, Aarhus 8000 C, Denmark. larslars@rm.dk
Telephone: +45-89492499 Fax: +45-89492500
Received: August 12, 2009 Revised: September 17, 2009
Accepted: September 24, 2009
Published online: January 27, 2010

Key words: Ultrasound; Contrast-enhanced ultrasound; Liver neoplasms; Metastasis; Ultrasound contrast agent

Peer reviewer: Seong Woo Jeon, Assistant Professor, Internal Medicine, Kyungpook National University Hospital, 50 Samduk-2Ga, Chung-gu, Daegu 700-721, South Korea

Larsen LPS. Role of contrast enhanced ultrasonography in the assessment of hepatic metastases: A review. *World J Hepatol* 2010; 2(1): 8-15 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/8.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.8

Abstract

Contrast enhanced ultrasonography (CEUS) has improved both the detection and characterization of focal liver lesions. It is now possible to evaluate in real time the perfusion of focal liver lesions in the arterial, portal and late contrast phases, and thus to characterize focal liver lesions with high diagnostic accuracy. As a result, CEUS has taken a central diagnostic role in the evaluation of focal liver lesions that are indeterminate upon computed tomography (CT) and magnetic resonance imaging. The combined use of second generation contrast agents and low mechanical index techniques is essential for the detection of liver metastases, and it now allows the examination of the entire liver in both the portal and late phases. Several studies have shown that using CEUS instead of conventional ultrasonography without contrast agents significantly improves sensitivity in detection of liver metastases. Furthermore, the detection rate with CEUS seems to be similar to that of CT. This review describes the clinical role of CEUS in detecting liver metastases, including details about examination techniques, features of metastases observed with CEUS, and clinical results and guidelines.

INTRODUCTION

The liver is a very common site for the spread of malignancy. Between 15%-25% of patients with colorectal cancer have synchronous liver metastases, and a similar proportion develop metachronous liver metastases after colorectal resection with curative intent[1-3]. Also, patients with gastric, pancreatic, breast and lung cancer have been shown to have a high frequency of liver metastases[4,5]. The frequent involvement of the liver is probably due to its inherent characteristics, such as its blood supply from both the portal vein and the hepatic artery, the high volume of blood flow, its major role in biochemical activities and its anatomy, which provides several different possibilities for tumor cells to become trapped. These factors all create an ideal environment for the rapid growth of malignant cells in the liver[6].

Early detection of liver metastases in patients with known malignancy is important for determining therapeutic strategy, and crucial to the prognosis for survival. In some patients with preoperatively detected liver metastases, synchronous therapy of the primary tumor and liver metastases is a possibility. Also, the detection of liver metastases in some patients with known malignancy influences the use of adjuvant

Table 1 Results of studies comparing CEUS and US in the detection of liver metastases

Study	*n*	Study group[1]	Gold standard	Type of analysis	Sensitivity		Specificity	
					US	CEUS	US	CEUS
Piscaglia *et al*[33], 2007	109	UK	CT, FNA, follow up	P-by-P[2]	0.77	0.95 (23%)[3]		
Konopke *et al*[23], 2007	100	K	IOUS	P-by-P	0.56	0.84 (50%)	0.93	0.84
Larsen *et al*[38], 2007	365	UK	FNA, CT, IOUS	P-by-P	0.69	0.80 (16%)	0.98	0.98
Janica *et al*[40], 2007	51	S or K	CT, FNA, follow up	P-by-P	0.63	0.90 (43%)		
Dietrich *et al*[32]	131	UK	CT, MRI, FNA, follow up	P-by-P	0.81	0.91 (12%)		
Quaia *et al*[31], 2006	253	S or K	FNA, CT, MRI, IOUS	P-by-P	0.40	0.83 (107%)	0.63	0.84
Konopke *et al*[39], 2005	56	S or K	IOUS, FNA, CT	P-by-P	0.53	0.86 (62%)	0.89	0.89
Oldenburg *et al*[15], 2005	40	S	CT, MRI	L-by-L[4]	0.69	0.90 (30%)		
Albrecht *et al*[51], 2003	123	S or K	CT (MRI, IOUS, FNA)	P-by-P	0.94	0.98 (4%)	0.60	0.88
Esteban *et al*[36], 2002	27	K	CT	L-by-L	Found 9.3 metastases pr. patient	Found 18.8 metastases pr. patient		
Solbiati *et al*[52], 2001	32	K	CT	L-by-L		Found in 21 out of 32 patients 10-94 more metastases than US		
Bertanik *et al*[53], 2001	28	K	CT	L-by-L	0.59	0.97 (64%)		
Albrecht *et al*[22], 2001	62	S or K	CT, MRI, IOUS, FNA	P-by-P	0.92	0.97 (5%)		
Harvey *et al*[37], 2000	11	K	CT	L-by-L	Found 9.0 metastases pr. patient	Found 21.8 metastases pr. patient		

[1]Included patients with known (K), suspected (S) or unknown (UK) liver metastases; [2]Patient-by-patient analysis; [3]Figures in brackets are percent changes in sensitivity; [4]Lesion-by-lesion analysis.

preoperative irradiation or chemo-irradiation. Finally, it is important to detect small liver metastases because the chances of radical treatment are related to the size and number of liver metastases. It is therefore crucial to have a preoperative imaging modality with a high sensitivity for the detection of liver metastases. Moreover, detection of all metastases and their localization is essential for the optimization of the therapeutic strategy, which may include liver surgery and radiofrequency ablation (RFA).

Furthermore, a high specificity in the preoperative imaging is required. The prevalence of solid benign liver tumors has been reported to be more than 20% in autopsy series[7,8]. In patients with malignancy, 25%-50% of lesions under 20 mm in size are benign[9,10], and about 80% of lesions less than 10 mm in size are benign[11].

Conventional transabdominal ultrasonography is still used in the detection of liver metastases, even though its sensitivity is known to be relatively low (53%-77%)[12-15]. The technique's sensitivity depends on the size of the metastasis, and it is only 20% effective for metastases smaller than 10 mm[13]. In addition, the echogenicity of the metastases is important. Isoechoic metastases are difficult to detect because they exhibit the same or similar acoustic behavior as the surrounding normal liver tissue (Figure 1), while hyperechoic metastases can mimic hemangiomas[16]. Finally, it is well known that the sensitivity of US is reduced in patients with obesity, a high lying diaphragm, interposition of the intestine, tissue-composition or even lack of co-operation.

With the abovementioned sensitivity of US in mind, the reported sensitivity of contrast enhanced CT (58%-85%)[12,17-20] and MRI (70%-98%)[19,21] is clearly superior.

However, during the 1990s, diagnostic ultrasound entered a new era with the introduction of microbubble contrast agents. Based on contrast-specific gray-scale US techniques, which are very sensitive to the non-linear signals from the microbubbles, the dynamic detection of tissue flow in both macro- and microvasculature was improved. This resulted in better detection of focal liver lesions. Several studies have shown that US techniques using intravenous contrast media (CEUS) have clearly improved sensitivity in detecting liver metastases to 80%-90% (Table 1), which is comparable with the best reported CT results. Some studies have found that CEUS improves sensitivity by more than 50%, and is especially helpful for metastases smaller than 10 mm[22,23].

CONTRAST AGENTS

This paper does not focus on the history of ultrasound contrast agents or their differences. In the case of liver metastasis detection, it is sufficient to emphasize the clear advantages of second generation contrast agents because they allow continuous real-time imaging of all the vascular contrast phases in the liver, and the scanning time (not more than 5 min) makes it possible to systematically scan the entire liver in the portal and late contrast phases. This is essential for liver metastasis detection[24,25]. In the following sections, the role of CEUS involving a second generation contrast agent in the detection of liver metastases will be reviewed.

BASIC PRINCIPLES

Performing CEUS for the detection of liver metastases always begins with a careful conventional B-mode US to assess the morphology of the lesions (i.e. fatty sparring, hemangioma or cyst), and the liver in general, including the assessment of diffuse parenchymal changes, such as steatosis or cirrhosis.

Then, before beginning the CEUS of the liver, it is

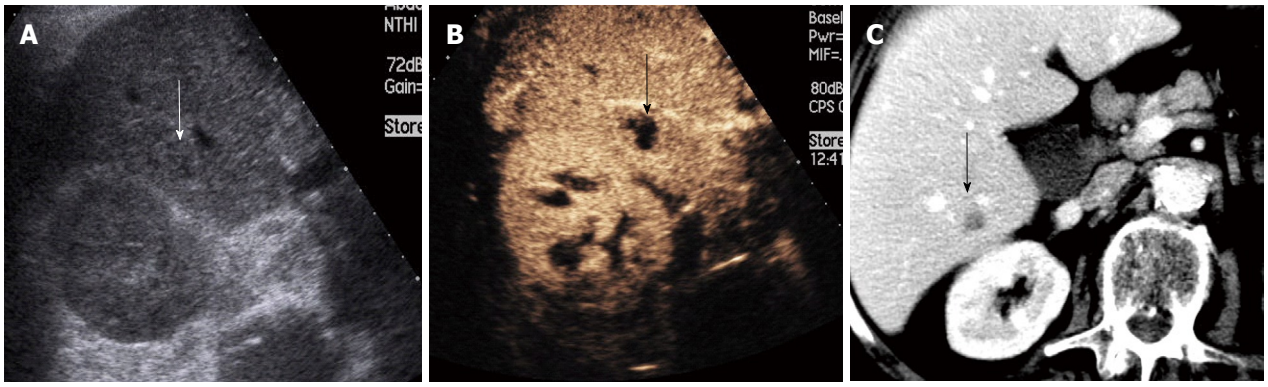


Figure 1 US, CEUS and CT visualizations of the same liver metastasis (indicated by an arrow). The lesion is not recognizable using US, but is visible by CEUS and CT. A: US; B: CEUS; C: CT.

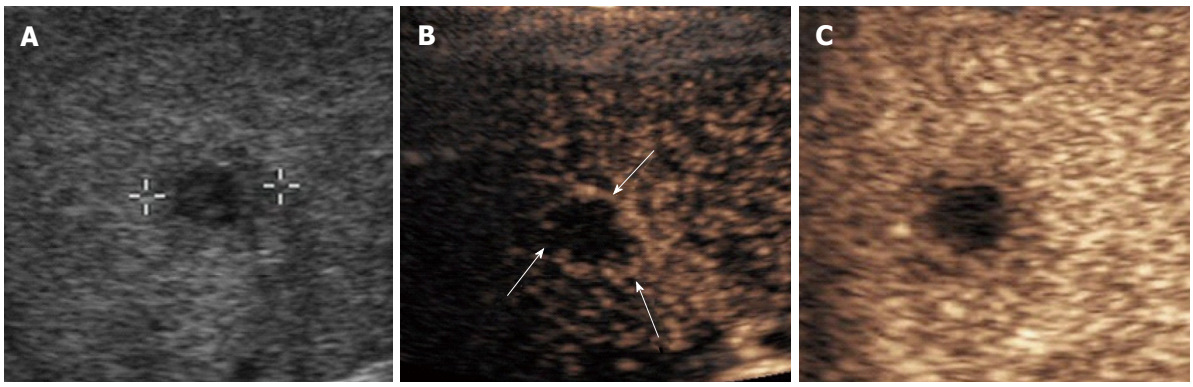


Figure 2 Hypovascular liver metastases visualized with US and CEUS. The same hypovascular liver metastasis is visualized by US and CEUS in both the arterial and portal phases. In the arterial phase a slight rim enhancement is seen (arrows). The non-enhancing area in the center represents a necrosis, and is demonstrated in both the arterial and late phases. A: B-mode US; B: Arterial phase; C: Late phase.

important to position the patient correctly, because due to the low MI, the penetration is limited to 12-14 cm. In order to overcome this limitation, some authors recommend placing the patient on the left side instead of the normal supine position, because in this position the liver moves forward toward the transducer at the anterior abdominal wall and usually improves the penetration by one to two cm[26].

When using Sonovue® (sulfur hexafluoride with phospholipid shell) (Bracco, Milan, Italy) for liver metastasis detection, a 2.4 mL bolus is given through a 20-gauge (minimum diameter) intravenous catheter and a three-way stopcock, which is followed by a flush with 5-10 mL of saline. Because of the specific blood supply to the liver, three phases of contrast enhancement appear, namely, the arterial, due to the supply from hepatic artery (10-20 s to 25-35 s after injection), the portal (30-45 s to 120 s), and the late (> 120 s) phases[27].

Video frames of the entire liver are recorded in all three contrast phases, but the portal and late phases are of greatest interest when detecting liver metastases. Finally, the examination can be evaluated on workstations.

FEATURES OF METASTASES ON CEUS

Both hypovascular and hypervascular liver metastases have a predominantly arterial blood supply, but the degree of arterial perfusion is variable, and the contrast enhancement in the arterial phase is related to this variation. Hypovascular metastases, which have relatively low arterial perfusion, are usually seen in patients with adenocarcinoma or squamous cell carcinoma, most likely related to colorectal cancer, gastric cancer, pancreatic cancer or ovarian cancer. In nearly all hypovascular metastases, contrast enhancement of varying degrees is seen in the arterial phase, typically in the periphery (rim enhancement) (Figure 2). However, some authors describe a more diffuse enhancement, especially when the tumor is small[28].

Hypervascular metastases, which frequently arise from neuroendocrine tumors, malignant melanoma, and sarcoma, as well as from renal, breast, or thyroid cancer, have a very high arterial perfusion and display a diffuse or inhomogeneous enhancement in the arterial phase. These metastases present a hyper-reflective signal compared to the surrounding normal liver parenchyma (Table 2 and Figure 3).

In the portal and delay phases, both hyper- and hypovascular metastases appear as dark defects, while the enhancement persists in normal liver parenchyma (Figure 4). The metastases do not retain the contrast agent like the normal liver parenchyma. This rapid and

Table 2 Enhancement patterns of liver metastases

Tumor entity	Arterial phase	Portal phase	Delayed phase
Hypovascular metastasis			
Typical features	Rim enhancement	Hypo-enhancement	Hypo-/non-enhancement
Additional features	Complete enhancement Non-enhancement areas (necrosis)	Non-enhancement areas	
Hypervascular metastasis			
Typical features	Hyper-enhancement, complete	Hypo-enhancement	Hypo-/non-enhancement
Additional features	Chaotic vessels		
Cystic metastasis			
Typical features	Hyper-enhancement nodular/rim component	Hypo-enhancement	Hypo-enhancement

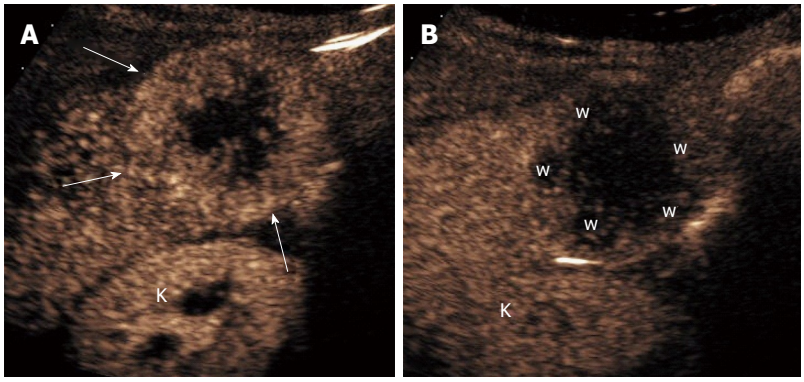


Figure 3 Hypervascular liver metastases visualized by CEUS. The patient is a woman, 77 years old, with renal cancer and hypervascular liver metastases in segment 6 as visualized with CEUS. In the arterial phase, the metastases present a hyper-reflective signal when compared to the surrounding normal liver parenchyma (arrows). The non- enhanced area in the center represents necrosis. In the late phase there is clearly a washout of contrast in the metastases (w). The kidneys are visualized below the liver (k). A: Arterial phase; B: Late phase.

complete "washout" in the metastases can be explained by a consistently lower fractional vascular volume when compared to normal liver parenchyma[29], and also by the absence of portal supply to the neoplastic lesions. The "washout" phenomenon in CEUS must not be confused with the "washout" of contrast agents seen on CT or MRI. The ultrasound contrast agent remains exclusively intravascular, which is not the case for the contrast agents used in CT and MRI.

Using CEUS, it is possible to differentiate cystic metastases from a non-neoplastic complex of cysts by demonstrating vascular flow in the cyst wall or in mural nodules[28].

The optimal time to scan for all types of metastases is from about 90 s to 5 min. This is the time when the contrast between the enhanced normal liver and the non-enhanced metastases is most pronounced, but some metastases can be detected in the arterial phase, especially hypervascular metastases.

RESULTS OF CLINICAL STUDIES

General considerations

One of the main problems in comparing the sensitivity of CEUS in liver metastasis detection to other imaging modalities is the choice of a gold standard. Theoretically, all detected liver lesions must be histologically proven, but for ethical and technical reasons, this is not possible. Due to a lack of verification, many studies on the topic have important limitations. Not all patients with liver metastases are histologically verified, and in cases where histology is obtained, it is usually only performed on one lesion per patient, even if multiple lesions are present. This is especially problematic in lesion-by-lesion analysis. However, in some studies, the preoperative CEUS findings have been compared with the histopathological specimens after liver resection, which is clearly the ideal gold standard[30]. Such results are correct for the particular specimen, but this practice does not exclude non-visualized metastases in the remainder of the liver.

In cases where the gold standard is based only on imaging modalities, like CT or MRI, the true prevalence of liver metastases remains unknown, since some of the smaller liver metastases will remain undetected and will not be included in the population of metastases[21]. Even the use of intraoperative ultrasonography (IOUS) as a gold standard cannot guarantee 100% reliability, primarily because it is unable to detect micrometastases[23]. Another problem is that many studies use CT (sometimes together with histology, MRI, and follow-up)[31-34] as the gold standard and then compare the sensitivity of CEUS and CT, which inevitably leads to an overestimate of CT's sensitivity. However, bearing these studies' limitations in mind, the results of the clinical studies concerning CEUS versus US and CEUS versus CT for the assessment of liver metastases are described in the next section.

Contrast enhanced US versus US

Several studies have shown that CEUS detects more liver metastases than US (Table 1). The first published studies were mostly based on lesion-by-lesion analysis. All of these studies showed that CEUS has better sensitivity in detecting liver metastases than US[35-37]. However, it is

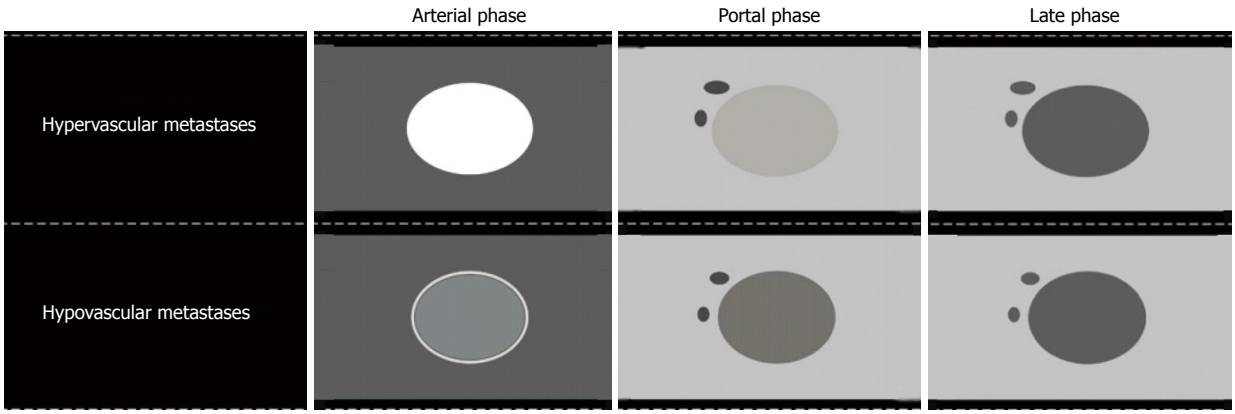


Figure 4 Typical contrast enhancement pattern of a hepatic metastasis by CEUS.

well known that it is often difficult to compare the same liver lesions by US and CEUS, despite their assignment to a Couinaud segment. Thus, when many liver lesions are present and are analyzed on a lesion-by-lesion basis, there is a potential source of error[37,38]. Nevertheless, these promising results have been confirmed in several studies based on a patient-by-patient analysis[23,32,33,38-40]. Most of these studies showed that the detection of liver metastases was significantly improved, by between 5% and 62%, with one study showing a 107% improvement. However, this study also found that US had a remarkably low sensitivity (0.40)[31]. The impact of CEUS on therapeutic strategies also seems to be significant. In one study involving[40] patients who underwent laparatomy for liver resection, the preoperative US findings would have led to an extension of the resections in 16 cases (40%). In a significant number of cases (9/40 patients, 22.5%), preoperative CEUS findings changed the surgical strategy[30]. Furthermore, in patients treated with chemotherapy, CEUS had significantly greater sensitivity than US as determined by both patient-by-patient analysis (79.5% *vs* 63.2%) and lesion-by-lesion analysis (82.0% *vs* 60.3%)[30]. Another study showed that the origin of the metastases seems not to influence the rate of detection in either US or CEUS (colorectal liver metastases were compared to metastases from cancers originating in the pancreas, kidneys, stomach, ovaries, breast, gallbladder and lungs)[23].

Contrast enhanced intraoperative US (CE-IOUS) versus intraoperative US (IOUS)

Two studies have found a further value for CE-IOUS, describing additional findings of colorectal liver metastases using contrast agents as compared to conventional IOUS[41,42]. Additional liver metastases were found in 19% and 13% of the patients, respectively, and in one of the studies, CE-IOUS altered the surgical plans in 30% of cases[41]. On the other hand, a recently published study involving 39 patients with 137 identified malignant lesions concluded that the use of CE-IOUS in addition to preoperative contrast enhanced CT and IOUS did not improve the ability to characterize previously detected lesions. This study also showed that only in a small number of patients did CE-IOUS facilitate the detection of new liver metastases or have implications on surgical strategy[43]. The differences in these results could be explained by the varying levels of skill with the techniques used in the studies, but further studies on the role of CE-IOUS seem to be needed.

Contrast enhanced US versus CT

The detection rate for liver metastases by CEUS seems to be similar to the best reported results by CT and MRI[15,31-33,39,44]. However, some of the studies must be evaluated with caution, due to the use of unclear gold standards[31] and because CEUS is sometimes compared to somewhat out-of-date CT equipment[31,33,39]. The technical advantages achieved with modern multidetector CT (MDCT) might show that the results from studies using single-slice CT and thick scan slices are no longer valid when compared to modern MDCT. None of the existing studies found significant differences between CEUS and CT with regard to sensitivity in detecting liver metastases (Table 3), but most studies found the sensitivity of CEUS to be slightly higher[33,39,40].

Contrast enhanced US versus MRI or positron emission tomography (PET)

To date, there have been no studies comparing the effectiveness of CEUS and MRI [with liver-specific contrast agents like SPIO (superparamagnetic iron oxide) or Gd-EOB-DTBA (gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid)] or PET in detecting liver metastases. However, in some studies, MRI is used in combination with other imaging modalities as the gold standard (Tables 1 and 3).

LIMITATIONS OF CEUS

In general, if an examination of the liver by US is insufficient, then examination by CEUS will also be insufficient. The limitations that apply to US are the same as those that apply to CEUS, so the quality of the

Table 3 Sensitivity of CEUS and CT in detecting liver metastases; an overview of studies

Study	*n*	Study group[1]	Type of CT	Analysis	Gold standard	Sensitivity		Statistic
						CEUS	CT	
Quaia *et al*[31], 2006	253	K or S	1-slice CT	P-by-P[3]	CT, FNA, Follow-up, MRI, IOUS	0.83	0.89	NS[2]
Larsen *et al*[34], 2007	365	S	4-slice CT	P-by-P	CT, IOUS, CEUS, FNA, surgery resection, follow-up	0.8	0.89	NS
Dietrich *et al*[32]	131	UK	Multislice CT in most cases[4]	P-by-P	CT, MRI, FNA, follow up	0.91	0.89	NS
Piscaglia *et al*[33], 2007	109	S	1-or 4-slice CT	P-by-P	CT, US, FNA, Follow-up	0.95	0.91	NS
Janica *et al*[40], 2007	51	K or S	Not described	L-by-L[5]	FNA, surgical resection, CT and follow-up	0.9	0.78	NS
Konopke *et al*[39], 2005	56	S or K	1-, 4- or 16-slice CT	P-by-P	IOUS, FNA, CT	0.86	0.76	NS

[1]Included patients with known (K), suspected (S) or unknown (UK) liver metastases; [2]Not significant; [3]Patient-by-patient analysis; [4]Details not described. [5]Lesion-by-lesion analysis.

examination still depends on the skill of the operator. In addition, as has been noted previously, CEUS has limited ability to observe certain parts of the liver, especially in obese patients and/or in cases of steatosis. Further limitations are related to the acoustic window and movement artifacts.

It is not possible to simultaneously examine multiple lesions in the arterial and early portal phases[45]. The characterization of suspected liver lesions can also be complicated by difficulties in interpreting results in cases of hypervascular metastases and hemangiomas with incomplete filling.

In addition, in patients with many cysts, metastases can be missed because it can be difficult to differentiate some of them from cysts in the late contrast phase, since both appear hypoechoic. Conversely, cysts can be misinterpreted as metastases. Also in cases with few and relatively small cysts, which have not been detected by US, the cysts can be misinterpreted as small metastases. They can usually be distinguished from metastases by their characteristically increased transmission[46]. However, it is recommended that cysts be confirmed by US before CEUS[38,47].

THE CLINICAL ROLE OF CEUS IN THE DETECTION OF LIVER METASTASES

The European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) in Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008[46] recommends that CEUS be used in the following cases: (1) All liver ultrasound scans to rule out liver metastases or abscess, unless conventional ultrasound shows clear evidence of these lesions; (2) In selected cases, when clinically relevant for treatment planning by helping to assess the number and location of liver metastases as a complement to contrast enhanced CT and/or contrast enhanced MRI; and (3) For surveillance of oncology patients in whom CEUS has previously been useful. These guidelines are based on literature surveys including results from several clinical trials, some of which have been mentioned earlier in this paper. However, it is important to emphasize that CEUS is complementary to CT/MRI in the preoperative staging before liver resection. It cannot replace the other imaging modalities in the preoperative work-up or in the follow-up of patients with liver metastases during chemotherapy, since CT and MRI give more comprehensive information about the liver and all other organs. Even the PACS-systems have been improved, so it is easier to manage digital cine-loops, and while some institutions have introduced the practice of doing a standard sweep of the entire liver when performing CEUS, there are still problems with reproducible image documentation. This limits the ability of CEUS to clearly show small changes over time. In most cancer centers, including ours, CT and MRI are therefore preferred for follow-up imaging. On the other hand, CEUS is a very useful and important non-invasive imaging modality for resolving problems, such as patients with or without known malignancies where CT has demonstrated an uncharacteristic appearance. It is also necessary to emphasize that, in patients who are known or strongly suspected of having a malignancy, an adequate US examination for the detection of liver metastases includes CEUS, even if the baseline US is normal. Numerous studies have demonstrated that CEUS clearly and significantly improved sensitivity and specificity in the detection of liver metastases (Table 1).

The economic advantage of the use of CEUS for characterization of focal liver lesions seems to be clear. Compared with CT and MRI, CEUS provides significant cost savings, both for a national health service and for hospitals[48,49]. However, there are still some barriers to using CEUS, like the costs of CEUS compared to US and the fees paid by health insurance companies to perform CEUS[50].

CONCLUSION

The use of second generation ultrasound contrast agents in combination with low MI contrast-specific US techniques has clearly improved US imaging of the liver, including the dynamic examination of focal liver lesions. Contrast enhanced US has improved the detection of liver metastases when compared to US itself, and it seems to have a diagnostic performance and accuracy similar to that of CT.

REFERENCES

1 **Tong D**, Russell AH, Dawson LE, Wisbeck W. Second laparotomy for proximal colon cancer. Sites of recurrence and implications for adjuvant therapy. *Am J Surg* 1983; **145**: 382-386
2 **Gilbert JM**, Jeffrey I, Evans M, Kark AE. Sites of recurrent tumour after 'curative' colorectal surgery: implications for adjuvant therapy. *Br J Surg* 1984; **71**: 203-205
3 **Russell AH**, Tong D, Dawson LE, Wisbeck W. Adenocarcinoma of the proximal colon. Sites of initial dissemination and patterns of recurrence following surgery alone. *Cancer* 1984; **53**: 360-367
4 **Pickren JW**, Tsuka Y, Lane WW. Liver metastases. In: Weiss L, Gilbert HA, editors. Liver metastasis. Boston: GK Hall Medical Publischers, 1982: 2-18
5 **Cosgrove DO**. Malignant liver disease. In: Meire HB, Cosgrove DO, Dewbury KC, Farrant P, editors. Clinical ultrasound: a comprehensive text, 2nd ed. London: Churchill Livingstone, 2001: 211-231.
6 **Robinson PJ**. Imaging liver metastases: current limitations and future prospects. *Br J Radiol* 2000; **73**: 234-241
7 **Karhunen PJ**. Benign hepatic tumours and tumour like conditions in men. *J Clin Pathol* 1986; **39**: 183-188
8 **Edmondson HA**, Craig JR. Neoplasms of the liver. In: Schiff L, editor. Diseases of the liver, 8th ed. Philadelphia: Lippincott, 1987
9 **Kreft B**, Pauleit D, Bachmann R, Conrad R, Krämer A, Schild HH. [Incidence and significance of small focal liver lesions in MRI] *Rofo* 2001; **173**: 424-429
10 **Jones EC**, Chezmar JL, Nelson RC, Bernardino ME. The frequency and significance of small (less than or equal to 15 mm) hepatic lesions detected by CT. *AJR Am J Roentgenol* 1992; **158**: 535-539
11 **Schwartz LH**, Gandras EJ, Colangelo SM, Ercolani MC, Panicek DM. Prevalence and importance of small hepatic lesions found at CT in patients with cancer. *Radiology* 1999; **210**: 71-74
12 **Ohlsson B**, Nilsson J, Stenram U, Akerman M, Tranberg KG. Percutaneous fine-needle aspiration cytology in the diagnosis and management of liver tumours. *Br J Surg* 2002; **89**: 757-762
13 **Wernecke K**, Rummeny E, Bongartz G, Vassallo P, Kivelitz D, Wiesmann W, Peters PE, Reers B, Reiser M, Pircher W. Detection of hepatic masses in patients with carcinoma: comparative sensitivities of sonography, CT, and MR imaging. *AJR Am J Roentgenol* 1991; **157**: 731-739
14 **Glover C**, Douse P, Kane P, Karani J, Meire H, Mohammadtaghi S, Allen-Mersh TG. Accuracy of investigations for asymptomatic colorectal liver metastases. *Dis Colon Rectum* 2002; **45**: 476-484
15 **Oldenburg A**, Hohmann J, Foert E, Skrok J, Hoffmann CW, Frericks B, Wolf KJ, Albrecht T. Detection of hepatic metastases with low MI real time contrast enhanced sonography and SonoVue. *Ultraschall Med* 2005; **26**: 277-284
16 **Bruneton JN**, Raffaelli C, Balu-Maestro C, Padovani B, Chevallier P, Mourou MY. Sonographic diagnosis of solitary solid liver nodules in cancer patients. *Eur Radiol* 1996; **6**: 439-442
17 **Valls C**, Andia E, Sanchez A, Guma A, Figueras J, Torras J, Serrano T. Hepatic metastases from colorectal cancer: preoperative detection and assessment of resectability with helical CT. *Radiology* 2001; **218**: 55-60
18 **Portugaller HR**, Stacher R, Komaz G, Aschauer M, Hausegger KA, Szolar DH. [The value of different spiral CT phases in the detection of liver metastases] *Rofo* 2002; **174**: 452-458
19 **Rappeport ED**, Loft A. Liver metastases from colorectal cancer: imaging with superparamagnetic iron oxide (SPIO)-enhanced MR imaging, computed tomography and positron emission tomography. *Abdom Imaging* 2007; **32**: 624-634
20 **Kim YK**, Ko SW, Hwang SB, Kim CS, Yu HC. Detection and characterization of liver metastases: 16-slice multidetector computed tomography versus superparamagnetic iron oxide-enhanced magnetic resonance imaging. *Eur Radiol* 2006; **16**: 1337-1345
21 **van Erkel AR**, Pijl ME, van den Berg-Huysmans AA, Wasser MN, van de Velde CJ, Bloem JL. Hepatic metastases in patients with colorectal cancer: relationship between size of metastases, standard of reference, and detection rates. *Radiology* 2002; **224**: 404-409
22 **Albrecht T**, Hoffmann CW, Schmitz SA, Schettler S, Overberg A, Germer CT, Wolf KJ. Phase-inversion sonography during the liver-specific late phase of contrast enhancement: improved detection of liver metastases. *AJR Am J Roentgenol* 2001; **176**: 1191-1198
23 **Konopke R**, Kersting S, Bergert H, Bloomenthal A, Gastmeier J, Saeger HD, Bunk A. Contrast-enhanced ultrasonography to detect liver metastases : a prospective trial to compare transcutaneous unenhanced and contrast-enhanced ultrasonography in patients undergoing laparotomy. *Int J Colorectal Dis* 2007; **22**: 201-207
24 **Hohmann J**, Skrok J, Puls R, Albrecht T. [Characterization of focal liver lesions with contrast-enhanced low MI real time ultrasound and SonoVue] *Rofo* 2003; **175**: 835-843
25 **Cosgrove D**. Ultrasound contrast agents: an overview. *Eur J Radiol* 2006; **60**: 324-330
26 **Albrecht T**. Detection and characterisation of liver metastases. In: Lencioni R, editor. Enhancing the role of ultrasound with contrast agents. Pisa: Springer-Verlag Italia, 2006: 53-67
27 **Albrecht T**, Blomley M, Bolondi L, Claudon M, Correas JM, Cosgrove D, Greiner L, Jäger K, Jong ND, Leen E, Lencioni R, Lindsell D, Martegani A, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines for the use of contrast agents in ultrasound. January 2004. *Ultraschall Med* 2004; **25**: 249-256
28 **Jang HJ**, Kim TK, Wilson SR. Imaging of malignant liver masses: characterization and detection. *Ultrasound Q* 2006; **22**: 19-29
29 **Cosgrove D**, Blomley M. Liver tumors: evaluation with contrast-enhanced ultrasound. *Abdom Imaging* 2004; **29**: 446-454
30 **Konopke R**, Bunk A, Kersting S. Contrast-enhanced ultrasonography in patients with colorectal liver metastases after chemotherapy. *Ultraschall Med* 2008; **29** Suppl **4**:S203-S209
31 **Quaia E**, D'Onofrio M, Palumbo A, Rossi S, Bruni S, Cova M. Comparison of contrast-enhanced ultrasonography versus baseline ultrasound and contrast-enhanced computed tomography in metastatic disease of the liver: diagnostic performance and confidence. *Eur Radiol* 2006; **16**: 1599-1609
32 **Dietrich CF**, Kratzer W, Strobe D, Danse E, Fessl R, Bunk A, Vossas U, Hauenstein K, Koch W, Blank W, Oudkerk M, Hahn D, Greis C. Assessment of metastatic liver disease in patients with primary extrahepatic tumors by contrast-enhanced sonography versus CT and MRI. *World J Gastroenterol* 2006; **12**: 1699-1705
33 **Piscaglia F**, Corradi F, Mancini M, Giangregorio F, Tamberi S, Ugolini G, Cola B, Bazzocchi A, Righini R, Pini P, Fornari F, Bolondi L. Real time contrast enhanced ultrasonography in detection of liver metastases from gastrointestinal cancer. *BMC Cancer* 2007; **7**: 171
34 **Larsen LP**, Rosenkilde M, Christensen H, Bang N, Bolvig L, Christiansen T, Laurberg S. Can contrast-enhanced ultrasonography replace multidetector-computed tomography in the detection of liver metastases from colorectal cancer? *Eur J Radiol* 2009; **69**: 308-313
35 **Dalla Palma L**, Bertolotto M, Quaia E, Locatelli M. Detection of liver metastases with pulse inversion harmonic imaging: preliminary results. *Eur Radiol* 1999; **9** Suppl **3**: S382- S387
36 **Esteban JM**, Molla MA, Tomas C, Maldonado L. Improved detection of liver metastases with contrast-enhanced wideband harmonic imaging: comparison with CT findings. *Eur J Ultrasound* 2002; **15**: 119-126

37 **Harvey CJ**, Blomley MJ, Eckersley RJ, Cosgrove DO, Patel N, Heckemann RA, Butler-Barnes J. Hepatic malignancies: improved detection with pulse-inversion US in late phase of enhancement with SH U 508A-early experience. *Radiology* 2000; **216**: 903-908

38 **Larsen LP**, Rosenkilde M, Christensen H, Bang N, Bolvig L, Christiansen T, Laurberg S. The value of contrast enhanced ultrasonography in detection of liver metastases from colorectal cancer: a prospective double-blinded study. *Eur J Radiol* 2007; **62**: 302-307

39 **Konopke R**, Kersting S, Saeger HD, Bunk A. [Detection of liver lesions by contrast-enhanced ultrasound -- comparison to intraoperative findings] *Ultraschall Med* 2005; **26**: 107-113

40 **Janica JR**, Lebkowska U, Ustymowicz A, Augustynowicz A, Kamocki Z, Werel D, Polaków J, Kedra B, Pepinski W. Contrast-enhanced ultrasonography in diagnosing liver metastases. *Med Sci Monit* 2007; **13** Suppl **1**: 111-115

41 **Leen E**, Ceccotti P, Moug SJ, Glen P, MacQuarrie J, Angerson WJ, Albrecht T, Hohmann J, Oldenburg A, Ritz JP, Horgan PG. Potential value of contrast-enhanced intraoperative ultrasonography during partial hepatectomy for metastases: an essential investigation before resection? *Ann Surg* 2006; **243**: 236-240

42 **Torzilli G**, Palmisano A, Del Fabbro D, Marconi M, Donadon M, Spinelli A, Bianchi PP, Montorsi M. Contrast-enhanced intraoperative ultrasonography during surgery for hepatocellular carcinoma in liver cirrhosis: is it useful or useless? A prospective cohort study of our experience. *Ann Surg Oncol* 2007; **14**: 1347-1355

43 **Fioole B**, de Haas RJ, Wicherts DA, Elias SG, Scheffers JM, van Hillegersberg R, van Leeuwen MS, Borel Rinkes IH. Additional value of contrast enhanced intraoperative ultrasound for colorectal liver metastases. *Eur J Radiol* 2008; **67**: 169-176

44 **Bipat S**, van Leeuwen MS, Comans EF, Pijl ME, Bossuyt PM, Zwinderman AH, Stoker J. Colorectal liver metastases: CT, MR imaging, and PET for diagnosis--meta-analysis. *Radiology* 2005; **237**: 123-131

45 **Seitz K**. Contrast-enhanced ultrasound in the diagnosis of hepatocellular carcinoma and liver metastases. *Ultraschall Med* 2005; **26**: 267-269

46 **Claudon M**, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, Correas JM, Darge K, Dietrich C, D'Onofrio M, Evans DH, Filice C, Greiner L, Jäger K, Jong N, Leen E, Lencioni R, Lindsell D, Martegani A, Meairs S, Nolsøe C, Piscaglia F, Ricci P, Seidel G, Skjoldbye B, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS)-update 2008. *Ultraschall Med* 2008; **29**: 28-44

47 **Dietrich CF**. Characterisation of focal liver lesions with contrast enhanced ultrasonography. *Eur J Radiol* 2004; **51** Suppl: S9-S17

48 **Romanini L**, Passamonti M, Aiani L, Cabassa P, Raieli G, Montermini I, Martegani A, Grazioli L, Calliada F. Economic assessment of contrast-enhanced ultrasonography for evaluation of focal liver lesions: a multicentre Italian experience. *Eur Radiol* 2007; **17** Suppl **6**: F99-F106

49 **Faccioli N**, D'Onofrio M, Comai A, Cugini C. Contrast-enhanced ultrasonography in the characterization of benign focal liver lesions: activity-based cost analysis. *Radiol Med* 2007; **112**: 810-820

50 **Mostbeck G**. [CEUS from a radiological standpoint: dream and reality] *Ultraschall Med* 2009; **30**: 125-127

51 **Albrecht T**, Blomley MJ, Burns PN, Wilson S, Harvey CJ, Leen E, Claudon M, Calliada F, Correas JM, LaFortune M, Campani R, Hoffmann CW, Cosgrove DO, LeFevre F. Improved detection of hepatic metastases with pulse-inversion US during the liver-specific phase of SHU 508A: multicenter study. *Radiology* 2003; **227**: 361-370

52 **Solbiati L**, Tonolini M, Cova L, Goldberg SN. The role of contrast-enhanced ultrasound in the detection of focal liver leasions. *Eur Radiol* 2001; **11** Suppl **3**: E15-E26

53 **Bernatik T**, Strobel D, Hahn EG, Becker D. Detection of liver metastases: comparison of contrast-enhanced wide-band harmonic imaging with conventional ultrasonography. *J Ultrasound Med* 2001; **20**: 509-515

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

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.16

World J Hepatol 2010 January 27; 2(1): 16-20
ISSN 1948-5182 (online)

GUIDELINES FOR BASIC SCIENCE

Targeting c-Myc as a novel approach for hepatocellular carcinoma

Che-Pin Lin, Chien-Ru Liu, Chun-Nin Lee, Tze-Sian Chan, H Eugene Liu

Che-Pin Lin, Chien-Ru Liu, Department of Medicine, Taipei City Hospital, Ren-Ai Branch, Taipei 106, Taiwan
Chun-Nin Lee, Tze-Sian Chan, Department of Medicine, Wanfang Hospital, Taipei Medical University, Taipei 116, Taiwan
H Eugene Liu, Department of Medicine, Department of Laboratory Medicine, and Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei 110, Taiwan
Author contributions: Lin CP and Liu CR contributed equally to this work; Both of them were responsible for manuscript writing; Lee CN and Chan TS were responsible for data collection and Liu HE for manuscript writing and editing.
Supported by the Grants from Taiwan Department of Health for the Center of Excellence for Clinical Trial and Research in Neuroscience (DOH99-TD-B-111-003)
Correspondence to: H Eugene Liu, MD, PhD, Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei 110, Taiwan. liuxx086@yahoo.com.tw
Telephone: +886-2-29307930-2548 Fax: +886-2-55583019
Received: August 8, 2009 Revised: January 8, 2010
Accepted: January 15, 2010
Published online: January 27, 2010

Abstract

Hepatocelluar carcinoma (HCC) is the most lethal cancer in the world. Most HCC over-express c-Myc, which plays a critical role in regulating cellular growth, differentiation and apoptosis in both normal and neoplastic cells. c-Myc is among the most frequently overexpressed genes in human cancers. Overexpression of c-Myc in hepatic cells leads to development of hepatocellular carcinoma. Here, we review the current progress in understanding physiologic function and regulation of c-Myc as well as its role in hepatic carcinogenesis and discuss the association of c-Myc activation in chronic hepatitis B infection and the upregulation of HIF-1/VEGF. We also explore the possibility of treating HCC by inhibiting c-Myc and examine the pros and cons of such an approach. Although this strategy is currently not available in clinics, with recent advances in better drug design, pharmacokinetics and pharmacogenetics, inhibition of c-Myc might become a novel therapy for HCC in the future.

Key words: Hepatocellular carcinoma; c-Myc; Novel therapy

Peer reviewers: Ryuichi Morisihita, Professor, Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita City, Osaka, 565-0871, Japan; Dr. Hironori Nakagami, Assisitant Professor, Division of Gene Therapy Science, Osaka University, 2-2 Yamada-oka, Suita 565-0871, Japan

Lin CP, Liu CR, Lee CN, Chan TS, Liu HE. Targeting c-Myc as a novel approach for hepatocellular carcinoma. *World J Hepatol* 2010; 2(1): 16-20 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/16.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.16

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide[1]. Each year, approximately 350 000 patients are diagnosed with HCC in China, representing half of the new cases in the world. Surgical resection is the only way to cure this disease, yet most patients are not suitable for surgery because of poor hepatic reserve, comorbidity, or the presence of infiltrative and metastatic nature lesions. With less than 20% response rate, chemotherapy is not a good option either. Therefore it is imperative to develop novel therapeutics. Genetic analyses have revealed that c-Myc over-expression, which is commonly caused by genomic amplification is present in up to 70% of viral and alcohol-related HCC[2]. Furthermore, the presence of c-Myc amplification portends a more advanced and aggressive phenotype, indicating that c-Myc plays a

critical role in pathogenesis of HCC[3,4]. In this review, we will focus on current understanding of c-Myc in hepatic carcinogenesis and its potential as a novel therapeutic target.

PHYSIOLOGIC ROLE OF C-MYC AND ITS REGULATION

c-Myc, together with L-Myc, and N-Myc in the family of c-Myc genes, was first discovered as the cellular homolog of the v-Myc oncogene[5]. The identification of c-Myc as a target for activation by chromosomal translocation in Burkitt's lymphoma resulted in the decade-long studies for its role in carcinogenesis[6]. In fact, c-Myc is the most commonly overexpressed gene in human cancers. In mammalian cells, c-Myc expression is highly regulated and closely tied to cell growth, apoptosis and differentiation[7]. The importance of c-Myc in development was exemplified by the embryonic lethality of c-Myc homologous knockouts[8].

c-Myc proteins consists of over 430 amino acids with 150 amino-terminal residues in the transactivation domain and 90 carboxy-terminal amino acid in the DNA binding and dimerization domain for binding to the obligate partner, Max[5]. To transactivate its downstream genes, c-Myc has to form heterodimers with Max to bind a consensus E-box site in the target promoter. In contrast to c-Myc, Max is a ubiquitous protein, thus the transactivating activity of c-Myc/Max heterodimers relies on the sophisticated control of c-Myc expression. Yet c-Myc is not the only protein that can partner with Max. Mad is another protein that forms heterodimers with Max to regulate c-Myc/Max transactivating activity. Upon differentiation, the binding of target DNA motif switches from c-Myc/Max to Mad/Max[9-11]. Mad protein contains a Sin3-containing domain that recruits Sin3, transcription repressor N-Cor, and histone deacetylase to repress target gene expression, thus adding another layer of control for c-Myc/Max mediated transactivation[12].

However, c-Myc also acts as a transcription repressor, especially for genes regarded to be differentiation markers. For example, when it is recruited by Miz-1 to target DNA binding motif as in the scenario found in p21[13]. Recent studies have found that c-Myc interacts with Miz-1 and recruit DNA methyltransferase DNMT3 to p21 promoter to silence p21 transcription, a critical step during tumorigenesis[14]. Along with the recruitment of DNA methyltransferases, c-Myc also acts as transcription repressor by interacting with histone deacetylases[15]. Other proteins related to cellular differentiation such as CCAAT/enhancer binding proteins and AP-2 have also been shown to be modulated by c-Myc-mediated transcription repression[16,17]. Both the transactivating and transcription-repressive properties are essential for c-Myc-mediated transforming activity.

In the past decades, various approaches have been used to identify c-Myc target genes[18-22]. So far, as many as 15%-20% of human genes can be regulated directly or indirectly by c-Myc. These genes are related to cell cycle control, protein synthesis, cytoskeleton and cell motility, cell metabolism, and microRNA- the small regulatory molecules that regulate the stability and translation of target mRNA[23]. How these genes interact with each other to modulate growth, differentiation, apoptosis, and survival is largely unknown, and it will require tremendous efforts to dissect the intricate networks and elucidate their role in tumorigenesis.

In order fine tune the sophisticated cellular network, the activity of c-Myc is tightly regulated at multiple levels. The half-life of c-Myc is as short as 20-30 min, meaning that its level changes dynamically in response to a broad range of cellular activities. But in cancer cells, the delicate balance of c-Myc expression is deranged by diverse mechanisms such as unidentified epigenetic aberration, dysregulated transcription, altered protein functionality, or resistance to modulation and proteasomal degradation. The story of c-Myc-mediated tumorigenesis is further complicated by a recent finding, indicating that it is not just its overexpression that matters, the levels of expression also determine its cellular response[24]. Low levels of deregulated c-Myc induce proliferation and sensitize cells to apoptotic signals; while high levels of c-Myc activate intrinsic ARF/p53 surveillance pathways. It is conceivable that different levels of c-Myc might trigger distinct subsets of target genes to determine the cell fate.

ROLE OF C-MYC DURING HEPATIC CARCINOGENESIS

The association of c-Myc with liver carcinogenesis was first identified by the high expression of c-Myc in chronic liver disease and HCC[25,26] and the frequent c-Myc amplification in liver cancer tissue, which is commonly seen in patients at younger age and with poor prognosis[3,4,25]. Using a chemically-induced liver cancer model, the expression of c-Myc is increased in proportion to hepatic injury but not in normal liver[27]. Studies on the HBV, whose chronic infection is often associated with HCC in Asian countries, also identified that HBx has been implicated in HBV-mediated HCC[28]. HBx transforms hepatocytes through multiple mechanisms. One of the critical genes activated by HBx is c-Myc[29,30]. In turn, activation of c-Myc accelerates HBx-mediated oncogenic potential[31], further underscoring the importance of c-Myc in HCC development. One of the downstream genes activated by c-Myc in HCC is human telomerase reverse transcriptase (hTERT), which has two c-Myc-binding E-boxes in its core promoter and is a direct target of c-Myc[32]. The activation of hTERT by c-Myc in HCC has important clinical significance. Inhibition of hTERT activity by either RNAi, or lipid-conjugated oligonucleotides leads to tumor regression in xenogenic HCC models[33, 34].

Another gene that interacts with c-Myc during hepatocarcinogenesis is HIF-1α, which is upregulated

during hypoxia and induces angiogenesis. HIF-1α cooperates with c-Myc to enhance the expression of vascular-endothelial growth factor-A (VEGFA), a critical gene for angiogenesis[35]. Both HBx and HCV infection have been found to stabilize HIF-1α expression in HCC cells[36,37]. Such stabilization could be critical in promoting hepatic carcinogenesis and be responsible for the drug resistance in HCC[38].

TARGETING C-MYC IN HEPATOCELLULAR CARCINOMA

Given the importance of c-Myc in HCC carcinogenesis, it is not surprising that c-Myc is an attractive target for developing novel therapies. The first evidence that down-regulation of c-Myc can be used as a strategy to treat HCC comes from an inducible c-Myc animal model, in which inactivation of c-Myc induced the regression and differentiation of liver tumors[39], yet could not eradicate them. This finding also echoes the recent discovery that, among the four factors required to maintain stem cell phenotypes, c-Myc is crucial[40-42]. Subsequent studies have indicated that in cells with intact p53, Rb and p16 signaling, inactivation of c-Myc leads to cell senescence[43]. This is also consistent with current knowledge on the relationship between cell senescence and hTERT. In addition, using antisense oligonucleotide strategies to downregulate c-Myc also inhibits HCC growth *in vitro*[44]. Recently small-molecule inhibitors that interfere with the c-Myc/Max heterodimerization have also been developed to block c-Myc-mediated transactivation[45]. Testing one of these small molecule c-Myc inhibitors, 10058-F4, in HCC reveals that 10058-F4 inhibited the growth of HCC cells *in vitro*, blocked the binding of E-box, and downregulated hTERT activity. Furthermore, c-Myc inhibition further sensitizes the chemotherapeutic agents against HCC[46]. However, the use of these small molecule c-Myc inhibitors *in vivo* has been less encouraging, probably due to rapid metabolism, resulting in low concentrations in tumors[47]. Subsequent development of c-Myc-Max inhibitors has tried to improve the activity with better pharmacokinetic profiles[48]. Hopefully these new compounds could better inhibit HCC in future *in vivo* studies.

Currently another small molecule compound, CX-3453 (Quarfloxin), which targets c-Myc by reducing c-Myc mRNA, is now in phase Ⅱ clinical trials (NCT00780663) for neuroendocrine carcinoma. Likewise, CX-3543 also inhibits VEGF expression. Since the small molecule VEGFR inhibitor, sorafenib, has been approved for treating advanced HCC[49]. Testing this compound in HCC might shed more light on its potential for future HCC therapy.

However, some caveats are noteworthy in targeting c-Myc in HCC. First, in a transgenic model, re-activation of c-Myc leads to regrowth of tumors, indicating that this approach might target more mature cancer cells, instead of cancer stem cells. A combination with other strategies, such as chemotherapy or agents that target other critical pathways might be needed to enhance anti-cancer effects. In addition, there is concern about systemic toxicity upon c-Myc inhibition, especially in patients with impaired hepatic reserve. In an animal model, knocking down c-Myc expression does not impair liver regeneration, but the architecture of c-Myc-deficient hepatic tissues is disorganized with hypertrophied hepatocytes[50]. The less-than-anticipated toxicity in adult animals indicates that c-Myc might be dispensable in adult but not in neonatal tissues. Further investigation is crucial to determine whether the disorganized hepatic tissues still function like normal tissues and whether disorganized hepatic cells are prone to transformation.

CONCLUSION

Since the first discovery of its oncogenic properties in Burkitt's lymphoma more than two decades ago, the role of c-Myc in normal and neoplastic cells has been extensively studied[51]. Although its critical functions in regulating cell physiology and in carcinogenesis have been well-recognized, the development of c-Myc as a therapeutic target lags far behind basic research. Reasons for such a slow progress are related to the sophisticated regulation of its expression and concerns of potential catastrophic events upon its inhibition. Indeed, even minor differences in its expression level might have divergent consequences[24]. Yet, with the advances in drug design, and in imaging tools to monitor cellular activity, it is now possible to better target c-Myc and investigate its potential as a novel therapeutic agent for HCC.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249
2 **Schlaeger C**, Longerich T, Schiller C, Bewerunge P, Mehrabi A, Toedt G, Kleeff J, Ehemann V, Eils R, Lichter P, Schirmacher P, Radlwimmer B. Etiology-dependent molecular mechanisms in human hepatocarcinogenesis. *Hepatology* 2008; **47**: 511-520
3 **Kawate S**, Fukusato T, Ohwada S, Watanuki A, Morishita Y. Amplification of c-myc in hepatocellular carcinoma: correlation with clinicopathologic features, proliferative activity and p53 overexpression. *Oncology* 1999; **57**: 157-163
4 **Peng SY**, Lai PL, Hsu HC. Amplification of the c-myc gene in human hepatocellular carcinoma: biologic significance. *J Formos Med Assoc* 1993; **92**: 866-870
5 **Dang CV**. c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol* 1999; **19**: 1-11
6 **Battey J**, Moulding C, Taub R, Murphy W, Stewart T, Potter H, Lenoir G, Leder P. The human c-myc oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. *Cell* 1983; **34**: 779-787
7 **Dang CV**, O'Donnell KA, Zeller KI, Nguyen T, Osthus RC, Li F. The c-Myc target gene network. *Semin Cancer Biol* 2006; **16**: 253-264
8 **Davis AC**, Wims M, Spotts GD, Hann SR, Bradley A. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. *Genes Dev* 1993; **7**: 671-682

9 **Larsson LG**, Pettersson M, Oberg F, Nilsson K, Lüscher B. Expression of mad, mxi1, max and c-myc during induced differentiation of hematopoietic cells: opposite regulation of mad and c-myc. *Oncogene* 1994; **9**: 1247-1252

10 **Sommer A**, Bousset K, Kremmer E, Austen M, Lüscher B. Identification and characterization of specific DNA-binding complexes containing members of the Myc/Max/Mad network of transcriptional regulators. *J Biol Chem* 1998; **273**: 6632-6642

11 **Xu D**, Popov N, Hou M, Wang Q, Björkholm M, Gruber A, Menkel AR, Henriksson M. Switch from Myc/Max to Mad1/Max binding and decrease in histone acetylation at the telomerase reverse transcriptase promoter during differentiation of HL60 cells. *Proc Natl Acad Sci USA* 2001; **98**: 3826-3831

12 **Harper SE**, Qiu Y, Sharp PA. Sin3 corepressor function in Myc-induced transcription and transformation. *Proc Natl Acad Sci USA* 1996; **93**: 8536-8540

13 **Wu S**, Cetinkaya C, Munoz-Alonso MJ, von der Lehr N, Bahram F, Beuger V, Eilers M, Leon J, Larsson LG. Myc represses differentiation-induced p21CIP1 expression via Miz-1-dependent interaction with the p21 core promoter. *Oncogene* 2003; **22**: 351-360

14 **Brenner C**, Deplus R, Didelot C, Loriot A, Viré E, De Smet C, Gutierrez A, Danovi D, Bernard D, Boon T, Pelicci PG, Amati B, Kouzarides T, de Launoit Y, Di Croce L, Fuks F. Myc represses transcription through recruitment of DNA methyltransferase corepressor. *EMBO J* 2005; **24**: 336-346

15 **Kurland JF**, Tansey WP. Myc-mediated transcriptional repression by recruitment of histone deacetylase. *Cancer Res* 2008; **68**: 3624-3629

16 **Zhang Y**, Sif S, DeWille J. The mouse C/EBPdelta gene promoter is regulated by STAT3 and Sp1 transcriptional activators, chromatin remodeling and c-Myc repression. *J Cell Biochem* 2007; **102**: 1256-1270

17 **Sakamuro D**, Prendergast GC. New Myc-interacting proteins: a second Myc network emerges. *Oncogene* 1999; **18**: 2942-2954

18 **Eilers M**, Picard D, Yamamoto KR, Bishop JM. Chimaeras of myc oncoprotein and steroid receptors cause hormone-dependent transformation of cells. *Nature* 1989; **340**: 66-68

19 **Haggerty TJ**, Zeller KI, Osthus RC, Wonsey DR, Dang CV. A strategy for identifying transcription factor binding sites reveals two classes of genomic c-Myc target sites. *Proc Natl Acad Sci USA* 2003; **100**: 5313-5318

20 **Guo QM**, Malek RL, Kim S, Chiao C, He M, Ruffy M, Sanka K, Lee NH, Dang CV, Liu ET. Identification of c-myc responsive genes using rat cDNA microarray. *Cancer Res* 2000; **60**: 5922-5928

21 **Elkon R**, Zeller KI, Linhart C, Dang CV, Shamir R, Shiloh Y. In silico identification of transcriptional regulators associated with c-Myc. *Nucleic Acids Res* 2004; **32**: 4955-4961

22 **Berns K**, Hijmans EM, Koh E, Daley GQ, Bernards R. A genetic screen to identify genes that rescue the slow growth phenotype of c-myc null fibroblasts. *Oncogene* 2000; **19**: 3330-3334

23 **Gao P**, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T, Zeller KI, De Marzo AM, Van Eyk JE, Mendell JT, Dang CV. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. *Nature* 2009; **458**: 762-765

24 **Murphy DJ**, Junttila MR, Pouyet L, Karnezis A, Shchors K, Bui DA, Brown-Swigart L, Johnson L, Evan GI. Distinct thresholds govern Myc's biological output in vivo. *Cancer Cell* 2008; **14**: 447-457

25 **Chan KL**, Guan XY, Ng IO. High-throughput tissue microarray analysis of c-myc activation in chronic liver diseases and hepatocellular carcinoma. *Hum Pathol* 2004; **35**: 1324-1331

26 **Yuen MF**, Wu PC, Lai VC, Lau JY, Lai CL. Expression of c-Myc, c-Fos, and c-jun in hepatocellular carcinoma. *Cancer* 2001; **91**: 106-112

27 **Fang CH**, Zhang GQ, Zhu XY, Gong JQ. Distribution of oval cells and c-myc mRNA expression in mouse hepatocarcinogenesis. *Hepatobiliary Pancreat Dis Int* 2004; **3**: 433-439

28 **Yu DY**, Moon HB, Son JK, Jeong S, Yu SL, Yoon H, Han YM, Lee CS, Park JS, Lee CH, Hyun BH, Murakami S, Lee KK. Incidence of hepatocellular carcinoma in transgenic mice expressing the hepatitis B virus X-protein. *J Hepatol* 1999; **31**: 123-132

29 **Balsano C**, Avantaggiati ML, Natoli G, De Marzio E, Will H, Perricaudet M, Levrero M. Full-length and truncated versions of the hepatitis B virus (HBV) X protein (pX) transactivate the cmyc protooncogene at the transcriptional level. *Biochem Biophys Res Commun* 1991; **176**: 985-992

30 **Wu CG**, Salvay DM, Forgues M, Valerie K, Farnsworth J, Markin RS, Wang XW. Distinctive gene expression profiles associated with Hepatitis B virus x protein. *Oncogene* 2001; **20**: 3674-3682

31 **Terradillos O**, Billet O, Renard CA, Levy R, Molina T, Briand P, Buendia MA. The hepatitis B virus X gene potentiates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* 1997; **14**: 395-404

32 **Wu KJ**, Grandori C, Amacker M, Simon-Vermot N, Polack A, Lingner J, Dalla-Favera R. Direct activation of TERT transcription by c-MYC. *Nat Genet* 1999; **21**: 220-224

33 **Zhang PH**, Zou L, Tu ZG. RNAi-hTERT inhibition hepatocellular carcinoma cell proliferation via decreasing telomerase activity. *J Surg Res* 2006; **131**: 143-149

34 **Djojosubroto MW**, Chin AC, Go N, Schaetzlein S, Manns MP, Gryaznov S, Harley CB, Rudolph KL. Telomerase antagonists GRN163 and GRN163L inhibit tumor growth and increase chemosensitivity of human hepatoma. *Hepatology* 2005; **42**: 1127-1136

35 **Huang LE**. Carrot and stick: HIF-alpha engages c-Myc in hypoxic adaptation. *Cell Death Differ* 2008; **15**: 672-677

36 **Nasimuzzaman M**, Waris G, Mikolon D, Stupack DG, Siddiqui A. Hepatitis C virus stabilizes hypoxia-inducible factor 1alpha and stimulates the synthesis of vascular endothelial growth factor. *J Virol* 2007; **81**: 10249-10257

37 **Yoo YG**, Na TY, Seo HW, Seong JK, Park CK, Shin YK, Lee MO. Hepatitis B virus X protein induces the expression of MTA1 and HDAC1, which enhances hypoxia signaling in hepatocellular carcinoma cells. *Oncogene* 2008; **27**: 3405-3413

38 **Han HK**, Han CY, Cheon EP, Lee J, Kang KW. Role of hypoxia-inducible factor-alpha in hepatitis-B-virus X protein-mediated MDR1 activation. *Biochem Biophys Res Commun* 2007; **357**: 567-573

39 **Shachaf CM**, Kopelman AM, Arvanitis C, Karlsson A, Beer S, Mandl S, Bachmann MH, Borowsky AD, Ruebner B, Cardiff RD, Yang Q, Bishop JM, Contag CH, Felsher DW. MYC inactivation uncovers pluripotent differentiation and tumour dormancy in hepatocellular cancer. *Nature* 2004; **431**: 1112-1117

40 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676

41 **Sridharan R**, Tchieu J, Mason MJ, Yachechko R, Kuoy E, Horvath S, Zhou Q, Plath K. Role of the murine reprogramming factors in the induction of pluripotency. *Cell* 2009; **136**: 364-377

42 **Cairo S**, Armengol C, De Reyniès A, Wei Y, Thomas E, Renard CA, Goga A, Balakrishnan A, Semeraro M, Gresh L, Pontoglio M, Strick-Marchand H, Levillayer F, Nouet Y, Rickman D, Gauthier F, Branchereau S, Brugières L, Laithier V, Bouvier R, Boman F, Basso G, Michiels JF, Hofman P, Arbez-Gindre F, Jouan H, Rousselet-Chapeau MC, Berrebi D, Marcellin L, Plenat F, Zachar D, Joubert M, Selves J, Pasquier D, Bioulac-Sage P, Grotzer M, Childs M, Fabre M, Buendia MA. Hepatic stem-like phenotype and interplay of Wnt/beta-catenin and Myc signaling in aggressive childhood liver cancer. *Cancer Cell* 2008; **14**: 471-484

43 **Wu CH**, van Riggelen J, Yetil A, Fan AC, Bachireddy P, Felsher DW. Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. *Proc Natl Acad Sci USA* 2007; **104**: 13028-13033

44 **Simile MM**, De Miglio MR, Muroni MR, Frau M, Asara G, Serra S, Muntoni MD, Seddaiu MA, Daino L, Feo F, Pascale RM. Down-regulation of c-myc and Cyclin D1 genes by antisense oligodeoxy nucleotides inhibits the expression of E2F1 and in vitro growth of HepG2 and Morris 5123 liver cancer cells. *Carcinogenesis* 2004; **25**: 333-341

45 **Yin X**, Giap C, Lazo JS, Prochownik EV. Low molecular weight inhibitors of Myc-Max interaction and function. *Oncogene* 2003; **22**: 6151-6169

46 **Lin CP**, Liu JD, Chow JM, Liu CR, Liu HE. Small-molecule c-Myc inhibitor, 10058-F4, inhibits proliferation, downregulates human telomerase reverse transcriptase and enhances chemosensitivity in human hepatocellular carcinoma cells. *Anticancer Drugs* 2007; **18**: 161-170

47 **Guo J**, Parise RA, Joseph E, Egorin MJ, Lazo JS, Prochownik EV, Eiseman JL. Efficacy, pharmacokinetics, tisssue distribution, and metabolism of the Myc-Max disruptor, 10058-F4 [Z,E]-5-[4-ethylbenzylidine]-2-thioxothiazolidin-4-one, in mice. *Cancer Chemother Pharmacol* 2009; **63**: 615-625

48 **Wang H**, Hammoudeh DI, Follis AV, Reese BE, Lazo JS, Metallo SJ, Prochownik EV. Improved low molecular weight Myc-Max inhibitors. *Mol Cancer Ther* 2007; **6**: 2399-2408

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

50 **Li F**, Xiang Y, Potter J, Dinavahi R, Dang CV, Lee LA. Conditional deletion of c-myc does not impair liver regeneration. *Cancer Res* 2006; **66**: 5608-5612

51 **Taub R**, Kirsch I, Morton C, Lenoir G, Swan D, Tronick S, Aaronson S, Leder P. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci USA* 1982; **79**: 7837-7841

S- Editor Zhang HN **L- Editor** Lalor PF **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.21

World J Hepatol 2010 January 27; 2(1): 21-31
ISSN 1948-5182 (online)

REVIEW

Minimizing liver uptake of cationic ^{99m}Tc radiotracers with ether and crown ether functional groups

Young-Seung Kim, Fan Wang, Shuang Liu

Young-Seung Kim, Shuang Liu, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, United States
Fan Wang, Medical Isotopes Research Center, Peking University, Beijing 100083, China
Author contritions: Kim YS, Liu S and Wang F contributed equally to this work. All of them were responsible for manuscript writing and revising.
Supported in part by Purdue University and research grants: R01 CA115883 A2 (S.L.) from National Cancer Institute, R21 EB003419-02 (S.L.) from National Institute of Biomedical Imaging and Bioengineering and R21 HL083961-01 from National Heart, Lung, and Blood Institute
Correspondence to: Shuang Liu, PhD, School of Health Sciences, Purdue University, 550 Stadium Mall Drive, West Lafayette, IN 47907, United States. lius@pharmacy.purdue.edu
Telephone: +1-765-4940236 Fax: +1-765-4961377
Received: September 1, 2009 Revised: January 11, 2010
Accepted: January 18, 2010
Published online: January 27, 2010

Abstract

Ischemia-related diseases, particularly coronary artery disease (CAD), account for the majority of deaths worldwide. Myocardial ischemia is a serious condition and the delay in reperfusion of ischemic tissues can be life-threatening. This is particular true in the aged population. Rapid and accurate early detection of myocardial ischemia is highly desirable so that various therapeutic regiments can be given before irreversible myocardial damage occurs. Myocardial perfusion imaging with radiotracers is an integral component in evaluations of patients with known or suspected CAD. ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin are commercial radiopharmaceuticals currently available for myocardial perfusion imaging. Despite their widespread clinical applications, both ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin do not meet the requirements of an ideal perfusion imaging agent, largely due to their high liver uptake. The intense liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall. Photon scattering from the high liver radioactivity accumulation remains a significant challenge for diagnosis of heart diseases. This review will summarize the most recent research efforts to minimize the liver uptake of cationic ^{99m}Tc radiotracers by using ether and crown ether-containing chelators. Fast liver clearance will shorten the duration of imaging protocols (< 30 min post-injection), and allow for early acquisition of heart images with high quality. Improvement of heart/liver ratio may permit better detection of the presence and extent of coronary artery disease. Identification of such a new radiotracer that allows for the improved noninvasive assessment of myocardial perfusion would be of considerable benefit in treatment of patients with suspected CAD.

Key words: Myocardial perfusion imaging; Cationic ^{99m}Tc radiotracers; Single photon emission computed tomography

Peer reviewer: Fei Chen, PhD, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, United States

Kim YS, Wang F, Liu S. Minimizing liver uptake of cationic ^{99m}Tc radiotracers with ether and crown ether functional groups. *World J Hepatol* 2010; 2(1): 21-31 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/21.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.21

INTRODUCTION

Coronary artery disease (CAD) is a leading cause of premature and permanent disability. CAD arises from gradual narrowing of coronary artery due to atherosclerotic deposits. The progressive narrowing of coronary artery

eventually predisposes the patient to myocardial ischemia, a condition in which coronary blood flow decreases to a level below what is needed to meet the demand for oxygen and nutrients. When coronary arterial lumen diameter is reduced by 50%, perfusion abnormalities can be detected but patients are usually asymptomatic. When the diameter is reduced by 70%, clinical symptoms occur during myocardial stress because tissue oxygenation is temporarily below what is needed for adequate heart function. In the advanced ischemic CAD, blood flow and tissue oxygenation are too low to sustain cardiac function at rest. As a result, myocardial infarction occurs. Therefore, rapid and accurate early detection of myocardial ischemia and infarction is highly desirable so that appropriate therapeutic regimens can be given before irreversible myocardial damage occurs.

Diagnostic radiotracers are small molecules labeled with a γ-emitter for single photon emission computed tomography (SPECT) or positron-emitter for positron emission tomography (PET). Nuclear cardiology plays a key role in CAD patient management[1-12]. Precise measurement of regional blood flow has significant clinical importance in identifying myocardial ischemia and infarction, defining the extent and severity of disease, assessing myocardial viability, establishing the need for medical and surgical intervention, and monitoring the effects of treatment. Thus, the radiotracer must be taken up by the myocardium proportionally to the blood flow in order to evaluate areas with reduced blood flow. If the patient has CAD, there will be an area of reduced radiotracer uptake in the myocardium, corresponding to the area of reduced blood flow. If the reduced uptake is worse under stress conditions than that at rest, the perfusion defect is most likely due to ischemia. Information gained during perfusion imaging studies can be used not only to identify CAD but also to give insight into the patient's prognosis, such as the probability of a hard cardiac event (myocardial infarction or cardiac-related death).

Despite recent development of new non-invasive imaging technologies, such as stress echocardiography and coronary CT (computed tomography) angiography, and wider availability of PET, myocardial perfusion imaging with SPECT radiotracers remains the mainstay of non-invasive evaluations in patients with known or suspected coronary artery disease (CAD)[1-9]. It is the only available imaging technology that assesses the physiological consequence of coronary stenosis and can be combined with exercise and pharmacological stress[10].

The introduction of ^{201}Tl in 1970's was the turning point of widespread clinical use of myocardial perfusion imaging, and had a profound impact on therapeutic decision-making in patients with CAD over the last three decades. However, the combination of long half-life ($t_{1/2}$ = 73 h), attenuation artifacts due to low abundance of γ-photons and the low count rate from dose constraints may result in suboptimal images in a significant proportion of perfusion imaging studies using ^{201}Tl. In addition, ^{201}Tl images should be taken as soon as it is injected into the patient due to its distribution and redistribution dynamics, which may not be suitable for situations where immediate imaging is not possible (for example, patients with acute myocardial infarction).

Compared to ^{201}Tl, ^{99m}Tc yields relatively high-energy photons (~140 keV) and can be used at high doses due to its short-half life ($t_{1/2}$ = 6.01 h). The use of ^{99m}Tc allows simultaneous assessment of myocardial perfusion and cardiac function in a single study[11]. The combination of half-life, optimal γ-energy and diverse coordination chemistry makes ^{99m}Tc the isotope of choice for development of myocardial perfusion radiotracers. In early 1980s, intensive efforts were focused on the development of ^{99m}Tc complex radioph armaceuticals[1,2,5,13]. As a result, ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin, and ^{99m}Tc-Teboroxime (Figure 1) have been approved as commercial radiopharmaceuticals for myocardial perfusion imaging in nuclear cardiology. These cationic ^{99m}Tc radiotracers are highly lipophilic with cationic or neutral charge, contain at least two ether-like linkages (N-O-R or C-O-R), and are excreted though the hepatobiliary system due to their high lipophilicity.

An ideal perfusion radiotracer should have a high heart uptake with stable myocardial retention, which linearly tracks myocardial blood flow over a wide range. The uptake in the liver and lungs should be minimal so that diagnostically useful images can be obtained within 30 min post-injection. Despite their widespread applications, both ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin do not meet the requirements of an ideal perfusion imaging agent due to their high liver uptake and inability to track the increase in the myocardial blood flow well with roll-off at higher blood flow levels[14]. The intense liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall[1,2,5,12,13]. Because of their enterohepatic clearance, the gut uptake is often aggravated by pharmacological stress[14]. Despite intensive efforts to reduce this interference, photon scattering from the liver activity remains a significant challenge for diagnosis of heart diseases. Thus, it would be of great benefit to develop a new ^{99m}Tc perfusion radiotracer that has high heart uptake with the heart/liver ratio substantially better than that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin. This review article will summarize recent research efforts to minimize the liver uptake of cationic ^{99m}Tc radiotracers, and will focus on the use of ether and crown ether groups to improve their liver clearance kinetics. The main objective is to illustrate that minimizing liver radioactivity accumulation is critically important for new ^{99m}Tc radiotracers. The ultimate goal is to develop a new ^{99m}Tc perfusion radiotracer that will satisfy the unmet medical need and serve a large population of patients with known or suspected CAD.

IMPROVING LIVER CLEARANCE BY ETHER GROUPS

The usefulness of ether groups to reduce radiotracer liver uptake was first observed in development of ^{99m}Tc-

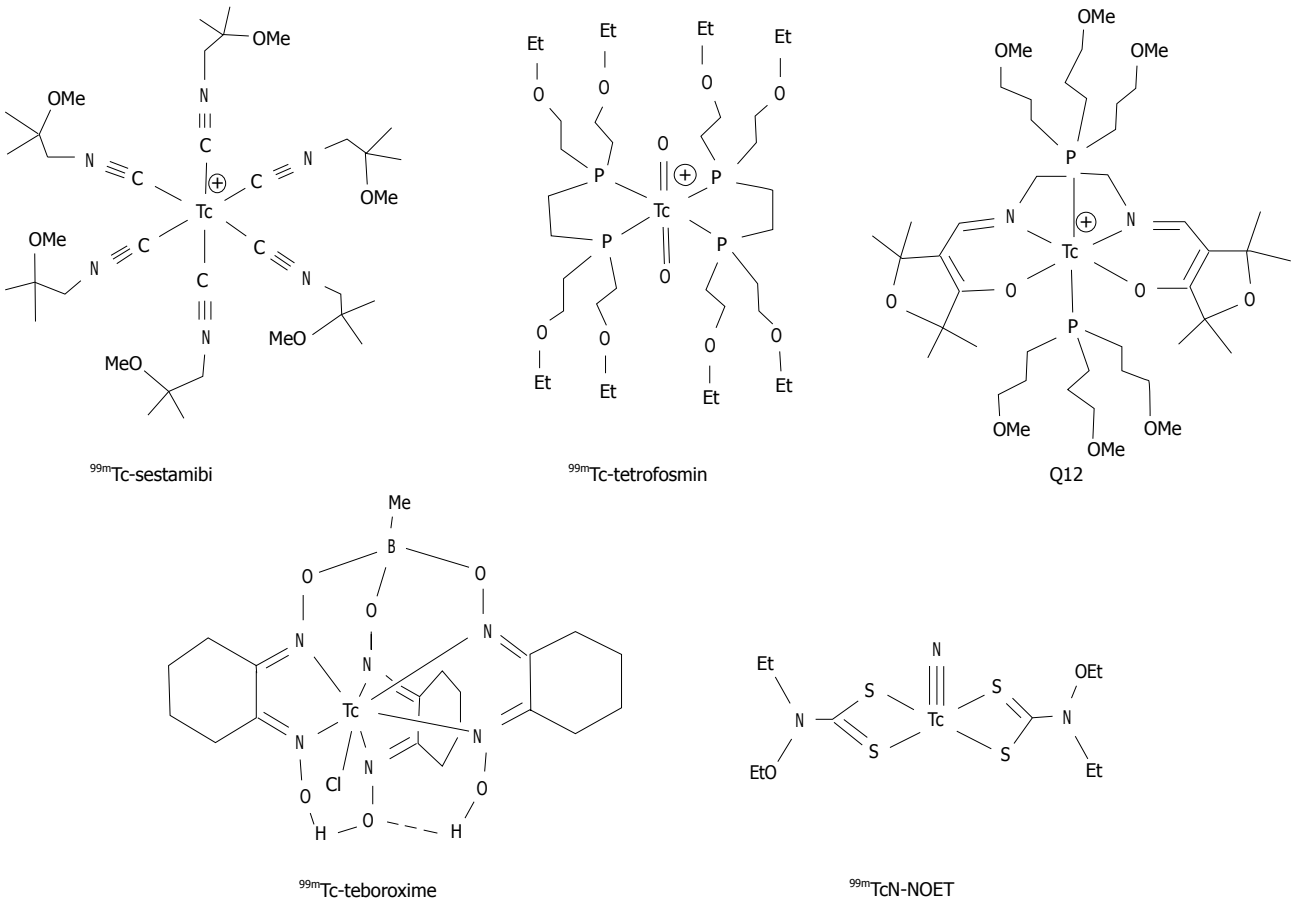


Figure 1 ^{99m}Tc radiotracers useful for heart imaging. ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin and ^{99m}Tc-Teboroxime have been approved as commercial radiopharmaceuticals for myocardial perfusion imaging.

Sestamibi[15-18]. Since then, many ether-containing ligands or chelators have been used to improve T/B ratios of cationic ^{99m}Tc complexes[19-22]. For example, studies on Q-series of ^{99m}Tc complexes showed that ethers on phosphine ligands could improve the heart uptake and imaging properties[19]. ^{99m}Tc-Sestamibi, ^{99m}Tc-Tetrofosmin and Q12 all contain six or more ether groups and show much less liver uptake as compared to ^{99m}TcN-Noet and ^{99m}Tc-Teboroxime (Figure 1).

Ether-containing cationic ^{99m}Tc-nitrido complexes.

Duatti's group reported a series of cationic ^{99m}Tc-nitrido complexes (Figure 2), which contain a $[^{99m}Tc\equiv N]^{2+}$ core, a bidentate dithiocarbamate (DTC), a tridentate PNP-type bisphosphine[24,25]. It was found that the amine-N donor atom in the PNP bisphosphine chelator invariably is trans to the Tc≡N triple bond[24,25]. The nitrogen heteroatom is important to provide stabilization for the $[^{99m}Tc\equiv N]^{2+}$ core. It is remarkable that the combination of DTCs and bisphosphines results in formation of cationic ^{99m}Tc-nitrido complexes of high yield and high radiochemical purity[24,25]. Results from animal studies show that DTCs and bisphosphines have significant impact on biodistribution properties of cationic ^{99m}Tc-nitrido complexes[26-29]. Among the cationic ^{99m}Tc-nitrido complexes evaluated in Sprague-Dawley (SD) rats, ^{99m}TcN-DBODC5 (Figure 2) had a high heart uptake and was able to retain in the rat myocardium for more than 2 h[26,27]. The liver radioactivity accumulation was almost completely eliminated at 2 h p.i. with the heart/liver ratios being 10 × better than that of ^{99m}Tc-Sestamibi[26,27]. Imaging studies also showed that ^{99m}TcN-DBODC5 had a fast liver clearance, and was able to give clear images of the heart as early as 15 min post-injection in SD rats[28]. The first-pass extraction fraction of ^{99m}TcN-DBODC5 was between that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin[29]. ^{99m}TcN-DBODC5 is currently under clinical investigation as a new myocardial perfusion radiotracer.

Other bidentate chelators have been used to replace DTC ligands in ^{99m}TcN-DBODC[30-33]. The use of these bidentate chelators offers a great structural diversity, and allows easy modification of the lipophilicity and biological properties of cationic ^{99m}Tc radiotracers. For example, 2-mercaptopyridine oxide was used to prepare ^{99m}TcN-MPO (Figure 2). ^{99m}TcN-MPO and ^{99m}TcN-DBODC5 shared the same basic structure, but differ in the bidentate π-donor chelating ligand. They also have very similar in vivo stabilities. Biodistribution studies in SD rats showed that ^{99m}TcN-MPO had a high initial heart uptake (2.45 ± 0.58 %ID/g at 5 min post-injection), with a long myocardial retention (2.44 ± 0.46 %ID/g at

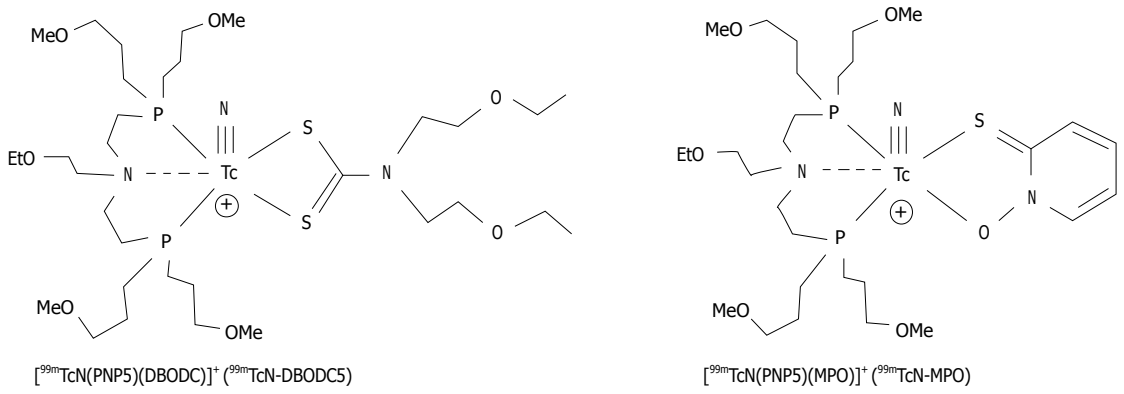


Figure 2 Cationic ^{99m}Tc-nitrido complexes with fast liver clearance and excellent heart/liver ratios. Both ^{99m}TcN-DBODC5 and ^{99m}TcN-MPO are under clinical investigation as new myocardial perfusion imaging agents.

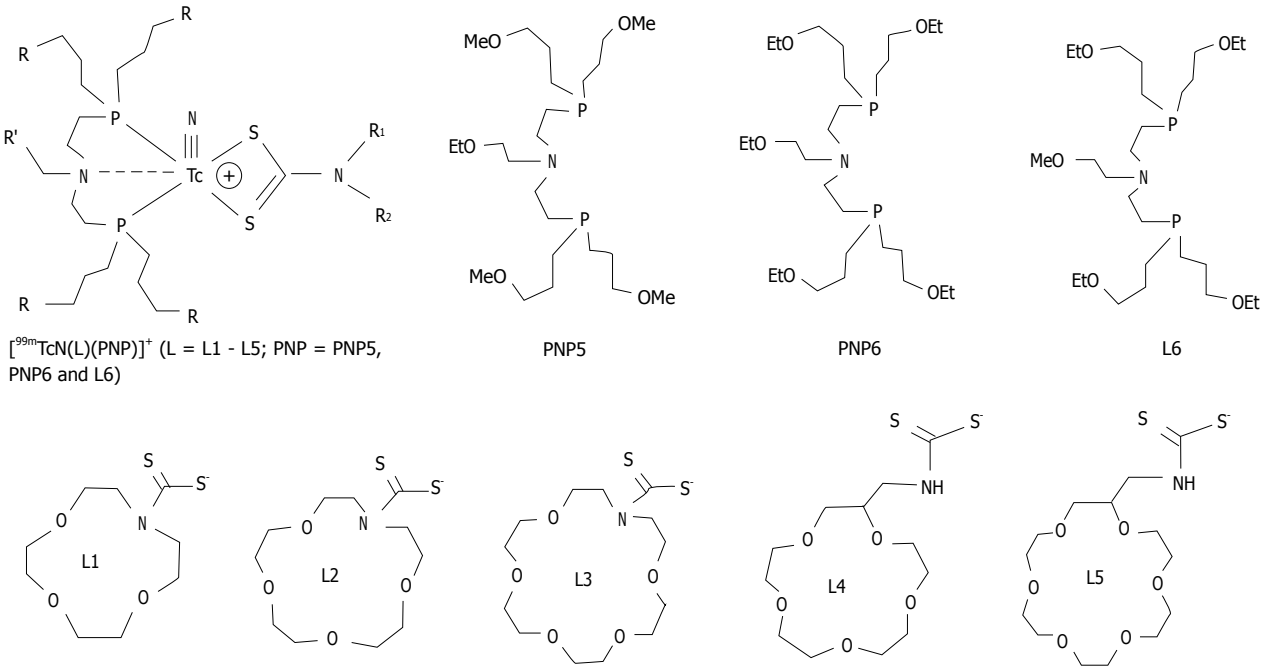


Figure 3 Crown ether-containing DTCs, bisphosphines and their cationic ^{99m}Tc-nitrido complexes.

120 min post-injection)[46]. The heart uptake of ^{99m}TcN-MPO was between that of ^{99m}Tc-sestamibi and that of ^{99m}TcN-DBODC. The liver clearance was fast, resulting in excellent heart/liver ratios. The heart/liver ratio of ^{99m}TcN-MPO at 30 min post-injection was 12.75 ± 3.34, which is ~4 × higher than that of ^{99m}Tc-sestamibi (2.90 ± 0.62) and 2 × higher than that of ^{99m}TcN-DBODC (6.01 ± 1.45). By 120 min post-injection, the heart/liver ratio of ^{99m}TcN-MPO increased to 27.60 ± 8.44, which is 8 × that of ^{99m}Tc-sestamibi (3.52 ± 0.34) and is slightly better than that of ^{99m}TcN-DBODC (21.20 ± 3.39) at the same time point, although this difference is not significant within the experimental error.

Crown ether-containing cationic ^{99m}Tc-nitrido complexes

The crown ether-containing DTCs (Figure 3) are of particular interest because they are able to form stable cationic ^{99m}Tc-nitrido complexes in combination with bisphosphines, and the crown ether groups are able to balance the lipophilicity of cationic ^{99m}Tc-nitrido complexes without changing their overall molecular charge[31,32]. Both crown ether-containing DTCs and bisphosphines have a significant impact on the lipophilicity of their corresponding cationic ^{99m}Tc-nitrido complexes. For example, [^{99m}TcN(L)(PNP6)]$^+$ is much more lipophilic than [^{99m}TcN(L)(PNP5)]$^+$ because of the high lipophilicity of the four ethoxy groups in PNP6[31]. Results from biodistribution studies showed that most of the crown ether-containing cationic ^{99m}Tc-nitrido complexes had a relatively high initial heart uptake with a long myocardial retention[31]. It was also demonstrated that [^{99m}TcN(L4)(L6)]$^+$ (^{99m}TcN-15C5) had the heart/liver ratios that were 4-5 times better than that of ^{99m}Tc-Sestamibi due to its faster liver clearance. The

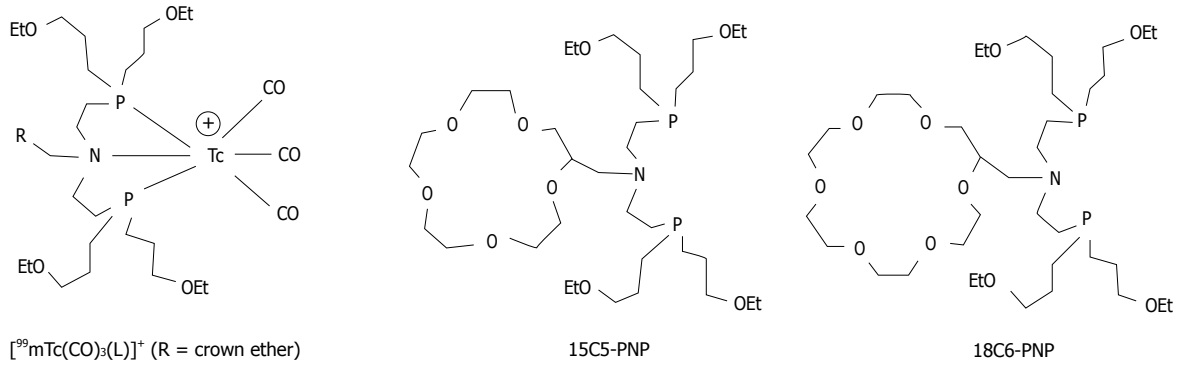


Figure 4 Cationic ^{99m}Tc(I)-tricarbonyl complexes with crown ether-containing PNP bisphosphines.

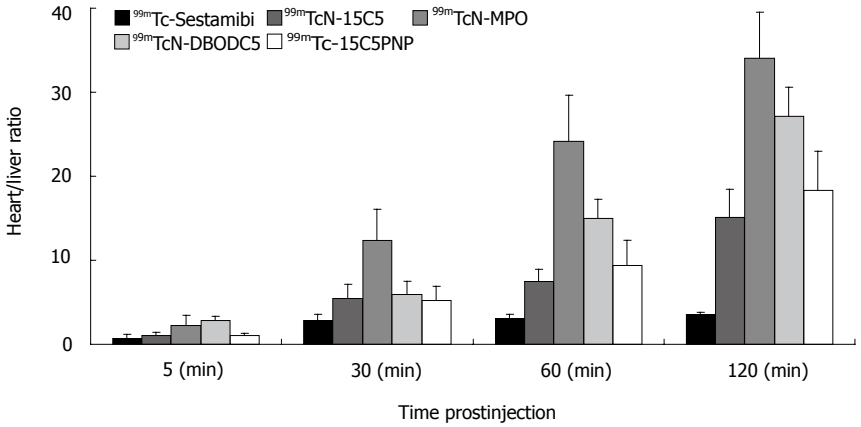


Figure 5 Comparison of heart/liver ratios between [^{99m}TcN(L4)(L6)]$^+$ (^{99m}TcN-15C5), [^{99m}TcN(mpo)(PNP5)]$^+$ (^{99m}TcN-MPO), [^{99m}Tc(CO)$_3$(15CPNP)]$^+$ (^{99m}Tc-15C5PNP), ^{99m}Tc-Sestamibi and ^{99m}TcN-DBODC5 in SD rats.

heart uptake and heart/liver ratios of ^{99m}TcN-15C5 are comparable to that of ^{99m}TcN-DBODC5[32]. The results from planar imaging and SPECT studies in dogs further confirm its faster liver clearance kinetics as compared to that of ^{99m}Tc-Sestamibi[33]. In dogs with acute myocardial infarction, ^{99m}TcN-15C5 was able to detect the perfusion defect as early as 15-30 min.

Crown ether-containing cationic ^{99m}Tc(I)-tricarbonyl complexes

The rich and diverse coordination chemistry of [^{99m}Tc(CO)$_3$]$^+$ offers a tremendous opportunity to develop new ^{99m}Tc(I)-tricarbonyl radiotracers[34-43]. The [^{99m}Tc(CO)$_3$]$^+$ core has also been widely used to prepare the target-specific ^{99m}Tc radiotracers[37-40]. In [^{99m}Tc(H$_2$O)$_3$(CO)$_3$]$^+$, all three water molecules are labile with respect to substitution[40,42]. Monodentate ligands, 2-methoxy-isobutylisonitriles (MIBI) and dimethyl-3-methoxypropylphosphine (DMMPP), have been used to prepare complexes [^{99m}Tc(L)$_3$(CO)$_3$]$^+$ (L = DMMPP and MIBI)[22]. In Sprague-Dawley rats, [^{99m}Tc(DMMPP)$_3$(CO)$_3$]$^+$ and [^{99m}Tc(MIBI)$_3$(CO)$_3$]$^+$ showed high heart uptake. However, their heart/liver and heart/lung ratios are not as good as that of ^{99m}Tc-Sestamibi due to slow hepatobiliary excretion[22]. The major challenge is to maintain the "true" cationic nature of [^{99m}Tc(L)$_3$(CO)$_3$]$^+$ (L = DMMPP and MIBI) in the blood circulation.

It has been reported that bidentate ligands form ^{99m}Tc(I)-tricarbonyl complexes with low solution stability, which may result in high protein binding and high background activity in blood[37,40]. The chloride in blood may react with [^{99m}Tc(L-L)(CO)$_3$]$^+$ to form neutral complexes [^{99m}Tc(L-L)(CO)$_3$Cl] that has low heart uptake and slow hepatobiliary excretion. In contrast, the PNP bisphosphines are able to form highly stable cationic complexes [^{99m}Tc(CO)$_3$(L)]$^+$ (Figure 4: L = 15C5-PNP and 18C6-PNP)[44,45]. The tridentate bisphosphines are critical to maintain the cationic nature of ^{99m}Tc(I)-tricarbonyl complexes. Among the radiotracers evaluated in SD rats, [^{99m}Tc(CO)$_3$(15C5-PNP)]$^+$ (^{99m}Tc-15C5-PNP) had a high initial heart uptake with a long myocardial retention. It also showed a rapid clearance from liver and lungs. The heart/liver ratio of ^{99m}Tc-15C5-PNP is ~2.5× better than that of ^{99m}Tc-Sestamibi at 30 min post-injection. ^{99m}Tc-15C5-PNP is almost identical to ^{99m}TcN-DBODC5 with respect to their heart uptake and heart/liver ratios[45]. Planar imaging studies also demonstrated that ^{99m}Tc-15C5-PNP had a much better liver clearance profile than ^{99m}Tc-sestamibi.

COMPARISON OF BIODISTRIBUTION CHARACTERISTICS

Figure 5 compares their heart/liver ratios in SD rats at 5, 30, 60 and 120 min post-injection. In general, the heart uptake of ^{99m}TcN-15C5, ^{99m}TcN-MPO, ^{99m}Tc-15C5PNP and ^{99m}TcN-DBODC5 are comparable within

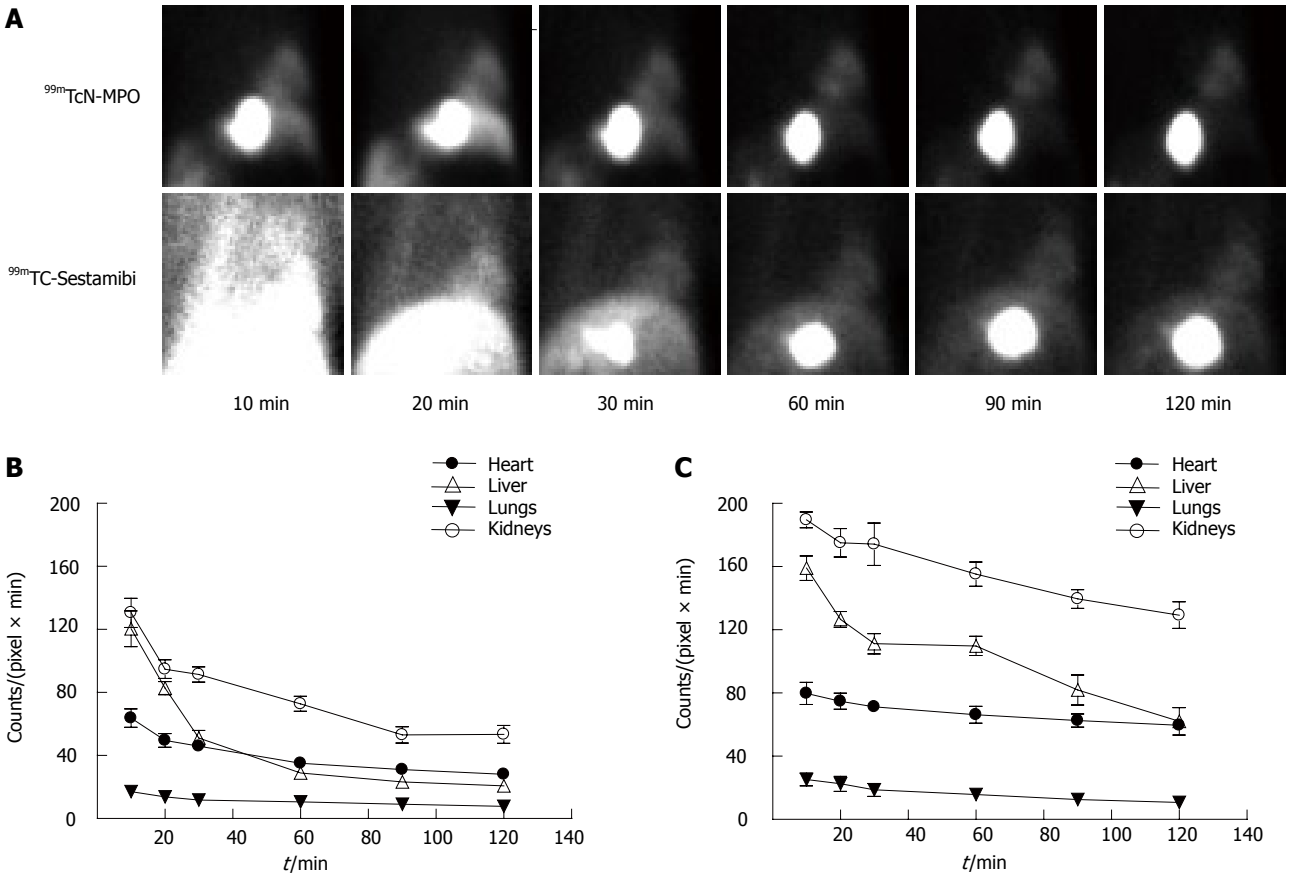


Figure 6 Planar images and organ clearance kinetics of normal dogs. A: Planar images of normal dogs administered with ^{99m}TcN-MPO and ^{99m}Tc-Sestamibi. Both radiotracers had similar initial myocardial uptake; **B: Imaging quantification in normal dogs administered with ^{99m}TcN-MPO; C: Imaging quantification in normal dogs administered with ^{99m}Tc-Sestamibi.** The liver radioactivity of ^{99m}TcN-MPO was markedly decreased within first 60 min whereas ^{99m}Tc-Sestamibi had a slower reduction in liver radioactivity over time. A mild myocardial washout was observed in the dogs administered with ^{99m}TcN-MPO. No significant myocardial washout was seen in dogs administered with ^{99m}Tc-Sestamibi.

the experimental error, and is slightly lower than that of ^{99m}Tc-Sestamibi, particularly at > 60 min post-injection. However, their heart/liver ratios are much better than that of ^{99m}Tc-Sestamibi at 30-120 min post-injection[46]. For example, the heart/liver ratio of ^{99m}TcN-MPO (12.75 ± 3.34) at 30 min post-injection is almost twice of that of ^{99m}TcN-DBODC5 (6.01 ± 1.45), and is ~4 times better than that of ^{99m}Tc-sestamibi (2.90 ± 0.22).

^{99m}TcN-MPO was further evaluated in normal dogs in comparison with ^{99m}Tc-sestamibi. It was found that ^{99m}TcN-MPO and ^{99m}Tc-sestamibi shared very similar blood clearance with < 50% of initial radioactivity remaining at 1 min, < 5% of initial radioactivity remaining at 30 min post-injection. The liver uptake in the dogs administered with ^{99m}TcN-MPO decreased rapidly whereas a prolonged liver uptake was seen in all images of the dogs administered with ^{99m}Tc-Sestamibi (Figure 6). The heart/liver ratio of ^{99m}TcN-MPO increased with time (0.53 ± 0.06 at 10 min and 1.22 ± 0.06 at 60 min post-injection), whereas the heart/liver ratio of ^{99m}Tc-Sestamibi remained relatively unchanged over the 2 h study period (0.50 ± 0.03 at 10 min and 0.60 ± 0.02 at 60 min post-injection). SPECT studies (Figure 7) in canines with acute myocardial infarction indicated that the perfusion defect could be visualized as early as 30 min after administration of ^{99m}TcN-MPO but not ^{99m}Tc-Sestamibi. The combination of fast blood clearance, relatively high heart uptake with prolonged myocardial retention makes ^{99m}TcN-MPO a better perfusion imaging radiotracer than ^{99m}Tc-Sestamibi. On the basis of preliminary results from the rats and dogs, ^{99m}TcN-MPO was selected as a clinical candidate for human studies.

Safety parameters measured up to 24 h after injection of ^{99m}TcN-MPO revealed no adverse reactions and clinically significant drug-related changes in healthy volunteers (unpublished data). The radiation dosimetry of ^{99m}TcN-MPO is comparable to that of ^{99m}Tc-Sestamibi[16] and ^{99m}Tc-Tetrofosmin[17]. Figure 8 shows the whole-body images of a healthy volunteer administered with ^{99m}TcN-MPO (~25 mCi) at 10, 30, 60 and 240 min post-injection. The radioactivity clearance was so fast that the heart was well separated from the left liver lobe at 10 min post-injection. Although its first-pass extraction fraction was between that of ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin[5,6], its fast liver clearance of ^{99m}TcN-MPO will allow early visualization of the heart in patients with CAD. The rapid liver clearance may shorten imaging protocols and permit a more precise determination of perfusion defects in the

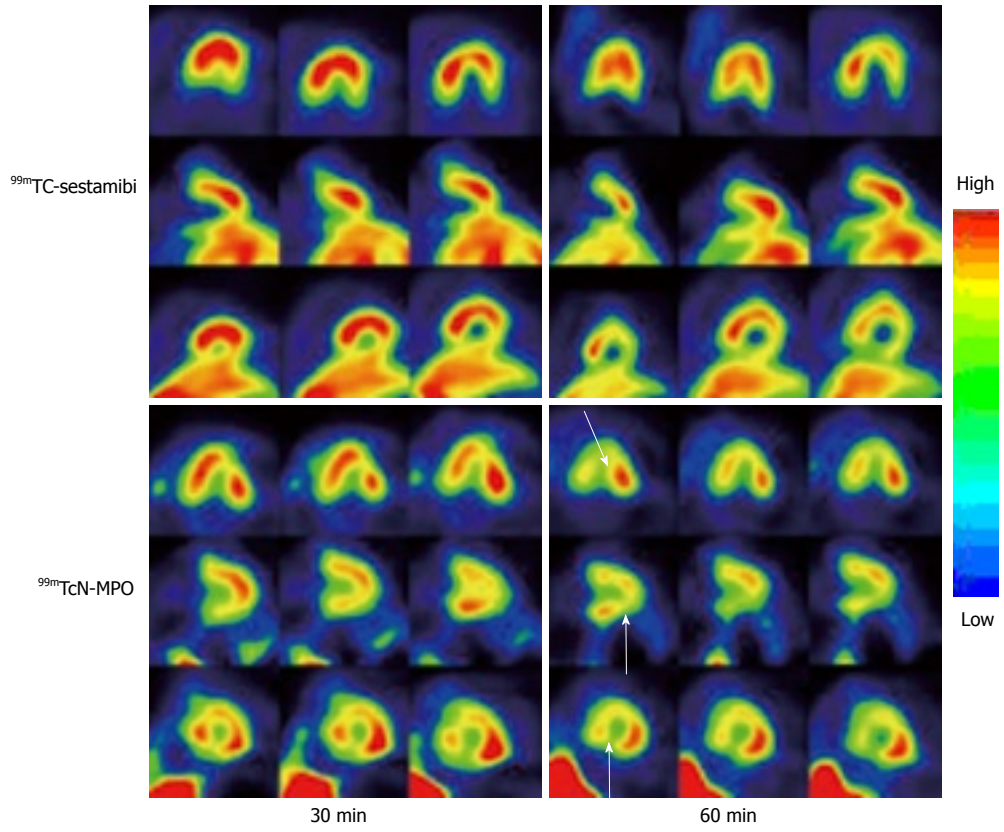


Figure 7 SPECT images of the same dog (with acute myocardial infarction) administered with ~10 mCi of ^{99m}Tc-Sestamibi and ^{99m}TcN-MPO at 30 and 60 min p.i. Arrows indicate the presence of perfusion defects due to myocardial infarction. For ^{99m}TcN-MPO, the perfusion defects were clearly seen as early as 30 min post-injection due to its fast liver clearance. For ^{99m}Tc-Sestamibi, there was a significant between the perfusion defects and the liver radioactivity.

inferoapical wall on myocardial images. ^{99m}TcN-MPO is an excellent alternative to ^{99m}Tc-Sestamibi for myocardial perfusion imaging when the liver uptake makes it difficult to interpret the heart activity in the inferior and left ventricular wall in patients with known or suspected CAD.

LOCALIZATION MECHANISM

Optimal log P values

Myocardium has the highest mitochondrial population that occupies up to 40% of the total volume of myocytes. Other mitochondria-rich organs include salivary glands, liver and kidneys. This may explain why most lipophilic ^{99m}Tc complex cations tend to have high uptake in mitochondria-rich organs, such as heart, liver and kidneys. For a ^{99m}Tc radiotracer to localize in mitochondria, it must be able to across plasma and mitochondrial membrane. Regardless of their charge, most small molecules are able to across the plasma membrane without significant difficulty. However, the mitochondrial membrane is only permeable to those molecules with appropriate molecular charge and molecular shape. While the contribution from mitochondrial potentials provides a driving force for mitochondrial localization of cationic ^{99m}Tc radiotracers, the lipophilicity might modulate their penetration capability across the lipophilic plasma and mitochondrial membranes. There is little information available with regard to the optimal lipophilicity for the heart-selectivity. Cationic ^{99m}Tc radiotracers with the log *P* values > 1.5 often shows a high protein binding and a slow clearance from non-cardiac organs while hydrophilic cationic radiotracers with log *P* < 0.5 usually show a low heart uptake with a fast washout from myocardium[30-32,45]. In both cases, the heart/liver ratio is low because of either high liver uptake or fast myocardial washout. On the basis of studies on cationic ^{99m}Tc-nitrido and ^{99m}Tc(I)-tricarbonyl complexes, it seems that cationic ^{99m}Tc radiotracers must have a log *P* value in the range of 0.9-1.2 in order to achieve a high heart uptake[30-32,45,46].

Subcellular distribution characteristics and mitochondrial localization

To understand the myocardial localization mechanism, the subcellular distribution characteristics of ^{99m}TcN-MPO[47] and ^{99m}TcN-DBODC5[48] in SD rat hearts were analyzed in comparison with ^{99m}Tc-Sestamibi according to the literature[49,50]. It was clearly demonstrated that more than 85% of myocardial radioactivity localized in the mitochondria of myocytes for both ^{99m}TcN-MPO and ^{99m}TcN-DBODC5. There was no significant difference between ^{99m}TcN-MPO, ^{99m}TcN-DBODC5 and ^{99m}Tc-Sestamibi with respect to their mitochondrial radioactivity accumulation, and subcellular distribution characteristics.

MULTIDRUG RESISTANCE GENE EXPRESSION AND LIVER CLEARANCE KINETICS

For the last two decades, many cationic ^{99m}Tc radiotracers have been evaluated for their potential as radiotracers

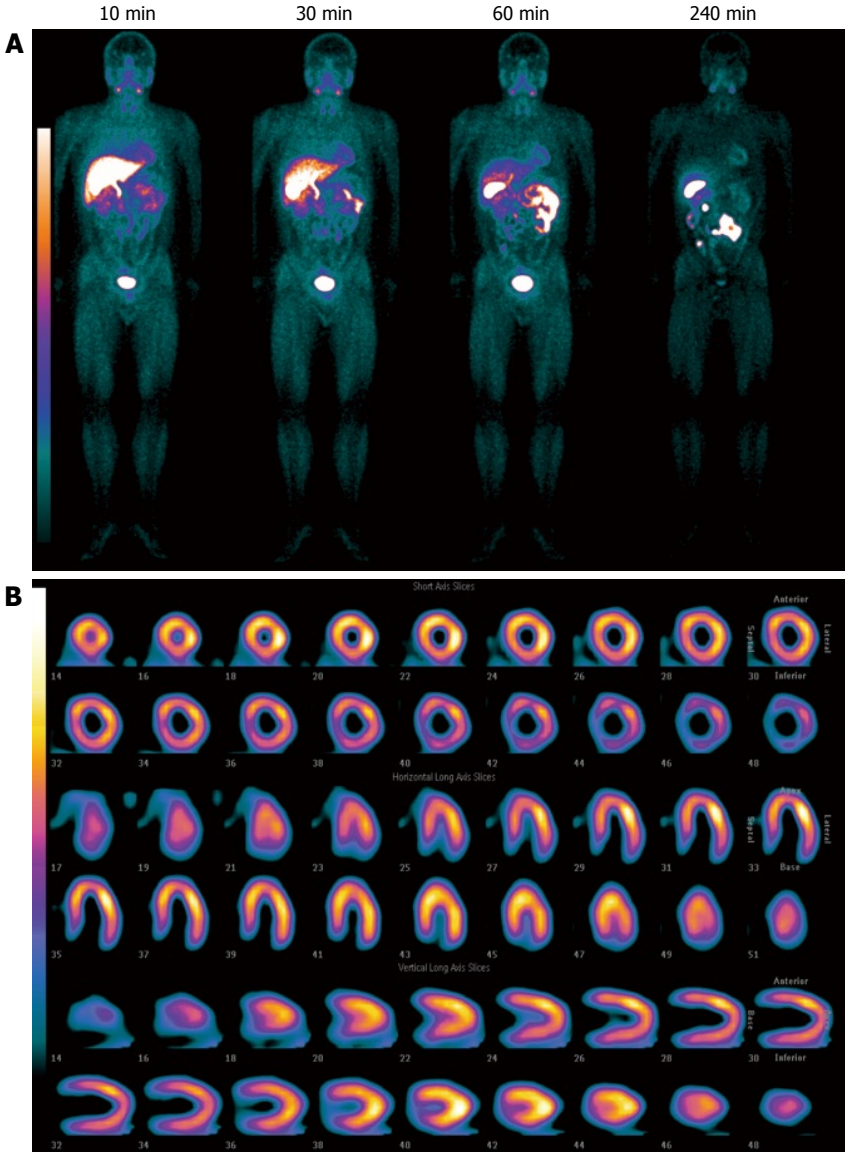


Figure 8 The whole-body images of representative a healthy volunteer administered with ^{99m}TcN-MPO. A: Administered with ^{99m}TcN-MPO (~25 mCi) at 10, 30, 60 and 240 min post-injection; B: Representative SPECT images of the heart **after administration of** ^{99m}TcN-MPO (~25 mCi) at 60 min post-injection.

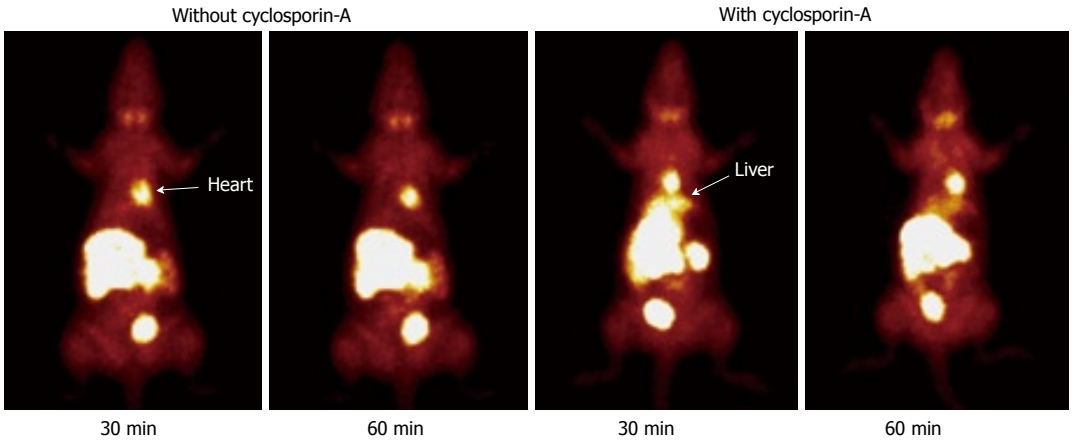


Figure 9 Planar images of the SD rats administered with ~1.0 mCi of ^{99m}TcN-MPO in the absence and presence of cyclosporin-A at 30 and 60 min post-injection. Pre-treatment with cyclosporin-A (14 mg/kg) results in more liver radioactivity accumulation and slower liver clearance.

for heart imaging. However, it is not clear why certain cationic ^{99m}Tc radiotracers show better liver clearance and heart/liver ratios than others, even though they may have a similar lipophilicity. It is well-documented that cationic ^{99m}Tc radiotracers, such as ^{99m}Tc-Sestamibi and ^{99m}Tc-Tetrofosmin, are also clinically useful for noninvasive imaging of multidrug resistance (*MDR*) in tumors of different origin[51-53,57,58]. It is well-documented that MDR1 P-glycoprotein (MDR1 Pgp) and multidrug resistance associated proteins (MRPs) are overexpressed in normal organs that are involved in excretory functions, including kidneys and liver[54-56]. For example, an increased ^{99m}Tc-

Sestamibi uptake in normal liver of cancer patients has been reported after administration of P-glycoprotein inhibitors[59,60]. To demonstrate the correlation between the MDR and MRP transport function of hepatocytes and liver excretion kinetics of ^{99m}TcN-MPO, both biodistribution and imaging studies were performed using the SD rats in the absence/presence of Cyclosporin A. It was found that the uptake of ^{99m}TcN-MPO in the kidneys and liver was significantly increased, and the radioactivity excretion was delayed, in the presence of Cyclosporin A. Similar results were also obtained in planar images (Figure 9) of SD rats administered with ^{99m}TcN-MPO with/without excess Cyclosporin A. Thus, the MDR/MRP transport function is most likely responsible of the fast efflux of ^{99m}TcN-MPO from kidneys and liver.

CONCLUSION

SPECT remains the modality of choice for myocardial perfusion imaging in current clinical practice. The success of SPECT in nuclear cardiology is largely due to the development of new ^{99m}Tc perfusion radiotracers. Studies on ether- and crown ether-containing cationic ^{99m}Tc complexes clearly show that it is possible to design cationic ^{99m}Tc radiotracers with heart/liver ratios substantially better than that of ^{99m}Tc-Sestamibi. Fast liver radioactivity clearance will shorten the duration of imaging protocols (< 30 min post-injection), and allow for early acquisition of images of high quality. Improvement of the heart/liver ratio will permit better detection of perfusion defects with improved noninvasive assessment of the myocardial perfusion. Preliminary studies on ^{99m}TcN-MPO show that it may have a lower first-pass extraction fraction than ^{99m}Tc-Sestamibi. Thus, future research should focus on cationic ^{99m}Tc perfusion radiotracers with both fast liver clearance and better first-pass extraction fraction. Identification of such a new radiotracer would be of considerable benefit in treatment of patients with suspected CAD.

REFERENCES

1 **Nunn AD**. Radiopharmaceuticals for imaging myocardial perfusion. *Semin Nucl Med* 1990; **20**: 111-118
2 **Saha GB**, Go RT, MacIntyre WJ. Radiopharmaceucticals for cardiovascular imaging. *Int J Rad Appl Instrum B* 1992; **19**: 1-20
3 **Opie LH, Hesse B**. Radionuclide tracers in the evaluation of resting myocardial ischaemia and viability. *Eur J Nucl Med* 1997; **24**: 1183-1193
4 **Berman DS**, Germano G, Shaw LJ. The role of nuclear cardiology in clinical decision making. *Semin Nucl Med* 1999; **29**: 280-297
5 **Jain D**. Technetium-99m labeled myocardial perfusion imaging agents. *Semin Nucl Med* 1999; **29**: 221-236
6 **Beller GA**, Bergmann SR. Myocardial perfusion imaging agents: SPECT and PET. *J Nucl Cardiol* 2004; **11**: 71-86
7 **Acampa W**, Di Benedetto C, Cuocolo A. An overview of radiotracers in nuclear cardiology. *J Nucl Cardiol* 2000; **7**: 701-707
8 **Dilsizian V**. The role of myocardial perfusion imaging in vascular endothelial dysfunction. *J Nucl Cardiol* 2000; **7**: 180-184
9 **Beller GA**, Zaret BL. Contributions of nuclear cardiology to diagnosis and prognosis of patients with coronary artery disease. *Circulation* 2000; **101**: 1465-1478
10 **Parker JA**. Cardiac nuclear medicine in monitoring patients with coronary heart disease. *Semin Nucl Med* 2001; **31**: 223-237
11 **Kapur A**, Latus KA, Davies G, Dhawan RT, Eastick S, Jarritt PH, Roussakis G, Young MC, Anagnostopoulos C, Bomanji J, Costa DC, Pennell DJ, Prvulovich EM, Ell PJ, Underwood SR. A comparison of three radionuclide myocardial perfusion tracers in clinical practice: the ROBUST study. *Eur J Nucl Med Mol Imaging* 2002; **29**: 1608-1616
12 **Kailasnath P**, Sinusas AJ. Comparison of Tl-201 with Tc-99m-labeled myocardial perfusion agents: technical, physiologic, and clinical issues. *J Nucl Cardiol* 2001; **8**: 482-498
13 **Banerjee S**, Pillai MR, Ramamoorthy N. Evolution of Tc-99m in diagnostic radiopharmaceuticals. *Semin Nucl Med* 2001; **31**: 260-277
14 **Llaurado JG**. The quest for the perfect myocardial perfusion indicator still a long way to go. *J Nucl Med* 2001; **42**: 282-284
15 **Jones AG**, Abrams MJ, Davison A, Brodack JW, Toothaker AK, Adelstein SJ, Kassis AI. Biological studies of a new class of technetium complexes: the hexakis(alkylisonitrile)technetium(I) cations. *Int J Nucl Med Biol* 1984; **11**: 225-234
16 **Wackers FJ**, Berman DS, Maddahi J, Watson DD, Beller GA, Strauss HW, Boucher CA, Picard M, Holman BL, Fridrich R. Technetium-99m hexakis 2-methoxyisobutyl isonitrile: human biodistribution, dosimetry, safety, and preliminary comparison to thallium-201 for myocardial perfusion imaging. *J Nucl Med* 1989; **30**: 301-311
17 **Higley B**, Smith FW, Smith T, Gemmell HG, Das Gupta P, Gvozdanovic DV, Graham D, Hinge D, Davidson J, Lahiri A. Technetium-99m-1,2-bis[bis(2-ethoxyethyl) phosphino]ethane: human biodistribution, dosimetry and safety of a new myocardial perfusion imaging agent. *J Nucl Med* 1993; **34**: 30-38
18 **Iskandrian AS**, Heo J, Kong B, Lyons E, Marsch S. Use of technetium-99m isonitrile (RP-30A) in assessing left ventricular perfusion and function at rest and during exercise in coronary artery disease, and comparison with coronary arteriography and exercise thallium-201 SPECT imaging. *Am J Cardiol* 1989; **64**: 270-275
19 **Marmion ME**, Woulfe SR, Neumann WL, Nosco DL, Deutsch E. Preparation and characterization of technetium complexes with Schiff base and phosphine coordination. 1. Complexes of technetium-99g and -99m with substituted acac$_2$en and trialkyl phosphines (where acac$_2$en = N,N'-ethylenebis[acetylacetone iminato]). *Nucl Med Biol* 1999; **26**: 755-770
20 **Lisic EC**, Heeg MJ, Deutsch E. ^{99m}Tc(L-L)$^{3+}$ complexes containing ether analogs of DMPE. *Nucl Med Biol* 1999; **26**: 563-571
21 **Tisato F**, Maina T, Shao LR, Heeg MJ, Deutsch E. Cationic [^{99m}TcIII(DIARS)$_2$(SR)$_2$]$^+$ complexes as potential myocardial perfusion imaging agents (DIARS = o-phenylenebis(dimethylarsine);SR- = thiolate). *J Med Chem* 1996; **39**: 1253-1261
22 **Marmion ME**, MacDonald JR. Preparation and biodistribution of ^{99m}Tc(I)-tricarbonyl complexes containing isonitrile and phosphine ligands. *J Nucl Med* 2000; **41**: 124P (abstract No. 489)
23 **Maresca KP**, Keith D, Graham-Coco WA, Banerjee SR, Zubieta JA, Babich JW. Novel ether containing ligand complexes of ^{99m}Tc(CO)$^{3+}$ for cardiac imaging. *J Nucl Med* 2004; **45**: 215P (abstract No. 703)
24 **Bolzati C**, Boschi A, Uccelli L, Tisato F, Refosco F, Cagnolini A, Duatti A, Prakash S, Bandoli G, Vittadini A. Chemistry of the strong electrophilic metal fragment [(99)Tc(N)(PXP)]$^{(2+)}$ (PXP

= diphosphine ligand). A novel tool for the selective labeling of small molecules. *J Am Chem* Soc 2002; **124**: 11468-11479
25 **Bolzati C**, Refosco F, Cagnolini A, Tisato F, Boschi A, Duatti A, Uccelli L, Dolmella A, Marotta E, Tubaro M. Synthesis, solution-state and solid-state structural characterization of monocationic nitrido heterocomplexes [M(N) (DTC)(PNP)]$^+$ (M = ^{99}Tc and Re; DTC = dithiocarbamate; PNP = heterodiphosphane). *Eur J Inorg Chem* 2004; 1902-1913
26 **Boschi A**, Bolzati C, Uccelli L, Duatti A, Benini E, Refosco F, Tisato F, Piffanelli A. A class of asymmetrical nitrido ^{99m}Tc heterocomplexes as heart imaging agents with improved biological properties. *Nucl Med Commun* 2002; **23**: 689-693
27 **Boschi A**, Uccelli L, Bolzati C, Duatti A, Sabba N, Moretti E, Di Domenico G, Zavattini G, Refosco F, Giganti M. Synthesis and biologic evaluation of monocationic asymmetric ^{99m}Tc-nitride heterocomplexes showing high heart uptake and improved imaging properties. *J Nucl Med* 2003; **44**: 806-814
28 **Hatada K**, Riou LM, Ruiz M, Yamamichi Y, Duatti A, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. ^{99m}Tc-N-DBODC5, a new myocardial perfusion imagingagent with rapid liver clearance: comparison with ^{99m}Tc-sestamibi and ^{99m}Tc-tetrofosmin in rats. *J Nucl Med* 2004; **45**: 2095-2101
29 **Hatada K**, Ruiz M, Riou LM, Lima RL, Goode AR, Watson DD, Beller GA, Glover DK. Organ biodistribution and myocardial uptake, washout, and redistribution kinetics of Tc-99m N-DBODC5 when injected during vasodilator stress in canine models of coronary stenoses. *J Nucl Cardiol* 2006; **13**: 779-790
30 **Liu S**. Ether and crown ether-containing cationic ^{99m}Tc complexes useful as radiopharmaceuticals for heart imaging. *Dalton Trans* 2007; 1183-1193
31 **Kim YS**, He Z, Hsieh WY, Liu S. Impact of bidentate chelators on lipophilicity, stability, and biodistribution characteristics of cationic ^{99m}Tc-nitrido complexes. *Bioconjug Chem* 2007; **18**: 929-936
32 **Liu S**, He Z, Hsieh WY, Kim YS. Evaluation of novel cationic (99m)Tc-nitrido complexes as radiopharmaceuticals for heart imaging: improving liver clearance with crown ether groups. *Nucl Med Biol* 2006; **33**: 419-432
33 **Fang W**, Liu Y, Zhu L, Kim YS, Liu S, He ZX. Evaluation of ^{99m}TcN-15C5 as a new myocardial perfusion imaging agent in normal dogs and canines with coronary stenosis. *Nucl Med Commun* 2008; **29**: 775-781
34 **Alberto R**, Schibli R, Egli A, Schubiger PA, Abram U, Kaden TA. A novel organometallic aqua complex of technetium for the labeling of biomolecules: synthesis of $[^{99m}Tc(H_2O)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in aqueous solution and its reaction with a bifunctional ligand. *J Am Chem Soc* 1998; **120**: 7987-7988
35 **Alberto R**, Ortner K, Wheatley N, Schibli R, Schubiger AP. Synthesis and properties of boranocarbonate: a convenient in situ CO source for the aqueous preparation of $[^{(99m)}Tc(OH_{(2)})_3(CO)_3]^+$. *J Am Chem Soc* 2001; **123**: 3135-3136
36 **Alberto R**, Schibli R, Abram U, Egli A, Knapp FF, Schubiger PA. Potential of the "$[M(CO)_3]^+$" (M = Re, Tc) moiety for the labeling of biomolecules. *Radiochim. Acta* 1997; **79**: 99-103
37 **Alberto R**, Schibli R, Waibel R, Abram U, Schubiger PA. Basic aqueous chemistry of $[M(H_2O)_3(CO)_3]^+$ (M = Re, Tc) directed towards radiopharmaceutical application. *Coord Chem Rev* 1999; **190-192**: 901-919
38 **Schubiger PA**, Grünberg J, Ametamey SM, Honer M, Garcia-Garayoa E, Bläuenstein P, Waibel R, Novak-Hofer I, Schibli R. Radiopharmaceuticals: from molecular imaging to targeted radionuclide therapy. *Chimia* 2004; **58**: 731-735
39 **Banerjee SR**, Maresca KP, Francesconi L, Valliant J, Babich JW, Zubieta J. New directions in the coordination chemistry of ^{99m}Tc: a reflection on technetium core structures and a strategy for new chelate design. *Nucl Med Biol* 2005; **32**: 1-20
40 **Schibli R**, Schubiger PA. Current use and future potential of organometallic radiopharmaceuticals. *Eur J Nucl Med Mol Imaging* 2002; **29**: 1529-1542
41 **Häfliger P**, Mundwiler S, Ortner K, Spingler B, Alberto R, Andócs G, Balogh L, Bodo K. Structure, stability, and biodistribution of cationic $[M(CO)_3]^+$ (M = Re, ^{99}Tc, ^{99m}Tc) complexes with tridentate amine ligands. *Synth React Inorg Met-Org Chem* 2005; **35**: 27-34
42 **Rattat D**, Eraets K, Cleynhens B, Knight H, Fonge H, Verbruggen A. Comparison of tridentate ligands in competition experiments for their ability to form a $[^{99m}Tc(CO)_3]^+$ complex. *Tetrahedron Lett* 2004; **45**: 2531-2534
43 **Schibli R**, La Bella R, Alberto R, Garcia-Garayoa E, Ortner K, Abram U, Schubiger PA. Influence of the denticity of ligand systems on the in vitro and in vivo behavior of (99m)Tc(I)-tricarbonyl complexes: a hint for the future functionalization of biomolecules. *Bioconjug Chem* 2000; **11**: 345-351
44 **Kim YS**, He Z, Schibli R, Liu S. Synthesis, characterization and X-ray crystal structure of $[Re(PNP)(CO)_3]Br \cdot 2CH_3OH$: a model compound for a novel cationic ^{99m}Tc radiotracers useful for heart imaging. *Inorg Chim Acta* 2006; **359**: 2479-2488
45 **He Z**, Hsieh WY, Kim YS, Liu S. Evaluation of novel cationic ^{99m}Tc(I)-tricarbonyl complexes as potential radiotracers for myocardial perfusion imaging. *Nucl Med Biol* 2006; **33**: 1045-1053
46 **Kim YS**, Wang J, Broisat A, Glover DK, Liu S. Tc-99m-N-MPO: novel cationic Tc-99m radiotracer for myocardial perfusion imaging. *J Nucl Cardiol* 2008; **15**: 535-546
47 **Kim YS**, Shi J, Zhai S, Hou G, Liu S. Mechanism for myocardial localization and rapid liver clearance of Tc-99m-N-MPO: a new perfusion radiotracer for heart imaging. *J Nucl Cardiol* 2009; **16**: 571-579
48 **Bolzati C**, Cavazza-Ceccato M, Agostini S, Tokunaga S, Casara D, Bandoli G. Subcellular distribution and metabolism studies of the potential myocardial imaging agent $[^{99m}Tc(N)(DBODC)(PNP_5)]^+$. *J Nucl Med* 2008; **49**: 1336-1344
49 **Carvalho PA**, Chiu ML, Kronauge JF, Kawamura M, Jones AG, Holman BL, Piwnica-Worms D. Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* 1992; **33**: 1516-1522
50 **Piwnica-Worms D**, Kronauge JF, Chiu ML. Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. *Mitochondrial and plasma membrane potential dependence. Circulation* 1990; **82**: 1826-1838
51 **Sharma V**, Piwnica-Worms D. Metal complexes for therapy and diagnosis of drug resistance. *Chem Rev* 1999; **99**: 2545-2560
52 **Sharma V**. Radiopharmaceuticals for assessment of multidrug resistance P-glycoprotein-mediated drug transport activity. *Bioconjug Chem* 2004; **15**: 1464-1474
53 **Vaidyanathan G**, Zalutsky MR. Imaging drug resistance with radiolabeled molecules. *Curr Pharm Des* 2004; **10**: 2965-2979
54 **Gatmaitan ZC**, Arias IM. Structure and function of P-glycoprotein in normal liver and small intestine. *Adv Pharmacol* 1993; **24**: 77-97
55 **Lee CH**, Bradley G, Zhang JT, Ling V. Differential expression of P-glycoprotein genes in primary rat hepatocyte culture. *J Cell Physiol* 1993; **157**: 392-402
56 **Mayer R**, Kartenbeck J, Büchler M, Jedlitschky G, Leier I, Keppler D. Expression of the MRP gene-encoded conjugate export pump in liver and its selective absence from the canalicular membrane in transport-deficient mutant hepatocytes. *J Cell Biol* 1995; **131**: 137-150
57 **Dyszlewski M**, Blake HM, Dahlheimer JL, Pica CM, Piwnica-Worms D. Characterization of a novel ^{99m}Tc-carbonyl complex as a functional probe of MDR1 P-glycoprotein transport activity. *Mol Imaging* 2002; **1**: 24-35
58 **Muzzammil T**, Ballinger JR, Moore MJ. ^{99}Tcm-sestamibi imaging of inhibition of the multidrug resistance transporter in a mouse xenograft model of human breast cancer. *Nucl Med Commun* 1999; **20**: 115-122
59 **Luker GD**, Fracasso PM, Dobkin J, Piwnica-Worms D. Mo-

dulation of the multidrug resistance P-glycoprotein: detection with technetium-99m-sestamibi in vivo. *J Nucl Med* 1997; **38**: 369-372

60 **Agrawal M**, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, Fojo T, Chen CC. Increased ^{99m}Tc-sestamibi accumulation in normal liver and drug-resistant tumors after the administration of the glycoprotein inhibitor, XR9576. *Clin Cancer Res* 2003; **9**: 650-656

S- Editor Zhang HN **L- Editor** Negro F **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.32

World J Hepatol 2010 January 27; 2(1): 32-41
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Differential expression of cell cycle regulators in HCV-infection and related hepatocellular carcinoma

Azza E El Bassiouny, Mona M Nosseir, Mona K Zoheiry, Noha A Ameen, Ahmed M Abdel-Hadi, Ibrahim M Ibrahim, Suher Zada, Abdel-Hakeem Saad El-Deen, Nora E El-Bassiouni

Azza E El Bassiouny, Mona K Zoheiry, Department of Immunology, Theodor Bilharz Research Institute, PO Box 30 Imbaba, Giza 12411, Egypt
Mona M Nosseir, Ahmed M Abdel-Hadi, Department of Pathology, TBRI, PO Box 30 Imbaba, Giza12411, Egypt
Noha A Ameen, Central Laboratory, Theodor Bilharz Research Institute, PO Box 30 Imbaba, Giza 12411, Egypt
Ibraheim M Ibrahim, Department of Hepato-Gastroenterology, Theodor Bilharz Research Institute, PO Box 30 Imbaba, Giza 12411, Egypt
Suher Zada, Biology Department, American University, PO Box 74, New Cairo 11835, Egypt
Abdel-Hakeem Saad El-Deen, Zoology Department, Faculty of Science, Cairo University, Giza 12613, Egypt
Nora E El-Bassiouni, Department of Hematology, Theodor Bilharz Research Institute, PO Box 30 Imbaba, Giza 12411, Egypt
Author contributions: El Bassiouny AE and El-Bassiouni NE: construction of the plan and design of work, interpretation and discussion of results, writing and revising the manuscript and financial support; Nosseir MM: interpretation and discussion of the results of histopathological and Immunohistochemical studies, close supervision of the different sets of immunohistochemistry, writing and revising the text; Zoheiry MK: help in performance of laboratory investigations, collection of data, writing and revising the manuscript; Ameen NA: collection of data, help in interpretation and discussion of the results. Performance of laboratory investigations and immunohistochemical staining; Abdel-Hadi AM: scientific interpretation of results of liver biopsies; Ibraheim IM: Providing clinical data and liver biopsy specimens; Zada S: scientific and financial support; Saad El-Deen AH: scientific supervision of the work.
Supported by Theodor Bilharz Research Institute (Grant # 74D) in collaboration with the American University of Cairo
Correspondence to: Dr. Mona M Nosseir, Professor of Pathology, Pathology Department, Theodor Bilharz Research Institute 12411, PO Box 30 Imbaba, Giza 12411, Egypt. drmonanosseir@yahoo.com
Telephone: +20-2-35401019 Fax: +20-2-35408125
Received: March 9, 2009 Revised: October 10, 2009
Accepted: October 17, 2009
Published online: January 27, 2010

Abstract

AIM: To investigate cell cycle proteins in chronic hepatitis C virus infection in order to analyze their role in the process of hepatocyte transformation and to characterize their prognostic properties.

METHODS: Subjects of the current study included 50 cases of chronic hepatitis C (CHC) without cirrhosis, 30 cases of CHC with liver cirrhosis (LC), and 30 cases of hepatitis C-related hepatocellular carcinoma (HCC) admitted to the Department of Hepato-Gastro-enterology, Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Fifteen wedge liver biopsies, taken during laparoscopic cholecystectomy, were also included as normal controls. Laboratory investigations including urine and stool analysis, liver function tests and prothrombin concentration; serologic markers for viral hepatitis and ultrasonography were done for all cases of the study together with immunohistochemical analysis using primary antibodies against Cyclin D1, Cyclin E, p21, p27 and Rb/p105 proteins.

RESULTS: Normal wedge liver biopsies didn't express Cyclin E or Rb/p105 immunostaining but show positive staining for Cyclin D1, p21 and p27. Cyclin D1 expressed nuclear staining that was sequentially increased from CHC to LC ($P < 0.01$) to HCC ($P < 0.001$) cases; meanwhile, Cyclin E revealed nuclear positivity only in the case of HCCs patients that was directly correlated to Rb/p105 immuno-reactivity. The expression of p21 and p27 was significantly increased in CHC and LC cases compared to normal controls and HCCs with no significant difference between well- and poorly-differentiated tumors. p21 showed only a nuclear pattern of staining, while, p27 presented with either cytoplasmic and/or nuclear reactivity in all studied cases. Correlation analysis revealed a direct relation between Cyclin D1 and p21 in CHC cases ($P < 0.001$), between Cyclin D1 and Cyclin E in HCCs ($P < 0.01$); however, an inverse

relationship was detected between Cyclin D1 and p21 or p27 ($P < 0.001$) and between p21 and Rb/p105 ($P < 0.05$) in HCCs.

CONCLUSION: Upregulation of Cyclin D1 in CHC plays a vital role in the development and differentiation of HCC; while, Cyclin E may be a useful marker for monitoring tumor behavior. p21 and p27 can be used as predictive markers for HCC. Furthermore, higher expression of Rb/p105 as well as inverse relation with p21 and histologic grades suggests its important role in hepatic carcinogenesis.

Key words: Chronic hepatitis C; Liver cirrhosis; Hepatocellular carcinoma; Cell cycle; Cyclin D1; Cyclin E; p21; p27; Rb/p105

Peer reviewers: Patricia Cristina Baré, PhD, Virology Section, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Pacheco de Melo 3081, Buenos Aires, 1425, Argentina; Melchiorre Cervello, Dr, Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy", Consiglio Nazionale delle Ricerche (CNR), Via Ugo La Malfa 153, Palermo 90146, Italy

El Bassiouny AE, Nosseir MM, Zoheiry MK, Ameen NA, Abdel-Hadi AM, Ibrahim IM, Zada S, Saad El-Deen AH, El-Bassiouni NE. Differential expression of cell cycle regulators in HCV-infection and related hepatocellular carcinoma. *World J Hepatol* 2010; 2(1): 32-41 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/32.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.32

INTRODUCTION

The cell cycle is divided into four sequential phases[1]. G1 is the first gap phase in which cells prepare for deoxyribonucleic acid (DNA) replication; S (synthesis) phase is the period of DNA synthesis for the reproduction of the whole genome; G2 is the second gap phase in which cells prepare mitosis; and M (mitosis) phase in which cell division occurs for the generation of two genetically identical daughter cells. Quiescent cells that have not entered the cell cycle are referred to as being in G0[2].

Cyclins are the prime cell cycle regulators that play a central role in the control of cell proliferation by forming complexes with different Cyclin-dependent kinases (Cdks)[3]. Members of Cyclin family are often quite distinct from each other in amino acid sequence[2]. At least, 15 different Cyclins and 10 Cdks have been identified[4]. In response to mitogenic signals, G1 Cyclins (Cyclin D1 and Cyclin E) participate in the initiation and progression of the cell cycle where Cyclin D1 is activated during the mid-G1, while Cyclin E is required for G1/S transition[5]. They can accelerate and shorten the G1-phase and reinforce the ability of cells to loose growth control[6] suggesting an oncogenic potential of G1 Cyclins. On the other hand, Cyclin-dependent kinase Inhibitors (CdkIs) are potent negative regulators of the cell cycle that inhibit the G1/S transition[6] and include two families on the basis of sequence homology: The Ink4 family including p16Ink4a, p15Ink4b, p18Ink4c and p19Ink4 that specifically binds to Cdk4 and Cdk6 and inhibits Cyclin binding[7] and the Cip/Kip family including p21Cip1, p27Kip1 and p57Kip2 that bind to and inhibit Cyclin-bound Cdks[8]. Moreover, the two main regulatory proteins of the cell cycle are the retinoblastoma proteins (pRb) and p53. The Rb gene family is composed of three members that share many structural and functional features and play a fundamental role in growth control. They include the Rb susceptibility gene which encodes a nuclear phosphoprotein (pRb/p105) and two related genes pRb/p107 and pRb2/p130[9]. The Rb/p105 gene maps to the 13q14 chromosome, where deletions and heterozygous mutations are frequent in many human malignancies[10,11]. The balance between cell cycle regulators and cell proliferation is an important determinant of tumor development and/or behavior[12].

It has been suggested that hepatocyte turnover is increased in chronic hepatitis C virus (HCV) infection as markers of cell proliferation are elevated[13] and telomere shortening is reported[14]. However, mitotic activity is usually sparse or absent as hepatocytes expressing "proliferation markers" could enter the cell cycle but have been arrested and unable to complete cell division or progress to S phase[15]. Viral replication is enhanced by induction of both cell cycle entry and cell cycle arrest by viral factors[16]. Accordingly, a relationship between viral replication and the host cell cycle state exists in HCV infection[15]. There are several potential consequences of cell cycle arrest and senescence for the liver. Cellular senescence is a risk factor for cancer development and senescent hepatocytes may act synergistically with oncogenic mutations in neighboring hepatocytes leading to the development of hepatocellular carcinoma (HCC)[17].

The present work was designed to investigate the hepatic expression of Cyclin D1, Cyclin E, p21, p27 and the retinoblastoma gene family member Rb/p105 as some regulatory molecules of the cell cycle in chronic HCV infection in a trial to assess the effect of these regulatory molecules on disease progression and development of complications in the form of liver cirrhosis and/or HCC.

PATIENTS AND METHODS

The current study enrolled 110 patients of chronic liver disease who had been admitted to Hepato-Gastroenterology Department of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. They were 75 males and 35 females with a mean age of 48.7 ± 7.5 (range 22-60 years). According to the guidelines of the Institution's Human Research Ethics Committee, all patients gave informed consents before inclusion in the study. After taking their full medical history, each was

subjected to a thorough clinical examination, subjected to ultrasonography and liver biopsies using ultrasound-guided percutaneous Menghini-needle.

Also, fifteen age- and sex-matched individuals who had undergone laparoscopic cholecystectomy were included in this study as controls. This group consisted of 10 males and 5 females with a mean age of 45.0 ± 7.5. After receiving their written consent, wedge liver biopsies were obtained from these cases.

Laboratory investigations

Urine and stool samples were collected and analyzed for all cases. Liver function tests were also done, including those for alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb) and prothrombin concentration (PT conc). Serological diagnosis of schistosomiasis and viral hepatitis were also carried out. Hepatitis B surface antigen and hepatitis B core antibodies were assayed using enzyme immunoassay kits (Abbott Laboratories; North Chicago, Illinois); while, circulating anti-HCV antibodies were detected using the Murex enzyme immunoassay kit (Murex anti-HCV, Version V; Murex Diagnostics; Dartford, England). Chronic hepatitis C (CHC) was confirmed by the presence of HCV-RNA viremia by reverse-transcriptase polymerase chain reaction[18].

Histopathologic study

Serial sections (5 μm thick) from formalin-fixed, paraffin-embedded blocks of either core or wedge liver biopsies which were stained with hematoxylin & eosin as well as Masson trichrome stains. Histopathologic examination of liver sections from control cases showed that they were histopathologically-free from any hepatic lesion. On the other hand, assessment of liver sections from the 110 HCV-infected patients showed features of chronic active hepatitis C in 50 biopsies, liver cirrhosis in 30 cases (according to the French METAVIR System)[19] and hepatocellular carcinoma in 30 specimens with features consistent with well-differentiated (15 cases) and poorly differentiated tumors (15 cases) according to Colecchia *et al*[20].

Immunohistochemistry for cell cycle markers

The 5-μm thick sections from formalin-fixed, paraffinem-bedded blocks were collected on microscopic slides which had been coated with 3-amino propyl triethoxysilane (Sigma Chemicals; St. Louis, USA) both for proper fixation of tissue sections on the slides and minimization of staining artifacts. Following deparaffinization, rehydration and endogenous peroxidase inactivation, antigen retrieval was performed by microwaving in 10 mmol/L citrate buffer, pH 6.0 (Dako, Denmark). Non-specific antibody binding was hindered by pre-incubation with 100 μL blocking serum for 30 min at room temperature. Liver sections were incubated overnight, at 4℃, with the primary mouse anti-human monoclonal antibodies for Cyclin D1, Cyclin E, p21, p27 (Santa Cruz Biotechnology, Inc, USA) and Rb1/p105 (BioGenex, USA) at 1:25, 1:40, 1:20, 1:20, and 1:20 dilution, respectively. After thorough rinsing in PBS, the biotinylated secondary antibody was applied, followed by streptavidin peroxidase conjugation. Peroxidase activity was developed, using diamino-benzidine as the chromogen, and Mayer's hematoxylin as the counterstain. Negative controls were stained appropriately with each setting.

Scoring was performed by counting 500 hepatocytes in each biopsy. Results are shown as labeling index (LI), which represents the percentage of the hepatocyte nuclei that were positive for the antigen under high power magnification of X40. For Cyclin D1, 5% nuclear positivity was considered as overexpression[21] and the immunohistochemical (IHC) reactivity was divided into mild (less than 5%), moderate (5%-30%) and marked expression (> 30%). If the positive rate for Cyclin E protein was over 5%, it was, also, defined as over expression[22]. The IHC reactivity of cyclin E was divided into mild (< 5%), moderate (5%-49%) and marked expression (50%-100%)[23]. These cut-off levels were chosen in order to achieve distinct separation between patients with high and low cyclin E expression[24]. For p21 only cells with distinct nuclear staining were considered positive[25]; while, those considered positive for p27 expressed either a nuclear and/or cytoplasmic staining pattern[7] that may have been either weak or marked. The results were therefore, stratified into cases showing p27 staining in less than 50% of cells (weak) and those with more than 50% positive cell staining (marked)[26]. Finally, for Rb1/p105, immunostaining was quantified by counting the cells exhibiting positive staining in 10 randomly selected high-power fields within the site of the most severe lesion in the biopsy, and the results were expressed as percentage of positive cells in these areas[27] as follows: 0, undetectable level; 1: Low expression level (positive cells = 1%-30%); 2: Medium expression level (positive cells = 31%-60%); and 3: High expression level (positive cells = 61%-100%)[9].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows (version 11) computer software was used for the statistical analysis. Means of different groups were compared using one-way ANOVA. A "*P*" value < 0.05 was considered statistically significant. Pearson correlation coefficient "*r*" was used to measure the relationship between 2 variables.

RESULTS

The one hundred and ten (110) HCV-infected patients had elevated liver enzymes, circulating anti-HCV antibodies and/or HCV-RNA viraemia. Moreover, they were sero-negative for hepatitis B virus or Schistosoma infection. The liver function tests of the fifteen control subjects were within normal range. They had no

Table 1 Clinical and laboratory data of all studied cases (mean ± SD) *n* (%)

Parameters	Control (*n* = 15)	CHC without cirrhosis (*n* = 50)	CHC with cirrhosis (*n* = 30)	HCV-related HCC (*n* = 30)
Age	45.0 ± 7.5	47.4 ± 9.3	51.3 ± 5.9	48.9 ± 7.2
Male/female ratio	2/1	7/3	2/1	2/1
Pallor	0 (0)	2 (4.0)	5 (16.5)	8 (26.6)
Jaundice	0 (0)	3 (6.0)	6 (20.0)	13 (43.3)
Palmer erythema	0 (0)	0 (0)	15 (50.0)	17 (56.6)
Spider naevi	0 (0)	0 (0)	13 (43.3)	16 (53.3)
Lower limb edema	0 (0)	0 (0)	16 (53.3)	10(33.3)
Child classification				
A	0 (0)	50 (100)	8 (26.6)	1 (3.3)
B		0 (0)	9 (30.0)	11 (36.6)
C		0 (0)	13 (43.3)	18 (60.0)
ALT (IU/L)	32.6 ± 4.2	63.2 ± 29.7	51.8 ± 4.3	78.1 ± 16.3
AST (IU/L)	31.1 ± 5.1	49.1 ± 18.3	46.4 ± 5.1	68.3 ± 12.4
Albumin (g/dL)	4.4 ± 0.5	3.80 ± 0.3	2.90 ± 0.7	2.80 ± 0.4
PT conc	97.6 ± 3.4	89.7 ± 5.8	53.4 ± 11.2	68.5 ± 3.8

serologic evidence of hepatitis B and/or C virus infection (Table 1).

In the current study, 3 normal wedge liver biopsies expressed mild Cyclin D1 immunostaining (Figure 1A). Positive nuclear expression was found in the hepatocytes of HCV-infected groups with sequential increase from CHC without cirrhosis (66%) to CHC with cirrhosis (70%) (Figure 1B and C) to HCV-related HCC (100%) (Table 2). Hepatic expression of Cyclin D1 in HCC was correlated with the histological grade, being significantly higher in the poorly differentiated tumors than the well-differentiated ones ($P < 0.001$) (Figure 1D). Marked staining intensity was only observed in poorly-differentiated neoplasms (Table 2).

For Cyclin E, no staining reaction was found in normal patients in the control group. CHC without cirrhosis and LC specimens showed a cytoplasmic pattern of staining that was considered negative. A strong nuclear staining was detected in cancerous livers (Figure 2A) with marked enhancement of expression in poorly differentiated cases (Table 3 and Figure 2B).

Immunoreactivity was detectable for p21 in all HCV-infected livers with minimal expression in the normal patients of the control group. In CHC without cirrhosis, p21 was expressed predominantly in hepatocytes, although occasional positive lymphocytes, sinusoidal lining cells and bile duct cells were also seen. p21 positive hepatocytes were more numerous in areas of intense inflammation and spotty necrosis as well as areas close to fibrosis. In biopsies with less inflammation or fibrosis, most p21 positive hepatocytes were located in the periportal areas rather than the central region. p21 expression in LC was higher than that observed in CHC without cirrhosis with no significant difference; however, it was significantly ($P < 0.01$) down-regulated in HCC cases, particularly in poorly-differentiated tumors (Table 4 and Figure 2C).

p27 was expressed in all cases of the study. The LI for p27 in HCC (Figure 2D) was 34.1 ± 2.7 which was significantly lower than that of the non-tumoral lesions ($P < 0.001$) and normal controls ($P < 0.01$). Furthermore, the LI of p27 in CHC without cirrhosis and LC were, also, significantly higher than those in normal patients in the control group ($P < 0.01$). The expression of p27 was either nuclear alone or mixed with cytoplasmic staining in HCC, LC and normal controls. While, the staining reaction was only cytoplasmic in CHC without cirrhosis (Table 5).

Immunoreactivity was detectable for Rb/p105 in some patients in the HCV-infected groups, and it was absent in normal control livers. The expression was mild to moderate in cases of CHC without cirrhosis and in LC cases (Figure 3A and B). Marked expression was found only in malignant cases. Hepatic expression of Rb/p105 was significantly lower in poorly differentiated HCCs than well-differentiated tumors (Table 6 and Figure 3C).

Correlation analysis revealed a highly significant correlation between Cyclin D1 and p21 in CHC without cirrhosis ($r = 0.70$, $P < 0.001$, Table 7).

In HCC, Cyclin E showed a direct correlation with Cyclin D1 ($P < 0.01$) and Rb/p105 ($P < 0.01$); however, Cyclin D1 showed an inverse correlation with both CdkIs p21 and p27 ($P < 0.001$). An inverse relation was, also, detected between p21 and Rb/p105 or Cyclin E in cancerous cells.

DISCUSSION

Hepatocyte cell cycle phase distribution is altered in chronic HCV infection[15]. Chronic necrosis and inflammation of the liver in HCV infection constituted an important driving force in the multistep process of hepatocarcinogenesis[28] and the majority of HCCs develop in cirrhotic livers[29].

In the current study, Cyclin D1 expression was found to be very low in control cases. However, the expression was significantly elevated in patients with chronic hepatitis C, emphasizing the increased hepatocyte turnover in chronic HCV infection[15]. The upregulation of Cyclin D1 in cirrhotic livers and HCC cases suggests that its expression may play an important role in the process of tumorigenesis. This goes in hand with the findings of other investigators[30] who reported that Cyclin D1 overexpression accelerates and shortens the G1-phase of the cell cycle, leading to a more rapid entry into the S-phase and also increases the number of cell cycle divisions. The upregulation of Cyclin D1, was also shown to be related to the histologic grade of HCC and reflects the aggressiveness of hepatic tumors. These data appear to be consistent with the results of previous studies[31, 32].

Immunolabeling localizes Cyclin E to the nucleus in the majority of human neoplasms. Although, the protein is synthesized and degraded in the cytoplasm, it is ordinarily transferred rapidly to the nucleus, where it carries out its functions[33]. This study revealed that Cyclin E was only expressed in the nuclei of cancerous hepatocytes and was marked in liver biopsies from patients with

Table 2 Immunohistochemical reactivity for Cyclin D1 in all studied cases (mean ± SE) *n* (%)

Groups *n* = 125	Staining pattern	Positive expression	Imunohistochemical reactivity			Hepatic expression
			Mild (< 5%)	Moderate (5%-30%)	Marked (> 30%)	
Control (*n* = 15)	Nuclear	3 (20.0)	3 (100)	0 (0)	0 (0)	1.33 ± 0.33
CHC without cirrhosis (*n* = 50)	Nuclear	33 (66.0)	25 (75.8)	8 (24.2)	0 (0)	6.60 ± 0.72[a]
CHC with cirrhosis (*n* = 30)	Nuclear	21 (70.0)	15 (71.4)	6 (28.6)	0 (0)	9.95 ± 1.70[a,b]
HCV-related HCC (*n* = 30)	Nuclear	30 (100)	12 (40.0)	12 (40.0)	6 (20.0)	19.85 ± 3.90[a,b,c]
Well-differentiated HCC (*n* = 15)	Nuclear	15 (100)	9 (60.0)	6 (40.0)	0 (0)	10.10 ± 2.20[a,b]
Poorly-differentiated HCC (*n* = 15)	Nuclear	15 (100)	3 (20.0)	6 (40.0)	6 (40)	31.60 ± 6.20[a,b,c d]

[a]$P < 0.001$ *vs* controls; [b]$P < 0.01$ *vs* CHC without cirrhosis; [c]$P < 0.001$ *vs* LC; [d]$P < 0.001$ *vs* well differentiated HCC.

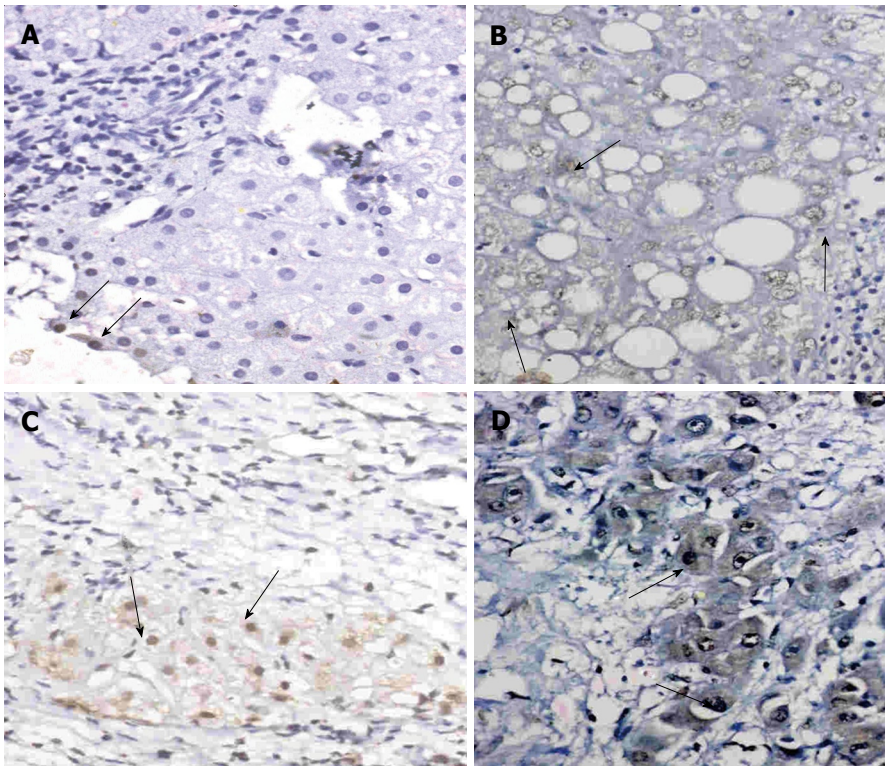


Figure 1 Immunohistochemical staining for Cyclin D1 in liver sections of patients from the control group as well as HCV-infected patients and HCV-related HCC patients. A: Liver section from a control case showing mild nuclear staining for Cyclin D1 (arrows) (Immunoperoxidase × 40); B: Liver section from a case of CHC showing mild nuclear expression of Cyclin D1 (arrows). The portal tract is completely free from immunostaining (Immunoperoxidase × 40); C: Liver section from a case of LC showing moderate number of positively stained hepatocytes in a cirrhotic nodule (arrows) (Immunoperoxidase × 40); D: Liver section from a case of poorly-differentiated HCC showing marked nuclear expression of Cyclin D1 (arrows) (Immunoperoxidase × 40).

poorly differentiated tumors. This indicates that Cyclin E overexpression is associated with tumor aggressiveness and may be considered as one of the markers for outcomes. Keyomarsi *et al*[34] suggested that high Cyclin E expression conveys additional negative consequences for the malignant cell, besides high proliferation. Our results corroborate the previous findings of Jung *et al*[5] who reported that Cyclin E protein was found to be overexpressed in HCC, whereas its expression was hardly detectable in their normal counterparts. Also, other investigators[23,26,32] observed that overexpression of Cyclin E was associated with poor differentiation, invasiveness and metastasis in HCC.

Deregulated cell-cycle progression is one of the most significant alterations in cancer cells. Because G1- to S-phase is the key target of tumorigenesis, it is, in part, negatively regulated by p21 protein, which is a universal inhibitor of Cyclin-dependent kinases and cell cycle progression[35]. In the present study, minimal p21 expression was detected in normal livers, which is consistent with a previous immunohistochemical study[36], but was significantly increased in HCV-infected patients. Wagayama *et al*[37] demonstrated that CdkIs which arrest or slow cell cycle progression are increased in chronic HCV infection. The upregulated p21 expression may play a role as a guard to prevent hepatocytes from tumorigenicity in HCV hepatitis. The highly expressed p21 may hold hepatocytes against transformation by inducing enough G1 span to evoke apoptosis or repair DNA mismatches under the activated cell cycle progression[38]. Stimuli causing increased hepatocyte p21 expression raise the threshold of Cyclin/Cdk inhibition and thereby, diminish mitogen-induced hepatocyte proliferation[25]. However, p21 expression induces transcription of profibrotic factors such as connective tissue growth factor and fibronectin-1[39], thus enhancing the progression to cirrhosis. Crary and Albrecht[25] and Wagayama *et al*[37,40] reported that the p21 labeling index in patients with liver

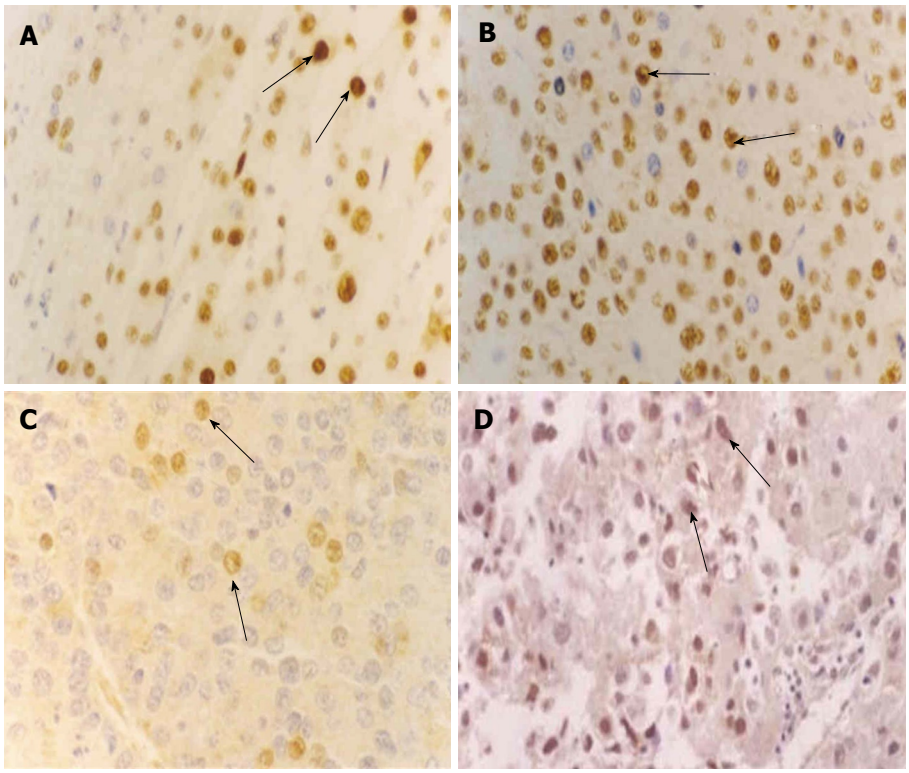


Figure 2 Immunohistochemical staining of liver sections from different differentiated HCC cases (Immunoperoxidase × 40). A: Well differentiated HCC showing moderate nuclear staining of cyclin E (arrows); B: Poorly-differentiated HCC showing marked nuclear staining of cyclin E (arrows); C: Well-differentiated HCC revealing nuclear expression of p21Cip1/Waf1 (arrows) in the hepatocytes; D: Poorly-differentiated HCC showing p27Kip1 expression in a mixed pattern (arrows).

Table 3 Immunohistochemical reactivity for cyclin E in patients with well-differentiated and poorly-differentiated (HCC) (mean ± SE) *n* (%)

Groups	Positive expression	Imunohistochemical reactivity			Stained cells
		Mild < 5%	Moderate 5%-49%	Marked 50%-100%	
Well-differentiated HCC (*n* = 15)	6 (40.0)	2 (33.3)	4 (66.7)	0 (0)	25.3 ± 5.9
Poorly-differentiated HCC (*n* = 15)	6 (40.0)	0 (0)	0 (0)	6 (100)	82.5 ± 2.1[a]

[a]*P* < 0.01 *vs* well differentiated HCC.

Table 4 Immunohistochemical reactivity for p21 in all studied cases (mean ± SE)

Groups	Staining pattern	Hepatic expression
Control (*n* = 15)	Nuclear	1.5 ± 0.5
CHC without cirrhosis (*n* = 50)	Nuclear	6.5 ± 0.2[a]
CHC with cirrhosis (*n* = 30)	Nuclear	7.2 ± 0.8[a]
HCV-related HCC (*n* = 30)	Nuclear	4.9 ± 0.1[a, b]
Well-differentiated HCC (*n* = 15)	Nuclear	5.6 ± 0.16
Poorly-differentiated HCC (*n* = 15)	Nuclear	4.2 ± 0.13

[a]*P* < 0.01 *vs* controls; [b]*P* < 0.05 *vs* LC.

cirrhosis was significantly higher than that in patients with chronic hepatitis, and concluded that p21 expression was upregulated by the stress of inflammation and fibrosis, and influenced by viral proteins. It was found that in normal cells, p21Cip1 is associated with quaternary complexes of most Cyclins, Cdks and proliferation cell nuclear antigen, but is absent from these complexes in most transformed cells[41]. These observations suggest that the reduction or loss of p21 expression plays an important role in the process of tumorigenesis that has also been shown in other reports[42] and supported in this study by the expression of elevated G1 cyclins. As a cause for decreased p21 mRNA expression in tumorous tissues of human cancers, p53 gene mutations are mostly suspected[43] because induction of the p53 tumor suppressor gene after DNA damage inhibits the G1 Cyclins/Cdk activity *via* p21Cip1[44,45] and this inhibition causes cell cycle arrest which, in turn, facilitates DNA repair[46,47].

In this study, expression of p27Kip1 in hepatic cells was significantly upregulated in patients with CHC and LC compared to control cases. On the other hand, it was significantly decreased in HCC cases compared to all groups. Matasuda *et al*[48] found that p27 is abundantly expressed in quiescent cells and is downregulated in many aggressive cancers. It has been recently found[49], that p27 is frequently inactivated in HCC, and is now considered to be a potent tumor suppressor as it is a negative regulator of G1-S phase transition through inhibition of the kinase activities of Cdk2/Cyclin E. Other series[7,23,50-52] have reported that the decreased expression of p27Kip1 is related to tumor progression and could be used as a potential predictor for HCC. The loss or decrease of p27

Table 5 Immunohistochemical reactivity for p27 in all studied cases (mean ± SE) *n* (%)

Groups *n* = 125	Staining pattern	Imunohistochemical reactivity		Hepatic expression
		Weak (< 50%)	Marked (> 50%)	
Control (*n* = 15)	Nuclear/mixed	9 (60.0)	6 (40.0)	44.4 ± 1.9
CHC without cirrhosis (*n* = 50)	Nuclear/cytoplasmic	5 (10.0)	45 (90.0)	59.8 ± 1.9[a]
CHC with cirrhosis (*n* = 30)	Nuclear/mixed	0 (0)	30 (100)	65.9 ± 1.6[a]
HCV-related HCC (*n* = 30)	Nuclear/mixed	21 (70.0)	9 (30.0)	34.1 ± 2.7[a,b,c]
Well-differentiated HCC (*n* = 15)	Nuclear	7 (46.7)	8 (53.3)	36.2 ± 5.2[a,b,c]
Poorly-differentiated HCC (*n* = 15)	Mixed	15 (100)	0 (0)	32.0 ± 1.7[a,b,c]

[a]$P < 0.01$ *vs* controls; [b]$P < 0.001$ *vs* CHC without cirrhosis; [c]$P < 0.001$ *vs* LC.

Table 6 Immunohistochemical reactivity for Rb/p105 in all studied cases (mean ± SE) *n* (%)

Groups *n* = 125	Staining pattern	Positive expression	Imunohistochemical reactivity			Hepatic expression
			Mild (1%-30%)	Moderate (31%-60%)	Marked (61%-100%)	
Control (*n* = 15)	Negative	0 (0)	0 (0)	0 (0)	0 (0)	0.00 ± 0.00
CHC without cirrhosis (*n* = 50)	Nuclear	30 (60.0)	27 (90.0)	3 (10.0)	0 (0)	28.7 ± .1
CHC with cirrhosis (*n* = 30)	Nuclear	15 (50.0)	5 (33.3)	10 (66.7)	0 (0)	40.9 ± 2.8[a]
HCV-related HCC (*n* = 30)	Nuclear	21 (70.0)	0 (0)	12 (57.1)	9 (42.9)	61 ± 3.7[a,b]
Well-differentiated HCC (*n* = 15)	Nuclear	12 (80.0)	0 (0)	12 (100)	0 (0)	67.3 ± 4.8[a,b]
Poorly-differentiated HCC (*n* = 15)	Nuclear	9 (60.0)	0 (0)	0 (0)	9 (100)	55.7 ± 1.4[a,b c]

[a]$P < 0.01$ *vs* CHC without cirrhosis; [b]$P < 0.001$ *vs* LC; [c]$P < 0.01$ *vs* well-differentiated carcinoma.

Table 7 Correlation analysis of different parameters in HCCs

Parameter	HCV-related HCC	
	r	*P*
Cyclin D1 # Cyclin E	0.61	< 0.01
Cyclin D1 # p21	-0.51	< 0.001
Cyclin D1 # p27	-0.65	< 0.001
Cyclin E # Rb/p105	0.62	< 0.01
Cyclin E # p21	-0.64	< 0.01
p21 # p27	0.47	< 0.001
p21 # Rb/p105	-0.42	< 0.05

protein may lead to reduction or disappearance of its cell cycle negative regulation; thus the cells pass the G1 into S-phase, resulting in division and autonomous program[53]. Moreover, in these studies, reduced p27Kip1 expression in HCC both at protein and mRNA levels was associated with tumor invasiveness, advanced clinical stage and poor cellular differentiation grade, as it may be involved in the anchor free survival of malignant cells resulting in viable metastatic tumor nests. However, cells with preserved or increased p27Kip1 expression are not able to proliferate and are driven to apoptotic death[26]. The protein p27 can bind to and inhibit the active Cyclin/Cdk complexes in the nucleus[7], but some tumors expressed increased level of p27 because of increased cytoplasmic expression of this protein, especially in their early stages[54]. However, this may be regulated by self stabilization through attenuating the activity of the proteasome pathway for p27, contributing to tumor development[54]. Results of the current study revealed the decreased p27 expression associated with increased Cyclin D1 expression that was previously explained[49] in some cases of HCC with increased cell proliferation, where p27 is overexpressed but inactivated by sequestration into Cyclin D1-Cdk4-containing complexes.

The retinoblastoma family of growth-inhibitory proteins act by binding and inhibiting several proteins with growth stimulatory activity, the most prominent of which is the cellular transcription factor E2F[55]. Phosphorylation of retinoblastoma family proteins by Cyclin-dependent kinases leads to release of the associated growth stimulatory proteins, which in turn mediate progression through the cell division cycle[56] that was supported in this study by the finding of a direct correlation between Rb/p105 and Cyclin E expression in HCCs. Bagui *et al*[57] suggested that Cyclin D1 combine with Cdk at mid- to late G1, forming complexes that phosphorylate the pRb and sequester p21Cip1 and p27Kip1 which when activated elicit additional events required for the initiation and execution of the S phase.

The p105 protein is important in the synthesis and transport of RNA[58]. It was detected in proliferating cells only where its concentration is elevated during G2-phase and mitosis[58]. Variable staining intensities of the tumor suppressor gene Rb/p105 have been demonstrated in different groups of the present study; and its presence in 60% of CHC and 50% of LC cases may help to protect cells against malignant transformation. When the degree of malignant potential (grading) of HCC cases was compared with the expression of this protein, lower expression was found in poorly differentiated tumors. The highest percentage of detectable levels of Rb/p105

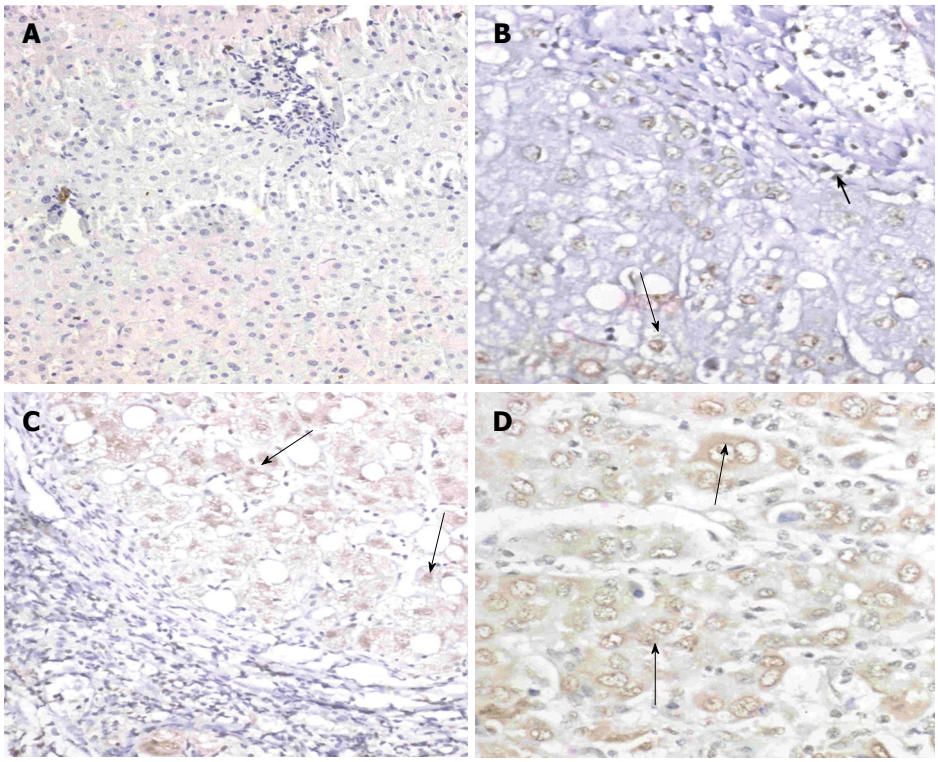


Figure 3 Immunohistochemical staining for Rb1/p105 in liver sections of the control group patients as well as HCV-infected patients and HCV-related HCC patients. A: Liver section from a case of the control showing negative staining for Rb1/p105 either in the cytoplasm or in the nuclei of hepatocytes (IP × 20); B: Liver section from a case with CHC showing moderate nuclear expression for Rb1/p105 in the hepatocytes (long arrow) and in few scattered inflammatory cells (short arrow) of the portal tract (IP × 40); C: Liver section from a case with LC showing moderate nuclear expression for Rb1/p105 in hepatocytes (arrows) and some inflammatory cells of the portal tract in LC (IP × 20); D: Liver section from a case with poorly-differentiated HCC showing marked nuclear expression for Rb1/p105 in the hepatocytes (arrows) (IP × 40).

in HCCs (70%) and the inverse correlation with p21 and histologic grading suggest an important role of Rb/p105 in the pathogenesis and progression of HCCs.

In conclusion, in HCV infection, the up-regulation of Cyclin D1 expression plays an important role in the development of tumorigenesis and in differentiation of HCC. Cyclin E; however, play no role in HCV infection and may be considered as a marker of differentiation and aggressiveness in HCCs. Further studies are needed to elucidate the mechanism of interaction among different Cyclins and the pathway for their regulation.

The p21 and p27 are independently increased in CHC and LC. They mediate hepatocyte cell cycle arrest and accumulation of growth-arrested hepatocytes which impairs hepatocellular function and limits hepatic regeneration. However, both of them are negative regulators of G1-S phase transition and may be considered as predictive factors in HCC. Decreased p21, p27Kip1, Rb/p105 and increased Cyclin D1 and Cyclin E stress the presence of invasive and highly proliferating tumors.

Our results offer insights into the highly complex mechanisms of cell cycle regulation and need to be confirmed by further large scale studies. Understanding the effect of interference at multiple points may well be the foundation upon which designing novel strategies for improving therapeutic approaches in CHC and HCC might begin.

ACKNOWLEDGMENTS

The authors are greatly indebted to Mrs. Hoda Abu-Taleb for performing the statistical analysis of this work.

COMMENTS

Background

An increased risk of hepatitis C virus(HCV)-related cirrhosis and/or hepatocellular carcinoma (HCC) can be explained by the highly complex mechanisms of cell cycle regulation.

Research frontiers

The current study was designed to investigate the hepatic expression of some regulatory molecules of the cell cycle in chronic HCV infection to assess their effect on disease progression and the development of complications.

Innovations and breakthroughs

The role of Cyclins, which are the prime cell cycle regulators, Cyclin-dependent kinase Inhibitors, which are the potent negative regulators of the cell cycle, and retinoblastoma proteins (pRb), as one of the main regulatory proteins of the cell cycle in HCV infection and associated cirrhosis or HCC were thoroughly addressed and correlated in this work. This is the first study to report high expression of Rb/p105 in HCV-induced liver disease and associated complications.

Applications

By understanding the interrelated factors involved in the expression or repression of cell cycle regulatory molecules, new strategies can be developed for improving the therapeutic treatment of chronic hepatitis C and its sequel.

Peer review

The authors have analyzed, through immunohistochemical staining, the expression of cell cycle regulatory molecules in the hepatic tissue of patients with different stages of hepatitis C-related disease, from chronic hepatitis to cirrhosis to hepatocellular carcinoma. Interestingly, they found that cyclin D1 expression increased from chronic hepatitis to cirrhosis to cancer, and thus its expression can be associated with the development of cancer, while cyclin E was over-expressed only in overt cancer with a strict correlation with cancer grading and Rb/p105. Cyclin-dependent kinase inhibitors, p21 and p27, showed an inverse correlation with Cyclin D1 in cancer and thus, as well as Cyclin D1, could be used as predictors of cancer onset. The article is well written, the population size, is large and data are convincing, also from a statistical point of view. The authors did a massive and great job by scrupulously analysing slides and counting positive stained cells.

REFERENCES

1 **Owa T,** Yoshino H, Yoshimatsu K, Nagasu T. Cell cycle

regulation in the G1 phase: a promising target for the development of new chemotherapeutic anticancer agents. *Curr Med Chem* 2001; **8**: 1487-1503
2 **Morgan DO**. The cell cycle control system. In: E Lawrence. The Cell Cycle: Principles of control. London: New Science press Ltd, 2007: 29-55
3 **Bornfeldt KE**. The cyclin-dependent kinase pathway moves forward. *Circ Res* 2003; **92**: 345-347
4 **Rosania GR**, Chang YT. Targeting hyperproliferative disorders with cyclin dependent kinase inhibitors. Expert Opinion on Therapeutic Patents 2000; **10:** 215-230. Available from: URL: http://www. Informapharmascience.com/ddi. Drug development and industrial pharmacy
5 **Jung YJ**, Lee KH, Choi DW, Han CJ, Jeong SH, Kim KC, Oh JW, Park TK, Kim CM. Reciprocal expressions of cyclin E and cyclin D1 in hepatocellular carcinoma. *Cancer Lett* 2001; **168**: 57-63
6 **Mann CD**, Neal CP, Garcea G, Manson MM, Dennison AR, Berry DP. Prognostic molecular markers in hepatocellular carcinoma: a systematic review. *Eur J Cancer* 2007; **43**: 979-992
7 **Nan KJ**, Jing Z, Gong L. Expression and altered subcellular localization of the cyclin-dependent kinase inhibitor p27Kip1 in hepatocellular carcinoma. *World J Gastroenterol* 2004; **10**: 1425-1430
8 **Polak J**, Pekova S, Schwarz J, Kozak T, Haskovec C. Expression of cyclin-dependent kinase inhibitors in leukemia. *Cas Lek Cesk* 2003; **142**: 25-28
9 **Baldi A**, Esposito V, De Luca A, Howard CM, Mazzarella G, Baldi F, Caputi M, Giordano A. Differential expression of the retinoblastoma gene family members pRb/p105, p107, and pRb2/p130 in lung cancer. *Clin Cancer Res* 1996; **2**: 1239-1245
10 **Nevins JR**. Toward an understanding of the functional complexity of the E2F and retinoblastoma families. *Cell Growth Differ* 1998; **9**: 585-593
11 **Sanseverino F**, Torricelli M, Petraglia F, Giordano A. Role of the retinoblastoma family in gynecological cancer. *Cancer Biol Ther* 2003; **2**: 636-641
12 **Lü ZL**, Luo DZ, Wen JM. Expression and significance of tumor-related genes in HCC. *World J Gastroenterol* 2005; **11**: 3850-3854
13 **Lake-Bakaar G**, Mazzoccoli V, Ruffini L. Digital image analysis of the distribution of proliferating cell nuclear antigen in hepatitis C virus-related chronic hepatitis, cirrhosis, and hepatocellular carcinoma. *Dig Dis Sci* 2002; **47**: 1644-1648
14 **Miura N**, Horikawa I, Nishimoto A, Ohmura H, Ito H, Hirohashi S, Shay JW, Oshimuraa M. Progressive telomere shortening and telomerase reactivation during hepatocellular carcinogenesis. *Cancer Genet Cytogenet* 1997; **93**: 56-62
15 **Marshall A**, Rushbrook S, Davies SE, Morris LS, Scott IS, Vowler SL, Coleman N, Alexander G. Relation between hepatocyte G1 arrest, impaired hepatic regeneration, and fibrosis in chronic hepatitis C virus infection. *Gastroenterology* 2005; **128**: 33-42
16 **Flemington EK**. Herpesvirus lytic replication and the cell cycle: arresting new developments. *J Virol* 2001; **75**: 4475-4481
17 **Krtolica A**, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA* 2001; **98**: 12072-12077
18 **Hodinka RL**. Detection of HCV RNA in serum by reverse transcriptase-PCR and radiolabeled liquid hybridization. *Method Mol Med* 1998; **19**: 29-45
19 **Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C**. The French METAVIR Cooperative Study Group. *Hepatology* 1994; **20**: 15-20
20 **Colecchia A**, Scaioli E, Vestito A, Grazi GL, Festi D. Tumor grading of hepatocellular carcinoma by preoperative needle biopsy: Is it useful for choosing the best therapeutic strategy? *Dig Liver Dis* 2009; **41**: A18
21 **Khan AA**, Abel PD, Chaudhary KS, Gulzar Z, Stamp GW, Lalani EN. Inverse correlation between high level expression of cyclin E and proliferation index in transitional cell carcinoma of the bladder. *Mol Pathol* 2003; **56**: 353-361
22 **Peng SY**, Chou SP, Hsu HC. Association of downregulation of cyclin D1 and of overexpression of cyclin E with p53 mutation, high tumor grade and poor prognosis in hepatocellular carcinoma. *J Hepatol* 1998; **29**: 281-289
23 **Zhou Q**, He Q, Liang LJ. Expression of p27, cyclin E and cyclin A in hepatocellular carcinoma and its clinical significance. *World J Gastroenterol* 2003; **9**: 2450-2454
24 **Lindahl T**, Landberg G, Ahlgren J, Nordgren H, Norberg T, Klaar S, Holmberg L, Bergh J. Overexpression of cyclin E protein is associated with specific mutation types in the p53 gene and poor survival in human breast cancer. *Carcinogenesis* 2004; **25**: 375-380
25 **Crary GS**, Albrecht JH. Expression of cyclin-dependent kinase inhibitor p21 in human liver. *Hepatology* 1998; **28**: 738-743
26 **Armengol C**, Boix L, Bachs O, Sole M, Fuster J, Sala M, Llovet JM, Rodes J, Bruix J. p27(Kip1) is an independent predictor of recurrence after surgical resection in patients with small hepatocellular carcinoma. *J Hepatol* 2003; **38**: 591-597
27 **Santopietro R**, Shabalova I, Petrovichev N, Kozachenko V, Zakharova T, Pajanidi J, Podistov J, Chemeris G, Sozaeva L, Lipova E, Tsidaeva I, Ivanchenko O, Pshepurko A, Zakharenko S, Nerovjna R, Kljukina L, Erokhina O, Branovskaja M, Nikitina M, Grunberga V, Grunberg A, Juschenko A, Cintorino M, Tosi P, Syrjänen K, Syrjänen S. Cell cycle regulators p105, p107, Rb2/p130, E2F4, p21CIP1/WAF1, cyclin A in predicting cervical intraepithelial neoplasia, high-risk human papillomavirus infections and their outcome in women screened in three new independent states of the former Soviet Union. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1250-1256
28 **Di Bisceglie AM**. Hepatitis C and hepatocellular carcinoma. *Semin Liver Dis* 1995; **15**: 64-69
29 **Bruno S**, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, Asti M, Rossi S, Larghi A, Cerino A, Podda M, Mondelli MU. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: A prospective study. *Hepatology* 2003; **25**: 754-758
30 **Fu M**, Wang C, Li Z, Sakamaki T, Pestell RG. Minireview: Cyclin D1: normal and abnormal functions. *Endocrinology* 2004; **145**: 5439-5447
31 **Nishida N**, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, Amenomori M, Shibagaki I, Nakao K, Ikenaga M. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994; **54**: 3107-3110
32 **Masaki T**, Shiratori Y, Rengifo W, Igarashi K, Yamagata M, Kurokohchi K, Uchida N, Miyauchi Y, Yoshiji H, Watanabe S, Omata M, Kuriyama S. Cyclins and cyclin-dependent kinases: comparative study of hepatocellular carcinoma versus cirrhosis. *Hepatology* 2003; **37**: 534-543
33 **Donnellan R**, Chetty R. Cyclin E in human cancers. *FASEB J* 1999; **13**: 773-780
34 **Keyomarsi K**, Tucker SL, Buchholz TA, Callister M, Ding Y, Hortobagyi GN, Bedrosian I, Knickerbocker C, Toyofuku W, Lowe M, Herliczek TW, Bacus SS. Cyclin E and survival in patients with breast cancer. *N Engl J Med* 2002; **347**: 1566-1575
35 **Shi YZ**, Hui AM, Takayama T, Li X, Cui X, Makuuchi M. Reduced p21WAF1/CIP1 protein expression is predominantly related to altered p53 in hepatocellular carcinomas. *British J Cancer* 2000; **83**: 50-55.
36 **Fredersdorf S**, Milne AW, Hall PA, Lu X. Characterization

of a panel of novel anti-p21Waf1/Cip1 monoclonal antibodies and immunochemical analysis of p21Waf1/Cip1 expression in normal human tissues. *Am J Pathol* 1996; **148**: 825-835

37 **Wagayama H**, Shiraki K, Yamanaka T, Sugimoto K, Ito T, Fujikawa K, Takase K, Nakano T. p21WAF1/CTP1 expression and hepatitis virus type. *Dig Dis Sci* 2001; **46**: 2074-2079

38 **Kobayashi S**, Matsushita K, Saigo K, Urashima T, Asano T, Hayashi H, Ochiai T. P21WAF1/CIP1 messenger RNA expression in hepatitis B, C virus-infected human hepatocellular carcinoma tissues. *Cancer* 2001; **91**: 2096-2103

39 **Chang BD**, Watanabe K, Broude EV, Fang J, Poole JC, Kalinichenko TV, Roninson IB. Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases. *Proc Natl Acad Sci USA* 2000; **97**: 4291-4296

40 **Wagayama H**, Shiraki K, Sugimoto K, Ito T, Fujikawa K, Yamanaka T, Takase K, Nakano T. High expression of p21WAF1/CIP1 is correlated with human hepatocellular carcinoma in patients with hepatitis C virus-associated chronic liver diseases. *Hum Pathol* 2002; **33**: 429-434

41 **Xiong Y**, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. *Nature* 1993; **366**: 701-704

42 **Kao JT**, Chuah SK, Huang CC, Chen CL, Wang CC, Hung CH, Chen CH, Wang JH, Lu SN, Lee CM, Changchien CS, Hu TH. P21/WAF1 is an independent survival prognostic factor for patients with hepatocellular carcinoma after resection. *Liver Int* 2007; **27**: 772-781

43 **Furutani M**, Arii S, Tanaka H, Mise M, Niwano M, Harada T, Higashitsuji H, Imamura M, Fujita J. Decreased expression and rare somatic mutation of the CIP1/WAF1 gene in human hepatocellular carcinoma. *Cancer Lett* 1997; **111**: 191-197

44 **Di Leonardo A**, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev* 1994; **8**: 2540-2551

45 **Gartel AL**, Serfas MS, Tyner AL. p21--negative regulator of the cell cycle. *Proc Soc Exp Biol Med* 1996; **213**: 138-149

46 **Pellegata NS**, Antoniono RJ, Redpath JL, Stanbridge EJ. DNA damage and p53-mediated cell cycle arrest: a reevaluation. *Proc Natl Acad Sci USA* 1996; **93**: 15209-15214

47 **Fotedar R**, Fitzgerald P, Rousselle T, Cannella D, Dorée M, Messier H, Fotedar A. p21 contains independent binding sites for cyclin and cdk2: both sites are required to inhibit cdk2 kinase activity. *Oncogene* 1996; **12**: 2155-2164

48 **Matsuda Y**, Ichida T, Genda T, Yamagiwa S, Aoyagi Y, Asakura H. Loss of p16 contributes to p27 sequestration by cyclin D(1)-cyclin-dependent kinase 4 complexes and poor prognosis in hepatocellular carcinoma. *Clin Cancer Res* 2003; **9**: 3389-3396

49 **Matsuda Y**. Molecular mechanism underlying the functional loss of cyclindependent kinase inhibitors p16 and p27 in hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 1734-1740

50 **Jing Z**, Nan KJ, Hu ML. Cell proliferation, apoptosis and the related regulators p27, p53 expression in hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 1910-1916

51 **Lei PP**, Zhang ZJ, Shen LJ, Li JY, Zou Q, Zhang HX. Expression and hypermethylation of p27 kip1 in hepatocarcinogenesis. *World J Gastroenterol* 2005; **11**: 4587-4591

52 **Shehata MA**, Nosseir HR, Nagy HM, Farouk G. Cyclin dependent kinase inhibitor p27(kip1) expression and subcellular localization in relation to cell proliferation in hepatocellualr carcinoma. *Egypt J Immunol* 2006; **13**: 115-130

53 **Filipits M**, Puhalla H, Wrba F. Low p27Kip1 expression is an independent prognostic factor in gallbladder carcinoma. *Anticancer Res* 2003; **23**: 675-679

54 **Sgambato A**, Ratto C, Faraglia B, Merico M, Ardito R, Schinzari G, Romano G, Cittadini AR. Reduced expression and altered subcellular localization of the cyclin-dependent kinase inhibitor p27(Kip1) in human colon cancer. *Mol Carcinog* 1999; **26**: 172-179

55 **Beijersbergen RL**, Bernards R. Cell cycle regulation by the retinoblastoma family of growth inhibitory proteins. *Biochim Biophys Acta* 1996; **1287**: 103-120

56 **Classon M**, Harlow E. The retinoblastoma tumour suppressor in development and cancer. *Nat Rev Cancer* 2002; **2**: 910-917

57 **Bagui TK**, Mohapatra S, Haura E, Pledger WJ. P27Kip1 and p21Cip1 are not required for the formation of active D cyclin-cdk4 complexes. *Mol Cell Biol* 2003; **23**: 7285-7290

58 **Yonemura Y**, Ohoyama S, Kimura H, Kamata T, Matsumoto H, Yamaguchi A, Kosaka T, Miwa K, Miyazaki I. The expression of proliferative-associated nuclear antigen p105 in gastric carcinoma. *Cancer* 1991; **67**: 2523-2528

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

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.42

World J Hepatol 2010 January 27; 2(1): 42-48
ISSN 1948-5182 (online)

BRIEF ARTICLE

Seroprevalence of HCV and its co-infection with HBV and HIV among liver disease patients of South Tamil Nadu

Ganesh Kumar Anbazhagan, Sridharan Krishnamoorthy, Thirunalasundari Thiyagarajan

Ganesh Kumar Anbazhagan, Sridharan Krishnamoorthy, Thirunalasundari Thiyagarajan 620024, Tamil Nadu, India
Ganesh Kumar Anbazhagan, National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India
Sridharan Krishnamoorthy, Department of Gastroenterology, KAP Viswanatham Government Medical College and Annal Gandhi Memorial Government Hospital, Tiruchirappalli 620024, Tamil Nadu, India
Thirunalasundari Thiyagarajan, Department of Biotechnology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India
Author contributions: Anbazhagan GK carried out the research; Krishnamoorthy S gave the samples, helped in identifying the patients and contributed to the clinical work; Thiyagarajan T had the idea and guided the work.
Correspondence to: Thirunalasundari Thiyagarajan, Professor, Department of Biotechnology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India. kayaljee@yahoo.com
Telephone: +91-0431-2407086
Received: August 18, 2009 Revised: January 14,2010
Accepted: January 21, 2010
Published online: January 27, 2010

Abstract

AIM: To determine the seroprevalence of hepatitis C virus (HCV) and its co-infection with hepatitis B virus (HBV), hepatitis delta agent (HDV) and human immunodeficiency virus (HIV) among liver disease patients of south Tamil Nadu.

METHODS: A total of 1012 samples comprising 512 clinically diagnosed cases of liver disease patients and 500 apparently healthy age and sex matched individuals were screened for Hepatitis C virus (anti HCV and HCV RNA), Hepatitis B virus (HBsAg), Hepatitis delta agent (anti HDV) and Human immuno virus (antibodies to HIV-1 and HIV-2) using commercially available enzyme linked immunosorbent assay kits. HCV RNA was detected by RT-PCR. Liver function tests like ALT, AST, GGT, ALP, bilirubin and albumin were also studied.

RESULTS: The seroprevalence of HCV was found to be 5.6% among liver disease patients by ELISA. 27/512, 49/512 and 12/512 patients were positive for HIV, HBV & HDV respectively. Co-infection of HCV & HBV was found in 8 patients, with 6 for HCV & HIV and 4 for HCV, HBV & HIV co-infections. Sex-wise analysis showed that HIV, HCV & HBV and HCV & HIV co-infection was high among females whereas for HBV it was high in males. The mean ALT and AST in HCV positive cases were 42.1 ± 8.3 and 49 ± 10.1. In people co-infected with HCV & HBV or HCV & HIV or HCV, HBV & HIV the mean ALT of 58.0 ± 03.16, 56.78 ± 4.401 and 64.37 ± 4.01 respectively.

CONCLUSION: We strongly recommend routine test of the blood for HCV in addition to HBV and HIV. We also recommend individualized counseling to identify those at risk and testing for those who want it. Improved surveillance and periodic epidemiological studies will have to be undertaken to monitor and prevent these blood-borne viruses.

Key words: Hepatitis C virus; Hepatitis B virus; Human immunodeficiency virus; Co-infection; Liver function test

Peer reviewer: Eva Herrmann, Professor, Department of Internal Medicine, Biomathematics Saarland University, Faculty of Medicine, Kirrberger Str., 66421 Homburg/Saar, Germany

Anbazhagan GK, Krishnamoorthy S, Thiyagarajan T. Seroprevalence of HCV and its co-infection with HBV and HIV among liver disease patients of South Tamil Nadu. *World J Hepatol* 2010; 2(1): 42-48 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/42.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.42

INTRODUCTION

Hepatitis C virus (HCV) infection is the most common chronic blood borne infection in the world[1]. HCV belongs to the family *flaviviridae* and the genus *hepacivirus*. It often causes lifelong persistent infection[2]. The prevalence of HCV infection worldwide has been estimated to be about 3% with 170 million people affected by HCV[3]. Meanwhile two billion people have been infected with hepatitis B virus (HBV). Of these, 360 million have chronic infection and 600 000 die each year due to HBV infection and related liver diseases[4]. HBV and HCV infections account for a substantial proportion of liver diseases worldwide. The majority of those with chronic HBV and/or HCV infection will develop complications i.e. 15%-40% may develop cirrhosis, liver failure and or hepatocellular carcinoma[5]. The exact number of patients infected with both HCV and HBV world wide is unknown[6]. It has been estimated that over the next 20 years, the proportion of HBV/HCV infected patients with cirrhosis will increase from 16% to 32% and that other complications will also increase dramatically including hepatic decompensation (increasing by 106%), HCC (increasing by 81%) and liver related deaths (increasing by 180%)[7]. In addition, prevalence of human immunodeficiency virus (HIV) is increasing everyday and it has become a disaster for humankind in certain areas. HIV accounted for 38.6 million infections world wide by the end of 2005[8]. These three viruses (HCV, HBV and HIV) have similar routes of transmission, namely through blood and blood products. Sharing of needles to inject drugs and sexual activity enables the co-infection of these viruses and thereby makes co-infection or super infection a common event[9]. End stage liver disease is currently a major concern among HIV positive individuals due to co-infection with hepatotropic viruses[10,11]. HIV infected patients with multiple hepatitis virus infections have a higher rate of liver related morbidity and mortality than patients with HIV infection alone or with a single hepatitis virus infection. The degree of immunodepression is an important factor in liver disease progression[12]. Current estimations indicate that approximately 1.8% to 2.5% of Indian population is presently infected by HCV[13]. The prevalence as well as the significance of HCV infection varies considerably from country to country, probably because of cultural factors and social habits that influence HCV transmission. A community based Indian study on HCV indicated a seroprevalence of 0.87% and that the rate reportedly increased from children < 10 years to 1.85% among subjects > 60 years of age[14]. Knowledge and awareness of HCV infection have been obtained from seroprevalence studies carried out in blood donors and hemodialysis patients from large cities[15-17]. Reports on the prevalence of HCV infection in the Indian subcontinent is scarce. Hence, this study was conducted to investigate the seroprevalence of HCV and its co-infection with HBV and HIV in liver disease patients in South Tamil Nadu.

MATERIALS AND METHODS

Study samples

The study was performed in 512 clinically diagnosed cases of liver disease patients and 500 apparently healthy age and sex matched controls. All the liver diseases and control samples were diagnosed and given by the Gastroenterologist based on signs, symptoms and examination. Blood samples were collected from them, serum was separated and stored at -20℃ until use. Among the liver disease patients clinical conditions including acute liver disease, chronic liver disease and cirrhosis were also diagnosed by the Gastroenterologist.

Serology

The blood samples were screened for markers of various hepatitis viruses and HIV by using third generation ELISA kits. Hepatitis B surface antigen (HBsAg) was screened by Hepalisa, supplied by M/s. J. Mitra and Co Pvt. Ltd, India; Hepatitis B envelop antigen (HBeAg) and antibody to envelop antigen (anti-HBe) using ELISA kits supplied by M/s. Biorad laboratories, USA.; Antibodies to HCV, HDV and HIV were screened by using Microlisa kits supplied by M/s. J. Mitra and Co Pvt. Ltd, India. All reactive analyses were repeated twice.

Biochemistry

Liver function tests studied were alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), serum alkaline phosphatase (SAP), bilirubin and albumin. The results were correlated with serological findings. The upper limits of normal for various tests were ALT 0-65 IU/L, AST 5-40 IU/L, GGT 0-61 IU/L, SAP 20-140 KA, Bilirubin Total 0.3 to 1.9 mg%, Direct *bilirubin*: 0 to 0.3 mg%, Indirect bilirubin 0.1-1.0 mg% and Albumin 35-50 gms%.

Molecular diagnosis of HCV

For the detection of the HCV genome, RNA was extracted from the samples and was subjected to reverse transcriptase polymerase chain reaction (RT-PCR). This was carried out for the constant HCV 5' untranslated region (5' UTR).

Statistical analysis

Statistical package for social sciences (SPSS, version 17.0) software was used for analyzing the data. Statistical tests used to find the significance were χ^2 for association of attributes and *t*-test for difference in the mean for independent samples.

RESULTS

Among the 512 liver disease patients investigated, 281 were male and 231 were female. Overall 29 (5.6%) were

Table 1 Incidence of blood borne viral infection (HCV, HBV, HDV and HIV) among liver disease patients *n* (%)

Category	Total number tested	Gender		HCV, HBV, HDV and HIV positive			
		Male	Female	HCV	HBV	HDV	HIV
Liver disease	512	281	231	29 (5.6)	49 (9.5)	12 (2.3)	27 (5.2)
Control	500	250	250	-	7 (1.4)	-	-
Total	1012	531	481	29	56	12	27

Table 2 Incidence of blood borne viral infection and co-infection among liver disease patients

Category	Clinical condition	Gender						Total
		Male			Female			
		Virus positive	Virus negative	Total	Virus positive	Virus negative	Total	
Liver disease	Acute liver disease	22	55	77	12	38	50	127
	Chronic liver disease	45	34	79	26	51	77	156
	Cirrhosis	14	31	45	7	33	40	85
	Others	16	64	80	2	62	64	144
	Total	97	184	281	47	184	231	512
Control	Without any signs or symptoms	4	246	250	3	247	250	500
Total		101	430	531	50	431	481	1012

$\chi^2 = 12.598$ for df = 1, $P < 0.01$ for gender *vs* viral infection. $\chi^2 = 41.33$ for df = 3, $P < 0.01$ for clinical condition *vs* viral infection.

Table 3 Clinical condition *vs* blood-borne viral infection

Clinical condition	Different viral infection positive									Total virus negative	Total number tested
	HCV	HBV	HIV	HDV	HCV and HBV	HCV and HIV	HBV and HIV	HCV, HBV and HIV	Total virus positive		
Acute liver disease	-	19	10	4	-	-	2	-	35	92	127
Chronic liver disease except cirrhosis	13	11	17	8	6	6	6	4	71	85	156
Cirrhosis	12	5	-	-	2	-	1	-	20	65	85
Others	4	14	-	-	-	-	-	-	18	126	144
Total	29	49	27	12	8	6	9	4	144	368	512

$\chi^2 = 41.631$ for df = 3, $P < 0.01$. χ^2 is calculated for clinical condition against total viral infection.

positive for HCV. This positivity was either for anti HCV by ELISA, for HCV RNA by RT-PCR or for both. Results based on other viral infection revealed 27 (5.2%) for HIV, 49 (9.5%) for HBsAg, and 12 (23%) for HDV (Table 1). Table 2 shows the incidence of blood borne viral infection and co-infection among liver disease patients. Co-infection of HCV & HBV was found in 8 patients, with 6 for HCV & HIV and 4 for HCV, HBV & HIV co-infections. In addition, clinical conditions of all viral infections and co-infections were significantly ($P < 0.01$) associated with liver disease (Table 3). Table 4 gives the age-wise distribution of patients with different kinds of viral infections. The total viral positivity was insignificant in various age groups ($P > 0.05$). Among the viral positive cases, HBV was most prevalent followed by other viral infections and co-infections whilst the rest were insignificant ($P < 0.05$) (Tables 5 and 6). The results of liver function tests in HCV infected and co-infected patients are presented in Table 7. All the parameters tested were found to increase significantly ($P < 0.05$) when compared to controls. Data collected based on risk factor for HCV seroconversion reveals, 47.6% of the patients had blood transfusion, 6 had surgical intervention (14.2%), 5 were intravenous drug users(11.9%) and 11 (26%) had unknown causes. Blood transfusion (65%) was observed as the predominant risk factors in the co-infected patients.

DISCUSSION

HIV, HBV and HCV are the three most common chronic blood borne viral infections documented worldwide[18]. Epidemiological studies of blood-borne viral disease such as HCV, HBV and HIV are important for revealing the risk groups and risk factors for these infections. Screening these groups of viruses helps us to solve difficulties in collecting information among healthy populations[19].

India has the second highest number of people living with HIV infection[20]. Co-infection of hepatotropic viruses with HIV infection reportedly leads to massive impairment of cell mediated responses and enhances

Table 4 Different viral infection *vs* age groups

Viral infection	Age group in years						Total
	11-20	21-30	31-40	41-50	51-60	61 and above	
HCV	2	6	7	7	4	3	29
HBV	5	19	6	10	7	2	49
HIV	-	5	4	8	7	3	27
HDV	-	1	5	6	-	-	12
HCV and HBV	1	2	1	1	2	1	8
HCV and HIV	1	1	2	1	-	1	6
HBV and HIV	1	1	4	2	-	1	9
HCV, HBV and HIV	1	-	1	1	-	1	4
All virus positive	11	35	30	36	20	12	144
All virus negative	42	83	82	94	51	16	369
Total	53	118	112	130	71	28	512

$\chi^2 = 4.689$ for df = 5, $P > 0.05$, χ^2 is calculated for age against total viral infection.

Table 5 Different viral infection *vs* sex

Viral infection	Gender		*P* value
	Male	Female	
HCV positive	18	11	0.423
HCV negative	263	220	
HBV positive	41	8	0
HBV negative	240	223	
HIV positive	14	13	0.745
HIV negative	267	218	
HDV positive	8	4	0.406
HDV negative	273	227	
HCV and HBV positive	5	3	0.663
HCV and HBV negative	276	228	
HCV and HIV positive	3	3	0.809
HCV and HIV negative	278	228	
HBV and HIV positive	6	3	0.474
HBV and HIV negative	275	228	
HCV, HBV and HIV positive	2	2	0.844
HCV, HBV and HIV negative	279	229	

χ^2 is calculated for gender *vs* different category of viral infections; $P < 0.05$ is significant, while $P > 0.05$ is insignificant.

the kinetics of hepatotropic viral replication[21-24]. The prevalence of HCV in this study is 8.2% in liver disease patients. A study conducted by Chowdhury *et al*[25] (2003) from eastern India showed a prevalence of 0.87%, which is ten times less than that observed in the present study. That study predominantly comprised blood-transfusion-acquired HCV infections rather than other modes of transmission. Age-wise analysis in the present study found HCV to be high among individuals belonging to the 41-50 years (28.5%) age group. HCV infections usually progress slowly to terminal liver disease[26]. It is, therefore, possible to recognize the impact that the disease may have in the future based on the knowledge of its previous incidence. It is also feasible to estimate the burden of late complications associated directly with the presence of chronic liver disease[27].

It was observed that males were more susceptible to HCV than female among the study population. This concurs with a previous report that male subjects were at a higher risk of developing HBV infection than females[28-29].

Out of the 512 liver disease patients tested 49 (9.5%) were positive for HBsAg. Similar results were obtained in an earlier study conducted on chronic liver disease in India[30]. A multicenter study in Italy showed that the subjects with dual HBV and HCV infection were more likely to be older than 42 years[31]. Similar results (1.5%) were found in the current study.

Sud *et al*[28] (2001) have reported 33.8% prevalence of HBV co-infection in HIV positive patients. Although the effect of HBV infection on HIV is uncertain, HIV appears to have marked influence on the natural history of HBV infection. Although HIV shares a common route of infection with HBV and HCV, its sexual transmission is known to be relatively efficient whereas the sexual transmission of HCV appears to be significantly less efficient than for HIV. Although detailed reports have documented HCV, HBV and HIV co-infection worldwide, only a few reports have been published regarding co-infection in India. Kumar *et al*[32] (2003) reported a 2.9% co-infection of HBV and HIV in patients with liver diseases. The increased viral replication of HBV in AIDS patients indicates that HIV significantly affects the HBV life cycle and the host ability to clear HBV infection. If this is true, more HBV infection and more chronic carriers would be expected as the AIDS epidemic expands in this part of the country. Such a profile would have worrying public health implications[33]. The frequency of HCV & HIV co-infection in this study (1.9%) is much lower than that reported previously among HCV & HIV co-infections in India[29,34-38] and higher than from the general Indian population[39]. In the HIV & HCV co-infected patients, the HCV RNA positivity was found to be higher. These observations were in agreement with previous reports of increased hepatotropic viral replication in immunocompromised subjects[40,41]. Moderate or severe chronic hepatitis or cirrhosis was more frequent in patients with HBV and HCV co-infections than in patients infected with HBV or with HCValone. Generally, HCV superinfection can cause a much more severe liver disease in patients with

Table 6 Clinical condition *vs* different viral infection *vs* sex

Sex	Clinical condition	Different viral infection positive								Total positive	Total negative
		HCV	HBV	HIV	HDV	HCV and HBV	HCV and HIV	HBV and HIV	HCV, HBV and HIV		
Male	Acute liver disease	-	14	5	3	-	-	-	-	22	55
	Chronic liver disease except cirrhosis	8	9	9	5	4	3	5	2	45	34
	Cirrhosis	7	5	-	-	1	-	1	-	14	31
	Others	3	13	-	-	-	-	-	-	16	64
	Total	18	41	14	8	5	3	6	2	97	184
Female	Acute liver disease	-	4	5	1	-	-	2	-	12	38
	Chronic liver disease except cirrhosis	5	2	8	3	2	3	1	2	26	51
	Cirrhosis	5	1	-	-	1	-	-	-	7	33
	Others	1	1	-	-	-	-	-	-	2	62
	Total	11	8	13	4	3	3	3	2	47	184
Total		29	49	27	12	8	6	9	4	144	368
P value		0.211	0	0.853	0.256	0.484	1	0.323	1	-	-

χ^2 is calculated for gender *vs* different viral infections positive cases; $P < 0.05$ is significant, while $P > 0.05$ is insignificant.

Table 7 Liver function test in HCV infected and co-infected patients (mean ± SD, *n* = 29)

Category	Liver function test							
	Bilirubin (mg%)			SAP (KA units)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Albumin (gms%)
	Total	Direct	Indirect					
Control	0.5340 ± 0.219	0.210 ± 0.055	0.386 ± 0.015	73.227 ± 21.622	37.76 ± 9.35	24.77 ± 07.65	69.70 ± 11.03	2.78 ± 0.219
HCV positive patients	3.461 ± 0.874	0.643 ± 0.1736	0.936 ± 0.016	144.630 ± 12.976	42.1 ± 8.3	49.00 ± 10.1	98.446 ± 09.57	3.87 ± 0.24
P value	0	0	0	0.005	0	0	0	0
HCV and HBV coinfected patients	3.70 ± 0.96	1.59 ± 0.06	1.641 ± 0.052	140.887 ± 10.922	58.0 ± 03.16	40.412 ± 02.008	99.362 ± 06.150	3.14 ± 0.66
P value	0	0	0	0	0	0	0	0
HCV and HIV coinfected patients	2.920 ± 1.032	1.975 ± 0.941	1.997 ± 0.078	134.53 ± 10.651	56.78 ± 4.401	39.65 ± 2.24	97.60 ± 5.94	3.12 ± 0.97
P value	0	0.001	0.001	0	0.001	0.001	0	0
HCV, HBV and HIV coinfected patients	3.295 ± 1.424	3.202 ± 0.488	0.265 ± 0.052	141 ± 17.301	64.37 ± 4.01	42.87 ± 2.84	99.95 ± 03.039	3.14 ± 0.02
P value	0.009	0	0	0	0.005	0.006	0.003	0.004

HCV positive patients: *P* values are based on students *t* test; $P < 0.05$ significant.

chronic HBV infection. This lends support to the notion that HBV superinfection may also aggravate the disease severity and increase the risk of fulminant hepatitis. In both HIV positive and negative cohorts, the presence of HBV & HCV, HBV & HDV or triple hepatitis infection was strongly associated with intravenous drug use (IDU). Overall, from 0.4% to more than 50% of HIV patients may carry more than one hepatitis virus[42-47]. The reported co-infection rates of HBV and HCV in HIV patients worldwide have varied, depending on the geographic regions, risk groups and the type of exposure involved[48,49]. Tankhiwale *et al*[50] (2003) reported 5.6% seroprevalence of HCV and 25.8% HBV in HIV infected patients. These co-infection rates were much higher than that of our findings. Four individuals co-infected with HCV, HBV & HIV had neither surgical intervention, blood transfusion or intravenous drug use, suggesting that sexual intercourse could have been the route of infection.

ACKNOWLEDGMENTS

The authors are grateful to Dr. A Rajathi, Associate Professor, Department of Zoology, Holly Cross College, Tiruchirappalli for helping us in performing the statistical analysis.

COMMENTS

Background

Hepatitis C virus (HCV) infection creates a significant burden on health care systems. HCV infection has probably been endemic in many populations for centuries. Despite a declining incidence of new infections, the burden of disease in terms of mortality is expected to increase over the next decade.

The complexity and uncertainty related to the geographic distribution of HCV infection and chronic HCV, determination of the associated risk factors and evaluation of cofactors that accelerate its progression, underscore the difficulties in global prevention and control of HCV. Thus, this study set out to determine the seroprevalence of HCV and its co-infection with other blood borne hepatitis group of viruses and human immunodeficiency virus.

Research frontiers

As HCV is a silent killer virus, diagnosis, treatment and prevention are very important. Hence the results of this study will, if further explored, benefit the public, health care workers, voluntary blood donors and liver disease patients.

Innovations and breakthroughs

Most earlier workers have studied the seroprevalence of HCV either by enzyme linked immunosorbent assay (Antigen or Antibody) specific for the virus or by RT-PCR (viral genome) alone. In this study the seroprevalence of HCV was evaluated by both the methods. This should help to both modify treatment and prevent infection.

Applications

The identification of a high prevalence of HCV in this study indicates the significance of screening HBV, HCV and HDV in addition to HIV, among liver disease patients. Hence we strongly recommend routine testing for health care workers, voluntary blood donors and others to include HBV, HCV, HDV and HIV. We also recommend individualized counseling to identify those at risk as well as testing for those who request it. Improved surveillance and periodic epidemiologic studies will have to be undertaken to monitor and prevent the spread of virulent and interferon-resistant strains. Given the current incidence and prevalence data cited above, HCV infection and its co-infection is expected to remain a problem. Further research in the virology, epidemiology, treatment and prevention of blood-borne viral infection is essential if better outcomes to be achieved.

Peer review

The present manuscript deals with an interesting epidemiologic data, and the topic is also interesting.

REFERENCES

1 **Gasiorowicz M**, Hurie M, Russell A, Hoxie N, Vergeront J. Epidemiologic trends in infection, mortality, and transplants related to hepatitis C in Wisconsin. *WMJ* 2006; **105**: 34-39

2 **Alter HJ**, Seeff LB. Recovery, persistence, and sequelae in hepatitis C virus infection: a perspective on long-term outcome. *Semin Liver Dis* 2000; **20**: 17-35

3 **Sy T**, Jamal MM. Epidemiology of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006; **3**: 41-46

4 **Shepard CW**, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev* 2006; **28**: 112-125

5 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539

6 **Liu Z**, Hou J. Hepatitis B virus (HBV) and hepatitis C virus (HCV) dual infection. *Int J Med Sci* 2006; **3**: 57-62

7 **Davis GL**, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl* 2003; **9**: 331-338

8 **Report on the Global AIDS Epidemic**. Available from: URL: http:// www.unaids.org/ en/ HIV_data/2006GlobalReport/ default.asp. Accessed on 2007-10-06

9 **Chung RT**. Hepatitis C and B viruses: the new opportunists in HIV infection. *Top HIV Med* 2006; **14**: 78-83

10 **Alter MJ**. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol* 2006; **44**: S6-S9

11 **Monica F**, Lirussi F, Pregun I, Vasile F, Fabris L, Okolicsanyi L. Hepatitis C virus infection in a resident elderly population: a 10-year follow-up study. *Dig Liver Dis* 2006; **38**: 336-340

12 **Gaeta GB**, Precone DF, Cozzi-Lepri A, Cicconi P, D'Arminio Monforte A. Multiple viral infections. *J Hepatol* 2006; **44**: S108-S113

13 **Chandra M**, Khaja MN, Farees N, Poduri CD, Hussain MM, Aejaz Habeeb M, Habibullah CM. Prevalence, risk factors and genotype distribution of HCV and HBV infection in the tribal population: a community based study in south India. *Trop Gastroenterol* 2003; **24**: 193-195

14 **Chowdhury A**, Santra A, Chaudhuri S, Dhali GK, Chaudhuri S, Maity SG, Naik TN, Bhattacharya SK, Mazumder DN. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. *Hepatology* 2003; **37**: 802-809

15 **Rivera-López MR**, Zavala-Méndez C, Arenas-Esqueda A. [Prevalence for seropositivity for HIV, hepatitis B and hepatitis C in blood donors] *Gac Med Mex* 2004; **140**: 657-660

16 **Carreto-Vélez MA**, Carrada-Bravo T, Martínez-Magdaleno A. [Seroprevalence of HBV, HCV, and HIV among blood donors in Irapuato, Mexico] *Salud Publica Mex* 2003; **45** Supp **5**: S690-S693

17 **Silva LK**, Silva MB, Rodart IF, Lopes GB, Costa FQ, Melo ME, Gusmão E, Reis MG. Prevalence of hepatitis C virus (HCV) infection and HCV genotypes of hemodialysis patients in Salvador, Northeastern Brazil. *Braz J Med Biol Res* 2006; **39**: 595-602

18 **Soriano V**, Barreiro P, Nuñez M. Management of chronic hepatitis B and C in HIV-coinfected patients. *J Antimicrob Chemother* 2006; **57**: 815-818

19 **Gunduz T**, Mumcuoglu I, Güray M. Evaluation of hepatitis B surface antigen, anti-hepatitis C virus and anti-human immunodeficiency virus antibodies and syphilis seropositivity in blood donors: six years' seropositivity. *Int J Clin Pharmacol Res* 2005; **25**: 155-158

20 **National AIDS Control Organization (NACO)**. HIV/AIDS epidemiological Surveillance & Estimation report for the year 2005. Available from: URL: http:// www.nacoonline.org/

21 **Yachimski P**, Chung RT. Update on Hepatitis B and C Coinfection in HIV. *Curr Infect Dis Rep* 2005; **7**: 299-308

22 **Schooley RT**. HIV and hepatitis C virus coinfection: bad bedfellows. *Top HIV Med* 2005; **13**: 112-116

23 **Koch S**, Göbels K, Oette M, Heintges T, Erhardt A, Häussinger D. [HIV-HBV-coinfection--diagnosis and therapy] *Dtsch Med Wochenschr* 2006; **131**: 1873-1877

24 **Murphy MJ**. Managing HIV/HBV coinfection can challenge some clinicians. *HIV Clin* 2003; **15**: 6-9

25 **Chowdhury A**, Santra A, Chaudhuri S, Dhali GK, Chaudhuri S, Maity SG, Naik TN, Bhattacharya SK, Mazumder DN. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. *Hepatology* 2003; **37**: 802-809

26 **Chen SL**, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med* Sci 2006; **3**: 47-52

27 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714

28 **Sud A**, Singh J, Dhiman RK, Wanchu A, Singh S, Chawla Y. Hepatitis B virus co-infection in HIV infected patients. *Trop Gastroenterol* 2001; **22**: 90-92

29 **Padmapriyadarsini C**, Chandrabose J, Victor L, Hanna LE, Arunkumar N, Swaminathan S. Hepatitis B or hepatitis C co-infection in individuals infected with human immunodeficiency virus and effect of anti-tuberculosis drugs on liver function. *J Postgrad Med* 2006; **52**: 92-96

30 **Arora DR**, Sehgal R, Gupta N, Yadav A, Mishra N, Siwach SB. Prevalence of parenterally transmitted hepatitis viruses in clinically diagnosed cases of hepatitis. *Indian J Med Microbiol* 2005; **23**: 44-47

31 **Gaeta GB**, Stornaiuolo G, Precone DF, Lobello S, Chiaramonte M, Stroffolini T, Colucci G, Rizzetto M. Epidemiological and clinical burden of chronic hepatitis B virus/hepatitis C virus infection. A multicenter Italian study. *J Hepatol* 2003; **39**: 1036-1041

32 **Kumar A**, Shukla I, Malik A. Co-infection with hepatitis B and human immunodeficiency viruses in patients of liver disease. *Indian J Med Microbiol* 2003; **21**: 141-142

33 **Shire NJ**, Sherman KE. Management of HBV/HIV-coinfected Patients. *Semin Liver Dis* 2005; **25** Suppl **1**: 48-57

34 **Kumarasamy N**, Solomon S, Flanigan TP, Hemalatha R, Thyagarajan SP, Mayer KH. Natural history of human immunodeficiency virus disease in southern India. *Clin Infect Dis* 2003; **36**: 79-85

35 **Bhattacharya S**, Badrinath S, Hamide A, Sujatha S. Co-infection with hepatitis C virus and human immunodeficiency virus among patients with sexually transmitted diseases in Pondicherry, South India. *Indian J Pathol Microbio*l 2003; **46**: 495-497

36 **Hussain T**, Kulshreshtha KK, Sinha S, Yadav VS, Katoch VM. HIV, HBV, HCV, and syphilis co-infections among patients attending the STD clinics of district hospitals in Northern India. *Int J Infect Dis* 2006; **10**: 358-363

37 **Gupta S**, Singh S. Hepatitis B and C virus co-infections in human immunodeficiency virus positive North Indian patients. *World J Gastroenterol* 2006; **12**: 6879-6883

38 **Poudel KC**, Jimba M, Okumura J, Wakai S. Emerging co-infection of HIV and hepatitis B virus in far western Nepal. *Trop Doct* 2006; **36**: 186-187

39 **Chandra M**, Khaja MN, Farees N, Poduri CD, Hussain MM, Aejaz Habeeb M, Habibullah CM. Prevalence, risk factors and genotype distribution of HCV and HBV infection in the tribal population: a community based study in south India. *Trop Gastroenterol* 2003; **24**: 193-195

40 **Lascar RM**, Gilson RJ, Lopes AR, Bertoletti A, Maini MK. Reconstitution of hepatitis B virus (HBV)-specific T cell responses with treatment of human immunodeficiency virus/HBV coinfection. *J Infect Dis* 2003; **188**: 1815-1819

41 **O'Leary JG**, Chung RT. Management of hepatitis C virus coinfection in HIV-infected persons. *AIDS Read* 2006; **16**: 313-316, 318-320

42 **Greub G**, Ledergerber B, Battegay M, Grob P, Perrin L, Furrer H, Burgisser P, Erb P, Boggian K, Piffaretti JC, Hirschel B, Janin P, Francioli P, Flepp M, Telenti A. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet* 2000; **356**: 1800-1805

43 **Sulkowski MS**, Moore RD, Mehta SH, Chaisson RE, Thomas DL. Hepatitis C and progression of HIV disease. *JAMA* 2002; **288**: 199-206

44 **Lincoln D**, Petoumenos K, Dore GJ. HIV/HBV and HIV/HCV coinfection, and outcomes following highly active antiretroviral therapy. *HIV Med* 2003; **4**: 241-249

45 **Anderson KB**, Guest JL, Rimland D. Hepatitis C virus coinfection increases mortality in HIV-infected patients in the highly active antiretroviral therapy era: data from the HIV Atlanta VA Cohort Study. *Clin Infect Dis* 2004; **39**: 1507-1513

46 **Law WP**, Duncombe CJ, Mahanontharit A, Boyd MA, Ruxrungtham K, Lange JM, Phanuphak P, Cooper DA, Dore GJ. Impact of viral hepatitis co-infection on response to antiretroviral therapy and HIV disease progression in the HIV-NAT cohort. *AIDS* 2004; **18**: 1169-1177

47 **Rockstroh J**, Konopnicki D, Soriano V, Krik O, Antunes F, Knysz B, Tural C, Wit SD, Mocroft A, Lundgren J. Hepatitis B and hepatitis C in the EuroSIDA Cohort: Prevalence and Effect on Mortality, AIDS Progression and Response to HAART. Eleventh conference on retroviruses and opportunistic infections 2004; abs 799

48 **Rockstroh JK**. Management of hepatitis B and C in HIV co-infected patients. *J Acquir Immune Defic Syndr* 2003; **34** Suppl **1**: S59-S65

49 **Tien PC**. Management and treatment of hepatitis C virus infection in HIV-infected adults: recommendations from the Veterans Affairs Hepatitis C Resource Center Program and National Hepatitis C Program Office. *Am J Gastroenterol* 2005; **100**: 2338-2354

50 **Tankhiwale SS**, Khadase RK, Jalgoankar SV. Seroprevalence of anti-HCV and hepatitis B surface antigen in HIV infected patients. *Ind J Med Microbial* 2003; **21**: 268-270

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

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.49

World J Hepatol 2010 January 27; 2(1): 49-51
ISSN 1948-5182 (online)

CASE REPORT

Combined approach for spontaneous rupture of hepatocellular carcinoma

Anna Rossetto, Gian Luigi Adani, Andrea Risaliti, Umberto Baccarani, Vittorio Bresadola, Dario Lorenzin, Giovanni Terrosu

Anna Rossetto, Gian Luigi Adani, Andrea Risaliti, Umberto Baccarani, Vittorio Bresadola, Dario Lorenzin, Giovanni Terrosu, Department of Surgery & Transplantation, University Hospital, P.Le S.M. della Misericordia, 33100 Udine, Italy
Author contributions: Rossetto A and Adani GL designed and wrote the paper, Baccarani U, Bresadola V and Lorenzin D collected and analysed data, Risaliti A and Terrosu G revised the paper.
Supported by Dipartimento di Scienze Chirurgiche, University Hospital, P.Le S.M. della Misericordia, 33100 Udine, Italy
Correspondence to: Anna Rossetto, MD, Department of Surgery & Transplantation, University Hospital, P.Le S.M. della Misericordia, 33100 Udine, Italy. rossettoannaar@libero.it
Telephone: +39-432-559902 Fax: +39-432-559552
Received: August 27, 2009 Revised: January 14, 2010
Accepted: January 21, 2010
Published online: January 27, 2010

Abstract

Ruptured hepatocellular carcinoma is a rare, emergency occurrence in western countries with high mortality risk. A number of hypotheses have been formulated in order to explain the precise mechanism that leads to hepatocellular carcinoma (HCC) rupture: sub-capsular location, dimensions, portal hypertension, tumour necrosis, local increase of venous pressure due to the outflow reduction caused by neoplastic invasion, and the presence of a previous vascular injury which might predispose to HCC rupture. There is still a debate in the literature concerning the best approach in cases of HCC rupture. Surgery is the first option for treatment of acute abdominal bleeding. However the advent of endovascular treatments widens the range of possible therapies for acute bleeding control and subsequent ablation purposes. We report a case of hemoperitoneum from spontaneous rupture of undiagnosed HCC, that was treated successfully by emergency surgical resection followed by transarterial chemo-embolization for local recurrence.

Key words: Hemoperitoneum; Acute abdominal bleeding; Bleeding hepatocellular carcinoma

Peer reviewer: Radha K Dhiman, Professor, Department of Hepatology,Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Rossetto A, Adani GL, Risaliti A, Baccarani U, Bresadola V, Lorenzin D, Terrosu G. Combined approach for spontaneous rupture of hepatocellular carcinoma. *World J Hepatol* 2010; 2(1): 49-51 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/49.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i1.49

INTRODUCTION

The first manifestation of unknown hepatocellular carcinoma (HCC) with hemoperitoneum caused by spontaneous rupture is a rare occurrence in western countries, with an incidence of less than 3%, but with a high mortality risk[1,2]. There is still a debate in the literature concerning the best approach in cases of HCC rupture.Surgery is the first option for treatment of acute abdominal bleeding. However the advent of endovascular treatments widens the range of possible therapies for acute bleeding control and subsequent ablation purposes. Short term survival benefit can be obtained with surgical treatment, associated or not with interventional radiology[3-5]. Long term survival seems to be unanimously correlated to the stage of the disease, its local spread after rupture and to the residual hepatic functionality[6,7]. We report a case of hemoperitoneum from spontaneous rupture of undiagnosed HCC successfully treated by emergency surgical resection

followed by transarterial chemo-embolization (TACE) two months later for tumour recurrence. At a follow-up of one year the patient is still alive, with no signs of HCC recurrence.

CASE REPORT

We report a 78-year-old male patient who came to the Emergency Room (ER) because of abdominal pain, hypotension (80/40 mmHg) associated with tachycardia, and hypothermia (35.7℃). Anamnesis was totally negative. Doppler ultrasound showed diffuse hemoperitoneum. Urgent abdomen Computed Tomography (CT) scan revealed an actively bleeding focal lesion of 3.5 centimeter diameter in the 6th hepatic segment, associated with perihepatic and perisplenic hemoperitoneum (Figure 1A). At emergency laparotomy, major hemoperitoneum of over 2000 mL was present. The source of bleeding was from a 3.5 cm, whitish, ligneous hepatic nodule in the 6th segment, which was lacerated in its central part. The liver was not cirrhotic. After cleaning of the abdomen, and liver mobilization, a 6th segment wedge resection was performed using Pringle manoeuvre (30 min of clamping). The post-operative course was uneventful, and at post-operative day 7 the patient was discharged from hospital in good general condition. Post-operative laboratory tests confirmed no evidence of hepatitis B or C positivity. Histological exam showed a lesion of 4.5 centimeter diameter partly covered with glissonian sheath compatible with HCC (grading 3, WHO 2001). Margins were free of HCC infiltration. Two months after the operation, local recurrence of HCC was evidenced by CT scan. Considering the general condition of the patient, and the high risk of a new hepatic resection, we performed TACE by selective catheterisation of the right hepatic artery, and injection of DC Bead 100-300 micron + 50 mg Doxorubicin (Figure 1B). The procedure was uneventful, and without evidence of liver failure. Two weeks later, an abdominal CT scan revealed complete devascularisation of the HCC (Figure 1C). At one year follow-up, no signs of tumour recurrence were evidenced: CT scan and Magnetic Resonance Imaging (MRI) were negative, and laboratory tests including alpha fetoprotein were in the normal range.

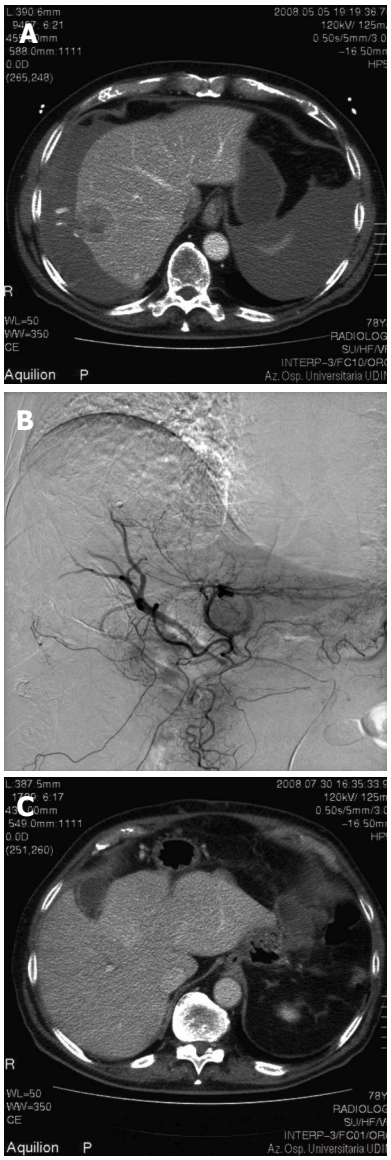


Figure 1 CT and TACE image of a 78-year-old male patients with hemoperitoneum from spontaneous rupture of undiagnosed HCC. A: Abdomen CT scan revealed a 3.5 cm focal lesion of the 6th hepatic segment actively bleeding, associated with hemoperitoneum; B: Trans-arterial chemoembolization (TACE) of the right hand side hepatic artery by means of selective catheterization; C: CT scan, performed two weeks after TACE, revealed an area of complete devascularzation.

DISCUSSION

The occurrence of a ruptured HCC is an unusual find in western countries with an incidence of < 3% compared to Eastern countries (12%-14%), with a reported mortality rate up to 50%[1,2]. A number of hypotheses have been formulated in order to explain the precise mechanism that leads to HCC rupture: subcapsular location, dimensions, portal hypertension, tumour necrosis, local increase of venous pressure due to the outflow reduction caused by neoplastic invasion[8], the presence of a previous vascular injury which might predispose to HCC rupture and to the rupture of smaller lesions in different locations[9]. With regard to diagnosis, according to the study of Yeh et al., abdominal pain was the only independent factor relating patients with ruptured HCC[3]. Preoperative diagnosis of HCC rupture is difficult in patients without a previous history of cirrhosis or HCC. Vergara *et al*[1], reported that an accurate pre-operative diagnosis of ruptured HCC was obtained in only 25% of cases, despite shock present in 33% to 90% of the patients. Doppler ultrasound and CT scan are useful to demonstrate the presence of hemoperitoneum and liver tumours. CT also has the advantage of demonstrating the patency of the portal vein but the site of active bleeding can seldom be demonstrated, conventional angiography demonstrates extravasations of contrast from the tumour only in 13%-35% of cases[10]. There is no single opinion about the best treatment for a ruptured HCC, even if patient's general conditions are considered in all cases. If hepatic insufficiency, liver cirrhosis and hemoperitoneum are present, trans-arterial embolization (TAE) is an effective and well-tolerated treatment for the emergency

management of hemoperitoneum[8]. In patients with normal liver function haemostasis followed by hepatic resection either in emergency or after staging has been suggested[2]. Literature reports such as[7] suggest that multidisciplinary management with TAE and postponed surgery in selected patients would improve the short term mortality. Liver resection has been advocated to achieve both haemostasis and to provide a definitive treatment in a single operation[1,4], even if liver resection should be reserved for patients with a small and easily accessible tumour in a non cirrhotic liver[4]. The studies on survival by Liu *et al*[5] and Yeh *et al*[3] supply contrasting data, therefore it is not clear whether there is a different survival expectation for the patients with ruptured HCC and the others[5,6]. The surgical treatment of intraperitoneal lesions caused by neoplastic dissemination during rupture can increase the long term survival.

In our case, the diagnosis of HCC was accidental in a patient with no history of hepatic disease. On his admission the patient had poor hemodynamic stability but normal liver function. After CT scan which confirmed hemoperitoneum secondary to hepatic rupture, we opted for emergency surgery, even because the lesion was small, and easily accessible.

A different approach could have been considered in cases of liver cirrhosis, with trans arterial embolization first, followed by surgical operation (laparoscopic/laparotomic approach) or percutaneous drainage of the hemoperitoneum to reduce any possible development of liver decompensation.

In our patient, HCC recurred two months after treatment. Considering the age of the patient and the risk of a new hepatic resection, we completed surgery with TACE. One year follow-up was regular, hepatic function was normal, as was the αFP values, and radiological examinations showed no HCC recurrence.

REFERENCES

1 **Vergara V**, Muratore A, Bouzari H, Polastri R, Ferrero A, Galatola G, Capussotti L. Spontaneous rupture of hepatocelluar carcinoma: surgical resection and long-term survival. *Eur J Surg Oncol* 2000; **26**: 770-772

2 **Battula N**, Madanur M, Priest O, Srinivasan P, O'Grady J, Heneghan MA, Bowles M, Muiesan P, Heaton N, Rela M. Spontaneous rupture of hepatocellular carcinoma: a Western experience. *Am J Surg* 2009; **197**: 164-167

3 **Yeh CN**, Lee WC, Jeng LB, Chen MF, Yu MC. Spontaneous tumour rupture and prognosis in patients with hepatocellular carcinoma. *Br J Surg* 2002; **89**: 1125-1129

4 **Lai EC**, Lau WY. Spontaneous rupture of hepatocellular carcinoma: a systematic review. *Arch Surg* 2006; **141**: 191-198

5 **Liu CL**, Fan ST, Lo CM, Tso WK, Poon RT, Lam CM, Wong J. Management of spontaneous rupture of hepatocellular carcinoma: single-center experience. *J Clin Oncol* 2001; **19**: 3725-3732

6 **Tanaka A**, Takeda R, Mukaihara S, Hayakawa K, Shibata T, Itoh K, Nishida N, Nakao K, Fukuda Y, Chiba T, Yamaoka Y. Treatment of ruptured hepatocellular carcinoma. *Int J Clin Oncol* 2001; **6**: 291-295

7 **Buczkowski AK**, Kim PT, Ho SG, Schaeffer DF, Lee SI, Owen DA, Weiss AH, Chung SW, Scudamore CH. Multidisciplinary management of ruptured hepatocellular carcinoma. *J Gastrointest Surg* 2006; **10**: 379-386

8 **Castells L**, Moreiras M, Quiroga S, Alvarez-Castells A, Segarra A, Esteban R, Guardia J. Hemoperitoneum as a first manifestation of hepatocellular carcinoma in western patients with liver cirrhosis: effectiveness of emergency treatment with transcatheter arterial embolization. *Dig Dis Sci* 2001; **46**: 555-562

9 **Zhu LX**, Geng XP, Fan ST. Spontaneous rupture of hepatocellular carcinoma and vascular injury. *Arch Surg* 2001; **136**: 682-687

10 **Marini P**, Vilgrain V, Belghiti J. Management of spontaneous rupture of liver tumours. *Dig Surg* 2002; **19:** 109-113

S- Editor Wang YR **L- Editor** Lalor PF **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i1.52

World J Hepatol 2010 January 27; 2(1): 52-54
ISSN 1948-5182 (online)

CASE REPORT

Two-stage treatment of an unusual haemobilia caused by intrahepatic pseudoaneurysm

Kazuhisa Takeda, Kuniya Tanaka, Itaru Endo, Shinji Togo, Hiroshi Shimada

Kazuhisa Takeda, Kuniya Tanaka, Itaru Endo, Shinji Togo, Hiroshi Shimada, Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, Yokohama 236-0004, Japan
Author contributions: Takeda K, Tanaka K, Endo I, Togo S, and Shimada H designed the study, performed the literature review, and analyzed the data; Takeda K wrote the paper.
Correspondence to: Kazuhisa Takeda, MD, Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. kazutake@yokohama-cu.ac.jp
Telephone: +81-45-7872650 Fax: +81-45-7829161
Received: August 21, 2009 Revised: January 14, 2010
Accepted: January 21, 2010
Published online: January 27, 2010

Abstract

A 84-year-old man with a surgical history of subtotal gastrectomy for gastric cancer was transferred to our department because of a disorder of consciousness. Septic shock due to obstructive suppurative cholangitis secondary to choledocholithiasis was diagnosed. Anemia was also present, and upper gastrointestinal tract endoscopy revealed blood emerging from the Papilla of Vater. The cause of the anemia was identified as haemobilia. Angiography showed a small aneurysm over the artery on segment 3 (A3). The cause of the haemobilia was suspected to be the bleeding into the biliary tree from this aneurysm. Because the patient's general condition was poor, minimally invasive therapy was needed. Transcatheter arterial embolization (TAE) was selected initally. Later, lateral sectionectomy was performed in order to remove the aneurysm on A3. No surgical complication occurred and, after surgery, no haemobilia was identified. In conclusion, a two-stage treatment, namely, surgery following TAE, is recommended for patients in a physically poor condition who have haemobilia due to intrahepatic aneurysm.

Key words: Two-stage treatment; Haemobilia; Intrahepatic pseudoaneurysm

Peer reviewer: Pierluigi Toniutto, Professor, Medical Liver Transplant Unit, Internal Medicine, University of Udine, Piazzale S.M. della Misericordia 1, Udine 33100, Iyaly

Takeda K, Tanaka K, Endo I, Togo S, Shimada H. Two-stage treatment of an unusual haemobilia caused by intrahepatic pseudoaneurysm. *World J Hepatol* 2010; 2(1): 52-54 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i1/52.htm
DOI: http://dx.doi.org/10.4254/wjh.v2.i1.52

INTRODUCTION

Bleeding into the biliary tree is a rare cause of gastrointestinal hemorrhage[1]. The majority of patients with hepatic artery aneurysms are asymptomatic[2] but once an aneurysm ruptures, haemobilia becomes a potentially life-threatening complication. Therefore, early diagnosis and certain hemostasis are essential. Furthermore, in patients in a poor physical condition or in elderly patients, minimally invasive therapy should be performed. In this report, we described a case of haemobilia resulting from severe obstructive suppurative cholangitis secondary to choledocholithiasis. Complete hemostasis was achieved by surgical treatment following transcatheter arterial embolization (TAE).

CASE REPORT

A 84-year-old-man with a surgical history of subtotal gastrectomy for gastric cancer was transferred to our department because of a disorder of consciousness. He had a fever of 39.5 degrees, yellowish conjunctivae and a blood pressure of 50/20 mmHg. Laboratory data on admission were as follows: Alanine/aspartate aminotransferase, 315/174 IU/L (respectively); total bilirubin, 4.8 mg/dL; direct bilirubin, 3.2 mg/dL;

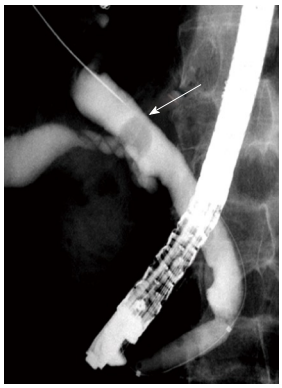


Figure 1 Emergency endoscopic retrograde cholangiopancreatography showing a solitary filling defect which was 12 mm in diameter (arrows).

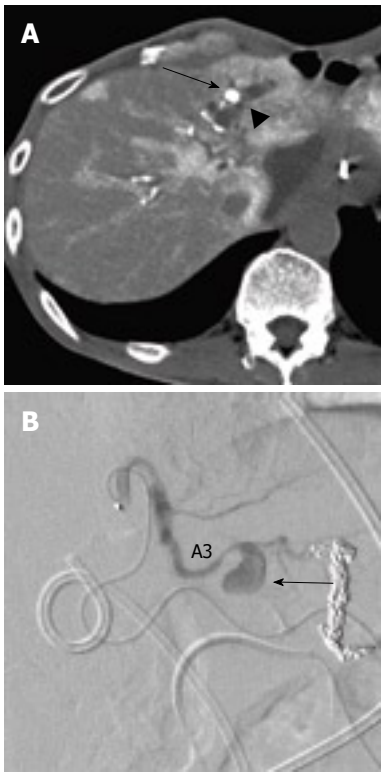


Figure 2 CT and Angiography image of an 84-year-old man with haemobilia. A: CT revealing a high-density area which was 1 cm in diameter at the lateral segment in the artery phase (arrows). It protruded into the intrahepatic duct (arrowhead). B: Angiography showing a small aneurysm over the A3, which was the second branch of the left hepatic artery (arrows).

alkaline phosphatase, 739 IU/L; C-reactive protein, 24.1 mg/dL; white blood cells, 1000 /μL; platelets, 0.9 × 10^4/μL; prothrombin time INR, 1.39. Septic shock due to severe infection accompanied by hepatobiliary dysfunction was diagnosed. The laboratory data also showed a decreased level of hemoglobin (7.1 g/dL). Computed tomography (CT) revealed an enlarged gallbladder and a dilated common bile duct. Emergency endoscopic retrograde cholangiopancreatography (ERCP) showed a solitary filling defect which was 12 mm in diameter (Figure 1). Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, and Clostridium species were detected both in both blood and bile. Therefore, the pathogenesis was considered to be obstructive suppurative cholangitis secondary to choledocholithiasis. After intensive conservative therapy including bile drainage which enabled the patient to recover from septic shock, a choledocholith was removed. During this process, bleeding from the Papilla of Vater was detected by upper

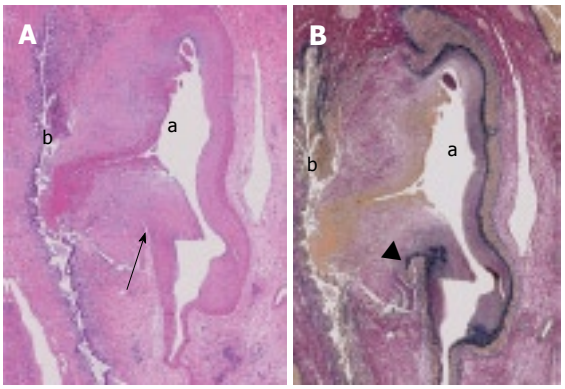


Figure 3 Histological findings revealing that the intrahepatic artery had ruptured into the intrahepatic bile duct (arrows). a: artery; b: bile duct. The inner membrane, elastic laminae, and tunica media of the artery could not be detected at the side of rupture (arrowhead). A: HE stain, ×10; B: Elastica van Gieson stain, ×10.

gastrointestinal tract endoscopy, enabling the cause of the anemia to be identified as haemobilia. Arterial phase CT revealed a high-density area 1 cm in diameter in the lateral segment of the artery that protruded into the intrahepatic duct (Figure 2A). Angiography showed a small aneurysm over the A3 segment, which was the second branch of the left hepatic artery (Figure 2B). These findings suggested that the haemobilia resulted from the bleeding into the biliary tree from this aneurysm. Because of the patient's advanced age and because Onodera's prognostic nutritional index[3] was extremely poor (20.2), we planned the two-stage treatment in order to minimize invasiveness. Transcatheter arterial embolization (TAE) was performed first and,after recovery of the patient's nutritional status, this was followed by hepatectomy in order to remove the aneurysmal intrahepatic artery. Because intermittent haemobilia persisted and the recovery of the patient's general condition made slow progress, TAE was required 3 times. After that, no massive biliary bleeding appeared, and the patient's nutritional status gradually improved during this period. Finally, lateral sectionectomy was performed safely. Histological findings indicated that the intrahepatic artery had ruptured into the intrahepatic bile duct. The inner membrane, the elastic laminae, and the tunica media of the artery were not detected at the site of rupture (Figure 3), and so rupture of an arterial pseudoaneurysm was diagnosed. In the 12 mo after surgical treatment, the patient has been attending our hospital as an outpatient, and no further anemia has come to light.

DISCUSSION

Haemobilia is the phenomenon of bleeding into the biliary tree, and is an unusual cause of obscure upper abdominal bleeding[4]. It is usually due to atherosclerotic aneurysm, trauma, pseudoaneurysm, or vascular anomalies[4]. In this case, choledocholithiasis in the common bile duct and a pseudoaneurysm in the liver were present. The pathogenesis in this case

was probably infiltration of the severe inflammatory reaction in the intrahepatic bile duct into the liver parenchyma because of choledocholithiasis, resulting in erosion of the wall of the hepatic artery. This was an unusual etiology, although the patient was 84 years old and histopathologic examination revealed evidence of severe arteriosclerosis. The walls of the arteries were fragile and a pseudoaneurysm was formed as a result[5]. The majority of patients with hepatic artery aneurysms are asymptomatic[2]. However, once such an aneurysm ruptures, haemobilia, which is potentially life-threatening, complicates the patient's condition. Therefore, all cases of intrahepatic aneurysm should be considered for immediate treatment[2], TAE being the accepted treatment of choice[1,2]. The hepatic artery should be embolized at both the proximal and the distal sides of the aneurysm. This technique is difficult when the aneurysm is peripheral to the second branch. When embolization is insufficient, normal flow in the portal vein or reflux in the multiple collateral pathways of the artery may appear through sinusoidal lesions[5], and intermittent haemobilia continues. Hepatectomy should be considered at the same time as TAE. In this patient, because of an advanced age and poor nutritional status, we planned a two-stage treatment; TAE first, followed by hepatectomy. After 3 repetitions of TAE, there was no massive biliary bleeding and the patient's status improved gradually, allowing surgical treatment to be performed safely. Even when the aneurysm is located on branches of the hepatic artery beyond the second, minimally invasive TAE should be performed first, although for hemostasis, TAE alone may be insufficient. In such cases, after improvement in the patient's condition following TAE, surgery is indispensable as a radical treatment.

In conclusion, we have reported an unusual case in which haemobilia resulted from the rupture of an intrahepatic pseudoaneurysm resulting from severe cholangitis. When the aneurysm is located distal to the second branch of the hepatic artery and the patient is of advanced age or in poor general condition, two-stage treatment comprising TAE and surgery should be performed.

REFERENCES

1 **Siablis D,** Papathanassiou ZG, Karnabatidis D, Christeas N, Vagianos C. Hemobilia secondary to hepatic artery pseudoaneurysm: an unusual complication of bile leakage in a patient with a history of a resected IIIb Klatskin tumor. *World J Gastroenterol* 2005; **11:** 5229-5231

2 **O'Driscoll D,** Olliff SP, Olliff JF. Hepatic artery aneurysm. *Br J Radiol* 1999; **72**: 1018-1025

3 **Onodera T,** Goseki N, Kosaki G. [Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients] *Nippon Geka Gakkai Zasshi* 1984; **85:** 1001-5

4 **Liu TT,** Hou MC, Lin HC, Chang FY, Lee SD. Life-threatening hemobilia caused by hepatic artery pseudoaneurysm: a rare complication of chronic cholangitis. *World J Gastroenterol* 2003; **9:** 2883-2884

5 **Akatsu T,** Hayashi S, Egawa T, Doi M, Nagashima A, Kitano M, Yamane T, Yoshii H, Kitajima M. Hepatic artery pseudoaneurysm associated with cholecystitis that ruptured into the gallbladder. *J Gastroenterol* 2004; **39:** 900-903

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

World Journal of Hepatology

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

World J Hepatol 2010 January 27; 2(1): I
ISSN 1948-5182 (online)

Acknowledgments to reviewers of *World Journal of Hepatology*

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

Patricia Cristina Baré, PhD, Virology Section, Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Pacheco de Melo 3081, Buenos Aires, 1425, Argentina

Fei Chen, PhD, Pathology and Physiology Research Branch, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, United States

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

Melchiorre Cervello, Dr, Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy", Consiglio Nazionale delle Ricerche (CNR), Via Ugo La Malfa 153, Palermo 90146, Italy

Radha K Dhiman, Professor, Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Eva Herrmann, Professor, Department of Internal Medicine, Biomathematics Saarland University, Faculty of Medicine, Kirrberger Str., 66421 Homburg/Saar, Germany

Seong Woo Jeon, Assistant Professor, Internal Medicine, Kyungpook National University Hospital, 50 Samduk-2Ga, Chung-gu, Daegu 700-721, South Korea

Wan Yee Joseph Lau, MD, Professor, Clinical Sciences Building, Prince of Wales Hospital, Rm 94005, 7th floor, Shatin, New Territories, Hong Kong SAR, China

Ryuichi Morisihita, Professor, Department of Clinical Gene Therapy, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita City, Osaka, 565-0871, Japan

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

Hironori Nakagami, Assisitant Professor, Division of Gene Therapy Science, Osaka University, 2-2 Yamada-oka, Suita 565-0871, Japan

Pierluigi Toniutto, Professor, Medical Liver Transplant Unit, Internal Medicine, University of Udine, Piazzale S.M. della Misericordia 1, Udine 33100, Iyaly

Fiona J Warner, Dr, Liver Cell Biology, Centenary Insitute, Locked Bag No. 6, Newtown, NSW, 2042, Australia

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

World Journal of Hepatology

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

World J Hepatol 2010 January 27; 2(1): I
ISSN 1948-5182 (online)

Meetings

Events Calendar 2010

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

World Journal of Hepatology

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

World J Hepatol 2010 January 27; 2(1): I-V
ISSN 1948-5182 (online)

Instructions to authors

GENERAL INFORMATION

World Journal of Hepatology (*World J Hepatol*, *WJH*, ISSN 1948-5182, DOI: 10.4254), is a monthly, open-access (OA), peer-reviewed online journal supported by an editorial board of 575 experts in hepatology from 45 countries.

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

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

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

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

CSSN
ISSN 1948-5182 (online)

Published by
Beijing Baishideng BioMed Scientific Co., Ltd.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages

consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Beijing Baishideng BioMed Scientific Co., Ltd, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

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

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-5182ffice. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-5182/g_info_17.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjh@wjgnet.com, or by telephone: +86-10-59080038. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

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

Title page

Title: Title should be less than 12 words.

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

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

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

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

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

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

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

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

Abstract

There are unstructured abstracts (no more than 256 words)

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

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words). Available from: http://www.wjgnet.com/1948-5182/g_info_20100107114338.htm.

Key words

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

Text

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

Illustrations

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

Tables

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

Notes in tables and illustrations

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

Acknowledgments

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

REFERENCES

Coding system

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

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

PMID and DOI

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

Style for journal references

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

Style for book references

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

Format

Journals

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

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

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

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

In press

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

Organization as author

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

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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

Units

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

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies

of *WJH*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office

World Journal of Hepatology

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

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

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

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107114601.htm.

Proof of financial support

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

Links to documents related to the manuscript

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

Science news releases

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

Publication fee

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