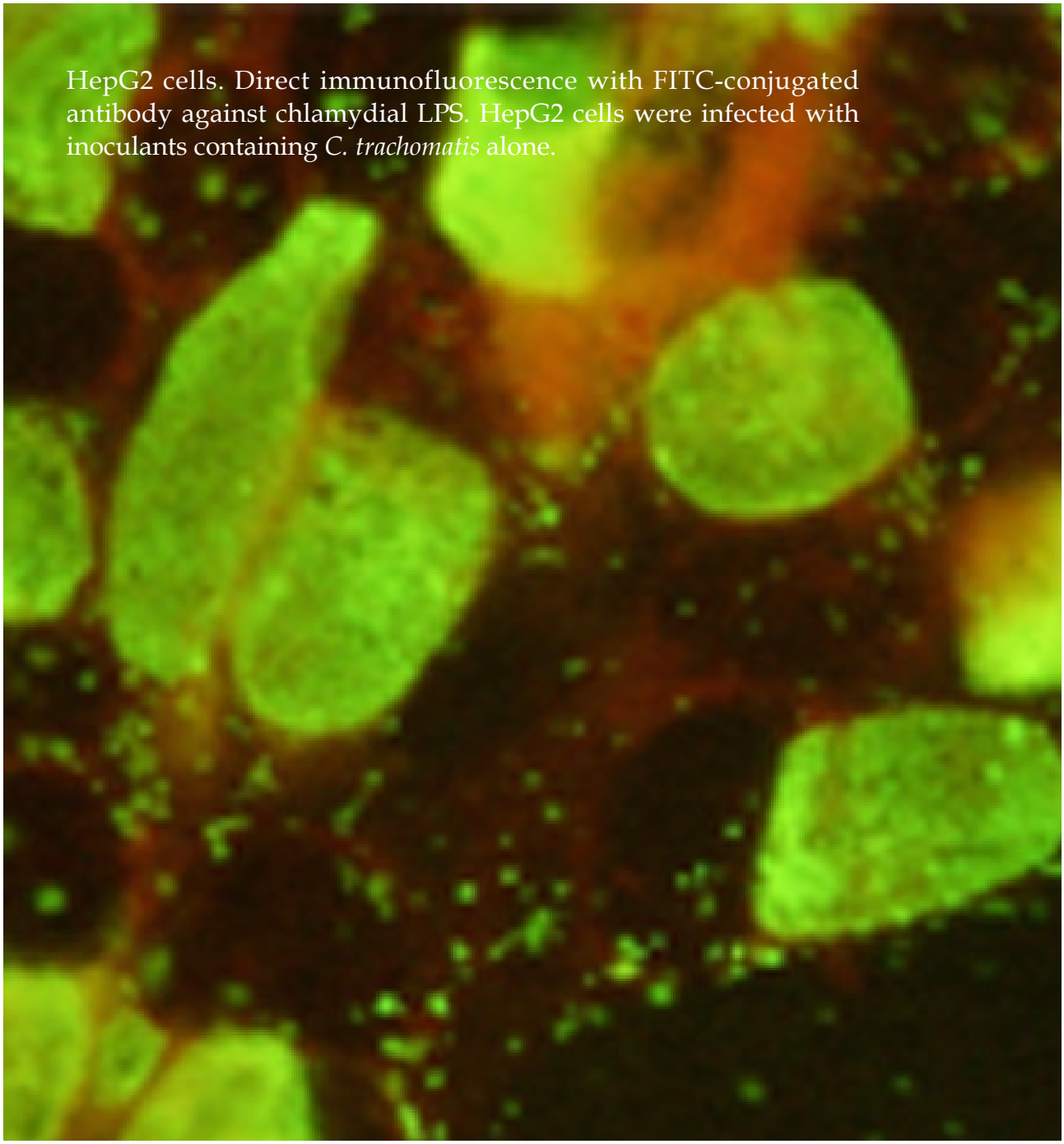
ISSN 1948-5182 (online)

World Journal of Hepatology

World J Hepatol 2010 February 27; 2(2): 55-90

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



HepG2 cells. Direct immunofluorescence with FITC-conjugated antibody against chlamydial LPS. HepG2 cells were infected with inoculants containing *C. trachomatis* alone.

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), Gambia (1), 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*

Yueming Tang, *Chicago*
John M Taylor, *Philadelphia*
Swee H The, *Springfield*
Chung-Jyi Tsai, *Lexington*
George P Tuszynski, *Pennsylvania*
Jean-Nicolas Vauthey, *Houston*
Michael E de Vera, *Pennsylvania*
Yu-Jui Yvonne Wan, *Kansas*
Jack R Wands, *Providence*
Hanlin L Wang, *Los Angeles*
Xin Wei Wang, *Maryland*
Wahid Wassef, *Worcester*

Ronald J Wong, *California*
George YH Wu, *Farmington*
Hai-Shan Wu, *New York*
Victor W Xia, *California*
Ximing J Yang, *Chicago*
Matthew M Yeh, *Seattle*
Mei Po Yip, *Seattle*
Min You, *Tampa*
Zobair M Younossi, *Falls Church*
Xiao-Fang Yu, *Maryland*
Yong Yuan, *Plainsboro*
Jian X Zhang, *Charlotte*

Jian-Ying Zhang, *El Paso*
Kezhong Zhang, *Michigan*
Yu-Jing Zhang, *New York*
Yuao Zhu, *Durham*
Saša Živković, *Pittsburgh*
William A Zule, *Research Triangle Park*

Venezuela

Flor Pujol de Freychet, *Caracas*

WJH World Journal of Hepatology

Contents

Monthly Volume 2 Number 2 February 27, 2010

EDITORIAL

55 Can proteomics lead to the discovery of real biomarkers for HCC?
Kuramitsu Y

GUIDELINES FOR CLINICAL PRACTICE

58 Surgery and chemotherapy for intrahepatic cholangiocarcinoma
Morise Z, Sugioka A, Tokoro T, Tanahashi Y, Okabe Y, Kagawa T, Takeura C

REVIEW

65 Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis
Kew MC

ORIGINAL ARTICLE

74 ApoB-containing lipoproteins promote infectivity of chlamydial species in human hepatoma cell line
Bashmakov YK, Zigangirova NA, Gintzburg AL, Bortsov PA, Petyaev IM

BRIEF ARTICLE

81 Prognosis of metastatic splenic hilum lymph node in patients with gastric cancer after total gastrectomy and splenectomy
Aoyagi K, Kouhuji K, Miyagi M, Imaizumi T, Kizaki J, Shirouzu K

CASE REPORT

87 Post-traumatic hepatic artery pseudoaneurysm treated with endovascular embolization and thrombin injection
Francisco LE, Asunción LC, Antonio CA, Ricardo RC, Manuel RP, Caridad MH

World Journal of Hepatology
Volume 2 Number 2 February 27, 2010

Contents

ACKNOWLEDGMENTS	I	Acknowledgments to reviewers of *World Journal of Hepatology*
APPENDIX	I	Meetings
	I-V	Instructions to authors
ABOUT COVER		Bashmakov YK, Zigangirova NA, Gintzburg AL, Bortsov PA, Petyaev IM. ApoB-containing lipoproteins promote infectivity of chlamydial species in human hepatoma cell line. *World J Hepatol* 2010; 2(2): 74-80 http://www.wjgnet.com/1948-5182/full/v2/i2/74.htm
AIM AND SCOPE		*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, open-access, peer-reviewed journal supported by an editorial board of 575 experts in hepatology from 45 countries. 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.
FLYLEAF	I-V	Editorial Board

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
February 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.i2.55

World J Hepatol 2010 February 27; 2(2): 55-57
ISSN 1948-5182 (online)

EDITORIAL

Can proteomics lead to the discovery of real biomarkers for HCC?

Yasuhiro Kuramitsu

Yasuhiro Kuramitsu, Yamaguchi University Graduate School of Medicine, Department of Biochemistry and Functional Proteomics, 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan
Author contributions: Kuramitsu Y solely contributed to this work.
Correspondence to: Dr. Yasuhiro Kuramitsu, MD, PhD, Yamaguchi University Graduate School of Medicine, Department of Biochemistry and Functional Proteomics 1-1-1 Minami-kogushi, Ube, Yamaguchi 755-8505, Japan. climates@yamaguchi-u.ac.jp
Telephone: +81-836-222213 Fax: +81-836-222212
Received: September 4, 2009 Revised: January 9, 2010
Accepted: January 16, 2010
Published online: February 27, 2010

Abstract

The development of proteomics technologies has lead to a great deal of effort being focused on the identification of biomarkers for cancers. Although many papers have reported candidate biomarkers for hepatocellular carcinomas (HCCs) in particular, so far none of these candidate biomarkers have been used either for diagnosis or therapy intreating patients. The question remains: Can proteomics identify real biomarkers for HCCs?

Key words: Hepatocellular carcinoma; Proteomics; Mass spectrometry; Two-dimensional polyacrylamide gel electrophoresis; Biomarker

Peer reviewers: Yusuf Yilmaz, MD, Department of Gastroenterology, Marmara University School of Medicine, Tophanelioglu cad. No:13/15, Altunizade, Istanbul 34662, Turkey; Xiu-Jie Wang, Professor, Laborotary of Geritrics, Laborotary of Experimental Oncology, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, No.37 Guoxue Xiang, Chengdu 610041, Sichuan Province, China

Kuramitsu Y. Can proteomics lead to the discovery of real biomarkers for HCC? *World J Hepatol* 2010; 2(2): 55-57 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i2/55.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.55

INTRODUCTION

During the past decade, proteomic technologies, including mass spectrometry, have developed considerably, and have been extensively applied to many fields of science, including medicine and pharmacy, as well as industry and agriculture. In the field of medicine in particular, a huge number of reports on the topic have been published. Above all, much effort has gone into proteomic analyses of tissues, cells and sera from cancer patients. The purpose of these studies has been the identification of biomarkers which could provide the development and identification of diagnostic and therapeutic targets for cancers. Many research labs and large pharmaceutical companies have been actively searching for new and effective biomarkers of cancers. Most applications use expression proteomics to determine expression profiles of proteins in tissues, cells and sera during normal or diseased states.

PROTEOMIC BIOMARKERS FOR HEPATOCELLULAR CARCINOMAS

Hepatocellular carcinoma (HCC) is the third most deadly cancer, and about one million patients with HCC die each year. Despite remarkable advances in diagnostic and therapeutic techniques, the incidence of HCC continues to increase. While some papers on the proteomic analysis and discovery of molecular diagnostic markers for the diagnoses against HCC have been reported[1-9], no complete molecular diagnostic markers specific to HCC have been revealed by proteomics.

So far, many proteins have been reported as candidates for new diagnostic biomarkers, and as therapeutic targets for HCC by proteomics from HCC tissues[10-20]. They are classified as (1) digestive enzymes, (2) growth factors, (3) cell adhesion molecules, (4) calcium-binding proteins, (5) proteases, (6) protease inhibitors, (7) transporter proteins, (8) structural molecules, (9) proteins related to cell growth, (10) proteins related to cell differentiation, (11) proteins related to cell transformation, (12) proteins related to tumor invasion, (13) apoptosis inhibitors, (14) proteins related to carcinogen metabolism, (15) molecular chaperone, and (16) others. However, up to now, unfortunately none of them have been able to be used for diagnostic purposes because of their sensitivity and specificity.

AUTOANTIBODIES AS BIOMARKERS

Although detection for autoantibodies as diagnostic markers in cancer patients' sera is useful, not many reports associating them with HCC have been published. Le Naour *et al*[21] identified autoantibodies reaction to calreticulin isoforms, cytokeratin 8, cytokeratin 18, creatine kinase B, HSP60, nucleoside diphosphate kinase A and F1-ATP synthase beta-subunit. Takashima *et al*[22] identified their reaction to HSP70, peroxiredoxin and Mn-SOD. Their sensitivity seems to be high, but their specificity is still not great enough.

METABOLOMIC BIOMARKERS FOR HCCS

Nowadays, in order to identify dramatically increased or decreased metabolites in cancer tissues, metabolomic profiling analyses have been used. Wu *et al*[23] and Xue *et al*[24] assayed endogenous metabolome in urine and sera from HCC patients using chemical derivatization followed by gas chromatography/mass spectrometry respectively, and many metabolites were shown to be significantly different between the HCC and control groups.

CONCLUSION

To exclude false positive biomarkers for hepatocellular carcinomas (HCCs), we need high specific biomarkers which show as increased biomarkers solely in HCCs, and not in hepatitis as well. Many reports have shown such high specific biomarker candidates, unfortunately they are still not enough.

Much time may still be needed for the identification of real biomarkers for HCC.

REFERENCES

1 **Chambers G**, Lawrie L, Cash P, Murray GI. Proteomics: a new approach to the study of disease. *J Pathol* 2000; **192**: 280-288

2 **Alaiya AA**, Franzén B, Auer G, Linder S. Cancer proteomics: from identification of novel markers to creation of artifical learning models for tumor classification. *Electrophoresis* 2000; **21**: 1210-1217

3 **Xu L**, Hui L, Wang S, Gong J, Jin Y, Wang Y, Ji Y, Wu X, Han Z, Hu G. Expression profiling suggested a regulatory role of liver-enriched transcription factors in human hepatocellular carcinoma. *Cancer Res* 2001; **61**: 3176-3181

4 **Hanash SM**. Biomedical applications of two-dimensional electrophoresis using immobilized pH gradients: current status. *Electrophoresis* 2000; **21**: 1202-1209

5 **Beretta L**. Comparative analysis of the liver and plasma proteomes as a novel and powerful strategy for hepatocellular carcinoma biomarker discovery. *Cancer Lett* 2009; **286**: 134-139

6 **Sun W**, Zhong F, Zhi L, Zhou G, He F. Systematic-omics analysis of HBV-associated liver diseases. *Cancer Lett* 2009; **286**: 89-95

7 **Orimo T**, Ojima H, Hiraoka N, Saito S, Kosuge T, Kakisaka T, Yokoo H, Nakanishi K, Kamiyama T, Todo S, Hirohashi S, Kondo T. Proteomic profiling reveals the prognostic value of adenomatous polyposis coli-end-binding protein 1 in hepatocellular carcinoma. *Hepatology* 2008; **48**: 1851-1863

8 **Chaerkady R**, Harsha HC, Nalli A, Gucek M, Vivekanandan P, Akhtar J, Cole RN, Simmers J, Schulick RD, Singh S, Torbenson M, Pandey A, Thuluvath PJ. A quantitative proteomic approach for identification of potential biomarkers in hepatocellular carcinoma. *J Proteome Res* 2008; **7**: 4289-4298

9 **Lee NP**, Chen L, Lin MC, Tsang FH, Yeung C, Poon RT, Peng J, Leng X, Beretta L, Sun S, Day PJ, Luk JM. Proteomic expression signature distinguishes cancerous and non-malignant tissues in hepatocellular carcinoma. *J Proteome Res* 2009; **8**: 1293-1303

10 **Park KS**, Kim H, Kim NG, Cho SY, Choi KH, Seong JK, Paik YK. Proteomic analysis and molecular characterization of tissue ferritin light chain in hepatocellular carcinoma. *Hepatology* 2002; **35**: 1459-1466

11 **Park KS**, Cho SY, Kim H, Paik YK. Proteomic alterations of the variants of human aldehyde dehydrogenase isozymes correlate with hepatocellular carcinoma. *Int J Cancer* 2002; **97**: 261-265

12 **Kim J**, Kim SH, Lee SU, Ha GH, Kang DG, Ha NY, Ahn JS, Cho HY, Kang SJ, Lee YJ, Hong SC, Ha WS, Bae JM, Lee CW, Kim JW. Proteome analysis of human liver tumor tissue by two-dimensional gel electrophoresis and matrix assisted laser desorption/ionization-mass spectrometry for identification of disease-related proteins. *Electrophoresis* 2002; **23**: 4142-4156

13 **Lim SO**, Park SJ, Kim W, Park SG, Kim HJ, Kim YI, Sohn TS, Noh JH, Jung G. Proteome analysis of hepatocellular carcinoma. *Biochem Biophys Res Commun* 2002; **291**: 1031-1037

14 **Li C**, Tan YX, Zhou H, Ding SJ, Li SJ, Ma DJ, Man XB, Hong Y, Zhang L, Li L, Xia QC, Wu JR, Wang HY, Zeng R. Proteomic analysis of hepatitis B virus-associated hepatocellular carcinoma: Identification of potential tumor markers. *Proteomics* 2005; **5**: 1125-1139

15 **Fujii K**, Kondo T, Yokoo H, Yamada T, Iwatsuki K, Hirohashi S. Proteomic study of human hepatocellular carcinoma using two-dimensional difference gel electrophoresis with saturation cysteine dye. *Proteomics* 2005; **5**: 1411-1422

16 **Zeindl-Eberhart E**, Haraida S, Liebmann S, Jungblut PR, Lamer S, Mayer D, Jäger G, Chung S, Rabes HM. Detection and identification of tumor-associated protein variants in human hepatocellular carcinomas. *Hepatology* 2004; **39**: 540-549

17 **Kim W**, Oe Lim S, Kim JS, Ryu YH, Byeon JY, Kim HJ, Kim YI, Heo JS, Park YM, Jung G. Comparison of proteome between hepatitis B virus- and hepatitis C virus-associated hepatocellular carcinoma. *Clin Cancer Res* 2003; **9**: 5493-5500

18 **Takashima M**, Kuramitsu Y, Yokoyama Y, Iizuka N, Toda T, Sakaida I, Okita K, Oka M, Nakamura K. Proteomic profiling of heat shock protein 70 family members as biomarkers for hepatitis C virus-related hepatocellular carcinoma. *Proteomics* 2003; **3**: 2487-2493

19 **Yokoyama Y**, Kuramitsu Y, Takashima M, Iizuka N, Toda T, Terai S, Sakaida I, Oka M, Nakamura K, Okita K. Proteomic

profiling of proteins decreased in hepatocellular carcinoma from patients infected with hepatitis C virus. *Proteomics* 2004; **4**: 2111-2116

20 **Takashima M**, Kuramitsu Y, Yokoyama Y, Iizuka N, Fujimoto M, Nishisaka T, Okita K, Oka M, Nakamura K. Overexpression of alpha enolase in hepatitis C virus-related hepatocellular carcinoma: association with tumor progression as determined by proteomic analysis. *Proteomics* 2005; **5**: 1686-1692

21 **Le Naour F**, Brichory F, Misek DE, Bréchot C, Hanash SM, Beretta L. A distinct repertoire of autoantibodies in hepatocellular carcinoma identified by proteomic analysis. *Mol Cell Proteomics* 2002; **1**: 197-203

22 **Takashima M**, Kuramitsu Y, Yokoyama Y, Iizuka N, Harada T, Fujimoto M, Sakaida I, Okita K, Oka M, Nakamura K. Proteomic analysis of autoantibodies in patients with hepatocellular carcinoma. *Proteomics* 2006; **6**: 3894-3900

23 **Wu H**, Xue R, Dong L, Liu T, Deng C, Zeng H, Shen X. Metabolomic profiling of human urine in hepatocellular carcinoma patients using gas chromatography/mass spectrometry. *Anal Chim Acta* 2009; **648**: 98-104

24 **Xue R**, Lin Z, Deng C, Dong L, Liu T, Wang J, Shen X. A serum metabolomic investigation on hepatocellular carcinoma patients by chemical derivatization followed by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2008; **22**: 3061-3068

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.i2.58

World J Hepatol 2010 February 27; 2(2): 58-64
ISSN 1948-5182 (online)

GUIDELINES FOR CLINICAL PRACTICE

Surgery and chemotherapy for intrahepatic cholangiocarcinoma

Zenichi Morise, Atsushi Sugioka, Takamasa Tokoro, Yoshinao Tanahashi, Yasuhiro Okabe, Tadashi Kagawa, Chinatsu Takeura

Zenichi Morise, Atsushi Sugioka, Takamasa Tokoro, Yoshinao Tanahashi, Yasuhiro Okabe, Tadashi Kagawa, Chinatsu Takeura, Department of Surgery, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsu-kake-cho, Toyoake, Aichi 470-1192, Japan

Author contributions: As principal investigator, Morise Z has full access to all of the data in the study and is responsible for the integrity of the data; Tokoro T, Tanahashi Y, Okabe Y, Kagawa T, and Takeura C collected data; Morise Z analyzed the data; Morise Z and Sugioka A played a supervising role.

Correspondence to: Zenichi Morise, MD, PhD, Department of Surgery, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukakecho, Toyoake, Aichi 470-1192, Japan. zmorise@aol.com

Telephone: +81-562-939248 Fax: +81-562-937060

Received: September 12, 2009 Revised: January 14, 2010
Accepted: January 21, 2010
Published online: February 27, 2010

Abstract

Cholangiocarcinoma, arising from bile duct epithelium, is categorized into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC), including hilarcholangiocarcinoma. Recently, there has been a worldwide increase in the incidence and mortality from ICC. Complete surgical resection is the only approach to cure the patients with ICC. However, locoregional extension of these tumors is usually advanced with intrahepatic and lymph-node metastases at the time of diagnosis. Resectability rates are quite low and variable (18%-70%). The five-year survival rate after surgical resection was reported to be 20%-40%. Median survival time after ICC resection was 12-37.4 mo. Only a small number of ICC cases, accompanied with ECC, gall bladder carcinoma, and ampullary carcinoma, have been reported in the studies of chemotherapy due to the rarity of the disease. However, in some reports, significant anti-cancer effects were achieved with a response rate of up to 40% and a median survival of one year. Although recurrence rate after hepatectomy is high for the patients with ICC, the residual liver and the lung are the main sites of recurrence after tentative curative surgical resection. Several patients in our study had a long-term survival with repeated surgery and chemotherapy. Repeated surgery, combined with new effective regimens of chemotherapy, could benefit the survival of ICC patients.

Key words: Intrahepatic cholangiocarcinoma; Surgery; Chemotherapy

Peer reviewers: Olivier Detry, MD, PhD, Department of Abdominal Surgery and Transplantation, CHU de Liège, University of Liège, Sart Tilman B35, B4000 Liège, Belgium; Xun-Di Xu, MD, PhD, Department of Gastroenterological Surgery, Xiangya 2nd Hospital, Central South University, Renmin Zhong Road 139, Changsha 410011, Hunan Province, China

Morise Z, Sugioka A, Tokoro T, Tanahashi Y, Okabe Y, Kagawa T, Takeura C. Surgery and chemotherapy for intrahepatic cholangiocarcinoma. *World J Hepatol* 2010; 2(2): 58-64 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i2/58.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.58

INTRODUCTION

Cholangiocarcinoma, arising from bile duct epithelium, is categorized into intrahepatic cholangiocarcinoma (ICC) and extrahepatic (ECC), including hilar cholangiocarcinoma. ICC, more than 90% of which is adenocarcinoma[1], is the second most, but relatively rare, common primary liver cancer after hepatocellular carcinoma, accounting for 5%-10% of the primary malignancies of the liver[2,3]. Recently, there has been a

Table 1 Recent reported series of hepatectomy for intrahepatic cholangiocarcinoma

Authors	Year	No	Survival rate (%) and MST				Significant prognostic factors for survival
			1-yr	3-yr	5-yr	MST (mo)	
Okabayashi	2001	60	68	35	29	19.6	Number of nodules, lymph-node involvement, vascular invasion, symptomatic tumor
Chen	1999	48	35.5	20.5	16.5[1]		
			27.2	8.8	7.8[2]		
DeOliveira	2007	44			40	28	Surgical margin
Palik	2008	97	74.9	51.8	31.1		Number of nodules, surgical margin
Inoue	2000	52	63	36	36	18	Surgical margin, lymph-node involvements, vascular invasion
Valverde	1999	30	86	22	22	28	Number of nodules, lymph-node involvements
Madariaga	1998	34	67	40	35	19	Number of nodules, surgical margin
Shimada	2007	94	69.5	35.5	31.1	24	Number of nodules, surgical margin, lymph-node involvements
Weber	2001	33		55	31	37.4	Vascular invasion

[1]Data from intrahepatic cholangiocarcinoma patients with hepatolithiasis; [2]Data from intrahepatic cholangiocarcinoma patients without hepatolithiasis; MST: Median survival time.

worldwide increase in the incidence and mortality from ICC, although the incidence and mortality from ECC are decreasing[4,5]. There has also been an increasing number of reports on the surgical treatment of ICC. Complete (R0) surgical resection is the only therapy for the cure of ICC patients. However, the resectability rate is still low and the prognosis following hepatectomy is poor, because the locoregional extension of these tumors is usually advanced with intrahepatic and lymph-node metastases at the time of diagnosis[6]. Although only a small number of ICC cases, accompanied with also ECC, gall bladder carcinoma, and ampullary carcinoma, have been reported in the studies of chemotherapy due to the rarity of the disease,several reports have demonstrated significant anti-cancer responses for ICC using new agents[7,8]. Recently, the treatment strategy for colorectal carcinoma (also adenocarcinoma) has been dramatically altered in the combination of surgery and chemotherapy with new anti-cancer agents, such as CPT-11 and oxaliplatin[9,10]. There is a need of considerations to improve the therapeutic approaches for ICC. It has been reported that ICC may include two pathologically and biologically different types of tumors, peripheral mass-forming (usually hepatitis-based) tumor and central periductal-infiltrating tumor without hepatitis[11,12].

In this article, the outcomes of the treatment with surgery and chemotherapy for ICC patients are reviewed and future prospects are discussed.

SURGICAL RESECTION

Currently, surgical resection of the involved liver segments is the only curative treatment for ICC. However, resectability rates have been quite low and variable (18%-70%), as most patients present at an advanced stage. Surgery has been successful in several reported series, with a 1-year survival rate of 35%-86%, 3-year survival of 20%-52%, and 5-year survival of 20%-40%. In our institute, 44 patients with ICC underwent hepatectomy by 2006 and their 3-, 5- and 10-year survival rates after the first hepatectomy were 51%, 29% and 22%, respectively. Disease-free survival at 5 years varied significantly -between 2% and 41%. Median survival after ICC resection was 12-37.4 mo[11,13-26]. Some of these studies report that peri-operative mortality was less than 5%, which is recently decreasing[11,12,15] (Table 1).

Since there is a limited number of patients in most reports of ICC cases few studies have specifically addressed surgical resection outcomes compared with non-operative treatment. Based on the available studies, surgery should be performed in patients with potentially resectable ICC regardless of its stage. DeOliveira *et al*[15] emphasized the importance of performing a complete resection because the 5-year survival rate among their 44 patients was 63% and the median survival was 80 mo in patients who could achieve an R0 resection. Nakeeb *et al*[18] demonstrated that resection was beneficial; and the 5-year survival was 44% and median survival was 26 mo in those who underwent resection versus 0% and 7 mo, respectively, in patients without surgical resections.

Indicators of poor prognosis noted in two or more studies included positive lymph nodes, positive margins, multiple nodules, vascular invasion, and large tumor size. Other indicators included capsular invasion, histological type, tumor spreading type, bilobar disease, mucobilia, left side involvement, and high CA 19-9[11,14,16,17,21-24]. Chen *et al*[14] also reported that patients with hepatolithiasis had higher rates of resection, and higher incidence of papillary tumors and postoperative complications, but no difference in survival was noted when compared with patients without hepatolithiasis. In a study of 33 patients, the recurrence rate was 61% at 12.4 mo. Liver was the most common site of recurrence, followed by the lung, lymph nodes, and bone[24,25].

In summary, surgical resection of the liver is the only curative treatment with a 5-year survival rate of around 30% and a median overall survival of 2-3 years for ICC patients. Strong prognostic factors after hepatectomy are number of nodules, surgical margin, and lymph-node involvement. Thus, further investigations should be carried out about the treatment for the recurrence after surgery and for patients with factors indicating poor prognosis.

CHEMOTHERAPY

There are not so much evidences for the evidence-based evaluation of the chemotherapeutic efficacy for ICC patients. Only a small number of ICC cases , accompanied with extrahepatic cholangiocarcinoma, gall bladder carcinoma, and ampullary carcinoma, have been reported due to the rarity of the disease. There are also a variety of factors, which influence the effect of chemotherapy and complicate the evaluation, such as control of cholangitis, liver function and performance status, except for tumor-related factors. In this article, studies in biliary tract carcinoma, including ICC, are reviewed.

To date, only two small randomized controlled trials have been published for biliary tract carcinoma[27,28]. An randomized controlled trial on chemotherapy and supportive treatment was conducted in patients with unresectable biliary tract cancer and pancreas cancer[30]. In this study, fluorouracil (5-FU) + leucovorin or 5-FU + leucovorin + etoposide were used for chemotherapy. For all the patients, significantly prolonged survival was observed in the group with chemotherapy [median survival time (MST), 6.0 mo] compared with the group with supportive treatment alone (MST, 2.5 mo). However, due to the small number of patients with biliary tract cancer (37), no significant difference was found between the groups (chemotherapy group MST, 6.5 mo; supportive treatment group 2.5 mo; $P = 0.1$). The rate of improvement in quality of life was also examined in this trial, and a significant difference was found in the chemotherapy group compared with the supportive treatment group (36% *vs* 10%; $P < 0.01$).

The single use of fluoropyrimidines such as 5-FU, or a combination of 5-FU with interferon, leucovorin, or hydroxyurea as biochemical modulators, was often used for advanced biliary tract cancer[27,29-34]. The response rates ranged from 7% to 34% and MST ranged from 6 to 14.8 mo with the combined use of 5-FU and these modulators. However, no difference was observed in survivals of patients with chemotherapy including 5-FU for unresectable biliary tract cancer and patients who received best supportive care.

Since 1999, clinical trials have been conducted with gemcitabine[35-41]. Although methods of administration are varied, relatively good results are reported. The response rates ranged from 0% to 36% and MST ranged from 4.6 to 14.0 mo. Toxicity-induced myelosuppression, such as leucopenia, as well as nausea and anorexia, was mainly observed, although they were well tolerable.

A clinical trial of tegafur/gimeracil/oteracil potassium (S-1), which is an oral anti-cancer drug consisting of tegafur as a prodrug of 5-FU, 5-chloro-2, 4 dihydroxypyridine and potassium oxonate, was conducted in Japan[42,43]. In a late phase Ⅱ trial, a favorable result was reported, with a response rate of 35% and MST of 9.4 mo in 40 patients.

Some reports used mitomycin C, cisplatin, taxanes, and irinotecan (CPT-11)[44-46], which showed a response rate of around 10% and MST of 4.5-6.1 mo.

For biliary tract cancer, since the chemotherapeutic effects are limited with a single agent, many modalities of combined chemotherapy have been carried out[47-65]. Compared with single-agent chemotherapy, the response rate of combined chemotherapy is generally high and the survival period is also inclined to be long. Although a regimen of combined 5-FU, anthracycline and platinum has often been employed, no standard regimen has been established. An attempt at a regimen focusing on the use of gemcitabine is currently being made and a favorable result has been achieved, with the response rate of 28%-38% and MST of 4.6-11.0 mo in patients treated with gemcitabine + cisplatin[66-69].

Therapeutic drugs targeting molecular biological characteristics (molecular targeting therapy) are now also under development. In view of a report suggesting the strong expression of epithelial growth factor receptor (EGFR) in biliary tract cancer, a phase Ⅱ trial using erlotinib, an EGFR-inhibiting drug, is being carried out (response rate 8% and MST 7.5 mo)[70] (Table 2).

In summary, recent advancement facilitates the chemotherapy to achieved a response rate of around-30% and a median survival of more than one year for ICC patients. Key drugs currently available for the therapy are gemcitabine, fluoropyrimidines, and platinums. Further investigations are required for the development of new agents, such as molecular-targeting drugs, and combined therapy with surgery.

POSSIBLE SUBTYPES OF ICC AND THEIR CHARACTERISTICS

Two studies categorized the ICC into subtypes and compared their prognoses. Shimada *et al*[11] categorized ICC into two types according to the classification of the Liver Cancer Study Group of Japan[71]: Mass-forming and mass-forming periductal-infiltrating types, which occurs with a definitive mass but also causes infiltration along the portal pedicle and bile ducts. The mass-forming periductal-infiltrating type was associated more with jaundice, bile duct invasion, portal invasion, lymph node involvement, and positive surgical margins. In their study of 74 patients, those with mass-forming ICC had less local recurrence (76.1% *vs* 92.9%) and a significantly higher median survival (32 *vs* 22 mo) than those with mass-forming periductal-infiltrating ICC. Aishima *et al*[12] classified 87 patients into hilar ICC and peripheral ICC and noted that hilar ICC was more likely to be associated with perineural invasion, lymph node metastases, and extrahepatic recurrence. 1-, 3-, and 5-year survival rates of the peripheral ICC patients were 88%, 72% and 60% compared with 66%, 41% and 36%, respectively, in the hilar ICC patients. The incidence of ICC has increased steadily over the past few decades and viral hepatitis, chronic liver disease, and fatty liver disease have been identified as possible contributing factors. Similar to the increased incidence of HCC in the last decade from the epidemic of hepatitis C, ICC may occur from viral hepatitis and metabolic syndrome-related

Table 2 Recent phase Ⅱ studies of chemotherapy for biliary tract carcinoma

Agents	*n*	RR (%)	PFS (M)	MST (M)	Authors	Year
Gemcitabine						
1000mg/m^2, 30-min infusion	25	36		6.9	Gallardo	2001
	24	12.5	2.5	7.2	Lin	2003
	40	17.5	2.6	7.6	Okusaka	2006
Fluoropyrimidine						
Capecitabine	26	19		8.1	Patt	2004
S-1	19	21	3.7	8.3	Ueno	2004
S-1	40	32.5	3.7	9.4	Furuse	2008
Others						
Docetaxel	24	20	6.0	8.0	Papakostas	2001
CPT-11	36	8	2.7	6.1	Alberts	2002
Erlotinib	42	8	2.6	7.5	Philip	2006
Gemcitabine + fluoropyrimidine						
Gem/5FU	27	33	3.7	5.3	Knox	2004
Gem/5FU/LV	30	20	3.7	4.7	Hsu	2004
Gem/5FU/LV	42	12	4.6	9.7	Alberts	2005
Gem/capecitabine	45	31	7.0	14.0	Knox	2005
Gem/capecitabine	45	32	6.0	14.0	Cho	2005
Gemcitabine + platinum						
Gem/cisplatin	30	37	4.1	4.6	Doval	2004
Gem/cisplatin	40	28	4.7	8.4	Thongprasert	2005
Gem/cisplatin	29	35	3.0	11.0	Kim	2006
Gem/cisplatin	27	33	5.6	10.0	Park	2006
Gem/oxaliplatin	33	33	5.7	15.4	Andre	2004
Gem/oxaliplatin/bevacizumab	26	29	7.6		Clark	2007
Fluoropyrimidine + platinum						
Capecitabine/oxaliplatin	65	20	6.5	12.8	Nehls	2006
S-1/cisplatin	51	30	4.8	8.7	Kim	2007

RR: Response rate; PFS: Median progression-free survival; ST: Median overall survival; FU: Fluorouracil.

liver disease[72]. There are some reports that suggest the relationship between chronic hepatitis and peripheral mass-forming ICC[73]. These facts may indicate that ICC includes two different types pathologically and biologically: Peripheral mass-forming type (usually hepatitis-based) and central periductal-infiltrating type without hepatitis. However, further investigations are needed.

LONG-TERM SURVIVAL WITH REPEATED SURGERY AND CHEMOTHERAPY

Although the results of surgical resection for ICC patients with lymph node metastases are thought to be especially poor, the outcome of hepatectomy for these patients is comparable to that for patients without lymph node metastases in our series.

Forty-four patients with ICC, including 13 patients with lymph node metastases, underwent hepatectomy before 2006 in our institute. The survival rates of those patients after first hepatectomy are 51%, 29% and 22% for 3, 5 and 10 years, respectively. The survival rates of the patients with and without lymph node metastases are 42% and 51% for 3 years, and 28% and 29% for 5 years, respectively. There was no significant difference in the survival curves between groups. However, 11 out of 13 patients with lymph node metastases have recurrences after first hepatectomy (7 in residual liver; 2 in lung, lymph node, each; and 1 each in bone, brain, peritoneum). Five patients with lymph node metastases and 11 patients without lymph node metastases actually survived more than 3 years, and 4 of those 5 patients with lymph node metastases underwent repeated surgery for recurrences in the residual liver or the lung. Three of them also underwent adjuvant and/or neo-adjuvant chemotherapy. One patient who underwent four hepatectomies and 1 pulmonary resection combined with chemotherapy survived 6 years and 9 mo[74].

We also examined the results of 12 consecutive patients with unresectable advanced biliary tract carcinoma, including 8 patients with ICC in the other series of our patients. They were treated with first-line chemotherapy of S1/cisplatin combined with surgical resection and second-line chemotherapy of gemcitabine. MST of the patients was 15.9 mo. With S1/cisplatin therapy, 6 patients had a partial response based on the Response Evaluation Criteria in Solid Tumors guideline and 4 had a stable disease. Two patients with surgical resection after the therapy survived more than 4 years[75].

FUTURE PROSPECTS

Surgical resection is the only therapy for the cure of ICC patients. However, current resectability and prognosis after hepatectomy are not satisfactory. Further investigations should be conducted about the treatment for the recurrence after surgery and for patients with poor

prognostic factors, such as multiple tumors and lymph-node metastases.

Although the recurrence rate after hepatectomy is still high for the patients with ICC, the residual liver and the lung are the main sites of recurrence. Repeated surgery for the lesions could contribute to the survival of the patients with recurrences. Combined repeated surgeries and new effective regimens of chemotherapy could facilitate ICC patients for a long-term survival, even though without complete cure.

REFERENCES

1 **Ishak KG**, Goodman ZD, Stocker JT. Tumors of the liver and intrahepatic bile ducts. (Atlas of Tumor Pathology, 3rd series, Fascicle 31.) Washington DC: Armed Forces Institute of Pathology; 2001

2 **Casavilla FA**, Marsh JW, Iwatsuki S, Todo S, Lee RG, Madariaga JR, Pinna A, Dvorchik I, Fung JJ, Starzl TE. Hepatic resection and transplantation for peripheral cholangiocarcinoma. *J Am Coll Surg* 1997; **185**: 429-436

3 **Ikai I**, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007; **37**: 676-691

4 **Patel T**. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001; **33**: 1353-1357

5 **Taylor-Robinson SD**, Toledano MB, Arora S, Keegan TJ, Hargreaves S, Beck A, Khan SA, Elliott P, Thomas HC. Increase in mortality rates from intrahepatic cholangiocarcinoma in England and Wales 1968-1998. *Gut* 2001; **48**: 816-820

6 **Roayaie S**, Guarrera JV, Ye MQ, Thung SN, Emre S, Fishbein TM, Guy SR, Sheiner PA, Miller CM, Schwartz ME. Aggressive surgical treatment of intrahepatic cholangiocarcinoma: predictors of outcomes. *J Am Coll Surg* 1998; **187**: 365-372

7 **Thongprasert S**. The role of chemotherapy in cholangiocarcinoma. *Ann Oncol* 2005; **16** Suppl 2: ii93-ii96

8 **Furuse J**, Takada T, Miyazaki M, Miyakawa S, Tsukada K, Nagino M, Kondo S, Saito H, Tsuyuguchi T, Hirata K, Kimura F, Yoshitomi H, Nozawa S, Yoshida M, Wada K, Amano H, Miura F. Guidelines for chemotherapy of biliary tract and ampullary carcinomas. *J Hepatobiliary Pancreat Surg* 2008; **15**: 55-62

9 **Bismuth H**, Adam R, Lévi F, Farabos C, Waechter F, Castaing D, Majno P, Engerran L. Resection of nonresectable liver metastases from colorectal cancer after neoadjuvant chemotherapy. *Ann Surg* 1996; **224**: 509-520; discussion 520-522

10 **Leonard GD**, Brenner B, Kemeny NE. Neoadjuvant chemotherapy before liver resection for patients with unresectable liver metastases from colorectal carcinoma. *J Clin Oncol* 2005; **23**: 2038-2048

11 **Shimada K**, Sano T, Sakamoto Y, Esaki M, Kosuge T, Ojima H. Surgical outcomes of the mass-forming plus periductal infiltrating types of intrahepatic cholangiocarcinoma: a comparative study with the typical mass-forming type of intrahepatic cholangiocarcinoma. *World J Surg* 2007; **31**: 2016-2022

12 **Aishima S**, Kuroda Y, Nishihara Y, Iguchi T, Taguchi K, Taketomi A, Maehara Y, Tsuneyoshi M. Proposal of progression model for intrahepatic cholangiocarcinoma: clinicopathologic differences between hilar type and peripheral type. *Am J Surg Pathol* 2007; **31**: 1059-1067

13 **Okabayashi T**, Yamamoto J, Kosuge T, Shimada K, Yamasaki S, Takayama T, Makuuchi M. A new staging system for mass-forming intrahepatic cholangiocarcinoma: analysis of preoperative and postoperative variables. *Cancer* 2001; **92**: 2374-2383

14 **Chen MF**, Jan YY, Jeng LB, Hwang TL, Wang CS, Chen SC, Chao TC, Chen HM, Lee WC, Yeh TS, Lo YF. Intrahepatic cholangiocarcinoma in Taiwan. *J Hepatobiliary Pancreat Surg* 1999; **6**: 136-141

15 **DeOliveira ML**, Cunningham SC, Cameron JL, Kamangar F, Winter JM, Lillemoe KD, Choti MA, Yeo CJ, Schulick RD. Cholangiocarcinoma: thirty-one-year experience with 564 patients at a single institution. *Ann Surg* 2007; **245**: 755-762

16 **Paik KY**, Jung JC, Heo JS, Choi SH, Choi DW, Kim YI. What prognostic factors are important for resected intrahepatic cholangiocarcinoma? *J Gastroenterol Hepatol* 2008; **23**: 766-770

17 **Inoue K**, Makuuchi M, Takayama T, Torzilli G, Yamamoto J, Shimada K, Kosuge T, Yamasaki S, Konishi M, Kinoshita T, Miyagawa S, Kawasaki S. Long-term survival and prognostic factors in the surgical treatment of mass-forming type cholangiocarcinoma. *Surgery* 2000; **127**: 498-505

18 **Nakeeb A**, Pitt HA, Sohn TA, Coleman J, Abrams RA, Piantadosi S, Hruban RH, Lillemoe KD, Yeo CJ, Cameron JL. Cholangiocarcinoma. A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; **224**: 463-473; discussion 473-475

19 **Schlinkert RT**, Nagorney DM, Van Heerden JA, Adson MA. Intrahepatic cholangiocarcinoma: clinical aspects, pathology and treatment. *HPB Surg* 1992; **5**: 95-101; discussion 101-102

20 **Yamamoto J**, Kosuge T, Takayama T, Shimada K, Makuuchi M, Yoshida J, Sakamoto M, Hirohashi S, Yamasaki S, Hasegawa H. Surgical treatment of intrahepatic cholangiocarcinoma: four patients surviving more than five years. *Surgery* 1992; **111**: 617-622

21 **Jan YY**, Jeng LB, Hwang TL, Wang CS, Chen MF, Chen TJ. Factors influencing survival after hepatectomy for peripheral cholangiocarcinoma. *Hepatogastroenterology* 1996; **43**: 614-619

22 **Valverde A**, Bonhomme N, Farges O, Sauvanet A, Flejou JF, Belghiti J. Resection of intrahepatic cholangiocarcinoma: a Western experience. *J Hepatobiliary Pancreat Surg* 1999; **6**: 122-127

23 **Madariaga JR**, Iwatsuki S, Todo S, Lee RG, Irish W, Starzl TE. Liver resection for hilar and peripheral cholangiocarcinomas: a study of 62 cases. *Ann Surg* 1998; **227**: 70-79

24 **Casavilla FA**, Marsh JW, Iwatsuki S, Todo S, Lee RG, Madariaga JR, Pinna A, Dvorchik I, Fung JJ, Starzl TE. Hepatic resection and transplantation for peripheral cholangiocarcinoma. *J Am Coll Surg* 1997; **185**: 429-436

25 **Weber SM**, Jarnagin WR, Klimstra D, DeMatteo RP, Fong Y, Blumgart LH. Intrahepatic cholangiocarcinoma: resectability, recurrence pattern, and outcomes. *J Am Coll Surg* 2001; **193**: 384-391

26 **Pichlmayr R**, Lamesch P, Weimann A, Tusch G, Ringe B. Surgical treatment of cholangiocellular carcinoma. *World J Surg* 1995; **19**: 83-88

27 **Glimelius B**, Hoffman K, Sjödén PO, Jacobsson G, Sellström H, Enander LK, Linné T, Svensson C. Chemotherapy improves survival and quality of life in advanced pancreatic and biliary cancer. *Ann Oncol* 1996; **7**: 593-600

28 **Takada T**, Nimura Y, Katoh H, Nagakawa T, Nakayama T, Matsushiro T, Amano H, Wada K. Prospective randomized trial of 5-fluorouracil, doxorubicin, and mitomycin C for non-resectable pancreatic and biliary carcinoma: multicenter randomized trial. *Hepatogastroenterology* 1998; **45**: 2020-2026

29 **Takada T**, Kato H, Matsushiro T, Nimura Y, Nagakawa T, Nakayama T. Comparison of 5-fluorouracil, doxorubicin and mitomycin C with 5-fluorouracil alone in the treatment of pancreatic-biliary carcinomas. *Oncology* 1994; **51**: 396-400

30 **Gebbia V**, Majello E, Testa A, Pezzella G, Giuseppe S, Giotta F, Riccardi F, Fortunato S, Colucci G, Gebbia N. Treatment

of advanced adenocarcinomas of the exocrine pancreas and the gallbladder with 5-fluorouracil, high dose levofolinic acid and oral hydroxyurea on a weekly schedule. Results of a multicenter study of the Southern Italy Oncology Group (G.O.I.M.). *Cancer* 1996; **78**: 1300-1307

31 **Patt YZ**, Jones DV Jr, Hoque A, Lozano R, Markowitz A, Raijman I, Lynch P, Charnsangavej C. Phase II trial of intravenous flourouracil and subcutaneous interferon alfa-2b for biliary tract cancer. *J Clin Oncol* 1996; **14**: 2311-2315

32 **Chen JS**, Jan YY, Lin YC, Wang HM, Chang WC, Liau CT. Weekly 24 h infusion of high-dose 5-fluorouracil and leucovorin in patients with biliary tract carcinomas. *Anticancer Drugs* 1998; **9**: 393-397

33 **Choi CW**, Choi IK, Seo JH, Kim BS, Kim JS, Kim CD, Um SH, Kim JS, Kim YH. Effects of 5-fluorouracil and leucovorin in the treatment of pancreatic-biliary tract adenocarcinomas. *Am J Clin Oncol* 2000; **23**: 425-428

34 **Malik IA**, Aziz Z. Prospective evaluation of efficacy and toxicity of 5-fu and folinic acid (Mayo Clinic regimen) in patients with advanced cancer of the gallbladder. *Am J Clin Oncol* 2003; **26**: 124-126

35 **Okusaka T**, Ishii H, Funakoshi A, Yamao K, Ohkawa S, Saito S, Saito H, Tsuyuguchi T. Phase II study of single-agent gemcitabine in patients with advanced biliary tract cancer. *Cancer Chemother Pharmacol* 2006; **57**: 647-653

36 **Tsavaris N,** Kosmas C, Gouveris P, Gennatas K, Polyzos A, Mouratidou D, Tsipras H, Margaris H, Papastratis G, Tzima E, Papadoniou N, Karatzas G, Papalambros E. Weekly gemcitabine for the treatment of biliary tract and gallbladder cancer. *Invest New Drugs* 2004; **22**: 193-198

37 **Gallardo JO**, Rubio B, Fodor M, Orlandi L, Yáñez M, Gamargo C, Ahumada M. A phase II study of gemcitabine in gallbladder carcinoma. *Ann Oncol* 2001; **12**: 1403-1406

38 **Lin MH**, Chen JS, Chen HH, Su WC. A phase II trial of gemcitabine in the treatment of advanced bile duct and periampullary carcinomas. *Chemotherapy* 2003; **49**: 154-158

39 **Raderer M**, Hejna MH, Valencak JB, Kornek GV, Weinländer GS, Bareck E, Lenauer J, Brodowicz T, Lang F, Scheithauer W. Two consecutive phase Ⅱ studies of 5-fluorouracil/leucovorin/mitomycin C and of gemcitabine in patients with advanced biliary cancer. *Oncology* 1999; **56**: 177-180

40 **Eng C**, Ramanathan RK, Wong MK, Remick SC, Dai L, Wade-Oliver KT, Mani S, Kindler HL. A Phase II trial of fixed dose rate gemcitabine in patients with advanced biliary tree carcinoma. *Am J Clin Oncol* 2004; **27**: 565-569

41 **Penz M**, Kornek GV, Raderer M, Ulrich-Pur H, Fiebiger W, Lenauer A, Depisch D, Krauss G, Schneeweiss B, Scheithauer W. Phase II trial of two-weekly gemcitabine in patients with advanced biliary tract cancer. *Ann Oncol* 2001; **12**: 183-186

42 **Furuse J**, Okusaka T, Boku N, Ohkawa S, Sawaki A, Masumoto T, Funakoshi A. S-1 monotherapy as first-line treatment in patients with advanced biliary tract cancer: a multicenter phase Ⅱ study. *Cancer Chemother Pharmacol* 2008; **62**: 849-855

43 **Ueno H**, Okusaka T, Ikeda M, Takezako Y, Morizane C. Phase Ⅱ study of S-1 in patients with advanced biliary tract cancer. *Br J Cancer* 2004; **91**: 1769-1774

44 **Taal BG**, Audisio RA, Bleiberg H, Blijham GH, Neijt JP, Veenhof CH, Duez N, Sahmoud T. Phase II trial of mitomycin C (MMC) in advanced gallbladder and biliary tree carcinoma. An EORTC Gastrointestinal Tract Cancer Cooperative Group Study. *Ann Oncol* 1993; **4**: 607-609

45 **Okada S**, Ishii H, Nose H, Yoshimori M, Okusaka T, Aoki K, Iwasaki M, Furuse J, Yoshino M. A phase II study of cisplatin in patients with biliary tract carcinoma. *Oncology* 1994; **51**: 515-517

46 **Alberts SR**, Fishkin PA, Burgart LJ, Cera PJ, Mahoney MR, Morton RF, Johnson PA, Nair S, Goldberg RM. CPT-11 for bile-duct and gallbladder carcinoma: a phase II North Central Cancer Treatment Group (NCCTG) study. *Int J Gastrointest Cancer* 2002; **32**: 107-114

47 **Harvey JH**, Smith FP, Schein PS. 5-Fluorouracil, mitomycin, and doxorubicin (FAM) in carcinoma of the biliary tract. *J Clin Oncol* 1984; **2**: 1245-1248

48 **Kajanti M**, Pyrhönen S. Epirubicin-sequential methotrexate-5-fluorouracil-leucovorin treatment in advanced cancer of the extrahepatic biliary system. A phase Ⅱ study. *Am J Clin Oncol* 1994; **17**: 223-226

49 **Chen JS**, Lin YC, Jan YY, Liau CT. Mitomycin C with weekly 24-h infusion of high-dose 5-fluorouracil and leucovorin in patients with biliary tract and periampullar carcinomas. *Anticancer Drugs* 2001; **12**: 339-343

50 **Ellis PA**, Norman A, Hill A, O'Brien ME, Nicolson M, Hickish T, Cunningham D. Epirubicin, cisplatin and infusional 5-fluorouracil (5-FU) (ECF) in hepatobiliary tumours. *Eur J Cancer* 1995; **31**: 1594-1598

51 **Morizane C**, Okada S, Okusaka T, Ueno H, Saisho T. Phase Ⅱ study of cisplatin, epirubicin, and continuous-infusion 5-fluorouracil for advanced biliary tract cancer. *Oncology* 2003; **64**: 475-476

52 **Ducreux M**, Rougier P, Fandi A, Clavero-Fabri MC, Villing AL, Fassone F, Fandi L, Zarba J, Armand JP. Effective treatment of advanced biliary tract carcinoma using 5-fluorouracil continuous infusion with cisplatin. *Ann Oncol* 1998; **9**: 653-656

53 **Taïeb J**, Mitry E, Boige V, Artru P, Ezenfis J, Lecomte T, Clavero-Fabri MC, Vaillant JN, Rougier P, Ducreux M. Optimization of 5-fluorouracil (5-FU)/cisplatin combination chemotherapy with a new schedule of leucovorin, 5-FU and cisplatin (LV5FU2-P regimen) in patients with biliary tract carcinoma. *Ann Oncol* 2002; **13**: 1192-1196

54 **Kim TW**, Chang HM, Kang HJ, Lee JR, Ryu MH, Ahn JH, Kim JH, Lee JS, Kang YK. Phase Ⅱ study of capecitabine plus cisplatin as first-line chemotherapy in advanced biliary cancer. *Ann Oncol* 2003; **14**: 1115-1120

55 **Patt YZ**, Hassan MM, Lozano RD, Waugh KA, Hoque AM, Frome AI, Lahoti S, Ellis L, Vauthey JN, Curley SA, Schnirer II, Raijman I. Phase Ⅱ trial of cisplatin, interferon alpha-2b, doxorubicin, and 5-fluorouracil for biliary tract cancer. *Clin Cancer Res* 2001; **7**: 3375-3380

56 **Park KH**, Choi IK, Kim SJ, Oh SC, Seo JH, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS. The efficacy of epirubicin, cisplatin, uracil/tegafur, and leucovorin in patients with advanced biliary tract carcinoma. *Cancer* 2005; **103**: 2338-2343

57 **Park SH**, Park YH, Lee JN, Bang SM, Cho EK, Shin DB, Lee JH. Phase Ⅱ study of epirubicin, cisplatin, and capecitabine for advanced biliary tract adenocarcinoma. *Cancer* 2006; **106**: 361-365

58 **Sanz-Altamira PM**, Ferrante K, Jenkins RL, Lewis WD, Huberman MS, Stuart KE. A phase II trial of 5-fluorouracil, leucovorin, and carboplatin in patients with unresectable biliary tree carcinoma. *Cancer* 1998; **82**: 2321-2325

59 **Nehls O**, Klump B, Arkenau HT, Hass HG, Greschniok A, Gregor M, Porschen R. Oxaliplatin, fluorouracil and leucovorin for advanced biliary system adenocarcinomas: a prospective phase Ⅱ trial. *Br J Cancer* 2002; **87**: 702-704

60 **Kuhn R**, Hribaschek A, Eichelmann K, Rudolph S, Fahlke J, Ridwelski K. Outpatient therapy with gemcitabine and docetaxel for gallbladder, biliary, and cholangio-carcinomas. *Invest New Drugs* 2002; **20**: 351-356

61 **Knox JJ**, Hedley D, Oza A, Siu LL, Pond GR, Moore MJ. Gemcitabine concurrent with continuous infusional 5-fluorouracil in advanced biliary cancers: a review of the Princess Margaret Hospital experience. *Ann Oncol* 2004; **15**: 770-774

62 **Hsu C**, Shen YC, Yang CH, Yeh KH, Lu YS, Hsu CH, Liu HT, Li CC, Chen JS, Wu CY, Cheng AL. Weekly gemcitabine plus 24-h infusion of high-dose 5-fluorouracil/leucovorin for locally advanced or metastatic carcinoma of the biliary tract. *Br J Cancer* 2004; **90**: 1715-1719

63 **Alberts SR**, Al-Khatib H, Mahoney MR, Burgart L, Cera PJ, Flynn PJ, Finch TR, Levitt R, Windschitl HE, Knost JA,

Tschetter LK. Gemcitabine, 5-fluorouracil, and leucovorin in advanced biliary tract and gallbladder carcinoma: a North Central Cancer Treatment Group phase II trial. *Cancer* 2005; **103**: 111-118

64 **André T**, Tournigand C, Rosmorduc O, Provent S, Maindrault-Goebel F, Avenin D, Selle F, Paye F, Hannoun L, Houry S, Gayet B, Lotz JP, de Gramont A, Louvet C. Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma: a GERCOR study. *Ann Oncol* 2004; **15**: 1339-1343

65 **Cho JY**, Paik YH, Chang YS, Lee SJ, Lee DK, Song SY, Chung JB, Park MS, Yu JS, Yoon DS. Capecitabine combined with gemcitabine (CapGem) as first-line treatment in patients with advanced/metastatic biliary tract carcinoma. *Cancer* 2005; **104**: 2753-2758

66 **Doval DC**, Sekhon JS, Gupta SK, Fuloria J, Shukla VK, Gupta S, Awasthy BS. A phase II study of gemcitabine and cisplatin in chemotherapy-naive, unresectable gall bladder cancer. *Br J Cancer* 2004; **90**: 1516-1520

67 **Thongprasert S**, Napapan S, Charoentum C, Moonprakan S. Phase Ⅱ study of gemcitabine and cisplatin as first-line chemotherapy in inoperable biliary tract carcinoma. *Ann Oncol* 2005; **16**: 279-281

68 **Kim ST**, Park JO, Lee J, Lee KT, Lee JK, Choi SH, Heo JS, Park YS, Kang WK, Park K. A Phase II study of gemcitabine and cisplatin in advanced biliary tract cancer. *Cancer* 2006; **106**: 1339-1346

69 **Park BK**, Kim YJ, Park JY, Bang S, Park SW, Chung JB, Kim KS, Choi JS, Lee WJ, Song SY. Phase II study of gemcitabine and cisplatin in advanced biliary tract cancer. *J Gastroenterol Hepatol* 2006; **21**: 999-1003

70 **Philip PA**, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, Donehower RC, Fitch T, Picus J, Erlichman C. Phase II study of erlotinib in patients with advanced biliary cancer. *J Clin Oncol* 2006; **24**: 3069-3074

71 **Liver Cancer Study Group of Japan**. General rules for the clinical and pathological study of primary liver cancer. Second English Edition. Tokyo: Kanehara; 2003

72 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. *Gastroenterology* 2004; **127**: 1372-80

73 **Kubo S**, Uenishi T, Yamamoto S, Hai S, Yamamoto T, Ogawa M, Takemura S, Shuto T, Tanaka H, Yamazaki O, Hirohashi K, Kinoshita H. Clinicopathologic characteristics of small intrahepatic cholangiocarcinomas of mass-forming type. *Hepatol Res* 2004; **29**: 223-227

74 **Morise Z**, Sugioka A, Hoshimoto S, Kato T, Tanahashi Y, Ikeda M, Kagawa T. Patient with advanced intrahepatic cholangiocarcinoma with long-term survival successfully treated with a combination of surgery and chemotherapy. *J Hepatobiliary Pancreat Surg* 2008; **15**: 545-548

75 **Morise Z**, Sugioka A, Tanahashi Y, Okabe Y, Ikeda M, Kagawa T, Takeura C. Treatment of patients with unresectable advanced carcinoma of biliary tract - chemotherapy and surgical resection. *Anticancer Res* 2009; **29**: 1783-1786

S- Editor Wang JL **L- Editor** Ma JY **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i2.65

World J Hepatol 2010 February 27; 2(2): 65-73
ISSN 1948-5182 (online)

REVIEW

Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis

Michael C Kew

Michael C Kew, Department of Medicine, Groote Schuur Hospital, Old Main Building, K Floor, Main Road, Observatory 7935, Cape Town, South Africa
Author contributions: Kew CM contributed solely to this paper.
Correspondence to: Michael C Kew, Professor, MD, PhD, Department of Medicine, Groote Schuur Hospital, Old Main Building, K Floor, Main Road, Observatory 7935, Cape Town, South Africa. michael.kew@uct.ac.za
Telephone: +27-21-4042126 Fax: +27-21-4486815
Received: June 4, 2009 Revised: January 14, 2010
Accepted: January 21, 2010
Published online: February 27, 2010

Abstract

Occult hepatitis B virus (HBV) infection was shown to be present in 75% of Black Africans with hepatocellular carcinoma (HCC) in whom the tumor was hitherto not thought to be caused by chronic HBV infection. The association between chronic HBV infection and the development of the tumor is thus even closer than was originally thought. HBV viral load was found to be significantly higher in patients with HCC than in Black African controls. As in other populations, HBV e antigen-positive patients with hepatocellular carcinoma had significantly higher viral loads than patients negative for this antigen. The significance of this finding is discussed. The risk for HCC development with genotype A of HBV, the predominant genotype in African isolates, has not been investigated. Genotype A was shown to be 4.5 times more likely than other genotypes to cause HCC in Black Africans, and tumours occurred at a significantly younger age. Increasing numbers of patients with human immunodeficiency virus (HIV) and HBV co-infection are being reported to develop HCC. A preliminary case/control comparison supports the belief that HIV co-infection enhances the hepatocarcinogenic potential of HBV. A study from The Gambia provides the first evidence that dietary exposure to aflatoxin B_1 may cause cirrhosis and that this may play a contributory role in the pathogenesis of aflatoxin-induced HCC. An animal model has provided experimental support for the clinical evidence that dietary iron overload in the African is directly hepatocarcinogenic, in addition to causing the tumor indirectly through the development of cirrhosis.

Key words: Hepatocellular carcinoma; Black Africans; Occult hepatitis B; Virus infection; Hepatitis B viral loads; Hepatitis B virus genotype A; Aflatoxin; Dietary iron overload

Peer reviewers: Feng-Zhi Li, Associate Professor of Oncology, Department of Pharmacology and Therapeutics, Director, FLI-78 Drug Discovery & Development Program, Roswell Park Cancer Institute (RPCI), Elm & Carlton Streets, Buffalo, New York 14263, United States; Claus J Fimmel, MD, Professor, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition Loyola University Medical Center Staff Physician, GI Section, Edward Hines, Jr. VA Medical Cente, Division of Gastroenterology, Hepatology, and Nutrition, 2160 S. First Avenue, Building 54, Room 007, Maywood, IL 60153, United States

Kew MC. Hepatocellular carcinoma in African Blacks: Recent progress in etiology and pathogenesis. *World J Hepatol* 2010; 2(2): 65-73 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i2/65.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.65

INTRODUCTION

Sub-Saharan Africa is one of three geographical regions where hepatocellular carcinoma (HCC) occurs very commonly. The high incidence of the tumor is confined to the Black population of the sub-continent. Published incidences of HCC in sub-Saharan Africa underestimate its true incidence because in many instances the tumor is

either not definitively diagnosed or is not recorded in a cancer registry.

A number of differences exist between HCC that occurs in sub-Saharan Africa and that seen in other parts of the world. The tumor generally presents at a younger age in African Blacks than it does in the populations of industrialized countries, and the male preponderance is more striking. Rural and rural-born Blacks have a higher incidence of the tumor than do urban-born Blacks. Although the prognosis of HCC is poor in all geographical regions, it is especially grave in African Blacks, in whom the annual fatality ratio of the tumor is 0.97. The fibrolamellar variant of HCC is rare in African Blacks.

Chronic hepatitis B virus (HBV) infection is the predominant cause of HCC in sub-Saharan Blacks, accounting for the great majority of the cases. The infection is almost always acquired in early childhood, usually by horizontal transmission of the virus. Recently infected and hence highly infectious young siblings or playmates are most often the source of the infection. Perinatal transmission of the virus plays a lesser but still important role. Rural and rural-born children and adults have a higher incidence of chronic HBV infection than do their urban counterparts. Chronic hepatitis C virus (HCV) infection is a less common cause of HCC in sub-Saharan Africa. Patients with HCV-induced tumors are generally about two decades older than those caused by HBV and the gender and rural-urban differences are less obvious. HCV and HBV act synergistically in causing HCC in African Blacks. Another important risk factor for the tumor in sub-Saharan Africa is prolonged heavy dietary exposure to the fungal toxin, aflatoxin B_1 (AFB_1), and there is a strong synergistic interaction between this toxin and HBV in causing the tumor. Heavy exposure to AFB_1 is virtually confined to rural areas. More recently, another important cause of HCC in African Blacks has been recognized. Originally referred to as Bantu visceral siderosis, the term dietary iron overload in the African is now preferred. Consumption of large volumes of a home-brewed traditional beer that has a high iron content is the cause of the condition, although a genetic predisposition may play a role. Over time, the resulting hepatic iron overload may be complicated by HCC development.

Some aspects of the recent progress in understanding the etiology and pathogenesis of HCC in African Blacks are summarised in this review.

OCCULT HBV INFECTION AND HCC

During recent years occult HBV infection has emerged as an important and challenging entity. It is defined as the presence of HBV DNA in low concentration in liver tissue of individuals whose serum persistently tests negative for HBV surface antigen with conventional serological assays, but in whom HBV DNA is usually but not invariably detectable in their serum using highly sensitive molecular assays[1]. When detectable in the serum, the amount of HBV DNA is usually very low (< 200 IU/mL). On the basis of the profile of HBV antibodies in serum, occult HBV infection may be divisible into seropositive (anti-HBc and/or anti-HBs positive) and seronegative (anti-HBc and anti-HBs negative)[1].

Because HBV infection is endemic in sub-Saharan Africa and chronic overt infection with this virus is the major cause of HCC in African Blacks, it is necessary to know the prevalence of occult HBV infection in this population and to ascertain if it plays a role in the aetiology of the tumor. Although early studies had hinted that low concentrations of HBV DNA in the serum of African Blacks with HCC may be undetected by conventional serological tests[2-4], occult HBV infection was first documented in this population in 2001[5]. The few studies published subsequently have indicated that occult HBV infection occurs in an additional 0.6% to 1.7% of sub-Saharan Blacks[6-8] over and above the average prevalence of overt HBV infection of 8% to 10%[9,10].

Because human immunodeficiency virus (HIV) is also endemic in sub-Saharan Africa and co-infection between this virus and HBV occurs in as many as 17% of the Black population[11,12], the prevalence of occult HBV infection in HBV/HIV co-infected individuals has recently been investigated. In the first study, a retrospective analysis of sera stored in a routine diagnostic laboratory, 5 of 15 HIV-positive individuals had occult HBV infection compared with none of 31 HIV-negative individuals[13]. In the second study, 4.8% of a large cohort of HIV-positive individuals had overt HBV infection and a further 7.4% had occult infection[14].

Sixty to 70% of African Blacks with HCC are overtly infected with HBV at the time the tumor is diagnosed[9,10]. The relative risk of HBV carriers developing HCC ranges from 9 to 23.3[15-17], with young Blacks carriers being at greater risk of tumor formation than their older counterparts[18]. The occurrence of occult HBV infection in HCC in southern African Blacks has recently been investigated[19]. A sensitive polymerase chain reaction (PCR) assay was used to amplify the DNA of the surface and precore/core genes from the serum of patients with HCC that was known to be negative for HBsAg but positive for HBV antibodies. Positive bands were confirmed by nucleotide sequencing. Surface gene HBV DNA was detected in a single PCR assay in 48.4% of the patients. Because of the known variability of the results of PCR assay at low concentrations of HBV DNA[20,21], a second assay was done, which increased the positivity rate to 57.7%. Two further assays increased the rate to 75.7%. A less sensitive PCR assay, of the precore/core region, yielded corresponding positive results in 23.7%, 32.2%, and 52% of the patients. The study concluded that seropositive occult HBV infection was present in the majority of African Black patients with HCC without overt HBV infection, and that the causal association between chronic HBV infection and HCC might therefore be even closer than was previously believed.

This study did not address the question of whether or not occult HBV infection *per se* causes malignant transformation of hepatocytes. In early studies of African Blacks with HCC the presence of HBV DNA as well as HBV RNA, indicating viral transcription, was demonstrated in the tumor tissue of some patients whose serum was negative for HBsAg[3,4]. With hindsight, this finding suggests that occult HBV infection might play a role in the pathogenesis of HCC in African Blacks. However, further investigation of the molecular genesis of the tumor and prospective molecular epidemiological studies in patients with occult HBV infection will be required to prove or disprove such a role[1].

HBV LOADS IN HCC

A number of host-specific variables and viral characteristics of chronic HBV infection are known to influence the risk of HBV-induced HCC. These include HBV e antigen (HBeAg) status, the presence of chronic necroinflammatory hepatic disease (particularly cirrhosis), gender and age, as well as viral load and genotypes and possibly subgenotypes of the virus. The availability during recent years of laboratory techniques that accurately quantitate the number of HBV particles in the serum of infected individuals has provided the opportunity to evaluate more fully the role of viral load in the pathogenesis of HCC. Long-term follow-up studies of HBV carriers in Taiwan, China, and Japan have shown that past high viral loads correlate with an increased risk of progression to malignant transformation[22-30]. This risk is influenced by a number of variables, including HBeAg status[30], genotype[31], male gender[30], and possibly age[32].

HBV-induced HCC in African Blacks differs from that in other populations in certain ways and these might either influence the viral load or be the result of differences in the viral load. To date, a single study of past HBV viral loads and involving only 14 Senegalese subjects has been reported in African Black patients with HCC[25]. The difficulty in performing meaningful long-term follow-up studies in southern African Blacks with chronic HBV infection precludes the possibility of directly determining the role played by past viral load in predicting the risk of malignant transformation in these individuals. In a recent attempt to partly circumvent this obstacle, viral loads measured by real-time PCR assay in a cohort of southern African Black male patients with HBV-induced HCC were compared with those in a cohort of asymptomatic Black male carriers of HBV[33]. The mean value of the viral loads in the HCC patients was appreciably higher than that in the asymptomatic controls. Of the HCC patients, 62% had viral loads greater than 1 × 105 copies per mL and 87% loads greater than 1 × 104 copies per mL, compared with 15% and 49.6%, respectively, of the carriers. Therefore, almost all of the HCC patients and one-half of the carriers had high viral loads.

As in other populations studied previously, HBeAg-positive HCC patients had significantly higher viral loads than HBeAg-negative patients. Of the HBeAg-positive patients, 84% had copy numbers per mL of greater than 1 × 105 and 96% greater than 1 × 104, compared with 57% and 85%, respectively, in the HBeAg-negative patients. Thus, although the values were lower in the HBeAg-negative patients, the majority of these patients had high viral loads. This finding may be relevant to the observation that African Black carriers of HBV seroconvert from HBeAg-positivity to negativity at a far earlier age than occurs in other populations (only 5% are positive in adulthood compared with 40% or more in Chinese carriers[34]) and yet are at high risk of developing HCC. Support for an association between HBeAg-negativity and the development of HCC in African Blacks, and perhaps other populations, is provided by the observations that a proportion of anti-HBe-positive European carriers retain high serum levels of HBV DNA[35,36], and that in a small series of Taiwanese patients HBeAg-negativity was shown to correlate with high viral loads and an increased risk of HCC, albeit a lesser risk than that associated with HBeAg-positivity[27]. The few Senegalese patients in whom viral loads were measured in the earlier study were equally likely to be HBeAg-positive and anti-HBe-positive[25] although, in a multivariate analysis, HBeAg-positivity alone carried an increased risk of HCC development. This finding is similar to the experience in Taiwanese patients in whom high viral loads in HBeAg-positive but not in anti-HBe-positive patients carried an increased risk of HCC development[31].

No differences in viral loads were found in relation to age, gender, or genotype in the African Black HCC patients. African Black and Chinese patients with HCC are often younger than their counterparts in industrialized countries. Little is known about the role of viral load in HBV-induced HCC occurring at a young age[37], although one recent study from Taiwan reported that HCC patients younger than 40 years of age had lower HBV DNA levels than older patients[33]. Despite the fact that male gender is one of the factors associated with an increased risk of HCC, studies on viral loads have either been comprised of men only or have not commented on any differences between the genders with respect to viral loads. Correlation between genotypes and viral loads has been performed in a single study only. Genotype C, particularly Ce, was shown in Taiwanese and Chinese patients to carry a greater risk of HCC development and to be associated with higher viral loads than genotype B[29,31,38].

Long-term follow-up studies are needed in Black African populations to further assess the role of past HBV viral loads as a risk factor for the subsequent development of HCC. A long-term study of chronic carriers of HBV is well advanced in The Gambia and should provide the required information in due course.

INCREASED HEPATOCARCINOGENIC POTENTIAL OF HBV GENOTYPE A

HBV is composed of 8 genotypes (A to H), each differing

from the others by a total nucleotide diversity of at least 8%[39-42]. Subgenotypes, each differing from the others by a total nucleotide diversity of at least 4%[42,43], have been described for 6 of the genotypes. The genotypes have different geographical distributions[39-42] and are proving to be an invaluable tool in tracing the molecular evolution and spread of HBV. Functional differences between the genotypes at the translational level may influence the course and severity of the disease caused and its response to treatment[42,44-46]. In regions where HBV is endemic and HBV-induced HCC common, recent evidence for differing risks of tumor development between the genotypes has emerged[47-49]. The early studies in this regard concerned patients infected with genotypes B, C, or D.

Genotype A predominates in southern Africa, constituting approximately 70% of isolates, with genotype D accounting for most of the remainder (approximately 20%)[42,50-52]. Genotype A comprises 3 subgenotypes, A1, A2, and A3[42,50-52], with A1 predominating in southern Africa. No studies of the hepatocarcinogenic potential of genotype A have been published until recently, and in the only study of genotype D Thakur and co-workers concluded that this genotype may predict the occurrence of HCC in young HBV carriers[49].

In a recent study of the hepatocarcinogenic potential of genotype A[52], the genotype and subgenotype patterns in unselected southern African Blacks with HBV-induced HCC were compared with those in apparently well chronic carriers of the virus matched for sex and approximately for age. The genotyping method of Lindh *et al*[53] was used to discriminate between the genotypes and an 'in-house' restriction fragment length polymorphism pattern assay between the subgenotypes[52]. Genotype A was shown to be present in 86.5% and genotype D in 8.1% of the HCC patients compared with genotype A in 68.5%, genotype D in 23.4% and genotype E in 2.7% of the carriers. Based on these findings, the relative risk for developing HCC in the patients with genotype A (compared with those with genotypes other than A) was calculated to be 4.5 (95% confidence limits 1.86; 10.9). Not only were African Blacks infected with genotype A at greater risk of developing HCC than those infected with other genotypes (mainly genotype D), but they also did so at a significantly younger age (mean age 39.0 ± 1.4 compared with 45.4 ± 4.2 yr). Subgenotype A1 was present in all of the HCC patients infected with genotype A and also in all but one of the controls with this genotype.

Although evidence for the hepatocarcinogenic potential of genotype A in African Blacks is now available, further studies on the hepatocarcinogenic potential of the A subgenotypes are needed.

INCREASING OCCURRENCE OF HCC IN HIV/ACQUIRED IMMUNODEFICIENCY SYNDROME (ADIS) PATIENTS CO-INFECTED WITH A HEPATITIS VIRUS

Chronic infection with HIV alone does not cause HCC in humans[54,55]. HIV is transmitted in similar ways to HBV and HCV, and co-infection between HIV and HBV or HCV is common in clinical practice[56,57]. Since the introduction of highly active antiretroviral therapy (HAART) for HIV/AIDS, increasing numbers of patients co-infected with HIV and HCV or HBV have been reported to develop HCC[54,58-64]. One plausible explanation for this observation is that the considerably longer survival of patients now treated with HAART compared with those who earlier had received largely ineffective anti-retroviral drugs allows sufficient time for hepatitis virus-induced HCC to develop[64]. Another possible explanation is that the immune deficiency caused by HIV infection results in higher HBV viral loads, which are known to increase the risk of malignant transformation of hepatocytes (*see section on HBV viral loads and HCC*). Alternatively, co-infection with HIV may directly enhance the hepatocarcinogenic potential of the hepatitis viruses. In this regard, the observation in transgenic mice that HIV *tat* protein expressed constitutively in the liver enhances the effect of a number of carcinogens[65] and results, after a long latency, in a high incidence of HCC[66] may be relevant. Moreover, the *tat*-binding protein interacts with HBV × gene, which is thought to play an important role in the pathogenesis of HBV-induced HCC, in such a way as to regulate HBV transcription[67], and the HBV × protein induces HIV-1 replication and transcription in synergy with *tat* protein and T-cell activation signals[68].

A retrospective analysis of the occurrence of HCC in HIV/AIDS patients with hepatitis virus co-infection before the HAART era would be of limited use because of the brief survival of these patients at that time, and a prospective study of the development of the tumor in HIV/AIDS patients with dual infection receiving or not receiving HAART is ethically unacceptable. A case/control comparison of the occurrence of HIV in patients with hepatitis virus-induced HCC with that in matched asymptomatic carriers of hepatitis virus might, however, provide some information in this regard.

In a recent such study the prevalence of HIV co-infection in a cohort of southern African Black patients with HBV-induced HCC from the time before HAART became available to these patients was compared with that in a cohort of age- and sex-matched apparently healthy Black carriers of HBV from the same time period[69]. HIV-1/HIV-2 antibodies were found to be present significantly more often in the HCC patients than in the controls. The prevalence of HIV in the carriers was in keeping with prevalences recorded in the South African Black population at that time[70]. This observation is compatible with the belief that HIV co-infection enhances the hepatocarcinogenic potential of HBV. However, relatively few HCC patients and controls were included in this study, and further and more comprehensive investigation into a possible hepatocarcinogenic interaction between these two viruses is needed.

Because the molecular genesis of HBV-induced and

HCV-induced HCC differs[71] an interaction between HBV and HIV in the development of HCC does not mean necessarily mean that a similar interaction would occur with HCV-induced HCC.

AFB1 EXPOSURE AND HCC

Cirrhosis, whatever its cause, is a premalignant condition. In most parts of the world 80% to 90% of HCCs occur in patients with underlying cirrhosis. The proportion of African Black patients in whom the HCC arises in a cirrhotic liver is generally less than that in other populations but is still high. Of the major risk factors for HCC in sub-Saharan Africa, HBV, HCV, and dietary iron overload are all capable of causing cirrhosis. Short-term heavy dietary exposure to AFB_1, the other major causal association of the tumor in sub-Saharan Africa, causes severe acute hepatic necrosis (acute aflatoxicosis), which is often complicated by acute hepatic failure and a fatal outcome. However, long-term exposure to the fungal toxin has not hitherto been thought to cause chronic necroinflammatory hepatic disease in the form of chronic hepatitis or cirrhosis. Cirrhosis has therefore not been incriminated in contributing to the pathogenesis of AFB_1-induced HCC.

A recent study from The Gambia provides the first evidence that this may not be true. Kuniholm[72] and co-workers used an ultrasound-based method to diagnose the presence of cirrhosis in 97 Black Africans. A score of at least 7 out of a possible 11 points on the ultrasound-based scale was the criterion for the diagnosis of cirrhosis. This method has a 77.8% sensitivity and 92.5% specificity in comparison with liver biopsy in identifying cirrhosis in HBV-infected patients[73,74]. Three hundred and ninety seven individuals with no evidence of liver disease and a normal serum AFB_1 concentration served as controls. Long-term exposure to AFB_1 was assessed in the patients with cirrhosis and the controls on the basis of two observations: A history of lifetime groundnut (peanut) intake or the finding in the serum of a genetic marker of heavy exposure to AFB_1, the 249^{ser} p53 mutation. An increased relative risk of cirrhosis development of 2.8 (95% confidence interval 1.1-7.7) was calculated using a history of life-time dietary intake of groundnuts as the criterion for significant exposure, and of 3.8 (95% confidence interval 1.5-9.6) using the finding of the 249^{ser} p53 mutation in serum as the criterion, allowing for the possible confounding effect of HBV and HCV infection in each instance. A synergistic interaction between AFB_1 intake and HBV infection in the development of cirrhosis was also shown. If confirmed, this finding will provide the first evidence that cirrhosis may play a contributory role in the pathogenesis of AFB_1-induced HCC.

Because contamination of staple crops by *Aspergillus* species occurs both during growth of the crops and as a result of their improper storage, attempts at primary prevention of AFB_1-induced HCC must be directed to minimizing both sources of fungal contamination.

The likelihood of contamination during storage is increased by excessive moisture and any form of damage to the crops.

The success of postharvest intervention measures has recently been demonstrated in a study carried out on subsistence farms in the lower Kindia region of Guinea, West Africa[75]. Farms from 20 villages were included, 10 of which implemented a package of postharvest measures to restrict AFB_1 contamination of the groundnut crop, and 10 followed the usual postharvest practices. Intervention measures included hand sorting of the groundnuts to identify and discard those that were visibly mouldy or had damaged shells; sun drying of the groundnuts on natural fibre mats rather than directly on the earth to lessen contact with damp soil and to facilitate gathering in the event of unexpected rain; complete sun-drying of groundnuts confirmed by shaking the kernels to listen for free movement of dried nuts; storage in natural fibre bags rather than plastic bags (which increase humidity); storing the groundnuts on wooden pallets rather than the floor and in well-ventilated, rain-proof storage facilities to reduce humidity; sprinkling of insecticides on the floor of the storage facilities to minimise insects, which produce humidity via metabolic activity, and spread the fungal spores.

The concentration of AFB_1-albumin adducts in the serum of 600 people was measured immediately after harvest and at 3 and 5 mo postharvest. In the control villages the adduct concentration increased from a mean of 5.5 pg/mg postharvest to 18.7 pg/mg 5 mo later. By contrast, in the intervention villages after 5 mo of storage the adduct concentration (7.2 pg/mg) was much the same as that immediately postharvest (8.0 pg/mg). At 5 mo 2% of the people in the control villages had non-detectable adduct concentrations compared with 20% of those in the intervention villages.

Thus, simple, low-technology, and inexpensive practices can result in a striking decrease in exposure to AFB_1. Provision of the means to improve storage facilities and training of subsistence farmers in their use will be required for these interventions to be successful on a wide scale in resource-poor countries.

DIETARY IRON OVERLOAD IN THE AFRICAN AS A CAUSE OF HCC

Dietary iron overload occurs in several sub-Saharan African countries, where it may affect as many as 15% of Black adult males[76]. The liver is the main organ of iron storage and high hepatic iron concentrations, comparable with those in the genetically determined iron storage disease, Hemochromatosis gene (HFE) hereditary hemochromatosis, result from the consumption over time of large volumes of home-brewed beer rich in iron. The high iron content results from the preparation of the beer in iron pots or drums, the iron leeching from

the container into the contents as a result of the very low pH generated during the process of fermentation. This iron is in an ionized, highly bioavailable form. The condition is more common in rural areas, where approximately 80% of the African Black population lives and where about two-thirds of adult males consume the traditional beverage. A genetic predisposition allowing an increased absorption of dietary iron in the presence of increased body iron stores is very likely to play a role in the pathogenesis of the condition, although a putative gene has yet to be identified.

Dietary iron overload is complicated by hepatic fibrosis and cirrhosis (in 58% and 10% of patients, respectively). In the early literature the excessive hepatic iron storage was not considered to be a cause of HCC, and no animal model of iron overload-induced HCC was produced. However, between 1996 and 1998 three large studies pathological or clinical concluded that African dietary iron overload was associated with an increased risk of malignant transformation of hepatocytes[77-79].

This observation has recently been supported by the induction of HCC in Wistar albino rats fed an iron-supplemented diet[80]. In the animal model, the iron accumulated in hepatocytes and macrophages, a pattern similar to that seen in African dietary iron overload, and by 16 mo the animals were heavily iron overloaded. At 20 mo iron-free altered hepatic foci were present in many of the animals, and by 28 mo these foci had become more plentiful and had changed in character, becoming indistinguishable from the iron-free preneoplastic nodules described by Deugnier and colleagues in patients with HFE hereditary hemochromatosis who went on to develop HCC[81]. The nodules had clear-cut boundaries, showed an expansive pattern with thickened trabeculae in a deranged pattern causing compression of the surrounding parenchyma, small and large cell dysplasia, and iron-positive intracytoplasmic globules. Further evidence of a proliferative preneoplastic lesion was provided by positive staining for placental glutathione sulphydryl transferase[82]. Moreover, the nodules showed additional features that would be considered dysplastic in the human liver, namely, an increased nuclear/cytoplasmic ratio and Mallory-like inclusions[83]. HCC was seen at 32 mo. Neither fibrosis nor cirrhosis was present in the rat livers, indicating that excessive hepatic iron can be directly hepatocarcinogenic.

The mechanisms by which excess iron induces malignant transformation of hepatocytes have yet to be fully characterized. The most important mechanism appears to be the generation of reactive oxygen species and oxidative stress, which leads to lipid peroxidation of unsaturated fatty acids in membranes of cells and organelles[84]. Cytotoxic by-products of lipid peroxidation, such as malondialdehyde and 4-hydroxy-2'-nonenol, are produced and these impair cellular function and protein synthesis, and damage DNA. Deoxyguanosine residues in DNA are also hydroxylated by reactive oxygen intermediates to form 8-hydroxy-2'-deoxyguanosine, a major promutagenic adduct that causes G:C to T:A transversions and DNA unwinding and strand breaks.

Subsequent studies in the same animal model have shown a synergistic interaction between iron overload and exposure to AFB_1[85] and iron overload and ingestion of alcohol[86] in hepatic mutagenesis.

REFERENCES

1 **Raimondo G**, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Villa E, Will H, Zanetti AR, Zoulim F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008; **49**: 652-657

2 **Kew MC**, Fujita Y, Takahashi H, Coppins A, Wands JR. Comparison between polyclonal and first and second generation monoclonal radioimmunoassays in the detection of hepatitis B surface antigen in patients with hepatocellular carcinoma. *Hepatology* 1986; **6**: 636-639

3 **Paterlini P**, Gerken G, Nakajima E, Terre S, D'Errico A, Grigioni W, Nalpas B, Franco D, Wands J, Kew M. Polymerase chain reaction to detect hepatitis B virus DNA and RNA sequences in primary liver cancers from patients negative for hepatitis B surface antigen. *N Engl J Med* 1990; **323**: 80-85

4 **Paterlini P**, Gerken G, Khemeny F, Franco D, D'Errico A, Grigioni W, Wands J, Kew MC, Pisi E, Tiollais P, Brechot C. Primary liver cancer in HBsAg-negative patients: a study of HBV genome using the polymerase chain reaction. In: Viral hepatitis and Liver Disease. Hollinger FB, Lemon S, Margolis HS, editoers. Baltimore, MD: Williams and Wilkins, 1991: 605-610

5 **Owiredu WK**, Kramvis A, Kew MC. Hepatitis B virus DNA in serum of healthy black African adults positive for hepatitis B surface antibody alone: possible association with recombination between genotypes A and D. *J Med Virol* 2001; **64**: 441-454

6 **Mphahlele MJ**, Moloto MJ. Detection of HBV DNA from serologically negative or 'silent' HBV infections--viral or host factors? *S Afr Med J* 2002; **92**: 613-615

7 **Zahn A**, Li C, Danso K, Candotti D, Owusu-Ofori S, Temple J, Allain JP. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. *J Gen Virol* 2008; **89**: 409-418

8 **Gibney KB**, Torresi J, Lemoh C, Biggs BA. Isolated core antibody hepatitis B in sub-Saharan African immigrants. *J Med Virol* 2008; **80**: 1565-1569

9 **Kew MC**. Chronic hepatitis-B virus infection and hepatocellular carcinoma in Africa. *S Afr J Sci* 1992; **88**: 524-528

10 **Kew MC**. Hepatocellular carcinoma in Africa. In: Malignant Liver Tumors: Current and Emerging Therapies. 2nd ed. Clavien PA, editor. Boston: Jones and Bartlett, 2003: 439-448

11 **Burnett RJ**, François G, Kew MC, Leroux-Roels G, Meheus A, Hoosen AA, Mphahlele MJ. Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation. *Liver Int* 2005; **25**: 201-213

12 **Firnhaber C**, Reyneke A, Schulze D, Malope B, Maskew M, MacPhail P, Sanne I, Di Bisceglie A. The prevalence of hepatitis B co-infection in a South African urban government HIV clinic. *S Afr Med J* 2008; **98**: 541-544

13 **Mphahlele MJ**, Lukhwareni A, Burnett RJ, Moropeng LM, Ngobeni JM. High risk of occult hepatitis B virus infection in HIV-positive patients from South Africa. *J Clin Virol* 2006; **35**: 14-20

14 **Firnhaber C**, Viana R, Reyneke A, Schultze D, Malope B,

Maskew M, Di Bisceglie A, MacPhail P, Sanne I, Kew M. Occult hepatitis B virus infection in patients with isolated core antibody and HIV co-infection in an urban clinic in Johannesburg, South Africa. *Int J Infect Dis* 2009; **13**: 488-492
15 **Kew MC**. The possible etiologic role of the hepatitis B virus in hepatocellular carcinoma: evidence from southern Africa. In: Advances in Hepatitis Research. Chisari FV, editor. New York: Masson Publishing USA Inc, 1984: 203-215
16 **Coursaget P**, Chiron JP, Barres JL, Barin F. Hepatitis B virus serological markers in Africans with liver cirrhosis and hepatocellular carcinoma. In: Virus-associated Cancers in Africa. Williams AO, O'Conor GT, De-The GB, Johnson CA, editors. Lyon: International Agency for Research on Cancer (IARC Scientific Publications, no.63), 1984: 181-198
17 **Vogel GL**, Anthony PP, Mody N, Barker LF. Hepatitis associated antigen in Ugandan patients with HCC. *Lancet* 1970; **2**: 621-624
18 **Kew MC**, Macerollo P. Effect of age on the etiologic role of the hepatitis B virus in hepatocellular carcinoma in Blacks. *Gastroenterology* 1988; **94**: 39-42
19 **Kew MC**, Welschinger R, Viana R. Occult hepatitis B virus infection in Southern African blacks with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2008; **23**: 1426-1430
20 **Bréchot C**, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203
21 **Torbenson M**, Thomas DL. Occult hepatitis B. *Lancet Infect Dis* 2002; **2**: 479-486
22 **Chen G**, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. *Am J Gastroenterol* 2006; **101**: 1797-1803
23 **Fung J**, Yuen MF, Yuen JC, Wong DK, Lai CL. Low serum HBV DNA levels and development of hepatocellular carcinoma in patients with chronic hepatitis B: a case-control study. *Aliment Pharmacol Ther* 2007; **26**: 377-382
24 **Liu TT**, Fang Y, Xiong H, Chen TY, Ni ZP, Luo JF, Zhao NQ, Shen XZ. A case-control study of the relationship between hepatitis B virus DNA level and risk of hepatocellular carcinoma in Qidong, China. *World J Gastroenterol* 2008; **14**: 3059-3063
25 **Tang B**, Kruger WD, Chen G, Shen F, Lin WY, Mboup S, London WT, Evans AA. Hepatitis B viremia is associated with increased risk of hepatocellular carcinoma in chronic carriers. *J Med Virol* 2004; **72**: 35-40
26 **Yuen MF**, Tanaka Y, Shinkai N, Poon RT, But DY, Fong DY, Fung J, Wong DK, Yuen JC, Mizokami M, Lai CL. Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer Ⅱ/core promoter/precore regions and HBV DNA levels. *Gut* 2008; **57**: 98-102
27 **Yang HI**, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, Hsiao CK, Chen PJ, Chen DS, Chen CJ. Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168-174
28 **Wu CF**, Yu MW, Lin CKL, Jiu CJ, Shih WL, Tsai KS, Chen CJ. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. *Carcinogenesis* 2008; **29**: 10-112
29 **Chan HL**, Tse CH, Mo F, Koh J, Wong VW, Wong GL, Lam Chan S, Yeo W, Sung JJ, Mok TS. High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol* 2008; **26**: 177-182
30 **Chu CJ**, Hussain M, Lok AS. Quantitative serum HBV DNA levels during different stages of chronic hepatitis B infection. *Hepatology* 2002; **36**: 1408-1415
31 **Yu MW**, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, Shih WL, Kao JI, Chen DS, Chen CJ. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: A prospective study in men. *J Natl Cancer Inst* 2005; **97**: 165-272
32 **Tsai FC**, Liu CJ, Chen CL, Chen PJ, Lai MY, Kao JH, Chen DS. Lower serum viral loads in young patients with hepatitis-B-virus-related hepatocellular carcinoma. *J Viral Hepat* 2007; **14**: 153-160
33 **Viana R**, Wang R, Yu MC, Welschinger R, Chen CY, Kew MC. Hepatitis B viral loads in southern African Blacks with hepatocellular carcinoma. *J Med Virol* 2009; **81**: 1525-1530
34 **Song E**, Dusheiko GM, Bowyer S, Kew MC. Hepatitis B virus replication in southern Africa blacks with HBsAg-positive hepatocellular carcinoma. *Hepatology* 1984; **4**: 608-610
35 **Hadziyannis SJ**, Lieberman HM, Karvountzis GG, Shafritz DA. Analysis of liver disease, nuclear HBcAg, viral replication, and hepatitis B virus DNA in liver and serum of HBeAg Vs. anti-HBe positive carriers of hepatitis B virus. *Hepatology* 1983; **3**: 656-662
36 **Bonino F**, Rosina F, Rizzetto M, Rizzi R, Chiaberge E, Tardanico R, Callea F, Verme G. Chronic hepatitis in HBsAg carriers with serum HBV-DNA and anti-HBe. *Gastroenterology* 1986; **90**: 1268-1273
37 **Sezaki H**, Kobayashi M, Hosaka T, Tsubota T, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Takagi K, Sato J, Kumada H. Hepatocellular carcinoma in non-cirrhotic young adult patients with chronic hepatitis B viral infection. *J Gastroenterol* 2004; **39**: 550-556
38 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143
39 **Norder H**, Couroucé AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. *Virology* 1994; **198**: 489-503
40 **Stuyver L**, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; **81**: 67-74
41 **Arauz-Ruiz P**, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; **83**: 2059-2073
42 **Kramvis A**, Kew M, François G. Hepatitis B virus genotypes. *Vaccine* 2005; **23**: 2409-2423
43 **Bowyer SM**, van Staden L, Kew MC, Sim JG. A unique segment of the hepatitis B virus group A genotype identified in isolates from South Africa. *J Gen Virol* 1997; **78**: 1719-1729
44 **Kramvis A**, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. *J Viral Hepat* 2005; **12**: 456-464
45 **Kao JH**, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000; **118**: 554-559
46 **Chan HL**, Wong ML, Hui AY, Hung LC, Chan FK, Sung JJ. Hepatitis B virus genotype C takes a more aggressive disease course than hepatitis B virus genotype B in hepatitis B e antigen-positive patients. *J Clin Microbiol* 2003; **41**: 1277-1279
47 **Sugauchi F**, Ohno T, Orito E, Sakugawa H, Ichida T, Komatsu M, Kuramitsu T, Ueda R, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the development of preS deletions and advanced liver disease. *J Med Virol* 2003; **70**: 537-544
48 **Tsubota A**, Arase Y, Ren F, Tanaka H, Ikeda K, Kumada H. Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. *J Med Virol* 2001; **65**: 257-265
49 **Thakur V**, Guptan RC, Kazim SN, Malhotra V, Sarin SK.

Profile, spectrum and significance of HBV genotypes in chronic liver disease patients in the Indian subcontinent. *J Gastroenterol Hepatol* 2002; **17**: 165-170
50 **Kramvis A**, Weitzmann L, Owiredu WK, Kew MC. Analysis of the complete genome of subgroup A' hepatitis B virus isolates from South Africa. *J Gen Virol* 2002; **83**: 835-839
51 **Kimbi GC**, Kramvis A, Kew MC. Distinctive sequence characteristics of subgenotype A1 isolates of hepatitis B virus from South Africa. *J Gen Virol* 2004; **85**: 1211-1220
52 **Kew MC**, Kramvis A, Yu MC, Arakawa K, Hodkinson J. Increased hepatocarcinogenic potential of hepatitis B virus genotype A in Bantu-speaking sub-saharan Africans. *J Med Virol* 2005; **75**: 513-521
53 **Lindh M**, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus--large-scale analysis using a new genotyping method. *J Infect Dis* 1997; **175**: 1285-1293
54 **Sitas F**, Bezwoda WR, Levin V, Ruff P, Kew MC, Hale MW, Carrara H, Beral V, Fleming G, Odes R, Weaving A. Association between human immunodeficiency virus type 1 infection and cancer in the Black population of Johannesburg and Soweto. *Br J Cancer* 1997; **78**: 1719-1729
55 **Giordano TP**, Kramer JR, Souchek J, Richardson P, El-Serag HB. Cirrhosis and hepatocellular carcinoma in HIV-infected veterans with and without the hepatitis C virus: a cohort study, 1992-2001. *Arch Intern Med* 2004; **164**: 2349-2354
56 **Alter MJ**. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol* 2006; **44**: S6-S9
57 **MacDonald DC**, Nelson M, Bower M, Powles T. Hepatocellular carcinoma, human immunodeficiency virus and viral hepatitis in the HAART era. *World J Gastroenterol* 2008; **14**: 1657-1663
58 **Bräu N**, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE, Trikha A, Sherman M, Sulkowski MS, Dieterich DT, Rigsby MO, Wright TL, Hernandez MD, Jain MK, Khatri GK, Sterling RK, Bonacini M, Martyn CA, Aytaman A, Llovet JM, Brown ST, Bini EJ. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a U.S.-Canadian multicenter study. *J Hepatol* 2007; **47**: 527-537
59 **Rosenthal E**, Poirée M, Pradier C, Perronne C, Salmon-Ceron D, Geffray L, Myers RP, Morlat P, Pialoux G, Pol S, Cacoub P. Mortality due to hepatitis C-related liver disease in HIV-infected patients in France (Mortavic 2001 study). *AIDS* 2003; **17**: 1803-1809
60 **Kramer JR**, Giordano TP, Souchek J, Richardson P, Hwang LY, El-Serag HB. The effect of HIV coinfection on the risk of cirrhosis and hepatocellular carcinoma in U.S. veterans with hepatitis C. *Am J Gastroenterol* 2005; **100**: 56-63
61 **Engels EA**, Frisch M, Lubin JH, Gail MH, Biggar RJ, Goedert JJ. Prevalence of hepatitis C virus infection and risk for hepatocellular carcinoma and non-Hodgkin lymphoma in AIDS. *J Acquir Immune Defic Syndr* 2002; **31**: 536-541
62 **Puoti M**, Bruno R, Soriano V, Donato F, Gaeta GB, Quinzan GP, Precone D, Gelatti U, Asensi V, Vaccher E. Hepatocellular carcinoma in HIV-infected patients: epidemiological features, clinical presentation and outcome. *AIDS* 2004; **18**: 2285-2293
63 **Clifford GM**, Rickenbach M, Polesel J, Dal Maso L, Steffen I, Ledergerber B, Rauch A, Probst-Hensch NM, Bouchardy C, Levi F, Franceschi S. Influence of HIV-related immunodeficiency on the risk of hepatocellular carcinoma. *AIDS* 2008; **22**: 2135-2141
64 **Thio CL**, Seaberg EC, Skolasky R, Phair J, Visscher B, Munoz A, Thomas DL. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet* 2002; **360**: 1921-1926
65 **Vogel J**, Hinrichs SH, Napolitano LA, Ngo L, Jay G. Liver cancer in transgenic mice carrying the human immunodeficiency virus tat gene. *Cancer Res* 1991; **51**: 6686-6690
66 **Altavilla G**, Caputo A, Lanfredi M, Piola C, Barbanti-Brodano G, Corallini A. Enhancement of chemical hepatocarcinogenesis by the HIV-1 tat gene. *Am J Pathol* 2000; **157**: 1081-1089
67 **Barak O**, Aronheim A, Shaul Y. HBV × protein targets HIV Tat-binding protein 1. *Virology* 2001; **283**: 110-120
68 **Gómez-Gonzalo M**, Carretero M, Rullas J, Lara-Pezzi E, Aramburu J, Berkhout B, Alcamí J, López-Cabrera M. The hepatitis B virus X protein induces HIV-1 replication and transcription in synergy with T-cell activation signals: functional roles of NF-kappaB/NF-AT and SP1-binding sites in the HIV-1 long terminal repeat promoter. *J Biol Chem* 2001; **276**: 35435-35443
69 **Kew MC**, Smuts H, Stewart A. Does human immunodeficiency virus infection enhance the hepatocarcinogenicity of chronic hepatitis B virus infection? *JAIDS* 2009; In press
70 **Dusheiko GM**, Brink BA, Conradie JD, Marimuthu T, Sher R. Regional prevalence of hepatitis B, delta, and human immunodeficiency virus infection in southern Africa: a large population survey. *Am J Epidemiol* 1989; **129**: 138-145
71 **Grisham JW**. Molecular genetic alterations in hepatocellular neoplasms: hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma. In: Coleman WB, Trongalis JG, editors. Molecular Basis of Human Cancer, Totawa, NJ: Harriman Press. 2001: 269-346
72 **Kuniholm MH**, Lesi OA, Mendy M, Akano AO, Sam O, Hall AJ, Whittle H, Bah E, Goedert JJ, Hainaut P, Kirk GD. Aflatoxin exposure and viral hepatitis in the etiology of liver cirrhosis in the Gambia, West Africa. *Environ Health Perspect* 2008; **116**: 1553-1557
73 **Lin DY**, Sheen IS, Chiu CT, Lin SM, Kuo YC, Liaw YF. Ultrasonographic changes of early liver cirrhosis in chronic hepatitis B: a longitudinal study. *J Clin Ultrasound* 1993; **21**: 303-308
74 **Hung CH**, Lu SN, Wang JH, Lee CM, Chen TM, Tung HD, Chen CH, Huang WS, Changchien CS. Correlation between ultrasonographic and pathologic diagnoses of hepatitis B and C virus-related cirrhosis. *J Gastroenterol* 2003; **38**: 153-157
75 **Turner PC**, Sylla A, Gong YY, Diallo MS, Sutcliffe AE, Hall AJ, Wild CP. Reduction in exposure to carcinogenic aflatoxins by post harvest intervention measures in west Africa: a community-based intervention study. *Lancet* 2005; **365**: 1950-1956
76 **Kew MC**, Asare GA. Dietary iron overload in the African and hepatocellular carcinoma. *Liver Int* 2007; **27**: 735-741
77 **Gordeuk VR**, McLaren CE, MacPhail AP, Deichsel G, Bothwell TH. Associations of iron overload in Africa with hepatocellular carcinoma and tuberculosis: Strachan's 1929 thesis revisited. *Blood* 1996; **87**: 3470-3476
78 **Moyo VM**, Makunike R, Gangaidzo IT, Gordeuk VR, McLaren CE, Khumalo H, Saungweme T, Rouault T, Kiire CF. African iron overload and hepatocellular carcinoma (HA-7-0-080). *Eur J Haematol* 1998; **60**: 28-34
79 **Mandishona E**, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, Rouault TA, Kew MC. Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. *Hepatology* 1998; **27**: 1563-1566
80 **Asare GA**, Paterson AC, Kew MC, Khan S, Mossanda KS. Iron-free neoplastic nodules and hepatocellular carcinoma without cirrhosis in Wistar rats fed a diet high in iron. *J Pathol* 2006; **208**: 82-90
81 **Deugnier YM**, Charalambous P, Le Quilleuc D, Turlin B, Searle J, Brissot P, Powell LW, Halliday JW. Preneoplastic significance of hepatic iron-free foci in genetic hemochromatosis: a study of 185 patients. *Hepatology* 1993; **18**: 1363-1369
82 **Nielsen P**, Heinrich HC. Metabolism of iron from (3,3,5-trimethyl hexanoyl ferrocene in rats. *Biochem Biopharmacol* 1993; **45**: 385-391

83 **Libbrecht L**, Desmet V, Roskams T. Preneoplastic lesions in human hepatocarcinogenesis. *Liver Int* 2005; **25**: 16-27
84 **Asare GA**, Mossanda KS, Kew MC, Paterson AC, Kahler-Venter CP, Siziba K. Hepatocellular carcinoma caused by iron overload: a possible mechanism of direct hepatocarcinogenicity. *Toxicology* 2006; **219**: 41-52
85 **Asare GA**, Bronz M, Naidoo V, Kew MC. Interactions between aflatoxin B1 and dietary iron overload in hepatic mutagenesis. *Toxicology* 2007; **234**: 157-166
86 **Asare GA**, Bronz M, Naidoo V, Kew MC. Synergistic interaction between excess hepatic iron and alcohol ingestion in hepatic mutagenesis. *Toxicology* 2008; **254**: 11-18

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.i2.74

World J Hepatol 2010 February 27; 2(2): 74-80
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

ApoB-containing lipoproteins promote infectivity of chlamydial species in human hepatoma cell line

Yuriy K Bashmakov, Nailia A Zigangirova, Alexander L Gintzburg, Petr A Bortsov, Ivan M Petyaev

Yuriy K Bashmakov, Ivan M Petyaev, Cambridge Theranostics Ltd, Babraham Research Campus, Babraham, Cambridge, CB2 4AT, United Kingdom
Nailia A Zigangirova, Alexander L Gintzburg, Petr A Bortsov, Gamaleya Institute for Epidemiology and Microbiology RAMS, 18 Gamaleya Str., Moscow 123098, Russia
Author contributions: Bashmakov YK and Zigangirova NA performed most of the experiments; Bortsov PA did flow cytometry analysis and immunostaining; Gintzburg AL and Petyaev IM were involved in study initiation, writing the manuscript and providing financial support.
Supported by Cambridge Theranostics Ltd
Correspondence to: Dr. Yuriy K Bashmakov, MD, PhD, Cambridge Theranostics Ltd, Babraham Research Campus, Cambridge, CB2 4AT, United Kingdom. yuriy@cammedica.com
Telephone: +44-797-1598348 Fax: +44-122-3240340
Received: August 14, 2009 Revised: January 4, 2010
Accepted: January 11, 2010
Published online: February 27, 2010

Abstract

AIM: To evaluate the direct binding of two main chlamydial biovars (*C. trachomatis* and *C. pneumoniae*) to plasma lipoproteins and its effect on chlamydial infection rate in human hepatoma cell line (HepG2 cells).

METHODS: Murine plasma lipoproteins were fractionated and isolated using fast-performance liquid chromatography (FPLC), spotted on nitrocellulose membrane and incubated with chlamydial suspensions. Direct binding of chlamydial particles to lipoprotein fractions has been studied using lipopolysaccharide-specific antibodies in immuno-dot blot binding assay and immunoprecipitation analysis. Immunostaining protocol as well as flow cytometry analysis have been employed to study the infectivity rate of chlamydial species in HepG2 cells.

RESULTS: Elementary bodies of both *C. trachomatis* and *C. pneumoniae* bind ApoB-containing fractions of plasma lipoproteins. That binding becomes stronger when heat-denatured FPLC fractions are used, suggesting a primary role of apolipoproteins in interaction between chlamydial particle and lipoprotein. Both chlamydial biovars efficiently propagate in human hepatoma cell line - HepG2 cells even in serum free conditions forming late-stage inclusion bodies and releasing extracellular elementary bodies. Preincubation of *C. trachomatis* and *C. pneumoniae* with native ApoB-containing lipoproteins enhances the rate of chlamydial infection in HepG2 cells.

CONCLUSION: A productive infection caused by *C. trachomatis* and *C. pneumoniae* may take place in human-derived hepatocytes revealing hepatic cells as possible target in chlamydial infection. Obtained results may suggest the participation of lipoprotein receptors in the mechanism of attachment and/or entry of chlamydial particles into target cells.

Key words: ApoB-containing lipoproteins; *Chlamydial trachomatis*; *Chlamydial pneumoniae*; Human hepatoma cell line; Liver infection

Peer reviewer: Lucia Malaguarnera, Associate Professor, MD, PhD, Department of Biomedical Sciences, University, Via E. De Amicis, 24 Trecastagni, Catania 95039, Italy

Bashmakov YK, Zigangirova NA, Gintzburg AL, Bortsov PA, Petyaev IM. ApoB-containing lipoproteins promote infectivity of chlamydial species in human hepatoma cell line. *World J Hepatol* 2010; 2(2): 74-80 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i2/74.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.74

INTRODUCTION

Although plasma constituents are known to have an

enormous impact on the initiation, development and outcomes of many infections, the role of plasma lipoproteins in the pathogenesis of infectious diseases remains poorly understood. However, at least in case of bacterial sepsis, the severity and clinical manifestation of the disease largely depends on the plasma lipoprotein profile of patients. Survival rate in bacterial sepsis is higher in hypercholesterolemic individuals presumably due to ability of plasma lipoproteins to scavenge lipopolysaccharide from the infected cells[1]. High density lipoproteins are considered to be a mayor acceptor of lipopolysaccharide (LPS) *in vivo* and *in vitro* systems preventing tissue damage in sepsis[2]. LPS avidly binds two major high density lipoproteins (HDL)-specific apolipoproteins - A1 and ApoC I [3,4]. Subsequent binding of HDL-LPS complexes to the scavenger receptor SR-BI in the liver promotes hepatic clearance of LPS from the blood stream[5].

Much less information is available about the possible role of plasma lipoproteins in dissemination mechanisms of infectious agents. Most of our knowledge in that field relies on the well characterized association between plasma lipoproteins and hepatitis C virus. The majority of *hepatitis C* viral particles are bound to ApoB-containing very low density lipoproteins (VLDL) and low density lipoproteins (LDL) and can be immunoprecipitated with ApoB-specific antibody[6]. Complexes LDL-Hepatitis C virus, elsewhere termed viral lipoparticles, interact with the LDL-receptor as well as with surface receptor CD81, providing a dual receptor mechanism for viral attachment and entry in the target cells[7].

However, interactions between chlamydial species and plasma lipoproteins remain completely unknown. A published paper on this issue[8] demonstrates that LDL promotes foam cell formation in the macrophage cell line preincubated with chlamydial trachomatis (*C. pneumoniae)*. The objective of this study was to initiate an investigation of direct interaction between chlamydial particles and plasma lipoproteins and its role in infecting host cells. Here we show, for the first time, that the elementary body of *C. trachomatis* and *C. pneumoniae* directly binds apoB-containing lipoproteins, promoting the infection rate in human hepatoma cell line (HepG2 cells).

MATERIALS AND METHODS

Reagents

All reagents were from Sigma-Aldrich unless otherwise stated. Fast-performance liquid chromatography (FPLC) was performed using Superose 6HR 10/30 column (Pharmacia, Sweden) as described[9,10]. Cholesterol content in the FPLC fractions was measured using Cholesterol/Cholesteryl Quantification Kit (Calbiochem, UK).

Gradient gel electrophoresis of FPLC fractions was performed as published by Ordovas JM[11]. Protein level was measured using BCA kit from Pierce (Cramlington, UK). HepG2 cells were obtained from "European Collection of Cell Cultures" (Salisbury, UK).

Genus-specific monoclonal antibodies against chlamydial LPS and chlamydial major outer membrane protein (MOMP) were described previously[12]. Polyclonal antibody against apolipoprotein B (ab20737) was purchased from Abcam (Cambridge, UK). Anti-mouse IgG horseradish-peroxidase linked secondary antibody was obtained from Amersham (Buckinghamshire, UK).

Cell culture and organisms

The following chlamydial organisms were used: *C. trachomatis* strain L2/Bu434 and *C. pneumoniae* strain *Kajjani-6*. Both of them were kindly provided by Dr. P. Saikku (University of Oulu, Finland).

Chlamydial strains were propagated in Hep2 cells and purified by Renografin gradient centrifugation as described[13]. Purified elementary bodies were suspended in sucrose-phosphate-glutamic acid buffer[13]. Chlamydial titers were determined by infecting Hep2 cells with 10-fold dilutions of thawed stock suspension.

HepG2 cells were maintained in Dulbecco's Modified Eagle Media (DMEM) with 10 % fetal calf serum until subconfluence was reached. Monolayers of HepG2 cells in 24-well plates containing cover slips were washed and infected with *C. trachomatis* or *C. pneumoniae* at multiplicity 1:1. Infected plates were centrifuged 1 h at 1500 g and kept in serum-free DMEM supplemented with 2 μg/mL of cycloheximide for 48 h (*C. trachomatis*) or 72 h (*C. pneumoniae*). Cover slips were fixed with acetone and stained by direct immunofluorescence using fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody against chlamydial LPS. To evaluate the contribution of ApoB-containing lipoproteins into infectivity rate of *C. trachomatis* and *C. pneumoniae*, titrated stock suspensions containing elementary bodies of each chlamydial type were incubated (1 h, 37℃) with 0.1 mg/mL of native ApoB-containing lipoproteins dissolved in Public Broadcasting Service (PBS). After removal of unbound lipoproteins (2 washes in PBS, 15000 g, 5 min each), the volume of the stock suspensions was readjusted to the initial value to commence immediately with the cell infection protocol.

Animals

C57BL/6 male mice 6-8 wk old from Pushino Animal Breeding Facility (Moscow, Russia) were kept in the animal facility in compliance with "Declaration of Helsinki and Guiding Principles in the Care and Use of Animals" under approved institutional protocol. Mice were kept on the synthetic atherogenic high-fat diet (ICN Biochemicals Inc, Aurora, Ohio) for 4 wk with free access to the food and water before collection of blood *via* retro orbital sinus puncture under anesthesia. Plasma obtained from inbred mice was considered as the preferred source of lipoproteins to avoid any variables related to the genetic background and/or dietary status of human individuals.

Isolation of native ApoB-containing lipoproteins

A low-density fraction of plasma lipoproteins was isolated

by centrifugation of mouse plasma at the density of 1.055 g/mL for 4 h, 4℃ and 543000 g TL100, Beckman Instruments, USA[14]. The upper layer was dialyzed overnight against PBS supplemented with 0.01% sodium EDTA (pH 7.4), filtered through 0.22 μm pore-sized membranes and stored at 4℃ for no longer than 3 wk.

FPLC and gel electrophoresis analysis

Pooled plasma (2.5 mL) obtained from 5 mice was subjected to ultracentrifugation at density of 1.215 g/mL. Purified lipoproteins were loaded on FPLC column equilibrated with PBS containing 0.01% EDTA and 0.01% sodium azide. Plasma lipoproteins were eluted from the column at room temperature and flow rate 0.2 mL/min with the same buffer. Elution fractions (0.3 mL each, 46 fractions total) were monitored at 280 nm and analyzed for cholesterol content. Plasma lipoprotein fractions were stored at 4℃ and used within 3 wk after preparation. For gel electrophoresis, each three consecutive FPLC fractions were pooled and delipidated with chloroform/methanol mixture (1:1). After centrifugation (5000 g, 10 min) the pellet was dissolved, vortexed and boiled in 50 mmol/L Tris-HCL (pH 7.8) containing 8 mol/L urea, 10% SDS, 10 % Glycerol and 0.05% bromophenol blue. Aliquots of reconstituted FPLC fractions were loaded on 4%-15% gradient SDS-polyacrilamide gel, which was stained after an overnight run with Coomassie Blue.

Immuno-dot blot binding assay

20 μL aliquotes of FPLC fractions (native or heat-denatured) containing 100 ng of total protein were loaded under vacuum on to nitrocellulose membrane (Amersham, UK). Crosslinked membranes were blocked in 3% gelatin in 20mmol/L Tris-buffer (pH 7.5) with 0.05% Tween 20 (TBST) for 1 h at room temperature and incubated overnight at 4℃ with suspensions of UV-inactivated *C. trachomatis* and *C. pneumoniae* in TBST (100 μg of total protein/mL). After three TBST washings (5 min each), the membranes were incubated with monoclonal antibodies against chlamydial LPS (2 h, 5 μg/mL) and blotted for 15 min with horse-radish peroxidase conjugated secondary antibody (Amersham, UK). After 3 final washes in TBST, membranes were developed in the Enhanced Chemiluminescent Substrate (Pierce, UK) and exposed to X-ray films for 5-7 s.

Immunoprecipitation analysis

Immunoprecipitation studies were done using Seize® X Bacterial Immunoprecipitation kit (Pierce, Cramlington, UK). Briefly, elementary bodies of *C. trachomatis* or *C. pneumoniae* (0.1 mg/mL in PBS) were thoroughly mixed with native LDL (0.1 mg/mL in PBS) and incubated at 37℃ for 1 h under continuous gentle shacking. Bacterial cells were pelleted by centrifugation (15000 g, 10 min) washed 3 times with ice-cold PBS to remove unbound lipoproteins and kept frozen at -80℃. After thawing, soluble proteins from the bacterial pellet were extracted with B-Per® reagent and applied to the affinity mini column with immobilized polyclonal ApoB-specific antibody. Uncoated beads and beads with irrelevant polyclonal antibody were used in control experiments. After 2 h of incubation at room temperature, the columns were centrifuged and the resulting flow-through was designated as supernatant. Following washing procedure, immunoprecipitated protein was eluted with 150 μL of the elution buffer and referred as a pellet fraction. Supernatant and pellet fractions were analyzed in 8% SDS-PAGE and further immunoblot analysis was conducted with monoclonal MOMP-specific antibody.

Flow Cytometry analysis

Flow Cytometry analysis was performed according to the method described by Dessus-Babus S[15]. Briefly, HepG2 monolayers were trypsinized and removed from the dishes, centrifuged at 400 g for 5 min. The cell pellet was resuspended and washed twice in PBS. After fixation in 70% ethanol, washed cells were incubated with chlamydial LPS-specific antibody labeled with FITC. The number of cells with fluorescent inclusions was determined with BD FACSCalibur Flow Cytometer (BD Biosciences, USA) with the support of CellQuest Pro Software. All experiments shown below were repeated at least twice. Representative sets of results are submitted here.

RESULTS

It is known[10] that normal mouse plasma contains almost undetectable amounts of ApoB-containing lipoproteins - VLDL, LDL and IDL (results not shown). As expected, dietary treatment of mice with high-fat diet promoted appearance in ApoB isoforms in FPLC fractions (Figure 1). As can be seen from the gradient gel image, ApoB-100 and ApoB-48 were almost equally represented in VLDL, LDL and IDL fractions, while ApoA1 was a major apolipoprotein present in HDL fractions. Cholesterol profile pinpoints the specific fractions and validates efficiency of FPLC protocol used in our study. The highest cholesterol content was detected in HDL fraction whereas the lowest cholesterol value was measured in VLDL fraction. Those tendencies are consistent with characteristics of the mouse plasma lipoprotein profile induced by high-fat diet published elsewhere[10].

Lipoprotein purification protocol implemented in this work allowed us to use pure murine lipoprotein fractions for dot-blot binding assay. Dot-blot immunodetection analysis showed that elementary bodies of *C. trachomatis* and *C. pneumoniae* directly interact with purified undenatured and denatured lipoproteins (Figure 2). As can be seen from our results, *C. trachomatis* predominantly binds native lipoproteins belonging to VLDL and LDL fractions. That interaction becomes much stronger and broader when lipoprotein fractions were denatured. This fact reveals that apolipoprotein component of plasma lipoproteins

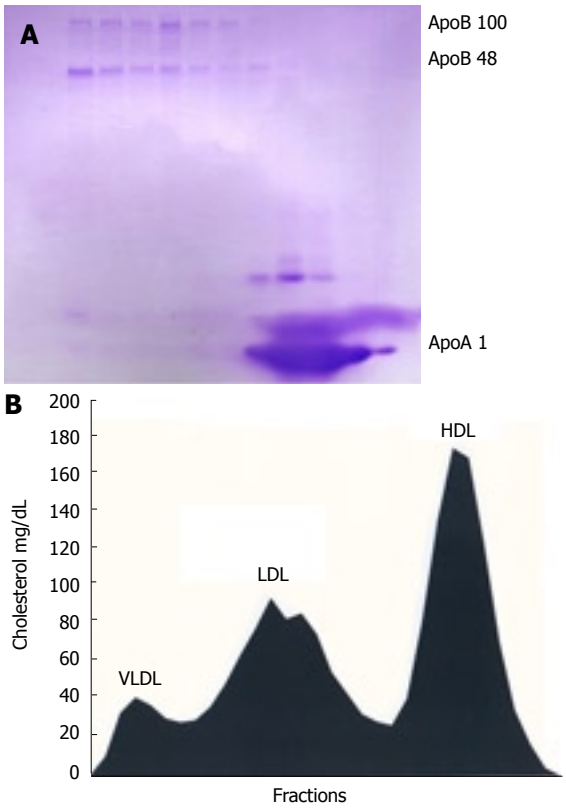


Figure 1 FPLC fractionation of mouse plasma. Mice kept on high-fat diet were bled and pooled plasma was adjusted with KBr to d = 1.215 g/mL and ultracentrifuged. Resulting lipoprotein fraction with density d < 1.215 g/mL was subjected to FPLC gel filtration and cholesterol content was measured in each fraction. Three of each consecutive fraction were pooled, delipidated, heat-denatured and subjected to SDS PAGE gel electrophoresis in gradient gel as described in "Material and Methods". A: Coomassie blue-stained gel with ApoB-100, ApoB-48 and ApoAI indicated by arrow; B: Cholesterol levels in FPLC fractions aligned to apolipoprotein profile of mouse plasma.

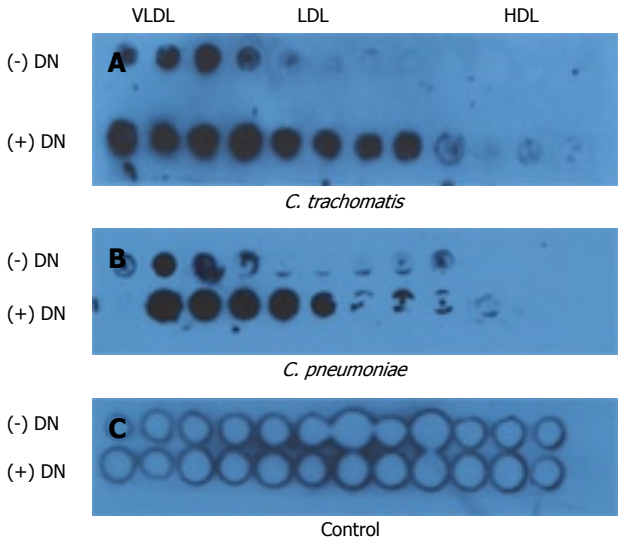


Figure 2 Immuno-dot blot binding assay. Heat-denatured (+ DN) or non-heat-denatured (- DN) FPLCS fractions of mouse plasma lipoproteins were applied to nitrocellulose membrane and incubated with elementary bodies of *C. trachomatis* (Panel A) or *C. pneumoniae* (Panel B) and blotted with chlamydial LPS-specific antibodies as described in "Material and Methods". Control membrane (Panel C) treated similarly except addition of chlamydial suspension.

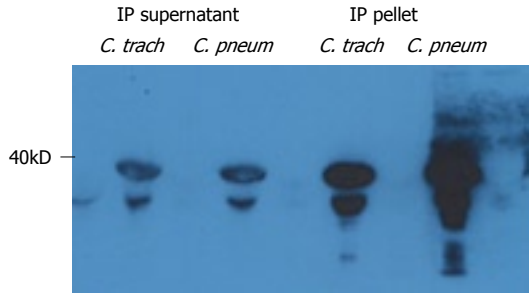


Figure 3 Immunoprecipitation analysis. Soluble membrane extracts of *C. trachomatis* and *C. pneumoniae* preincubated with native Apo-B containing lipoproteins were immunoprecipitated with Apo-B specific polyclonal antibody and subjected to SDS PAGE. Immunoprecipitated (IP) supernatant and pellet were obtained as described in Materials and Methods. Membranes were immunoblotted with MOMP-specific monoclonal antibodies.

is crucial for lipoprotein attachment to the chlamydial particles. Similar pattern of binding has been found for *C. pneumoniae*. That interaction appears to be very similar at exactly the same conditions of binding assay used (duration of incubation and exposure time, concentration of basic reagents). Therefore, the major feature of chlamydial-lipoprotein interaction is clear. Both chlamydial species bind ApoB-containing lipoproteins, with no detectable affinity to HDL fraction. To confirm our observation we employed immunoprecipitation analysis. Protein extracts obtained from *C. trachomatis* and *C. pneumoniae* after preincubation with native LDL were immunoprecipitated with ApoB-specific antibody. As can be seen from Figure 3, ApoB-specific antibody pulls down MOMP from protein extracts of both chlamydial species, when bacterial particles were pre-exposed to LDL.

The validity of immunoprecipitation analysis was confirmed in the control experiments by immunoblotting of supernatant fractions (Figure 3) as well as by using irrelevant antibody and uncoated beads (results not shown).

The results presented above showed that there is distinct binding of chlamydial species to the ApoB-containing plasma lipoproteins. Therefore, we decided to test if complexes containing chlamydial particles and plasma lipoproteins may infect LDL-receptor expressing cell line. HepG2 cells were used for that purpose. As can be seen from Figure 4, both *C. trachomatis* and *C. pneumoniae* may efficiently propagate in immortalized human hepatocytes infected in serum-free conditions. After infecting the cells with chlamydial species, late stage inclusions and extracellular elementary bodies were seen in approximately 60% of the cells. Inoculums containing chlamydial particles preincubated with ApoB-containing lipoproteins promoted intensity of staining and number of infected HepG2 cells.

To quantify the effect of native Apo-B containing lipoproteins on infectivity rate of *C. trachomatis* and *C .pneumoniae*, flow cytometric analysis has been used (Figure 5). As follows from our results, ApoB-containing

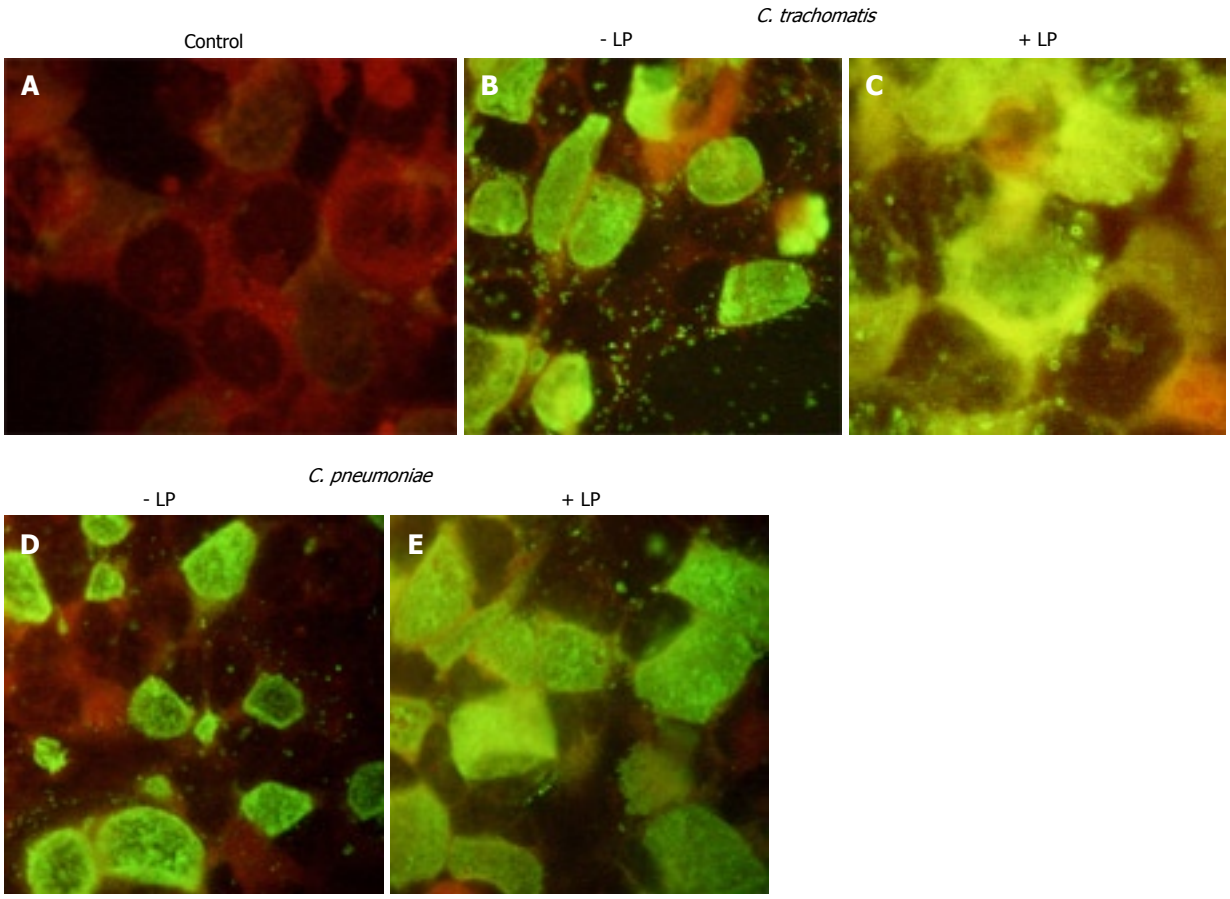


Figure 4 HepG2 cells. Direct immunofluorescence with FITC-conjugated antibody against chlamydial LPS. HepG2 cells were infected with inoculants containing: A: No chlamydial particles, B: *C. trachomatis* alone; C: *C. trachomatis* preincubated with native Apo-B containing lipoproteins; D: *C. pneumoniae* alone; E: *C. pneumoniae* preincubated with native Apo-B containing lipoproteins. Immunofluorescence protocol is detailed in Materials and Methods. Original magnification × 40.

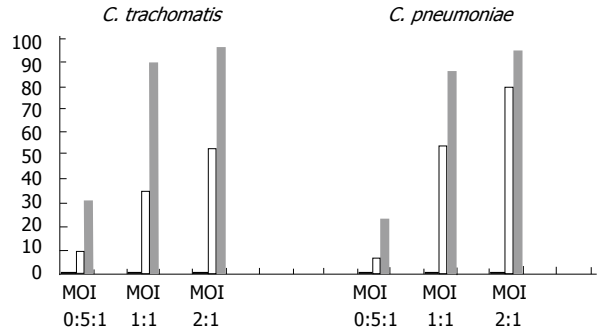


Figure 5 Flow Cytometry Analysis of HepG2 cells. Infectivity of *C. pneumoniae* and *C. trachomatis* preincubated with native ApoB-containing lipoproteins. HepG2 cells were infected with *C. pneumoniae* (left side of diagram) or *C. trachomatis* (right side of diagram) with or without preincubation of bacteria with native ApoB-containing lipoproteins as described in Material Methods. Three different levels of infection multiplicity were studied for each pathogen (MOI 0.5:1; 1:1; 2:1). Percentage of the infected cells shown on value Y axis was determined by flow cytometry (see Materials and Methods) for control (uninfected) HepG2 suspensions (hardly seen black columns), HepG2 suspensions infected with chlamydia bacteria alone (white columns) and HepG2 suspensions infected with chlamydial bacteria preincubated with native ApoB-containing lipoproteins (gray columns).

lipoproteins consistently enhanced the infectivity rate of *C. trachomatis* at different levels of infection multiplicity (MOI 0.5:1; 1:1; 2:1). The same tendency has been seen in the case of *C. pneumoniae*. HDL fraction did not affect infection rate in HepG2 cells incubated with chlamydial biovars (results are not shown).

DISCUSSION

In the present paper we show for the first time that both *C. trachomatis* and *C. pneumoniae* interact directly with plasma lipoproteins and may infect a novel cell target type for chlamydial infection - hepatic cells. That interaction enhances the ability of chlamydial particles to enter and/or propagate inside of the hepatocytes, increasing the infection rate in the hepatic cell line HepG2. The ability of Chlamydia trachomatis to propagate in HepG2 cells has been reported recently by G Wang *et al*[16].

The precise molecular mechanisms responsible for chlamydial invasion of the host cells have yet to be identified. Several receptors, including mannose 6-phosphate/insulin-like growth factors (IGF-2)[17] and eukaryotic lipid membrane domains[18], are proposed to play a significant role in the entrance of chlamydial particles in the host cells. On the other hand, a variety of other polyvalent interactions between chlamydial particles and host cell membrane deserves detailed considerations[19].

An initially surprising finding about direct interaction between chlamydial particles and plasma lipoproteins may represent a case of metabolic mimicry, when a bacterial particle acquires mammalian ligand for specific membrane receptor, allowing subsequent endocytosis and initiation of infectious cycle inside of the host cell. Association of chlamydial particles with plasma lipoproteins does not seem to be an absolute requirement for initiation of the chlamydial infection in the hepatocytes since chlamydial infection in HepG2 cells can be initiated and sustained in serum-free medium. However, if binding chlamydial particles to plasma lipoproteins takes place in vivo situation, it may have significant pathophysiological consequences especially in hyperlipidemic and atherosclerotic patients. ApoB-containing lipoproteins are known to be efficiently internalized not only by hepatocytes, but also by macrophages and endothelial cells residing in atherosclerotic lesions[20]. Therefore, persistent increase in plasma ApoB - containing lipoproteins may promote targeted delivery of chlamydial particles and aggravate inflammatory response in the atherosclerotic plaque. This hypothesis and a molecular mechanism of interaction between chlamydial biovars and lipoproteins are currently under investigation.

ACKNOWLEDGMENTS

The authors are thankful to Drs E Fedina, E Tokarskaya and N Kolkova for challenging discussions. Ms Agni Roce is appreciated for invaluable help during experimental work and manuscript preparation.

COMMENTS

Background

Two major chlamydial biovars - *C. trachomatis* and *C. pneumoniae* cause a wide range of urogenital and respiratory infections. Possible role of *C. pneumoniae* in atherosclerosis is currently discussed. Participation of chlamydial species in hepatic diseases is suspected but not proven yet.

Research frontiers

It has been shown recently that *C. trachomatis* may propagate in hepatocytes. On the other hand, a productive infection by *C. pneumoniae* is reported in Kupffer cells isolated from liver. The relevance of these observations for hepatobiliary pathology remains to be elucidated.

Innovations and breakthroughs

This study is first to show that two major chlamydial species (*C. trachomatis* and *C. pneumoniae*) can bind plasma lipoproteins and efficiently propagate in immortalized human hepatocytes. Plasma ApoB-containing lipoproteins promote the infection rate in human hepatoma cell line (HepG2 cells), suggesting possible role of lipoproteins in the mechanism of attachment and/or entry of bacteria into the host cell.

Applications

Understanding of the precise molecular mechanism predetermining interaction of chlamydial biovars with the host cells is essential for the development of new strategies in treatment of chlamydial infections.

Peer review

The authors showed that *C. trachomatis* and *C. pneumoniae* can bind ApoB-containing plasma lipoproteins. Both chlamydial biovars are shown to propagate in human hepatoma cell line - HepG2 cells. Preincubation of chlamydial particles with ApoB-containing lipoproteins promotes the infectivity rate of chlamydial biovars in HepG2 cells, suggesting possible participation of lipoprotein receptor in interaction of chlamydial species with the host cells. The results are interesting and may bring new insight into pathogenesis of chlamydial infections.

REFERENCES

1 **von Haehling S**, Schefold JC, Springer J, Anker SD. The cholesterol paradox revisited: heart failure, systemic inflammation, and beyond. *Heart Fail Clin* 2008; **4**: 141-151
2 **Murch O**, Collin M, Hinds CJ, Thiemermann C. Lipoproteins in inflammation and sepsis. I. Basic science. *Intensive Care Med* 2007; **33**: 13-24
3 **Berbee JF**, van der Hoogt CC, Kleemann R, Schippers EF, Kitchens RL, van Dissel JT, Bakker-Woudenberg IA, Havekes LM, Rensen PC. Apolipoprotein CI stimulates the response to lipopolysaccharide and reduces mortality in gram-negative sepsis. *FASEB J* 2006; **20**: 2162-2164
4 **Ma J**, Liao XL, Lou B, Wu MP. Role of apolipoprotein A-I in protecting against endotoxin toxicity. *Acta Biochim Biophys Sin* (Shanghai) 2004; **36**: 419-424
5 **Cai L**, Ji A, de Beer FC, Tannock LR, van der Westhuyzen DR. SR-BI protects against endotoxemia in mice through its roles in glucocorticoid production and hepatic clearance. *J Clin Invest* 2008; **118**: 364-375
6 **André P**, Perlemuter G, Budkowska A, Bréchot C, Lotteau V. Hepatitis C virus particles and lipoprotein metabolism. *Semin Liver Dis* 2005; **25**: 93-104
7 **Wunschmann S**, Muller HM, Stipp CS, Hemler ME, Stapleton JT. In vitro interaction between hepatitis C virus (HCV) envelope glycoprotein E2 and serum lipoproteins (LPs) results in enhanced cellular binding of both HCV E2 and LPs. *J Infect Dis* 2006; **194**: 1058-1067
8 **Cao F**, Castrillo A, Tontonoz P, Re F, Byrne GI. Chlamydia pneumoniae--induced macrophage foam cell formation is mediated by Toll-like receptor 2. *Infect Immun* 2007; **75**: 753-759
9 **Garber DW**, Kulkarni KR, Anantharamaiah GM. A sensitive and convenient method for lipoprotein profile analysis of individual mouse plasma samples. *J Lipid Res* 2000; **41**: 1020-1026
10 **Gallou-Kabani C**, Vigé A, Gross MS, Boileau C, Rabes JP, Fruchart-Najib J, Jais JP, Junien C. Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. *Am J Physiol Endocrinol Metab* 2007; **292**: E1095-E1100
11 **Rashid S**, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA, Horton JD. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. *Proc Natl Acad Sci USA* 2005; **102**: 5374-5379
12 **Kutlin AV**, Drobyshevskaia EI, Shatkin AA. [The immunochemical and biological properties of monoclonal antibodies to Chlamydia trachomatis] *Zh Mikrobiol Epidemiol Immunobiol* 1996; 3-6
13 **Caldwell HD**, Kromhout J, Schachter J. Purification and partial characterization of the major outer membrane protein of Chlamydia trachomatis. *Infect Immun* 1981; **31**: 1161-1176
14 **Diaz O**, Delers F, Maynard M, Demignot S, Zoulim F, Chambaz J, Trépo C, Lotteau V, André P. Preferential association of Hepatitis C virus with apolipoprotein B48-containing lipoproteins. *J Gen Virol* 2006; **87**: 2983-2991
15 **Dessus-Babus S**, Belloc F, Bébéar CM, Poutiers F, Lacombe F, Bébéar C, de Barbeyrac B. Antibiotic susceptibility testing for Chlamydia trachomatis using flow cytometry. *Cytometry* 1998; **31**: 37-44
16 **Wang G**, Burczynski F, Anderson J, Zhong G. Effect of host fatty acid-binding protein and fatty acid uptake on growth of Chlamydia trachomatis L2. *Microbiology* 2007; **153**: 1935-1939
17 **Puolakkainen M**, Kuo CC, Campbell LA. Chlamydia pneumoniae uses the mannose 6-phosphate/insulin-like

growth factor 2 receptor for infection of endothelial cells. *Infect Immun* 2005; **73**: 4620-4625

18 **Jutras I**, Abrami L, Dautry-Varsat A. Entry of the lymphogranuloma venereum strain of Chlamydia trachomatis into host cells involves cholesterol-rich membrane domains. *Infect Immun* 2003; **71**: 260-266

19 **Dautry-Varsat A**, Balañá ME, Wyplosz B. Chlamydia--host cell interactions: recent advances on bacterial entry and intracellular development. *Traffic* 2004; **5**: 561-570

20 **Moore KJ**, Freeman MW. Scavenger receptors in atherosclerosis: beyond lipid uptake. *Arterioscler Thromb Vasc Biol* 2006; **26**: 1702-1711

S- Editor Wang YR **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i2.81

World J Hepatol 2010 February 27; 2(2): 81-86
ISSN 1948-5182 (online)

Prognosis of metastatic splenic hilum lymph node in patients with gastric cancer after total gastrectomy and splenectomy

Keishiro Aoyagi, Kikuo Kouhuji, Motoshi Miyagi, Takuya Imaizumi, Junya Kizaki, Kazuo Shirouzu

Keishiro Aoyagi, Kikuo Kouhuji, Motoshi Miyagi, Takuya Imaizumi, Junya Kizaki, Kazuo Shirouzu, Department of Surgery, Kurume University School of Medicine, Fukuoka 830-0011, Japan
Author contributions: Aoyagi K, Management of the research and analysis of the data; Kouhuji K Chief of gastric cancer group and instructor of the research, Miyagi M, Clinical work, Imaizumi T, Clinical work, Kizaki J, Clinical work, Shirouzu K, Chairman of department of surgery and adviser of the research.
Correspondence to: Dr. Keishiro Aoyagi, MD, Department of Surgery, Kurume University School of Medicine, 67 Asahi-mach, Kurume City, Fukuoka 830-0011, Japan. keishiro@med.kurume-u.ac.jp
Telephone: +81-942-353311 Fax: +81-942-340709
Received: March 13, 2009 Revised: September 10, 2009
Accepted: September 17, 2009
Published online: February 27, 2010

Abstract

AIM: To clarify the significance of combined resection of the spleen to dissect the No. 10 lymph node (LN).

METHODS: We studied 191 patients who had undergone total gastrectomy with splenectomy, excluding non-curative cases, resection of multiple gastric cancer, and those with remnant stomach cancer. Various clinicopathological factors were evaluated for any independent contributions to No. 10 LN metastasis, using χ^2 test. Significant factors were extracted for further analysis, carried out using a logistic regression method. Furthermore, lymph node metastasis was evaluated for any independent contribution to No. 10 LN metastasis, using the same methods. The cumulative survival rate was calculated using the Kaplan-Meier method. The significance of any difference between the survival curves was determined using the Cox-Mantel test, and any difference was considered significant at the 5% level.

RESULTS: From the variables considered to be potentially associated with No. 10 LN metastasis, age, depth, invasion of lymph vessel, N factor, the number of lymph node metastasis, Stage, the number of sites, and location were found to differ significantly between those with metastasis (the Positive Group) and those without (the Negative Group). A logistic regression analysis showed that the localization and Stage were significant parameters for No. 10 LN metastasis. There was no case located on the lesser curvature in the Positive Group. The numbers of No. 2, No. 3, No. 4sa, No. 4sb, No. 4d, No. 7, and No. 11 LN metastasis were each found to differ significantly between the Positive Group and the Negative Group. A logistic regression analysis showed that No. 4sa, No. 4sb, and No. 11 LN metastasis were each a significant parameter for No. 10 LN metastasis. There was no significant difference in survival curves between the Positive Group and the Negative Group.

CONCLUSION: Splenectomy should be performed to dissect No. 10 LN for cases which have No. 4sa, No. 4sb or No. 11 LN metastasis. However, in cases where the tumor is located on the lesser curvature, splenectomy can be omitted.

Key words: Gastric cancer; Lymph node metastasis; Lymphadenectomy, Splenectomy; Total gastrectomy

Peer reviewer: Dr. Kelvin K Ng, PhD, Department of Surgery, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China

Aoyagi K, Kouhuji K, Miyagi M, Imaizumi T, Kizaki J, Shirouzu K. Prognosis of metastatic splenic hilum lymph node in patients with gastric cancer after total gastrectomy and splenectomy. *World J Hepatol* 2010; 2(2): 81-86 Available from: URL: http://www.wjgnet.com/wjh/full/v2/i2/81.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.81

INTRODUCTION

The lymph nodes at the splenic hilum (No. 10 LN) belo-

ng to the Group 2 lymph nodes of gastric cancer that involve the upper third of the stomach, according to the Japanese Classification of Gastric Carcinoma[1], and D2 lymph node dissection is the standard operation for gastric cancer in Japan. Therefore, splenectomy is widely performed to achieve complete D2 lymphadenectomy in Japan for macroscopically advanced gastric cancer located at the proximal part of the stomach. Extended lymphadenectomy, splenectomy, or a combination of both might theoretically improve prognosis by achieving better lymph node clearance[2,3], but none of these was associated with improved outcome in randomized trials specifically addressing this issue[4-7]. Moreover some complications in splenectomy, for example pancreatic fistula and left subdiaphragmatic abscess, sometimes occur[2,8-10], and splenectomy has been associated with increased morbidity after gastrectomy for gastric cancer[8,9,11-13]. Although most authors have recommended splenic preservation in the surgical treatment of gastric cancer[4,11,12], splenectomy is still considered for proximal gastric and gastroesophageal junction cancer, because the incidence of lymph node metastasis at the splenic hilum is thought to be higher in these tumors[2,3,14,15]. The aim of the present study was to clarify the significance of combined resection of the spleen with total gastrectomy to dissect No. 10 LN. Splenectomy has been more commonly performed in the resection of a proximal tumor, and therefore previous analyses may be biased. Here, we compared gastric cancer cases which underwent curative surgery with positive and negative No. 10 LN metastasis to total gastrectomy cases with splenectomy, clinicopathologically. The indication for splenectomy in patients with proximal and middle gastric cancer remain controversial. Here, we have investigated the characteristic findings in patients with lymph node metastasis to the splenic hilus, and report the indication for splenectomy.

MATERIALS AND METHODS

Patients

Between 1990 and 2004, 2064 patients with gastric cancer underwent surgical resection at Kurume University Hospital. Here, we retrospectively studied the 191 cases of total gastrectomy with splenectomy and D2 or D3 dissection, excluding the cases of non-curative resection, those cases involving resection of multiple gastric cancer, and those of remnant stomach cancer. One hundred and twenty-four patients were men, and 67 were women. The mean age was 60.6 years, with an age range from 40 to 84 years. There were 7 patients at Stage ⅠA, 18 patients at Stage ⅠB, 40 patients at Stage Ⅱ, 46 patients at Stage ⅢA, 38 patients at Stage ⅢB, and 42 patients at Stage Ⅳ. There were 20 cases with positive No. 10 LN metastasis (the Positive Group), and 171 cases with none (the Negative Group), as defined by the Japanese Classification of Gastric Carcinoma[1].

Statistical analysis

Various factors, including age, sex, site, location, tumor size, macroscopic type, histological type, depth of invasion, stromal volume, pattern of tumor infiltration, invasion of lymph vessel, venous invasion, N factor, Stage, and the number of lymph nodes with positive metastasis were evaluated for any independent contributions to No. 10 LN metastasis, using the χ^2 test. The number of lymph node metastases was classified dependent on TNM staging, as follows, N0: no regional lymph nodes metastasis, N1: metastasis in 1 to 6 regional lymph nodes, N2: metastasis in 7 to 15 regional lymph nodes, and N3: metastasis in more than 15 regional lymph nodes. Significant factors were extracted for further analysis, carried out using a logistic regression method. Furthermore, lymph node metastasis was evaluated for any independent contribution to No. 10 LN metastasis, using similar methods as above. The statistical analyses were performed using a statistical analysis computer program (SPSS Ⅱ for Windows, SPSS Japan Inc., Tokyo, Japan). The *P* value level of significance was set at 0.05.

The cumulative survival rate was calculated using the Kaplan-Meier method. The significance of any difference between the survival curves was determined using the Cox-Mantel test, and any difference was considered significant at the 5% level.

RESULTS

Clinicopathological findings

From the variables considered to be potentially associated with No. 10 LN metastasis, age, the number of sites, location, depth, invasion of lymph vessel, N factor, the number of lymph node metastases, and Stage were found to differ significantly between the Positive Group and the Negative Group ($P = 0.017$, $P = 0.008$, $P < 0.001$, $P = 0.017$, $P = 0.008$, $P < 0.001$, $P = 0.003$, and $P < 0.001$, respectively) (Table 1).

A logistic regression analysis was conducted for the eight parameters (age, the number of site, location, depth, invasion of lymph vessel, N factor, the number of lymph node metastases, and Stage) that had been found to be significant using the χ^2 test. A logistic regression analysis showed that the location and Stage were each significant parameters of No. 10 LN metastasis ($P = 0.003$ and $P = 0.006$, respectively) (Table 2). There was no case locating on the lesser curvature in the Positive Group (Table 1).

Lymph node metastasis

The numbers of No. 2, No. 3, No. 4sa, No. 4sb, No. 4d, No. 7, and No. 11 LN metastasis were found to differ significantly between the Positive Group and the Negative Group ($P < 0.001$, $P = 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.019$, and $P < 0.001$, respectively) (Table 3). A logistic regression analysis showed that No. 4sa, No. 4sb, and No. 11 LN metastasis were each significant parameters for No. 10 LN metastasis ($P < 0.001$, $P = 0.006$, and $P = 0.002$, respectively) (Table 4) (Figure 1).

Survival curves

There was no significant difference in survival rate be-

Table 1 Demographics and clinicopathological data on 191 patients with gastric cancer with respect to No. 10 LN metastasis *n* (%)

		Metastasis positive	Metastasis negative	Total	*P* value
		20 (10.5)	171 (89.5)	191 (100)	
Gender	Male	14 (70.0)	111 (64.9)	125 (65.4)	0.651
	Female	6 (30.0)	60 (35.1)	66 (34.5)	
Age (years)	≤ 60	7 (35.0)	107 (62.6)	114 (59.7)	0.017
	> 60	13 (65.0)	64 (37.4)	77 (40.3)	
Site	L	1 (5.0)	11 (6.4)	12 (6.3)	0.616
	M	9 (45.0)	58 (33.9)	67 (35.1)	
	U	10 (50.0)	102 (59.6)	112 (58.6)	
Number of site	1	5 (25.0)	41 (24.0)	46 (24.1)	0.008
	2	7 (35.0)	78 (45.6)	85 (44.5)	
	≤ 3	8 (40.0)	52 (30.4)	60 (31.4)	
Location	Less	0 (0.0)	73 (42.7)	73 (38.2)	< 0.001
	Ant	1 (5.0)	15 (8.8)	16 (8.4)	
	Post	3 (15.0)	32 (18.7)	35 (18.3)	
	Great	8 (40.0)	13 (7.6)	21 (11.0)	
	Cir	8 (40.0)	38 (22.2)	46 (24.1)	
Number of location	1	7 (35.0)	90 (52.6)	97 (50.8)	0.200
	2	2 (10.0)	28 (16.4)	30 (15.7)	
	3	3 (15.0)	15 (8.8)	18 (9.4)	
	4	8 (40.0)	38 (22.2)	46 (24.1)	
Tumor size (mm)	≤ 100	11 (55.0)	65 (38.0)	76 (39.8)	0.142
	> 100	9 (45.0)	106 (62.0)	115 (60.2)	
Macroscopic type	0	2 (10.0)	13 (7.6)	15 (7.9)	0.503
	1	1 (5.0)	5 (2.9)	6 (3.1)	
	2	2 (10.0)	44 (25.7)	46 (24.1)	
	3	10 (50.0)	69 (40.4)	79 (41.4)	
	4	5 (25.0)	30 (17.5)	35 (18.3)	
	5	0 (0.0)	10 (5.8)	10 (5.2)	
Histological type	Differentiated	3 (15.0)	53 (31.0)	56 (29.3)	0.137
	Undifferentiated	17 (85.0)	118 (69.0)	135 (70.7)	
Depth	M, SM	1 (5.0)	10 (5.8)	11 (5.6)	0.020
	MP	0 (0.0)	12 (7.0)	12 (6.3)	
	SS	2 (10.0)	17 (9.9)	19 (9.9)	
	SE	9 (45.0)	111 (64.9)	120 (62.8)	
	SI	8 (40.0)	21 (12.3)	29 (15.2)	
Ly	0, 1	1 (5.0)	48 (28.1)	49 (25.7)	0.025
	2, 3	19 (95.0)	123 (71.9)	142 (74.3)	
V	-	2 (10.0)	50 (29.2)	52 (27.2)	0.067
	+	18 (90.0)	121 (70.8)	139 (72.8)	
Stromal volume	Med	3 (15.0)	60 (35.1)	63 (33.0)	0.147
	Int	8 (40.0)	62 (36.3)	70 (36.6)	
	Sci	9 (45.0)	49 (28.7)	58 (30.4)	
INF	α	1 (5.0)	42 (24.6)	43 (22.5)	0.127
	β	6 (30.0)	47 (27.5)	53 (27.7)	
	γ	13 (65.0)	82 (48.0)	95 (49.7)	
N	0	0 (0.0)	60 (35.1)	60 (31.4)	< 0.001
	1	2 (10.0)	50 (29.2)	52 (27.2)	
	2	12 (60.0)	40 (23.4)	52 (27.2)	
	3	6 (30.0)	18 (10.5)	24 (12.6)	
	M	0 (0.0)	3 (1.8)	3 (1.6)	
Number of N	0	0 (0.0)	60 (35.1)	60 (31.4)	0.003
	1-6	7 (35.0)	55 (32.2)	62 (32.5)	
	7-15	6 (30.0)	34 (19.9)	40 (20.9)	
	16-	7 (35.0)	22 (12.9)	29 (15.2)	
Stage	ⅠA, ⅠB	0 (0.0)	25 (14.6)	25 (13.1)	< 0.001
	Ⅱ	1 (5.0)	39 (22.8)	40 (20.9)	
	ⅢA	1 (5.0)	45 (26.3)	46 (24.1)	
	ⅢB	4 (20.0)	35 (20.5)	39 (20.4)	
	Ⅳ	14 (70.0)	27 (15.8)	41 (21.5)	

Various clinicopathological factors, including gender, age, site, the number of sites (Number of sites), location, the number of locations (Number of locations), tumor size, macroscopic type, and histological type were evaluated for any independent contributions to No. 10 LN metastasis, depth of invasion (Depth), invasion of lymph vessel (Ly), venous invasion (V), stromal volume, pattern of tumor infiltration (INF), N factor (N), the number of lymph nodes with positive metastasis (Number of N), and stage using the χ^2 test. Age, Number of sites, Location, Depth, Ly, N, Number of N, and Stage were found to differ significantly between the Positive Group and the Negative Group ($P = 0.017$, $P = 0.008$, $P < 0.001$, $P = 0.017$, $P = 0.008$, $P < 0.001$, $P = 0.003$, and $P < 0.001$, respectively).

Table 2 Logistic regression analysis of clinicopathological data for No. 10 LN metastasis

Parameters	Disease progression		
	OR	95% CI	*P* value
Age (yr)	2.697	0.852-8.538	0.092
Depth	0.636	0.264-1.532	0.313
Ly	2.269	0.220-23.407	0.491
N	0.801	0.277-2.319	0.683
Number of N	1.062	0.446-2.531	0.892
Stage	4.840	1.576-14.864	0.006
Number of sites	0.541	0.246-1.191	0.127
Location	2.027	1.269-3.238	0.003

A logistic regression analysis was conducted for eight parameters (Age, Number of sites, Location, Depth, Ly, N, Number of N, and Stage) that had been found to be significant using the χ^2 test. A logistic regression analysis showed that the location and Stage were each significant parameter of No. 10 LN metastasis ($P = 0.003$ and $P = 0.006$, respectively).

Table 3 Demographics and LN metastasis in 191 patients with gastric cancer with respect to No. 10 LN metastasis *n* (%)

	Metastasis positive	Metastasis negative	Total	*P* value
No. 1	8 (40.0)	41 (24.0)	49 (25.7)	0.121
No. 2	11 (55.0)	28 (16.4)	39 (20.4)	< 0.001
No. 3	18 (90.0)	87 (50.9)	105 (55.0)	0.001
No. 4sa	12 (60.0)	10 (5.8)	22 (11.5)	< 0.001
No. 4sb	10 (50.0)	16 (9.4)	26 (13.6)	< 0.001
No. 4d	12 (60.0)	34 (19.9)	46 (24.1)	< 0.001
No. 5	1 (5.0)	10 (5.8)	11 (5.8)	0.878
No. 6	4 (20.0)	19 (11.1)	23 (12.0)	0.248
No. 7	10 (50.0)	43 (25.1)	53 (27.7)	0.019
No. 8a	5 (25.0)	19 (11.1)	24 (12.6)	0.076
No. 8p	1 (5.0)	8 (4.7)	9 (4.7)	0.949
No. 9	2 (10.0)	13 (7.6)	15 (7.9)	0.706
No. 11	11 (55.0)	19 (11.1)	30 (15.7)	< 0.001
No. 12a	0 (0.0)	3 (1.8)	3 (1.6)	0.550
No. 12b	1 (5.0)	2 (1.2)	3 (1.6)	0.192
No. 12p	0 (0.0)	3 (1.8)	3 (1.6)	0.550
No. 13	1 (5.0)	1 (0.6)	2 (1.0)	0.066
No. 14v	1 (5.0)	3 (1.8)	4 (2.1)	0.337
No. 16	4 (20.0)	16 (9.4)	20 (10.5)	0.141
Etc.	0 (0.0)	4 (2.3)	4 (2.1)	0.489

Lymph node metastasis was evaluated for any independent contribution to No. 10 LN metastasis, using the χ^2 test. The numbers of No. 2, No. 3, No. 4sa, No. 4sb, No. 4d , No. 7, and No. 11 LN metastasis were found to differ significantly between the Positive Group and the Negative Group ($P < 0.001$, $P = 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.001$, $P = 0.019$, and $P < 0.001$, respectively).

tween the Positive Group and the Negative Group in Stage ⅢB (Figure 2A), and in Stage Ⅳ cases (Figure 2B).

DISCUSSION

Resection of the spleen en bloc with the stomach for gastric cancer is still widely performed for a curative resection (R0). Fatouros *et al*[4] reported that an overestimation of the risk of residual disease in the splenic hilum nodes in the case of spleen preservation was seen

Table 4 Logistic regression analysis of LN metastasis for No. 10 LN metastasis

Parameters	Disease progression		
	OR	95% CI	*P* value
No. 2	0.791	0.181-3.452	0.755
No. 3	1.414	0.219-9.117	0.716
No. 4sa	18.377	4.071-82.962	< 0.001
No. 4sb	8.447	1.844-38.688	0.006
No. 4d	1.098	0.267-4.518	0.897
No. 7	1.085	0.277-4.253	0.907
No. 11	10.096	2.320-43.941	0.002

Significant lymph nodes were extracted for further analysis, carried out using a logistic regression method. A logistic regression analysis showed that No. 4sa, No. 4sb, and No. 11 LN metastasis were each significant parameters for No. 10 LN metastasis ($P < 0.001$, $P = 0.006$, and $P = 0.002$, respectively).

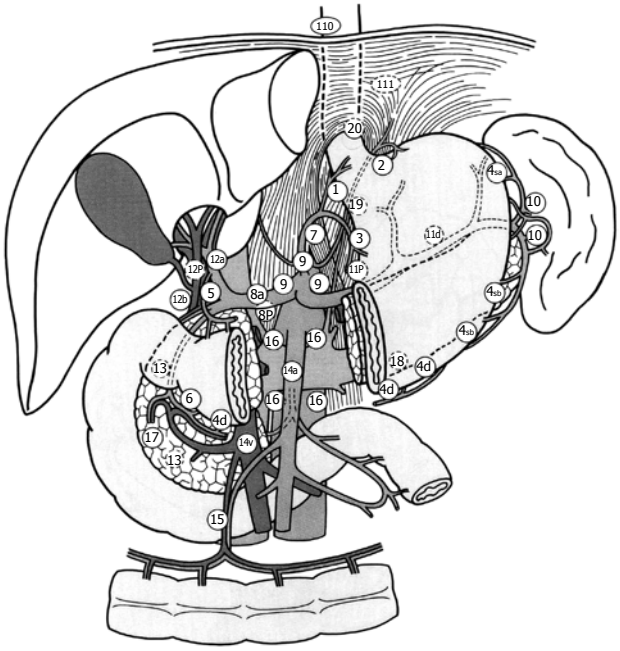


Figure 1 Location of regional lymph nodes. No. 2: Left Pericardial LN, No. 3: LN along the lesser curvature, No. 4sa: LN along the short gastric vessels, No. 4sb: LN along the left gastroepiploic vessels, No. 4d: LN along the right gastroepiploic vessels, No. 7: LN along the left gastric artery, No. 11: LN along the splenic artery.

in 94% of splenectomized patients, and preservation of the spleen may be associated with a reduced risk of early and overall recurrence, translating into a better survival in patients receiving curative surgery for gastric cancer. Pancreas-related abscess formation remains a strong factor in the mortality and morbidity rates[9,12,13]. The dissection of nodes along the distal splenic artery and nodes in the splenic hilum is an intraoperative risk factor for pancreas-related abscess formation. Distal pancreatectomy with splenectomy has a high risk of abscess formation. Pancreas-preserving splenectomy is a standard operation in Japan. However, splenectomy without dissection along the distal splenic artery also has a high risk

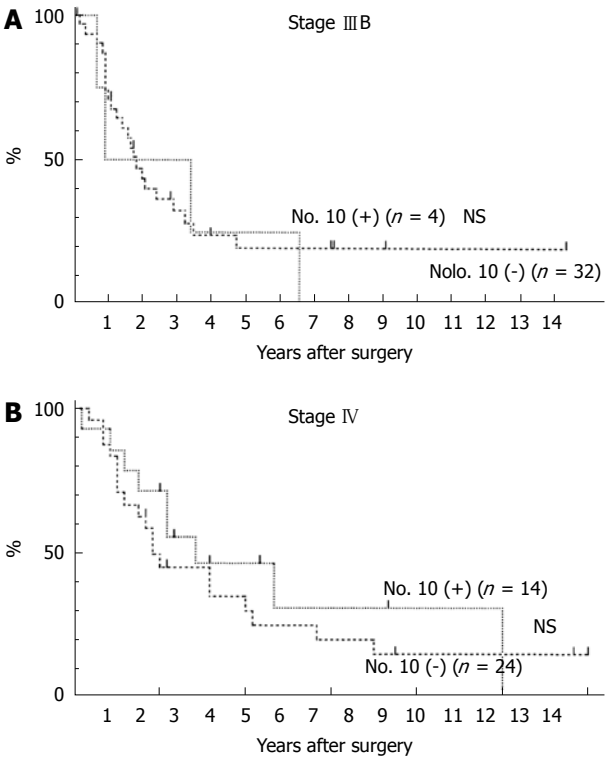


Figure 2 Overall survival curves in patients with gastric cancer. No. 10 (+): Patients with No. 10 LN metastasis; No. 10 (-): Patients without No. 10 LN metastasis. The Cox-Mantel test was not significant. A: Stage ⅢB; B: Stage Ⅳ.

to abscess formation. Some authors[9,13] have reported that splenectomy (with or without pancreatectomy) and nodal dissection are risk factors for operative morbidity but not mortality. Hartgrink *et al*[16] reported that the risk for morbidity and mortality was significant for pancreactomy and splenectomy, and that splenectomy and pancreatectomy should be performed only in case of direct invasion from the tumor into these organs. In considering the lymphatic pathway from the primary tumor to No 10 LN, metastasis in the lymph nodes along the lesser curvature (No. 3), the short gastric vessels, or gastroepiploic vessels (No. 4) may be good indicators of No. 10 LN metastasis[3]. In our study, there was no case with the cancer located on the lesser curvature in the Positive Group, and metastasis in the No. 4sa, No. 4sb, or No. 11 LN was a good indicator for No. 10 LN metastasis. The frequency of No. 10 LN metastasis was high in cases with cancer located on the greater curvature or posterior wall of the stomach. These results suggested that the lymphatic pathway along the posterior gastric artery, splenic artery, short gastric vessels, or gastroepiploic vessels were important for No. 10 LN metastasis. In our study, there was no significant difference in survival curves between the Positive Group and the Negative Group. These data show that dissection of the No. 10 LN has a survival benefit when curative surgery was performed. Ikeguchi *et al*[3] reported when curative surgery was performed, the survival of No. 10 positive patients was not different from that of No. 10 negative patients; therefore, for patients with an advanced gastric cancer located in the proximal part of the stomach, then D2 lymphadenectomy with splenectomy is recommended when patients show macroscopic evidence of serosal invasion with regional lymph node metastasis. Hartgrink *et al*[16] reported that the relevance of the dissection of the No. 10 and 11 LN has to be questioned since the survival benefit is small and the hospital mortality is significantly increased. Splenectomy and pancreatectomy are important risk factors for morbidity and mortality after D2 dissection, with adverse effects on survival as well[5]. A Japanese prospective randomized study on spleen preservation might be beneficial in patients with advanced gastric cancer who receive postoperative immunochemotherapy after total gastrectomy[7]. A randomized trial in Chile found no survival benefit from a splenectomy in patients with total gastrectomy, whereas morbidity was significantly increased[6]. Yamamoto *et al*[17] reported that total gastrectomy with splenectomy should be done for patients with T3 advanced gastric cancer and T2 advanced gastric cancer with multiple lymph node metastasis (more than 7 nodes), and recognized lymph node metastasis to the splenic hilus. Ikeguchi *et al*[3] reported that all cases with No. 10 LN positivity were macroscopically diagnosed as positive for serosal invasion or regional lymph node metastasis at the time of surgery. Sakaguchi *et al*[14] reported that splenectomy should be conducted in T2 cases with gross serosal change and T3, 4 cases. In our study, all cases with No. 10 LN metastasis showed regional lymph node metastasis. However, we detected No. 10 LN metastasis even in early gastric cancer, or cases with few lymph node metastases (less than 7 nodes), The effect of splenectomy on prognosis in patients with gastric cancer remains controversial. Splenectomy might facilitate a more complete lymph adenectomy by thorough clearance of the lymph nodes from the splenic hilum. Other surgeons have recommended splenectomy in patients with proximal or gastroesophageal junction cancer to address the increased likelihood of lymph node metastasis in the splenic hilum[2,3,14]. The method of No. 10 LN dissection without splenectomy is very difficult. Injury of the spleen and high bleeding volume occurred in many cases where No. 10 LN was dissected without splenectomy. Even if splenectomy was not performed, movement of the pancreas tail and spleen was needed to dissect No. 10 LN, so pancreatic fistula and pancreas related abscess formation were recognized in some cases. Moreover, the dissection of No. 10 LN may be incomplete in cases without splenectomy. Numerous retrospective as well as prospective randomized trials, however, have not demonstrated a prognostic benefit for splenectomy or extended lymph adenectomy. Some retrospective studies even demonstrated a worse survival after splenectomy[4,12]. Many cases with No. 10 LN were far advanced cancer which had peritoneal metastasis, liver metastasis, numerous lymph node metastasis, and distant metastasis. In these cases the benefit of dissection of No, 10 LN is small. However, dissection of No. 10 LN has survival benefit when curative surgery

was performed. Therefore, we recommend splenectomy to dissect No. 10 LN in gastric cancer which involves the upper third of the stomach that is curatively resected, especially, when the tumor is located on the greater curvature or posterior wall of the stomach, or has No. 4sa, No. 4sb, or No. 11 LN metastasis. However, when the tumor is located on the lesser curvature, then splenectomy can be omitted.

COMMENTS

Background

The lymph nodes at the splenic hilum (No. 10 LN) belong to the Group 2 LN of gastric cancer that involves the upper third of the stomach, according to the Japanese Classification of Gastric Carcinoma, and D2 LN dissection is the standard operation for gastric cancer in Japan. Therefore, splenectomy is widely performed to achieve complete D2 lymphadenectomy in Japan for macroscopically advanced gastric cancer located at the proximal part of the stomach. Extended lymphadenectomy, splenectomy, or a combination of both might theoretically improve prognosis by achieving better lymph node clearance, but none of these was associated with improved outcome in randomized trials specifically addressing this issue. Moreover some complications in splenectomy, for example pancreatic fistula and left subdiaphragmatic abscess, sometimes occur, and splenectomy has been associated with increased morbidity after gastrectomy for gastric cancer. Although most authors have recommended splenic preservation in the surgical treatment of gastric cancer, splenectomy is still considered for proximal gastric and gastroesophageal junction cancer, because the incidence of lymph node metastasis at the splenic hilum is thought to be higher in these tumors.

Research frontiers

The present study was to clarify the significance of combined resection of the spleen with total gastrectomy to dissect No. 10 LN.

Innovations and breakthroughs

This research clarified that splenectomy can be omitted in cases with the tumor located on the lesser curvature.

Application

If we perform total gastrectomy curatively for proximal gastric cancer which is located on the lesser curvature, we can omit splenectomy to dissect No. 10 LN.

Peer review

This is a retrospective study on the outcome of patients with gastric cancer after total gastrectomy and splenectomy. The authors have highlighted the survival benefit of dissection of No. 10 LN and the predictive factors for the metastatic No. 10 LN in patients with gastric cancer. The authors need to study a cohort of patients with histologically proven metastatic No. 10 LN with and without splenectomy.

REFERENCES

1 Japanese Gastric Cancer Association: Japanese classification of gastric carcinoma. 13th ed. Tokyo: Kanehara, 1999: 6-16
2 **Chikara K**, Hiroshi S, Masato N, Hirotoshi A, Goro M, Hidetaka O. Indications for pancreaticosplenectomy in advanced gastric cancer. *Hepatogastroenterology* 2001; **48**: 908-912
3 **Ikeguchi M**, Kaibara N. Lymph node metastasis at the splenic hilum in proximal gastric cancer. *Am Surg* 2004; **70**: 645-648
4 **Fatouros M**, Roukos DH, Lorenz M, Arampatzis I, Hottentrott C, Encke A, Kappas AM. Impact of spleen preservation in patients with gastric cancer. *Anticancer Res* 2005; **25**: 3023-3030
5 **Degiuli M**, Sasako M, Ponzetto A, Allone T, Soldati T, Calgaro M, Balcet F, Bussone R, Olivieri F, Scaglione D, Danese F, Morino M, Calderini P, Capussotti L, Fronda G, Garavoglia M, Locatelli L, Dellepiane M, Rossini FP, Calvo F. Extended lymph node dissection for gastric cancer: results of a prospective, multi-centre analysis of morbidity and mortality in 118 consecutive cases. *Eur J Surg Oncol* 1997; **23**: 310-314
6 **Csendes A**, Burdiles P, Rojas J, Braghetto I, Diaz JC, Maluenda F. A prospective randomized study comparing D2 total gastrectomy versus D2 total gastrectomy plus splenectomy in 187 patients with gastric carcinoma. *Surgery* 2002; **131**: 401-407
7 **Okinaga K**, Iinuma H, Kitamura Y, Yokohata T, Inaba T, Fukushima R. Effect of immunotherapy and spleen preservation on immunological function in patients with gastric cancer. *J Exp Clin Cancer Res* 2006; **25**: 339-349
8 **Otsuji E**, Yamaguchi T, Sawai K, Okamoto K, Takahashi T. Total gastrectomy with simultaneous pancreaticosplenectomy or splenectomy in patients with advanced gastric carcinoma. *Br J Cancer* 1999; **79**: 1789-1793
9 **Weitz J**, Jaques DP, Brennan M, Karpeh M. Association of splenectomy with postoperative complications in patients with proximal gastric and gastroesophageal junction cancer. *Ann Surg Oncol* 2004; **11**: 682-689
10 **Sasako M**, Saka M, Fukagawa T, Katai H, Sano T. Surgical treatment of advanced gastric cancer: Japanese perspective. *Dig Surg* 2007; **24**: 101-107
11 **Roukos D**, Schmidt-Mathiesen A, Encke A. Adenocarcinoma of the gastric antrum: does D2 total gastrectomy with splenectomy improve prognosis compared to D1 subtotal gastrectomy? A long-term survival analysis with emphasis on Lauren classification. *Surg Oncol* 1995; **4**: 323-332
12 **Brady MS**, Rogatko A, Dent LL, Shiu MH. Effect of splenectomy on morbidity and survival following curative gastrectomy for carcinoma. *Arch Surg* 1991; **126**: 359-364
13 **Wu CW**, Chang IS, Lo SS, Hsieh MC, Chen JH, Lui WY, Whang-Peng J. Complications following D3 gastrectomy: post hoc analysis of a randomized trial. *World J Surg* 2006; **30**: 12-16
14 **Sakaguchi T**, Sawada H, Yamada Y, Fujimoto H, Emoto K, Takayama T, Ueno M, Nakajima Y. Indication of splenectomy for gastric carcinoma involving the proximal part of the stomach. *Hepatogastroenterology* 2001; **48**: 603-605
15 **Kitamura K**, Nishida S, Yamamoto K, Ichikawa D, Okamoto K, Taniguchi H, Yamaguchi T, Sawai K, Takahashi T. Lymph node metastasis in gastric cancer in the upper third of the stomach--surgical treatment on the basis of the anatomical distribution of positive node. *Hepatogastroenterology* 1998; **45**: 281-285
16 **Hartgrink HH**, van de Velde CJ. Status of extended lymph node dissection: locoregional control is the only way to survive gastric cancer. *J Surg Oncol* 2005; **90**: 153-165
17 **Yamamoto M**, Baba H, Kakeji Y, Endo K, Ikeda Y, Toh Y, Kohnoe S, Okamura T, Maehara Y. Postoperative morbidity/mortality and survival rates after total gastrectomy, with splenectomy/pancreaticosplenectomy for patients with advanced gastric cancer. *Hepatogastroenterology* 2004; **51**: 298-302

S- Editor Zhang HN **L- Editor** Lalor PF **E- Editor** Ma WH

World Journal of Hepatology

Online Submissions: http://www.wjgnet.com/1948-5182office
wjh@wjgnet.com
doi:10.4254/wjh.v2.i2.87

World J Hepatol 2010 February 27; 2(2): 87-90
ISSN 1948-5182 (online)

CASE REPORT

Post-traumatic hepatic artery pseudoaneurysm treated with endovascular embolization and thrombin injection

Lloret Estañ Francisco, López Conesa Asunción, Capel Alemán Antonio, Robles Campos Ricardo, Reus Pintado Manuel, Marín Hernández Caridad

Lloret Estañ Francisco, Capel Alemán Antonio, Reus Pinado Manuel, Section of Vascular and Interventional Radiology, Department of Radiology, University General Hospital Virgen de la Arrixaca. Ctra Murcia-Cartagena, 30120 El Palmar, Murcia, Spain
López Conesa Asunción, Robles Campos Ricardo, Marín Hernández Caridad, Division of Hepatobiliary Surgery, Department of General Surgery, University General Hospital Virgen de la Arrixaca, Ctra Murcia-Cartagena, 30120 El Palmar, Murcia, Spain
Author contributions: Francisco LE, wrote the paper; Francisco LE, Antonio CA, and Manuel RP, performed the arteriography, the endovascular embolization and the percutaneous thrombin injection; Asunción LC, Ricardo RC, and Caridad MH, are the surgeons who performed the operations and followed up the patient.
Supported by Departments of Radiology and Surgery at University general hospital Virgen de la Arrixaca
Correspondence to: Lloret Estañ Francisco, MD, Radiology Department, Section of Vascular and Interventional Radiology, University General Hospital Virgen de la Arrixaca, 30120 El Palmar, Murcia, Spain. pakolloret13@hotmail.com
Telephone: +34-968-369366 Fax: +34-968-369347
Received: August 15, 2009 Revised: January 14, 2010
Accepted: January 21, 2010
Published online: February 27, 2010

Abstract

Post-traumatic hepatic artery pseudoaneurysm is uncommon, appearing in approximately 1% of hepatic trauma cases. Most are extrahepatic (80%) and have a late onset. Although they are usually asymptomatic, they should always be treated becasue of the high risk of complications, especially breakage. Currently the treatment of choice is endovascular embolization with coils or the exclusion of the pseudoaneurysm using other intravascular devices. Recently there have been accounts of a treatment that combines embolization with coils and image-guided percutaneous human thrombin injection. We present a case of post-traumatic hepatic artery pseudoaneurysm that was successfully treated using this combined technique.

Key words: Pseudoaneurysm; Hepatic trauma; Hepatic artery; Endovascular treatment; Thrombin

Peer reviewer: Dr. Patrick Veit-Haibach, MD, University Hospital Zürich, Department of Nuclear Medicine, Raemistrasse 100, Zürich 80941, Switzerland

Francisco LE, Asunción LC, Antonio CA, Ricardo RC, Manuel RP, Caridad MH. Post-traumatic hepatic artery pseudoaneurysm treated with endovascular embolization and thrombin injection. *World J Hepatol* 2010; 2(2): 87-90 Available from: URL: http://www.wjgnet.com/1948-5182/full/v2/i2/87.htm DOI: http://dx.doi.org/10.4254/wjh.v2.i2.87

INTRODUCTION

Post-traumatic hepatic artery pseudoaneurysms are rare complications of blunt abdominal trauma. Patients are generally asymptomatic and diagnosis is an incidental finding. Occasionally they can be symptomatic and the most common clinical manifestations are abdominal pain, hematemesis, anemia, hypovolemia and jaundice[1,2].

The classical surgical management of these patients is changing because of the introduction of new endovascular and percutaneous approaches.

We report a case of post-traumatic hepatic artery pseudoaneurysm which was embolized with coils as a first therapeutic choice. However, due to its partial closure, the occlusion was completed using ultrasound-guided percutaneous human thrombin injection.

CASE REPORT

In an 18 year-old male patient who suffered a motorcycle

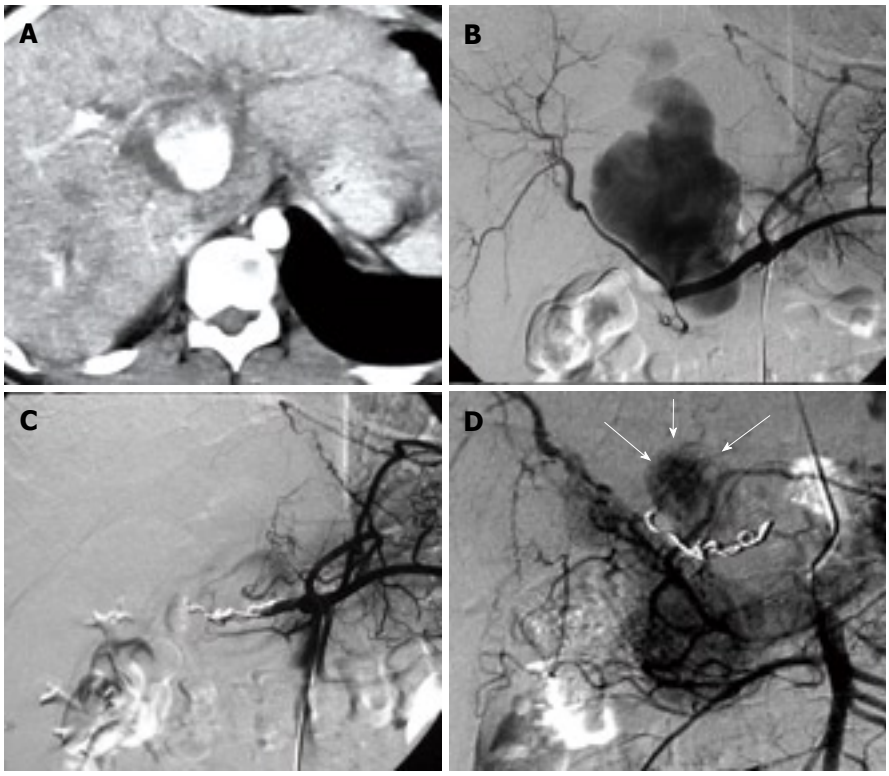


Figure 1 CT and angiography images. A: contrast enhanced CT revealing a collection filled of contrast at the hepatic hilium compatible with pseudoaneurysm and dilated intrahepatic bile duct; B: Selective arteriography of the celiac trunk showing the pseudoaneurysm arising from the common hepatic artery; C: Angiography of the common hepatic artery after embolization. No fill of the pseudoaneurysm is visible from this artery; D: Selective superior mesenteric artery arteriography three days after embolization. The pseudoaneurysm (white arrows) is partly thrombosed with persistence of filling from thin branches. The superselective catheterization of these vessel wasn´t possible due its tortuosity and narrow caliber.

accident,chest and abdominal computed tomography (CT) showed multiple pulmonary contusions, vertebral fractures, hemoperitoneum and splenic fracture. During an operation for splenectomy, a tiny hepatic laceration which was observed near the falciform ligament was electrocoagulated. The patient was admitted into the intensive care unit (ICU) and was discharged 2 mo later without relevant complications.

A CT during the ICU stay showed hepatic left lobe contusions, not seen in initial CT, which evolved favorably. The hepatic artery was normal in this control study and there was not any evident pseudoaneurysm.

Six months after the liver trauma the patient went to the emergency department complaining of colic-type abdominal pain, nausea, vomiting, pruritus and choluria. On physical examination jaundice was observed and blood tests indicated that there was an increase in total and direct bilirubin levels.

Emergency ultrasound showed moderate intrahepatic biliary dilation and a well defined hypoechogenic mass of 10 cm × 5 cm located in the hepatic hilum with features of pseudoaneurysm. Abdominal CT with intravenous contrast (120 mL; 4 mL/s; 80 s delay; pitch 1.5) confirmed the findings described in the ultrasound examination (Figure 1A).

Angiography of the celiac axis revealed the presence of a pseudoaneurysm which was joined to the hepatic artery through a short, narrow neck. A significant difference in caliper between the afferent and efferent arterial segment was evident (Figure 1B). Superior mesenteric artery angiography showed collateral branches that connected with the right hepatic artery. In indirect portography the permeability of the portal vein was confirmed.

Using selective catheterization of the common hepatic artery with a 4F cobra catheter (Cordis, L Roden, Holland) the afferent and efferent artery segment was embolized with 3 coils of 5 cm × 5 mm (Cook-coil for MREYE embolization, IMWCE 35-5-5; William Cook Europe). Closure of the pseudoaneurysm was confirmed in the immediate post-embolization test from the celiac axis (Figure 1C) and from the superior mesenteric artery.

Three days later, the patient presented with hematemesis, hematochezia, hypotension, decreased hematocrit and increased bilirubin. An endoscopy of the upper gastro-intestinal tract was normal. Given that there was suspicion of pseudoaneurysm breakage to the biliary tract, a new contrast enhanced abdominal CT was carried out. This revealed partial thrombosis of the pseudoaneurysm and persistent dilated intrahepatic bile ducts without free peritoneal fluid or collections. We carried out a second angiography, finding this time that the residual light of the pseudoaneurysm was fed by narrow vessels from the upper mesenteric artery (Figure 1D). We tried to carry out another embolization, but this time the thin branches could not be catheterized supraselectively.

The next step we considered to achieve the total occlusion of the pseudoaneurysm was the direct injection of human thrombin with ultrasound guidance. We used a 22 G spinal needle (Boston Scientific Medi-tech) and 2 mL (500 UI/mL) of human thrombin was injected (Tissucol Duo; Baxter Health Care Corporation) controlling the entire procedure with Doppler ultrasound until the absence of flow in the lesion was confirmed (Figure 2).

In follow-up at two years, the patient was asymptomatic and had totally normal bilirubin levels. Abdominal CT showed thrombosis of the pseudoaneurysm, decreased size and normal-sized bile ducts (Figure 3).

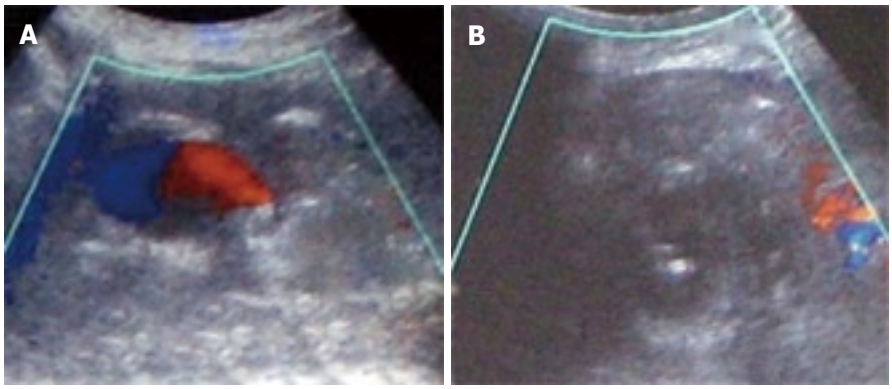


Figure 2 Ultrasound guided thrombin injection. A: Color doppler ultrasound. A small cavity persists with flow in the pseudoaneurysm; B: Needle inside the pseudoaneurysm immediately after the injection of thrombin showing the absence of flow (thrombosis).

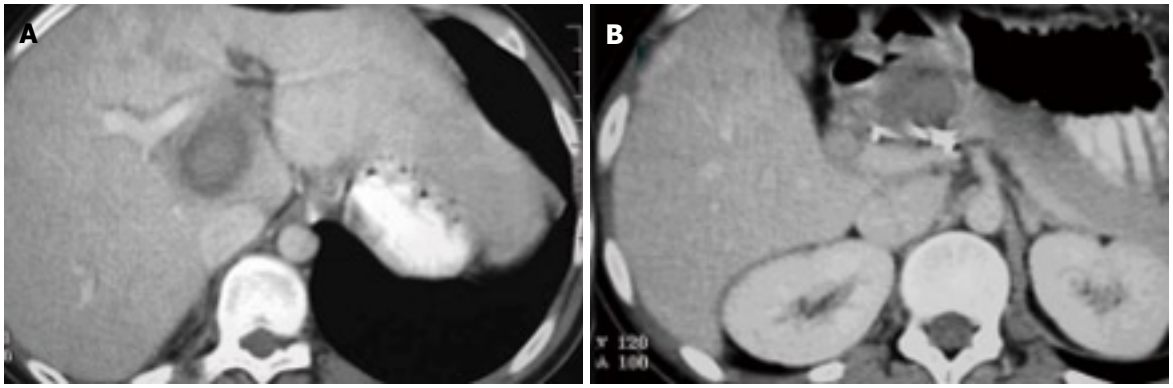


Figure 3 CT scan 12 mo after treatment. Complete thrombosis of the pseudoaneurysm and no dilation of the biliary tract are observed.

DISCUSSION

Conservative treatment has become the standard of care in hemodynamically stable patients with blunt liver trauma. Approximately 71%-89% of these patients are treated conservatively with a success rate of between 85%-94%[3-5]. Contrast enhanced abdominal CT is the imaging modality of choice for diagnosis and follow-up of these patients. It facilitates the evaluation of hepatic parenchyma and other abdominal and retroperitoneal organs; the presence and estimation of the quantity of hemoperitoneum. It also monitors the healing process and the evaluation of possible complications[2].

Delayed complications can occur from weeks to months after the trauma and they include delayed hemorrhage, post-traumatic pseudoaneurysm, abscesses, hemobilia and biliary complications such as biloma and biliary peritonitis[2].

Post-traumatic hepatic artery pseudoaneurysm is an uncommon delayed complication. Although they are usually asymptomatic and found at follow-up by CT or ultrasound, they should be treated as early as possible because they have a high risk of rupture and are associated with high morbidity[1,6,7].

Conventional treatments include either endovascular embolization or surgery. Whereas surgery is associated with significant morbidity and mortality, endovascular embolization is safe and effective and has emerged as the primary line of treatment and the most commonly used option[2,8,9]. Coils are the most commonly used materials and the afferent and efferent artery segments should be embolized to avoid retrograde filling, achieving a success rate of 70%-100%. On occasions, the pseudoaneurysm cannot be totally thrombosed by this way because the occlusion of the vessel has not been complete or because it is fed by collaterals. On these occasions, embolization should be attempted again through the endovascular route. Distal coil migration, hepatic abscess and hepatic ischemia are some of the reported complications of endovascular hepatic artery embolization[9].

Another percutaneous treatment options available are described below.

Image guided human thrombin injection: Image guided human thrombin injection has been widely used in peripheral false aneurysms that occur mainly as a consequence of angiographies. The recurrence rate is 2.08% after thrombin treatment for femoral pseudoaneurysms[10,11], almost always when the neck is wide. Unwanted effects of thrombin injection in arterial false aneurysms are infrequent and can be classified into thrombotic and immunologic. Distal arterial thrombosis after thrombin injection occurs in approximately 0.95% of cases for femoral pseudoaneurysms, generally when the neck is wide, the flow is very fast or the thrombin is injected near the neck and without enough imaging control[12]. The consequences are rarely serious. Thrombin has also been used in another locations such as the common iliac artery[13], radial[14] and visceral arteries without complications[9,15]. The immunologic complications where

described when using bovine thrombin and include the development of antibodies with the potential risk of bleeding and coagulopathy. A number of recent studies have demonstrated that human thrombin does not seem to incur any risk of immunologic sensitization[16].

Endovascular treatment: There is also the possibility of endovascular treatment by placing a stent and using liquid agents like glue. With uncovered stents, the false aneurysm is not excluded from blood flow and it is possible that it might not thrombose, especially when the neck is wide. Through the mesh of these stents it is possible to access into the pseudoaneurysm and embolize it with microcoils, achieving better results than when only the prosthesis alone is used[17,18]. With covered stents the pseudoaneurysm is excluded although these could have potential disadvantages because of technical difficulties in negotiating tortuous vessels and the low flexibility of the endoprosthesis[16].

In our patient, after carrying out an angiography and seeing the features of the pseudoaneurysm as well as hepatic arterial and portal vascularization, it was decided to embolize the afferent and efferent arterial segments with coils. The possibility of positioning a covered stent was considered but, due to the difference in caliber between the afferent and efferent arterial segments, the existence of collaterals for the right hepatic lobe from the upper mesenteric artery and the normal flow in the portal vein we believed it was safer to carry out the embolization of the hepatic artery. After the embolization we checked correct closure of the false aneurysm from the celiac axis and from the superior mesenteric artery. Two days later, a small residual cavity still remained. On this occasion it was not possible repeat the endovascular embolization with coils because the afferent vessel were thin and tortuous and we therefore decided to inject human thrombin percutaneously and with color doppler ultrasound control[9]. The effects were immediate after the injection of human thrombin, leaving the residual light closed. Although we could have used a direct injection with thrombin from the start, we believe that the slow-down in flow provoked by the coils improved the success of the thrombin treatment.

To summarize, for the treatment of visceral pseudoaneurysms it is necessary to know all therapeutic options and to evaluate the convenience of each one. In the absence of studies with a large number of patients to validate one or other treatment as the preferred option, we contribute by adding our experience to the cases reported in the bibliography.

REFERENCES

1 **Croce MA**, Fabian TC, Spiers JP, Kudsk KA. Traumatic hepatic artery pseudoaneurysm with hemobilia. *Am J Surg* 1994; **168**: 235-238

2 **Yoon W**, Jeong YY, Kim JK, Seo JJ, Lim HS, Shin SS, Kim JC, Jeong SW, Park JG, Kang HK. CT in blunt liver trauma. *Radiographics* 2005; **25**: 87-104

3 **Malhotra AK**, Fabian TC, Croce MA, Gavin TJ, Kudsk KA, Minard G, Pritchard FE. Blunt hepatic injury: a paradigm shift from operative to nonoperative management in the 1990s. *Ann Surg* 2000; **231**: 804-813

4 **Velmahos GC**, Toutouzas K, Radin R, Chan L, Rhee P, Tillou A, Demetriades D. High success with nonoperative management of blunt hepatic trauma: the liver is a sturdy organ. *Arch Surg* 2003; **138**: 475-480; discussion 480-481

5 **David Richardson J**, Franklin GA, Lukan JK, Carrillo EH, Spain DA, Miller FB, Wilson MA, Polk HC Jr, Flint LM. Evolution in the management of hepatic trauma: a 25-year perspective. *Ann Surg* 2000; **232**: 324-330

6 **Pachter HL**, Knudson MM, Esrig B, Ross S, Hoyt D, Cogbill T, Sherman H, Scalea T, Harrison P, Shackford S. Status of nonoperative management of blunt hepatic injuries in 1995: a multicenter experience with 404 patients. *J Trauma* 1996; **40**: 31-38

7 **Basile KE**, Sivit CJ, Sachs PB, Stallion A. Hepatic arterial pseudoaneurysm: a rare complication of blunt abdominal trauma in children. *Pediatr Radiol* 1999; **29**: 306-308

8 **Sun L**, Guan YS, Wu H, Pan WM, Li X, He Q, Liu Y. Post-traumatic hepatic artery pseudo-aneurysm combined with subphrenic liver abscess treated with embolization. *World J Gastroenterol* 2006; **12**: 2798-2799

9 **Dambrin C**, Marcheix B, Birsan T, Cron C, Muscari F, Suc B, Cérène A, Rousseau H. Posttraumatic pseudoaneurysm of the hepatic artery: treatment with ultrasound-guided percutaneous transhepatic thrombin injection. *J Trauma* 2005; **59**: 239-242

10 **Vázquez V**, Reus M, Morales MD, Abellán J, Piñero A, Soria F, Parrilla P. [Usefulness of sonographically guided thrombin injection of iatrogenic femoral pseudoaneurysms] *Med Clin (Barc)* 2003; **121**: 53-57

11 **Vázquez V**, Reus M, Piñero A, Abellán D, Canteras M, Espinosa de Rueda M, Morales D, Parrilla P. Human thrombin for treatment of pseudoaneurysms: comparison of bovine and human thrombin sonogram-guided injection. *AJR Am J Roentgenol* 2005; **184**: 1665-1671

12 **Kang SS**, Labropoulos N, Mansour MA, Michelini M, Filliung D, Baubly MP, Baker WH. Expanded indications for ultrasound-guided thrombin injection of pseudoaneurysms. *J Vasc Surg* 2000; **31**: 289-298

13 **Reus M**, Morales D, Vázquez V, Llorente S, Alonso J. Ultrasound-guided percutaneous thrombin injection for treatment of extrarenal pseudoaneurysm after renal transplantation. *Transplantation* 2002; **74**: 882-884

14 **Reus M**, Vázquez V, Alonso J, Morales D, Rodríguez JM. Treatment of a radial artery pseudoaneurysm with ultrasound-guided percutaneous thrombin injection in a patient with Behçet's syndrome. *J Clin Ultrasound* 2003; **31**: 440-444

15 **Krueger K**, Zaehringer M, Lackner K. Percutaneous treatment of a splenic artery pseudoaneurysm by thrombin injection. *J Vasc Interv Radiol* 2005; **16**: 1023-1025

16 **Paci E**, Antico E, Candelari R, Alborino S, Marmorale C, Landi E. Pseudoaneurysm of the common hepatic artery: treatment with a stent-graft. *Cardiovasc Intervent Radiol* 2000; **23**: 472-474

17 **Ichihara T**, Sato T, Miyazawa H, Shibata S, Hashimoto M, Ishiyama K, Yamamoto Y. Stent placement is effective on both postoperative hepatic arterial pseudoaneurysm and subsequent portal vein stricture: a case report. *World J Gastroenterol* 2007; **13**: 970-972

18 **Hartnell GG**, Gates J. Treatment of gastroduodenal artery hemorrhage with a conventional stent. *J Vasc Interv Radiol* 1999; **10**: 172-174

S-Editor Wang YR 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 Feburary 27; 2(2): 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.

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

Olivier Detry, MD, PhD, Department of Abdominal Surgery and Transplantation, CHU de Liège, University of Liège, Sart Tilman B35, B4000 Liège, Belgium

Claus J Fimmel, MD, Professor, Department of Internal Medicine, Division of Gastroenterology, Hepatology and Nutrition Loyola University Medical Center Staff Physician, GI Section, Edward Hines, Jr. VA Medical Cente, Division of Gastroenterology, Hepatology, and Nutrition, 2160 S. First Avenue, Building 54, Room 007, Maywood, IL 60153, United States

Feng-Zhi Li, Associate Professor of Oncology, Department of Pharmacology and Therapeutics, Director, FLI-78 Drug Discovery & Development Program, Roswell Park Cancer Institute (RPCI), Elm & Carlton Streets, Buffalo, NY 14263, United States

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

Lucia Malaguarnera, Associate Professor, MD, PhD, Department of Biomedical Sciences, University, Via E. De Amicis, 24 Trecastagni, Catania 95039, Italy

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

Dr. Kelvin K Ng, PhD, Department of Surgery, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong, China

Dr. Patrick Veit-Haibach, MD, University Hospital Zürich, Department of Nuclear Medicine, Raemistrasse 100, Zürich 80941, Switzerland

Xiu-Jie Wang, Professor, Laborotary of Geritrics, Laborotary of Experimental Oncology, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, No.37 Guoxue Xiang, Chengdu 610041, Sichuan Province, China

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

Yusuf Yilmaz, MD, Department of Gastroenterology, Marmara University School of Medicine, Tophanelioglu cad. No:13/15, Altunizade, Istanbul 34662, Turkey

World Journal of Hepatology

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

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

Instructions to authors

GENERAL INFORMATION

World Journal of Hepatology (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a monthly, 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.