ISSN 2220-6132 (online)

World Journal of *Translational Medicine*

World J Transl Med 2014 April 12; 3(1): 1-36

Baishideng Publishing Group
www.wjgnet.com

WJTM World Journal of Translational Medicine

A peer-reviewed, online, open-access journal of translational medicine

Editorial Board

2012-2016

The *World Journal of Translational Medicine* Editorial Board consists of 525 members, representing a team of worldwide experts in translational medicine. They are from 47 countries, including Argentina (2), Australia (18), Austria (6), Belgium (1), Brazil (6), Canada (11), Chile (1), China (47), Cuba (2), Czech Republic (2), Denmark (1), Egypt (2), Finland (1), France (16), Germany (17), Greece (11), Hungary (2), India (20), Iran (3), Israel (13), Italy (45), Japan (20), Jordan (1), Malaysia (4), Mexico (6), Netherlands (12), Nigeria (2), Peru (1), Poland (3), Portugal (1), Russia (1), Saudi Arabia (1), Senegal (1), Singapore (3), South Africa (1), South Korea (6), Spain (22), Sri Lanka (2), Sweden (6), Switzerland (5), Thailand (2), Tunisia (1), Turkey (9), United Arab Emirates (1), United Kingdom (28), United States (158), and Venezuela (1).

Yu-Ping Ran, *Chengdu*
Jian-Yong Shao, *Guangzhou*
Xue-Jun Sun, *Shanghai*
Chi-Chiu Wang, *Hong Kong*
Gang Wang, *Chengdu*
Min Wang, *Guangzhou*
Ning Wang, *Beijing*
Shu-Lin Wang, *Shanghai*
Yu-Lan Wang, *Wuhan*
Zhan-You Wang, *Shenyang*
Mian Wu, *Hefei*
Wei Wu, *Beijing*
Dong Xie, *Shanghai*
Chuan-Shan Xu, *Hong Kong*
Shun-Qing Xu, *Wuhan*
Hong Yu, *Hangzhou*
Zhi-Ling Yu, *Hong Kong*
Peng Zhang, *Shenzhen*
Shi-Cui Zhang, *Qingdao*
Xiao-Nong Zhou, *Shanghai*
Xiao-Feng Zhu, *Guangzhou*
Xue-Qiong Zhu, *Wenzhou*

Cuba

Maria G Guzman, *Havana*
Yasser Iturria Medina, *Ciudad Habana*

Czech Republic

Martin Huser, *Brno*
Kamil Kuca, *Hradec Kralove*

Denmark

Claus Yding Andersen, *Copenhagen*

Egypt

Olfat Gamil Shaker, *Cairo*
Mohamed AFM Youssef, *Cairo*

Finland

Riitta Anneli Suhonen, *Turku*

France

Mouad Alami, *Chatenay Malabry*
Nadia Alfaidy, *Grenoble*
Bernard Binetruy, *Marseille*
Alain Braillon, *Amiens*
Bruno Dubois, *Paris*
Philippe Gervois, *Lille*
Gilles Gosselin, *Montpellier*
Cyril Goudet, *Montpellier*
Nicholas Huntington, *Saint Ouen*
Bechir Jarraya, *Paris*
Guido Kroemer, *Villejuif*
Claire Lugnier, *Illkirch*
Jean-Luc Martinot, *Orsay*
Anna Patrikidou, *Villejuif*
Zuzana Saidak, *Paris*
Georgios Stamatas, *Issy-les-Moulineaux*

Germany

Wolf-Rainer Abraham, *Braunschweig*
Heike Allgayer, *Mannheim*
Thomas Bock, *Berlin*
Stefan R Bornstein, *Dresden*
Guo Cheng, *Dortmund*
Rupert Conrad, *Bonn*
Hassan Dihazi, *Goettingen*
Joachim Drevs, *Herdecke*
Thomas Efferth, *Mainz*
Benedikt Fritzsching, *Heidelberg*
Frank Norbert Gellerich, *Magdeburg*
Ioanna Gouni-Berthold, *Cologne*
Axel M Gressner, *Aachen*
Wolfgang E Jelkmann, *Luebeck*
Walter Paulus, *Gottingen*
Joerg F Schlaak, *Essen*
Klaus Schulze-Osthoff, *Tubingen*

Greece

Evangelos C Alexopoulos, *Athens*
Demosthenes Bouros, *Alexandroupolis*
Ioannis Kotsianidis, *Alexandroupolis*
Vaios Karanikas, *Larissa*
Dimitrios H Roukos, *Ioannina*
Lazaros Sakkas, *Larissa*
Andreas Scorilas, *Athens*
Alexandros Sotiriadis, *Panorama*
Nicholas Tentolouris, *Athens*
Andrew Tsotinis, *Athens*
Sotirios G Zarogiannis, *Larissa*

Hungary

Istvan Hermecz, *Budapest*
Bela Melegh, *Pecs*

India

Ritesh Agarwal, *Chandigarh*
Manjeshwar Shrinath Baliga, *Mangalore*
Prakash S Bisen, *Gwalior*
Koel Chaudhury, *West Bengal*
Srinivas Gopala, *Kerala*
Sudeep Gupta, *Mumbai*
Vivekanand Jha, *Chandigarh*
Guruprasad Kalthur, *Manipal*
Indu Pal Kaur, *Chandigarh*
Anisur R Khuda-Bukhsh, *Kalyani*
Sudhir Krishna, *Mumbai*
Nirmal Kumar Lohiya, *Jaipur*
Saumen Kumar Maitra, *Santiniketan*
P Manikandan, *Tamil Nadu*
Ida Parwati, *Bandung*
Yenamandra S Prabhakar, *Lucknow*
HS Randhawa, *New Delhi*
Shaival K Rao, *Wadhwan*
Syed Ibrahim Rizvi, *Allahabad*
Shyam Sundar, *Varanasi*

Iran

Leila Azadbakht, *Isfahan*
Seyed Javad Mowla, *Tehran*
Amirhossein Sahebkar, *Mashhad*

Israel

Rachel Bar-Shavit, *Jerusalem*
Zeev Blumenfeld, *Haifa*
Eliezer Flescher, *Tel Aviv*
Vadim Fraifeld, *Beer Sheva*
Oren Froy, *Rehovot*
Eva Gak, *Tel Hashomer*
Jacob George, *Rehovot*
Rafael Gorodischer, *Beer-Sheva*
Ariel Miller, *Haifa*
Zvi Naor, *Tel Aviv*
Rachel Sarig, *Rehovot*
Moshe Schaffer, *Zefat*
Yosef Shiloh, *Tel Aviv*

Italy

Walter Arancio, *Palermo*
Luca Arcaini, *Pavia*
Giuseppe Argenziano, *Naples*
Elisabetta Baldi, *Florence*
Tiziano Barbui, *Bergamo*
Saverio Bettuzzi, *Parma*
Giuseppe Biondi-Zoccai, *Latina*
Giampaolo Bresci, *Pisa*
Giuseppe Maurizio Campo, *Messina*
Andrea Cavalli, *Bologna*
Mario Cazzola, *Roma*
Massimo Chello, *Rome*
Graziamaria Corbi, *Portici*
Silvia Deaglio, *Turin*
Paolo Francesco Fabene, *Verona*
Alfio Ferlito, *Udine*
Daniela Galimberti, *Milan*
Giuseppe Giannini, *Pomezia*
Paolo Gisondi, *Verona*
Fabio Grizzi, *Milan*
Massimo Guidoboni, *Meldola*
Pietro Invernizzi, *Rozzano*
Giuseppe Ippolito, *Rome*
Angelo A Izzo, *Naples*
Francesco Landi, *Rome*
Paolo Lanzetta, *Udine*
Michele Malaguarnera, *Catania*
Francesco Marotta, *Milano*
Nicola Micale, *Messina*
Simone Mocellin, *Padova*
Francesco Novelli, *Turin*
Stefano Palomba, *Catanzaro*
Francesco Perticone, *Catanzaro*
Elia Ranzato, *Alessandria*
Graziano Riccioni, *San Severo*
Luigi Fabrizio Rodella, *Brescia*
Pantaleo Romanelli, *Pozzilli*
Giovanna Romeo, *Latina*
Sergio Rutella, *Rome*
Daniele Santini, *Rome*
Luca Sigalotti, *Aviano*
Claudiu T Supuran, *Firenze*
Giovanni Tarantino, *Naples*
Bruno Vincenzi, *Rome*

Japan

Takaaki Arigami, *Kagoshima*

Katsuya Dezaki, *Tochigi*
Shotaro Enomoto, *Wakayama*
Ryuji Fukuzawa, *Fuchu*
Akira Hokama, *Okinawa*
Kenji Kabashima, *Kyoto*
Terumi Kamisawa, *Tokyo*
Takeshi Maruo, *Kobe*
Tomoshige Matsumoto, *Osaka*
Tatsuya Mimura, *Tokyo*
Koh-ichi Nagata, *Aichi*
Kazuaki Nishio, *Tokyo*
Nobuhiko Oridate, *Sapporo*
Katsutoshi Ozaki, *Tochigi*
Keizo Takenaga, *Izumo*
Hirokazu Tsukahara, *Okayama*
Noboru Uchide, *Tokyo*
Kiyotsugu Yoshida, *Tokyo*
Naohisa Yoshida, *Kyoto*
Hitoshi Yoshiji, *Nara*

Jordan

Moamar Al-Jefout, *Karak*

Malaysia

Hean Teik Ong, *Penang*
Mong How Ooi, *Kuching*
Azarisman MS Shah, *Kuantan*
Chandrashekhar T Sreeramareddy, *Kajang*

Mexico

Gabriel Gutierrez-Ospina, *Mexico Cty*
Jorge Morales-Montor, *Mexico Cty*
Martha Sonia Morales Rios, *Mexico Cty*
Marvin A Soriano-Ursua, *Mexico Cty*
Julio Sotelo, *Mexico Cty*

Netherlands

Dahan Albert, *Leiden*
Ronny de Nijs, *Eindhoven*
Pim van der Harst, *Groningen*
Harish C Gugnani, *Kratendijk*
Pim MW Janssens, *Arnhem*
Cornelis Melief, *Leiden*
Gerard Pasterkamp, *Utrecht*
Timothy Radstake, *Nijmegen*
Joris JTH Roelofs, *Amsterdam*
Ronit Shiri-Sverdlov, *Maastricht*
Paul Peter Tak, *Amsterdam*
Charles J Vecht, *Hague*

Nigeria

Ayodele Samuel Jegede, *Ibadan*
Akintunde Sowunmi, *Ibadan*

Peru

Luis Huicho, *Lima*

Poland

Alicja Kasperska-Zahac, *Katowice*
Krzysztof Ksiazek, *Poznan*
Jolanta Slowikowska-Hilczer, *Lodz*

Portugal

Natesan Balasubramanian, *Ponta Delgada*

Russia

Alexander Khalyavkin, *Moscow*

Saudi Arabia

Jaffar Al Tawfiq, *Dhahran*

Senegal

Badara Cisse, *Dakar*

Singapore

Anqi Qiu, *Singapore*
Rob M van Dam, *Singapore*
Shu Wang, *Singapore*

South Africa

Marlon Eugene Cerf, *Cape Town*

South Korea

Jae Chan Kim, *Seoul*
Dong Ryul Lee, *Seoul*
Jung Eun Lee, *Seoul*
Myeong Soo Lee, *Daejeon*
Yong Chul Lee, *Jeonju*
Hong-Gyun Wu, *Seoul*

Spain

Ignacio Torres Aleman, *Madrid*
Jaime Arias, *Madrid*
Pablo Avanzas, *Oviedo*
Jose Marco Contelles, *Madrid*
Martin Bermudo Francisco, *Seville*
Carmen Gomez-Guerrero, *Madrid*
Francisco J Lopez-Hernandez, *Salamanca*
Jose Luno, *Madrid*
Miguel Marcos, *Salamanca*
Luis Menendez-Arias, *Madrid*
Faustino Mollinedo, *Salamanca*
Alberto Ortiz, *Madrid*
Jesus Pintor, *Madrid*
Jesus Prieto, *Pamplona*
Eugenia Resmini, *Barcelona*
Juan Pablo Rodrigo, *Asturias*
Diego Ruano, *Sevilla*
Juan Sastre, *Valencia*
Rafael Simo, *Barcelona*
Sergio Vano-Galvan, *Madrid*
Fernando Vidal-Vanaclocha, *Madrid*
Enrique Zapater-Latorre, *Valencia*

Sri Lanka

Suneth B Agampodi, *Anuradhapura*
Preethi V Udagama-Randeniya, *Colombo*

Sweden

Stefan R Hansson, *Lund*
Stefan Karlsson, *Lund*
Marek J Los, *Linkoping*
Roger Olsson, *Lund*
Uffe Ravnskov, *Lund*
Shao-Nian Yang, *Stockholm*

Switzerland

Pieter Borger, *Basel*
Gilbert Lefevre, *Basel*
Joan Muela Ribera, *Neuchatel*
Norman Sartorius, *Geneva*
Xiao Yan Zhong, *Basel*

Thailand

Somchai Pinlaor, *Khon Kaen*
Viroj Wiwanitkit, *Bangkok*

Tunisia

Fekri Abroug, *Monastir*

Turkey

Ozkan Ates, *Tekirdag*
Mehmet Baykara, *Bursa*
Gulbeyaz Can, *Istanbul*
Ayse Kalkanci, *Ankara*
Suleyman Kaplan,*Samsun*
Oral Oncul, *Uskudar Istanbul*
Selda Secginli, *Istanbul*
Asli Gamze Sener, *Izmir*
Fatih Tanriverdi, *Kayseri*

United Arab Emirates

Taleb H Al-Tel, *Sharjah*

United Kingdom

Sabine Bahn, *Cambridge*
Dominique Bonnet, *London*
David A Brindley, *Oxford*
Darren R Brooks, *Salford*
Vincent P Collins, *Cambridge*
William Davies, *Cardiff*

Mohamed El-Tanani, *Belfast*
Lars-Peter Erwig, *Aberdeen*
Anthony R Fooks, *Addlestone*
Christopher L Jackson, *Bristol*
Royston Jefferis, *Birmingham*
Marios Kyriazis, *London*
Kenneth Edward Louis McColl, *Glasgow*
Hugh P McKenna, *Coleraine*
Ghulam Nabi, *Dundee*
Gladys Onambele-Pearson, *Cheshire*
Anthony Paul, *London*
Alberto Pertusa, *London*
Stefano Pluchino, *Cambridge*
Marios Politis, *London*
Camillo Porcaro, *Newcastle*
Emmanuel Stamatakis, *Hove*
Gijsbert Stoet, *Leeds*
Ying Sun, *London*
Solomon Tesfaye, *Sheffield*
Bijay Vaidya, *Exeter*
Ping Wang, *London*
Roger Stanley Williams, *London*

United States

Edward Abraham, *Birmingham*
Ron A Adelman, *Hamden*
Hossam M Ashour, *Detroit*
Iris Asllani, *New York*
Aline M Betancourt, *New Orleans*
Vineet Bhandari, *New Haven*
Guoyin Bing, *Lexington*
Philip John Brooks, *Bethesda*
David L Brown, *Stony Brook*
Lawrence P Carter, *Little Rock*
Manuel F Casanova, *Louisville*
Pietro Ceccato, *Palisades*
Amy Suzon Chappell, *Indianapolis*
Georgia Zhuo Chen, *Atlanta*
Xiaozhuo Chen, *Athens*
Undurti N Das, *Shaker Heights*
Shuo Dong, *Houston*
Raimon Duran-Struuck, *Boston*
Guo-Chang Fan, *Cincinnati*
Bingliang Fang, *Houston*
Timothy S Fenske, *Milwaukee*
Christopher Robert Flowers, *Atlanta*
Felipe Fregni, *Boston*
Richard A Gatti, *Los Angeles*
Leonid A Gavrilov, *Chicago*
Yubin Ge, *Detroit*
Irene M Ghobrial, *Boston*
Antonio Giordano, *Philadelphia*
Shannon S Glaser, *Temple*
Elbert D Glover, *College Park*
Ajay Goel, *Dallas*
Ahmet Gokce, *New Orleans*
Daniel Mordechai Goldenholz, *Sacramento*
Michael P Goodman, *Davis*
Mitchell H Grayson, *Milwaukee*
Valentina Grishko, *Mobile*
Zongsheng Guo, *Pittsburgh*
Qian Han, *Blacksburg*
ShouWei Han, *Atlanta*
Kuzhuvelil B Harikumar, *Richmond*
Biyu Jade He, *Bethesda*
David W Hein, *Louisville*
Zdenek Hel, *Birmingham*
Nuria Homedes, *El Paso*
Matthew Owen Howard, *Chapel Hill*
Robert H Howland, *Pittsburgh*
Victor J Hruby, *Tucson*
Xianxin Hua, *Philadelphia*
Shile Huang, *Shreveport*
Wendong Huang, *Duarte*
Hao Jiang, *La Jolla*
Kristopher T Kahle, *Boston*
Toshiaki Kawakami, *La Jolla*
Mark S Kindy, *Charleston*
Siva Kumar Kolluri, *Corvallis*
Thomas R Kosten, *Houston*
Gregory Luke Larkin, *New Haven*
Yun-Zheng Le, *Oklahoma City*
Chun-Ting David Lee, *Baltimore*
Mong-Hong Lee, *Houston*
Peng Lee, *New York*
Nathan Woolf Levin, *New York*
Benyi Li, *Kansas City*
Jie Jack Li, *Wallingford*
Xing-Cong Li, *Oxford*
Yong Li, *Houston*
Shiaw-Yih Lin, *Houston*
Andrea Lisco, *Bethesda*
Peng Liu, *Chapel Hill*
Shujun Liu, *Columbus*
Yang Liu, *Ann Arbor*
Yuchuan Liu, *Philadelphia*
Shi-Jiang Lu, *Marlborough*
Liangsuo Ma, *Houston*
Stephen Magura, *Kalamazoo*
Kenneth Maiese, *Newark*
Francesco M Marincola, *Bethesda*
Tarik F Massoud, *Stanford*
Stuart Maudsley, *Baltimore*
Murielle Mimeault, *Omaha*
Gene D Morse, *Buffalo*
Chulso Moon, *Luthervill*e
Shaker A Mousa, *Albany*
Mihai D Niculescu, *Kannapolis*
Shuji Ogino, *Boston*
SangKon Oh, *Dallas*
Diana S Woodruff Pak, *Philadelphia*
Minggui Pan, *Santa Clara*
Deric M Park, *Charlottesville*
Haydeh Payami, *Albany*
Dominique J Pepper, *Jackson*
David L Perkins, *La Jolla*
Andras Perl, *Syracuse*
Ilona Petrikovics, *Huntsville*
Holly Gwen Prigerson, *Boston*
Wenqing Qi, *Tucson*
Qi Qian, *Rochester*
Xuebin Qin, *Cambridge*
Venky Ramakrishna, *Phillipsburg*
Veena N Rao, *Atlanta*
Suraiya Rasheed, *Los Angeles*
P Hemachandra Reddy, *Beaverton*
Richard E Rothman, *Baltimore*
Thorsten Rudroff, *Boulder*
Irwin J Russell, *San Antonio*
Wolfgang Sadee, *Columbus*
Ahmad Salehi, *Palo Alto*
Michael L Salgaller, *Rockville*
Gaetano Santulli, *New York*
Tor C Savidge, *Galveston*
Bassel E Sawaya, *Philadelphia*
Matthew A Schaller, *Ann Arbor*
Paul Schoenhagen, *Cleveland*
Robert J Schwartzman, *Philadelphia*
Sanjay Sethi, *Buffalo*
Rong Shao, *Springfield*
Behrooz G Sharifi, *Los Angeles*
Hamid Shokoohi, *Washington*
Jasvinder A Singh, *Birmingham*
Ravinder J Singh, *Rochester*
Subhash C Sinha, *La Jolla*
Ilke Sipahi, *Cleveland*
Wanli Smith, *Baltimore*
Xuemei Sui, *Columbia*
Jun Sun, *Chicago*
Xue-Long Sun, *Cleveland*
Charles Buz Swanik, *Newark*
Ming Tan, *Mobile*
Weihong Tan, *Gainesville*
Hirofumi Tanaka, *Austin*
Hugh S Taylor, *New Haven*
Patricia Ann Thistlethwaite, *San Diego*
Robin Thurmond, *San Diego*
Stephen Tomlinson, *Charleston*
Yaping Tu, *Omaha*
Barbara Van Der Pol, *Bloomington*
Chiayeng Wang, *Chicago*
Jieyi Wang, *Abbott Park*
Mingyi Wang, *Baltimore*
Sergio Waxman, *Burlington*
Georg F Weber, *Cincinnati*
Ellen Lori Weisberg, *Boston*
Gregory Thomas Wolf, *Ann Arbor*
Karen Y Wonders, *Dayton*
Savio Lau-Yuen Woo, *Pittsburgh*
Li-Tzy Wu, *Durham*
Zhongcong Xie, *Boston*
Phillip Chung-Ming Yang, *Stanford*
Yihong Yao, *Gaithersburg*
Kyoung-Jin Yoon, *Ames*
John S Yu, *Los Angeles*
Lydia B Zablotska, *San Francisco*
Robert Yuk-Lung Zee, *Boston*
Chang-Guo Zhan, *Lexington*
Hongtao Zhang, *Philadelphia*
Qunwei Zhang, *Louisville*
Xuanping Zhang, *Atlanta*
Guofa Zhou, *Irvine*

Venezuela

Leonor Chacin-Bonilla, *Maracaibo*

WJTM

World Journal of Translational Medicine

Contents

Four-monthly Volume 3 Number 1 April 12, 2014

AIM AND SCOPE

World Journal of Translational Medicine (*World J Transl Med*, *WJTM*, online ISSN 2220-6132, DOI: 10.5528) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJTM publishes articles that report the results of translational medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs. Priority publication will be given to articles concerning diagnosis and treatment of translational medicine diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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

NAME OF JOURNAL
World Journal of Translational Medicine

ISSN
ISSN 2220-6132 (online)

LAUNCH DATE
June 12, 2012

FREQUENCY
Four-monthly

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Translational Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai,
Hong Kong, China
Fax: +852-6555-7188
Telephone: +852-3177-9906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
April 12, 2014

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2220-6132/g_info_20100722180909.htm.

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

World Journal of Translational Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5528/wjtm.v3.i1.1

World J Transl Med 2014 April 12; 3(1): 1-8
ISSN 2220-6132 (online)

REVIEW

Epithelial ovarian cancer: An overview

Arpita Desai, Jingyao Xu, Kartik Aysola, Yunlong Qin, Chika Okoli, Ravipati Hariprasad, Ugorji Chinemerem, Candace Gates, Avinash Reddy, Omar Danner, Geary Franklin, Anachebe Ngozi, Guilherme Cantuaria, Karan Singh, William Grizzle, Charles Landen, Edward E Partridge, Valerie Montgomery Rice, E Shyam P Reddy, Veena N Rao

Arpita Desai, Department of Internal Medicine, University of Buffalo, Erie County Medical Center, Buffalo, NY 14215, United States
Jingyao Xu, Kartik Aysola, Yunlong Qin, Chika Okoli, Candace Gates, Avinash Reddy, Geary Franklin, Anachebe Ngozi, Valerie Montgomery Rice, E Shyam P Reddy, Veena N Rao, Department of OB/GYN, Cancer Biology Program, Morehouse School of Medicine, Georgia Cancer Center for Excellence, Grady Health System, Atlanta, GA 30303, United States
Ravipati Hariprasad, Ugorji Chinemerem, Department of Internal Medicine, Morehouse School of Medicine, Grady Health System, Atlanta, GA 30303, United States
Omar Danner, Department of Surgery, Morehouse School of Medicine, Grady Health System, Atlanta, GA 30303, United States
Guilherme Cantuaria, NCI Cancer Program, Northside Hospital, Atlanta, GA 30342, United States
Karan Singh, William Grizzle, Charles Landen, Edward E Partridge, University of Alabama at Birmingham, Comprehensive Cancer Center, Birmingham AL 35294, United States
Author contributions: All the authors contributed to this paper.
Supported by In part by Georgia Cancer Coalition Distinguished Cancer Scholar award, NIH-NCRR-RCMI, No. G-12-RR003034, No. U54 RR02613, and No. 5P20RR11104; NIHMD research endowment, No. 2S21MD000101, and No. U54CA118638; and ING foundation grant to Rao VN
Correspondence to: Veena N Rao, Professor and Co-Director Cancer Biology Program, GCC Distinguished Cancer Scholar, Department of OB/GYN, Cancer Biology Program, Morehouse School of Medicine, Georgia Cancer Center for Excellence, Grady Health System, Room 10C011, 80 Jesse Hill Jr. Drive, Atlanta, GA 30303, United States. vrao@msm.edu
Telephone: +1-404-4899993 Fax: +1-404-4899220
Received: November 6, 2013 Revised: February 17, 2014
Accepted: Feburary 20, 2014
Published online: April 12, 2014

Abstract

Ovarian cancer is the second most common gynecological cancer and the leading cause of death in the United States. In this article we review the diagnosis and current management of epithelial ovarian cancer which accounts for over 95 percent of the ovarian malignancies. We will present various theories about the potential origin of ovarian malignancies. We will discuss the genetic anomalies and syndromes that may cause ovarian cancers with emphasis on Breast cancer type 1/2 mutations. The pathology and pathogenesis of ovarian carcinoma will also be presented. Lastly, we provide a comprehensive overview of treatment strategies and staging of ovarian cancer, conclusions and future directions.

Key words: Epithelial ovarian cancer; Breast cancer type 1; Chemotherapy

Core tip: Epithelial ovarian cancer (EOC) is one of the most common gynecological cancers. We present a number of theories on the origin of EOC including the recent hypothesis that the fallopian tube is the primary site of most serous carcinomas. We also discuss genetic anomalies that may cause ovarian cancer. The pathology of ovarian cancer by malignant transformation of the epithelium of the ovarian surface, peritoneum or fallopian tube is also presented. Finally we provide an overview of ovarian cancer treatment options, comparing various chemotherapy regimens and future predictive biomarkers and functional assays for targeted therapy for breast cancer type 1 associated EOC.

Desai A, Xu J, Aysola K, Qin Y, Okoli C, Hariprasad R, Chinemerem U, Gates C, Reddy A, Danner O, Franklin G, Ngozi A, Cantuaria G, Singh K, Grizzle W, Landen C, Partridge EE, Rice VM, Reddy ESP, Rao VN. Epithelial ovarian cancer: An overview.

World J Transl Med 2014; 3(1): 1-8 Available from: URL: http://www.wjgnet.com/2220-6132/full/v3/i1/1.htm DOI: http://dx.doi.org/10.5528/wjtm.v3.i1.1

INTRODUCTION

Amongst gynecologic malignancies ovarian cancer is the second most common and the #1 cause of death[1]. In this article we review the diagnosis and current management of epithelial ovarian cancer (EOC) which accounts for over 95 percent of the ovarian malignancies[2]. The clinical presentation of EOC can either be acute or subacute. Acute cases present with conditions such as pleural effusion, small bowel obstruction and venous thromboembolism. Subacute cases may present with non specific symptoms such as abdominal fullness, bloating, an adnexal mass, vague pelvic or abdominal pain and gastrointestinal symptoms.

In the following review, we initially present various intriguing theories about the potential origin of ovarian malignancies. Subsequently, we discuss in depth genetic anomalies and syndromes that may cause ovarian cancers with emphasis on breast cancer type 1 (BRCA1)/2 mutations (section Ⅱ). Next, the pathology and pathogenesis of ovarian carcinoma is presented (Section Ⅲ). Finally, we provide a comprehensive overview of treatment strategies and staging of ovarian cancer (Section Ⅳ), conclusions (Section Ⅴ) and future directions (Ⅵ).

ORIGIN

The cellular origin of EOC is not well known thus hampering the development of effective early detection methods. Sir Spencer Wells in 1872 claimed that EOCs arose from ovarian surface epithelial (OSE) cells[3]. This hypothesis is supported by Cheng's work on *HOX* genes[4], which shows that ectopic expression of *HOXA9*, *HOXA10* and *HOXA11* genes in mouse OSE cells followed by intraperitoneal (IP) injection into mice resulted in the establishment of cancers of serous, endometrioid and mucinous subtypes, respectively. Inclusion cysts lined by OSE were considered as precursors for EOCs in the past[4]. However the above theory has been refuted by a number of studies[5]. For example, a prospective study of 48230 postmenopausal women who had no family history of ovarian cancer but had annual pelvic ultrasounds was conducted in 2010. Investigators found that after a median follow-up of 6 years there was no increase in the development of EOC in women who had inclusion cysts in their initial study. Yet in another study[6] it has been proposed that ovarian cancers arise from secondary Mullerian tract structures as they are indistinguishable from adjacent mesothelial cells lining the peritoneum.

However, in the last 5-10 years, several studies have proposed that EOCs should no longer be considered as a single disease entity. For example, high-grade serous ovarian cancers (HGSOC), which represent the most common type of invasive EOC, are exemplified by the ubiquitous presence of TP53 mutations[7]. In a detailed analysis of the fallopian tubes of patients who underwent prophylactic salpingo-oophorectomies, histologic studies showed that tubal lesions were present in almost 100% of cases of early serous cancers associated with familial BRCA mutations. This lends evidence to the hypothesis that fallopian tube cells may play an important role in the genesis of BRCA-associated HGSOC. Kindelberger *et al*[8] have proposed that cancer cells are shed from the tubal epithelium and implanted on the surface of the ovary and most likely become trapped within the surface inclusion cysts to produce ovarian or primary peritoneal carcinomas.

Recently Nik *et al*[9] hypothesized that the fallopian tube is the primary site of most serous carcinomas. Evidence supporting this hypothesis includes: (1) more than 50% of sporadic pelvic high grade serous carcinomas have serous tubal intra-epithelial cancers (STIC); (2) STICs have also been found in 10%-15% of fallopian tubes prophylactically removed from women with BRCA mutations; (3) 92% of STICs have been shown to have TP53 mutation similar to those found in concordant high grade serous carcinoma samples; (4) STIC oncogene productions such as cyclin E1, Rsf-1, and fatty acid synthase, are also overexpressed in HGSCs; and (5) STICs have been found to be present in prophylactic salpingectomy specimens in the absence of carcinoma which lends evidence against the view that STICs are formed due to metastasis from adjacent HGSCs.

GENETIC ANOMALIES AND SYNDROMES

Individuals from families with multiple cancers, cancers occurring at an early age, and two or more primary cancers in a single individual have a higher risk of hereditary ovarian cancer syndromes. A recent Cancer Genomic Atlas project analyses of mRNA expression, miRNA expression, promoter methylation, and DNA copy number in 489 high-grade serous ovarian adenocarcinomas revealed TP53 mutations in almost all tumors (65). Approximately 13% had germ line mutations in BRCA1 or BRCA2 and a small percentage had somatic mutations in NF1, RB1 and CDK12[10]. Analysis also showed four ovarian cancer transcriptional subtypes, three miRNA subtypes, four promoter methylation subtypes and signatures associated with survival (65). Furthermore pathway analyses showed defect in homologous recombination, Notch and FOXM1 signaling was involved in the pathophysiology of serous ovarian cancer[10].

The majority of hereditary ovarian cancers are associated with mutations in tumor suppressor genes, *BRCA1* and *BRCA2*. BRCA1 (susceptibility protein) encoded by *BRCA1* gene (located on the long arm of chromosome 17) is a nuclear-cytoplasmic shuttling protein, and many cancer-associated mutations have altered subcellular localization of BRCA1 protein[11]. BRCA1 also plays a role in DNA repair by transcription regulation, chromatin

remodeling, homologous recombination, and cell cycle regulation. BRCA1 mutations function as either truncating or missense mutations interfering with critical regions of the gene such as the *RING* finger motif or *BRCT* region of the gene. BRCA2 (susceptibility protein) is encoded by *BRCA2* gene (located on long arm of chromosome 13), plays a crucial role in repair of double stranded DNA breaks. It has been observed that the risk of ovarian cancer is higher in women with BRCA1 mutation as compared to BRCA2 mutation[12,13]. The cumulative risk of ovarian cancer by age 70 is estimated to be 40%-50% for BRCA1 mutation-carriers and 10%-25% for BRCA2 mutation carriers[14]. Furthermore, BRCA1 mutations are also associated with earlier onset[12]. In one study, the average age at diagnosis of ovarian cancer in BRCA1 and BRCA2 mutation carriers was 52 and 62 years respectively[15].

BRCA associated cancer risks are determined by the mutation location and genetic variation of the *BRCA1* and *BRCA2* gene function. Mutations occurring within the central region of the *BRCA2* gene, called the ovarian cancer cluster region, compared to mutations in the 5' or 3' region, may be associated with a significantly higher risk of ovarian cancer in women[16].

Research has also shown that the BRCA1 Associated Ring Domain 1 (BARD1) protein which binds to the BRCA1 protein stabilizes both proteins and targets the BRCA1 to sites where DNA strands are broken. Mutations in the *BARD1* gene may prevent the BARD1 protein from helping repair damaged DNA. Thus, BARD1 isoform expression is required for cancer cell proliferation, thereby making them cancer maintenance genes[17]. Studies also suggest that the BARD1 protein has other functions different from its partnership with the BRCA1 protein. The BARD1 protein interacts with another protein, p53 (which is produced from the *TP53* gene) to promote controlled cell death (apoptosis) and regulate cell division. Other potential functions of the BARD1 protein are under study.

Specific genetic modifiers of cancer penetrance, sometimes called modifier genes, may influence the expression of genes like *BRCA1* or *BRCA2*. Genetic variation, particularly in the genes comprising endocrine signaling and DNA repair pathways, may modify BRCA-associated cancer risks. Another potential modifier gene in BRCA1 and BRCA2 mutation carriers is CYP1A1, which encodes an enzyme involved in the metabolism of polyaromatic hydrocarbons and in the hydroxylation of estradiol[18]. A particular allelic variant, found in 14 percent of carriers with cancer and 22 percent of unaffected carriers, reduced the risk of breast cancer by about 40 percent. Recently Qin *et al*[19] have shown that BRCA1 inhibits the growth of ovarian cancers by regulating Ubc9 binding. With the aid of live imaging of YFP, RFP-tagged BRCA1 and BRCA1a proteins they showed enhanced cytoplasmic localization of mutant BRCA1 proteins in certain types of ovarian cancer cells. The mutant BRCA1 proteins were found to be impaired in their capacity to inhibit growth of ES-2 ovarian cancer cells. Less commonly hereditary non polyposis colorectal cancer which is caused by mutations in mismatch repair genes, MSH2, MLH1, MSH6, PMS1, and PMS2 is also associated with ovarian cancer[20]. Jones *et al*[21] analyzed 42 cases of ovarian clear cell carcinoma and found that 57% of these cases had mutations in ARID1A which suggests that aberrant chromatin remodeling contributes to the pathogenesis of ovarian clear cell carcinoma.

PATHOLOGY

Epithelial cancer of the ovary is thought to derive from malignant transformation of the epithelium of the ovarian surface, peritoneum, or fallopian tube. The exact molecular transformation events causing EOC are not known. Baylin *et al*[22] have shown that epigenetic phenomenon may also play a role. Mutations of the oncogenes HER2, C-myc, K-ras, Akt, and the tumor suppressor gene p53 have been observed[23-25]. The molecular pathways underlying cancer progression are also not well understood. Most research to date have shown that factors associated with reproduction and ovulation have the largest impact[26,27]. Some studies have shown that somatic mutations in *BRCA1*, *BRCA2* and other genes can also lead to cancer progression.

Some of the theories for the pathogenesis of EOC include: (1) repeated ovulation with trauma[28,29]; (2) increased estrogen concentrations as a result of excess gonadotropin secretion[30]; (3) high androgen concentrations[30]; and (4) stromal hyperactivity[31].

Histopathology

The classification of EOC is usually based on the origin of the tumor[32]: (1) About 75% of EOCs are of the serous type. They simulate the lining of the fallopian tube. This histologic variant is often associated with concentric rings of calcification known as psammoma bodies. While there is no established grading scheme for serous ovarian cancer, recent work has suggested a two-tiered system-low-grade and high-grade[33,34]. The work of Meinhold-Heerlein *et al*[35] has shown that low-grade serous carcinomas and serous tumors of low malignant potential involve similar genes and pathways that are distinct from high-grade serous carcinomas. These findings have led some to hypothesize that low-grade carcinomas represent the natural progression of an undetected serous ovarian tumor of low malignant potential; (2) Mucinous tumors usually resemble intestinal or endocervical epithelium. Mucinous tumors are usually large with a median diameter of 18 to 20 cm and tend to remain confined to the ovaries[36]. Furthermore, primary ovarian mucinous tumors can be difficult to distinguish from metastatic mucinous tumors from the colon/rectum, appendix, cervix or pancreas; (3) Endometrioid tumors are hypothesized to sometimes arise from the foci of endometriosis. They are associated with slightly better survival when compared to serous adenocarcinoma, regardless of disease stage[37];

(4) Clear cell carcinomas can also sometimes arise from endometriosis. They derive their name as their histologic features include "clear cells". Prognosis is poor due to an increased risk of venous thromboembolism and decreased response to platinum-and-taxane based chemotherapy regimens[38]; and (5) Brenner tumors, also known as transitional cell tumors because histologically, they resemble transitional epithelium of the bladder. Majority of Brenner tumors are benign. Histologically, there are nests of transitional-type epithelial cells with longitudinal nuclear grooves lying in abundant fibrous stroma.

TREATMENT

There are basically three forms of treatment of ovarian cancer: (1) Surgery; (2) Chemotherapy; and (3) Radiation (rarely used). Surgery is considered the mainstay of treatment for ovarian cancer. Treatment is mainly based on the histologic subtype and the stage at diagnosis, which is determined surgically by a tumor reductive procedure including hysterectomy, removal of ovaries, removal of the omentum, and any other sites of disease feasible to remove. The goal of surgery is to reduce the tumor burden to no visible disease, though an "optimal" status is assigned if no residual lesions are larger than 1 cm. However, other studies[39] designate the status of maximal cytoreduction for tumors less than 3 cm. The following table lists the different stages of EOC: stage I : Cancer is confined to one or both of the ovaries; stage Ⅱ: Either one or both the ovaries are involved and the cancer has spread to uterus and/or fallopian tubes or other sites in the pelvis; stage Ⅲ: Either one or both the ovaries are involved and the cancer has spread to lymph nodes and/or sites outside the pelvis but is still within the abdominal cavity; and stage Ⅳ: Either one or both the ovaries are involved with distant metastasis.

Stages I and II

For stage I epithelial cancers, surgery alone is adequate. Here surgery is used for both staging and therapeutic purposes. Surgery includes hysterectomy, bilateral salpingo-oophorectomy, and omentectomy. Samples of the under surface of the diaphragm, peritoneal washings, pelvic/para-aortic lymph node samples are also examined for completeness. In high-grade ovarian cancer, adjuvant chemotherapy has led to better overall survival rates. Large clinical trials such as EORTC-ACTION and MRC-ICON1 involved patients with stage I and II ovarian cancer who were randomly assigned to adjuvant chemotherapy or observation[40,41]. In EORTC-ACTION, at least 4 cycles of carboplatin/cisplatin based chemotherapy were administered whereas in MRC-ICON1 patients received 6 cycles of single agent carboplatin or cisplatin or platinum-based chemotherapy. Data from both studies showed significant improvement in recurrence-free-survival and overall survival (5 year survival figures were 82% with chemotherapy and 74% with observation with a 95% confidence-interval in the difference of 2%-12%).

Stages Ⅲ and Ⅳ

In the case of stages Ⅲ and Ⅳ cancers, surgery includes total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and debulking of any grossly visible tumor. Cytoreduction is an independent prognostic variable for survival[39,42]. In patients with optimal cytoreduction the median survival was 39 mo as opposed to 17 mo if the surgery was suboptimal[43].

Treatment options for patients with optimally cytoreduced stage Ⅲ disease include conventional intravenous and/or IP chemotherapy. The use of IP cisplatin has shown better progression free survival (PFS) and overall survival[44]. One of the recent studies gynecologic oncology group (GOG)-0172 showed a higher median survival rate of 66 mo for patients receiving chemotherapy *via* the IP route as opposed to 50 mo in patients who received intra-venous cisplatin and paclitaxel ($P = 0.03$)[44]. Another study concluded that IP cisplatin works better on small tumors (< 1 cm) that are platinum responsive[45].

Therapeutic strategies for patients with sub-optimally cytoreduced stage Ⅲ and Ⅳ disease include intravenous and/or IP chemotherapy plus additional cytoreductive surgery in some circumstances. The benefits of cytoreductive surgery have not been well established in advanced disease states. While one study performed by the EORTC showed better survival rates in patients who had debulking surgery after 4 cycles of cyclophosphamide and cisplatin[46]. Another study, GOG-0162, in patients who received paclitaxel and cisplatin with interval cytoreductive surgery did not show any survival benefit[47]. GOG 182-ICON5[48] compared the effectiveness of various combination chemotherapy regimens as first line therapy for patients with stage Ⅲ/Ⅳ EOC who have undergone either optimal or sub-optimal cytoreductive surgery. The study shows that combining either carboplatin or paclitaxel with one of the follow new cytotoxic agents-gemcitabine, poly-ethylene glycol liposomal doxorubicin and topotecan gave favorable results.

A combination of carboplatin and a taxane (paclitaxel or docetaxel) is the standard chemotherapeutic agent for ovarian cancer with clinical response rates > 60% and median time to recurrence usually > 1 year. The combination of carboplatin and paclitaxel regimen is the standard worldwide and is sometimes used as induction chemotherapy, if patients are poor surgical candidates, or the surgeon feels that the primary surgery is not likely to be optimal. The SCOTROC trial[49] compared the use of either docetaxel or paclitaxel in combination with carboplatin as first line chemotherapy and found similar PFS in both cases therefore offering an alternative to paclitaxel.

Recently there have been a number of studies investigating the role of bevacizumab in first-line therapy for ovarian cancer following surgical cytoreduction. Bevacizumab is a humanized-monoclonal antibody against vascular endothelial growth factor (angiogenesis inhibitor). Two trials GOG-0218 and ICON7 have shown improvement in PFS when bevacizumab was added to initial chemotherapy and continued every three weeks for

16 and 12 additional cycles respectively[50,51]. However, overall survival has not been demonstrated, and therefore has not been adopted outside of recurrent disease or the clinical trial setting.

Eskander *et al*[52] have recently proposed a new strategy for combining maximal cytoreductive surgery with intra-operative hyperthermic intraperitoneal chemotherapy (HIPEC) for treatment of advanced stage EOC resulting in promising oncologic outcomes. Currently phase Ⅲ clinical trials are ongoing to test the efficacy of combining cytoreductive surgery with HIPEC.

Recurrent or persistent ovarian epithelial cancer

Recurrent cancer has a wide range of presentations. Patients may present with abdominal distention or pain or symptoms related to sites where the cancer cells have spread to. The most common follow-up procedures include bimanual pelvic examination, serial measurement of CA125, laparotomy and repeating imaging studies. Platinum sensitive cancers (recurrence more than 6 mo since completion of primary therapy) are usually re-treated with a combination of carboplatin and a taxane. The OCEANS trial[53] was conducted to test the efficacy and safety of bevacizumab with gemcitabine and carboplatin (GC) compared with GC alone (*i.e.*, bevacizumab replaced by a placebo) in platinum sensitive recurrent ovarian cancers. Results show a significant improvement in PFS when bevacizumab was used in combination with GC. Platinum resistant disease can be treated with one of several agents that typically provide response rates in the range of 10%-20%. These include bevacizumab, topotecan, gemcitabine, liposomal doxorubicin, and others. The AURELIA trial[54] was one of the first randomized phase Ⅲ trials to demonstrate the benefit of combining bevacizumab with standard chemotherapy regimen resulting in increased PFS (6.7 mo *vs* 3.4 mo) in patients with platinum resistant ovarian cancer.

The NOVEL trial[55] proposed the idea of a "dose-dense" therapy for treating primary ovarian cancer. It is based on the hypothesis that a shortening of the time interval between the administrations of cytotoxic agents is able to achieve better cell kill. They compared the standard 3-wk paclitaxel and carboplatin combination *vs* dose-dense weekly paclitaxel and 3-wk carboplatin for advanced EOC and found that the PFS was significantly better in the former (28 mo *vs* 17.2 mo).

CONCLUSION

Among gynecologic malignancies ovarian cancer is the most lethal. The cellular origin of EOC is not well known. Early theories hypothesized that EOC arises from OSE cells, while more recent ones have proposed that it should no longer be considered as a single disease entity but rather a diverse group of tumors with specific morphologic and genetic characteristics.

Histological differences in the ovarian cancers define a number of subtypes of EOC. Subtypes are named based on the tissue that they closely resemble and include serous, mucinous, endometrioid, clear cell, and transitional cell. Ovarian cancer is also classified as benign, borderline or malignant depending on the degree of epithelial proliferation and stromal invasion.

Treatment of EOC is based on the combination of surgery and chemotherapy. Over the past three decades, optimal surgical cytoreduction, followed by platinum-based chemotherapy has become the standard treatment for advanced ovarian cancer.

FUTURE DIRECTIONS

A recent national institute of health study discovered genomic similarities between basal-like subtype of breast cancer (triple negative breast cancer) and serous ovarian cancer[56]. Computational analyses show that both these cancers are susceptible to agents inhibiting blood vessel growth and chemotherapeutic drugs targeting DNA repair. However, more work is needed in this direction to determine how these findings may be used functionally and clinically.

Another area that has received attention recently is the impact of oxidative stress and BRCA1 mutations in ovarian cancer. Oxidative stress induces DNA damage (as evidenced by increased 8-hydroxydeoxyguanosine) correlates with poor outcomes in ovarian cancer[57]. Martinez-Outschoorn *et al*[58] found that UWB1.289 cells, which contain mutant BRCA1, produce large amounts of hydrogen peroxide leading to oxidative stress and catabolic processes in adjacent stromal fibroblasts *via* stromal NF-κB activation. Catabolism in stromal fibroblasts was also accompanied by the up regulation of MCT4 and decreased Cav-1 expression which signify tumor microenvironment. Furthermore, the study also showed the effect of UWB1.289 could be negated by using the antioxidant N-acetyl-cysteine or by *BRCA1* gene replacement. This suggests that new trials are needed for cancer prevention using antioxidants in hereditary BRCA1 mutations.

Another recent breakthrough in the treatment of recurrent ovarian cancer is in the use of vaccines derived from lysate-pulsed dendritic cells. Kandalaft *et al*[59] reported a study encompassing dendritic cell based autologous whole tumor vaccination and anti-angiogenesis therapy. They took the tumor lysate from patients with recurrent ovarian cancer. The patients were initially treated with intravenous bevacizumab and oral metronomic cyclophosphamide. This was followed by bevacizumab and the vaccination of dendritic cells with autologous tumor lysate, lymphodepletion and administration of a large number of vaccine primed T-cells. The study found that cellular immunotherapy may be used for treatment of recurrent ovarian cancer. However the study included only six subjects and the therapy was found to work for four of them. Thus more work is required in this direction to ascertain the benefits of such vaccines.

Some studies such as those by Bryant *et al*[60] and Farmer *et al*[61] have shown that BRCA-deficient cells are

especially sensitive to chemical inhibitors of poly (ADP-ribose) polymerase (PARP), which plays a critical role in single stranded DNA break repair. The most studied PARP inhibitor Olaparib has shown good results[62] in initial studies in BRCA1 mutated and sporadic ovarian cancer but further studies are needed.

There are a no clear indications for ovarian cancer screening. Testing is recommended for women at high risk such as those with a significant family history, but consultation with a genetic counselor is recommended to discuss limitations and alternatives to genetic testing. Potential screening tests include the blood test for CA-125 marker and transvaginal ultrasound. However these tests are neither sensitive nor specific for ovarian cancer. They may be abnormal in benign conditions such as endometriosis, menstruation, pregnancy, and cancers of fallopian tube, breast or the GI tract. Early results from the United Kingdom Collaborative Trial of Ovarian Cancer Screening showed that combining annual CA-125 tests with ultrasound imaging was useful for detecting ovarian cancers at an earlier stage[63]. The full results of the trial are expected in 2015. Lastly the key to developing targeted therapies for EOC will depend on understanding the biological pathways and targets involved in the development of these cancers. Our results (unpublished work) suggest Ubc9 to be expressed at elevated levels in several ovarian cancers and BRCA1-mutant serous epithelial ovarian cancer cells[19]. We have developed BRCA1 function-based cellular assays (Patent number United States 8372580) where loss of Ubc9 binding by BRCA1 mutants can not only predict the risk for developing Triple Negative Breast Cancers and EOC, but it may lead to the development of targeted therapies for these cancers.

ACKNOWLEDGMENTS

We thank Ms Wimes and Mr. Hill for editorial assistance.

REFERENCES

1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10 [DOI: 10.3322/caac.20138]

2 **Lacey JV**, Sherman ME. Ovarian neoplasia. In: Robboy's Pathology of the Female Reproductive Tract, 2nd ed. Robboy SL, Mutter GL, Prat J eds. Churchill Livingstone Elsevier, Oxford, 2009. p601 [DOI: 10.1016/B978-0-443-07477-6.50028-7]

3 **Hamilton TC**. Ovarian cancer, Part I: Biology. *Curr Probl Cancer* 1992; **16**: 1-57 [PMID: 1547635]

4 **Cheng W**, Liu J, Yoshida H, Rosen D, Naora H. Lineage infidelity of epithelial ovarian cancers is controlled by HOX genes that specify regional identity in the reproductive tract. *Nat Med* 2005; **11**: 531-537 [PMID: 15821746 DOI: 10.1038/nm1230]

5 **Ahmed AA**, Etemadmoghadam D, Temple J, Lynch AG, Riad M, Sharma R, Stewart C, Fereday S, Caldas C, Defazio A, Bowtell D, Brenton JD. Driver mutations in TP53 are ubiquitous in high grade serous carcinoma of the ovary. *J Pathol* 2010; **221**: 49-56 [PMID: 20229506 DOI: 10.1002/path.2696]

6 **Dubeau L**. The cell of origin of ovarian epithelial tumors and the ovarian surface epithelium dogma: does the emperor have no clothes? *Gynecol Oncol* 1999; **72**: 437-442 [PMID: 10053122 DOI: 10.1006/gyno.1998.5275]

7 **Finch A**, Shaw P, Rosen B, Murphy J, Narod SA, Colgan TJ. Clinical and pathologic findings of prophylactic salpingo-oophorectomies in 159 BRCA1 and BRCA2 carriers. *Gynecol Oncol* 2006; **100**: 58-64 [PMID: 16137750 DOI: 10.1016/j.ygyno.2005.06.065]

8 **Kindelberger DW**, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, Callahan MJ, Garner EO, Gordon RW, Birch C, Berkowitz RS, Muto MG, Crum CP. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol* 2007; **31**: 161-169 [PMID: 17255760 DOI: 10.1097/01.pas.0000213335.40358.47]

9 **Nik NN**, Vang R, Shih IM, Kurman RJ. Origin and Pathogenesis of Pelvic (Ovarian, Tubal, and Primary Peritoneal) Serous Carcinoma. *Annu Rev Pathol* 2014; **9**: 27-45 [PMID: 23937438]

10 **The Cancer Genome Atlas Research Network.** Integrated genomic analyses of ovarian carcinoma. *Nature* 2011; **474**: 609-615 [PMID: 21720365 DOI: 10.1038/nature10166]

11 **Habashy HO**, Powe DG, Rakha EA, Ball G, Paish C, Gee J, Nicholson RI, Ellis IO. Forkhead-box A1 (FOXA1) expression in breast cancer and its prognostic significance. *Eur J Cancer* 2008; **44**: 1541-1551 [PMID: 18538561 DOI: 10.1016/j.humpath.2007.10.011]

12 **van der Kolk DM**, de Bock GH, Leegte BK, Schaapveld M, Mourits MJ, de Vries J, van der Hout AH, Oosterwijk JC. Penetrance of breast cancer, ovarian cancer and contralateral breast cancer in BRCA1 and BRCA2 families: high cancer incidence at older age. *Breast Cancer Res Treat* 2010; **124**: 643-651 [PMID: 20204502 DOI: 10.1007/s10549-010-0805-3]

13 **Malone KE**, Begg CB, Haile RW, Borg A, Concannon P, Tellhed L, Xue S, Teraoka S, Bernstein L, Capanu M, Reiner AS, Riedel ER, Thomas DC, Mellemkjaer L, Lynch CF, Boice JD, Anton-Culver H, Bernstein JL. Population-based study of the risk of second primary contralateral breast cancer associated with carrying a mutation in BRCA1 or BRCA2. *J Clin Oncol* 2010; **28**: 2404-2410 [PMID: 20368571 DOI: 10.1200/JCO.2009.24.2495]

14 **Schorge JO**, Modesitt SC, Coleman RL, Cohn DE, Kauff ND, Duska LR, Herzog TJ. SGO White Paper on ovarian cancer: etiology, screening and surveillance. *Gynecol Oncol* 2010; **119**: 7-17 [PMID: 20692025 DOI: 10.1016/j.ygyno.2010.06.003]

15 **Meijers-Heijboer EJ**, Verhoog LC, Brekelmans CT, Seynaeve C, Tilanus-Linthorst MM, Wagner A, Dukel L, Devilee P, van den Ouweland AM, van Geel AN, Klijn JG. Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation. *Lancet* 2000; **355**: 2015-2020 [PMID: 10885351 DOI: 10.1016/S0140-6736(00)02347-3]

16 **Thompson D**, Easton D; Breast Cancer Linkage Consortium. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. *Am J Hum Genet* 2001; **68**: 410-419 [PMID: 11170890 DOI: 10.1086/318181]

17 **Li L**, Ryser S, Dizin E, Pils D, Krainer M, Jefford CE, Bertoni F, Zeillinger R, Irminger-Finger I. Oncogenic BARD1 isoforms expressed in gynecological cancers. *Cancer Res* 2007; **67**: 11876-11885 [PMID: 18089818]

18 **Brunet JS**, Ghadirian P, Rebbeck TR, Lerman C, Garber JE, Tonin PN, Abrahamson J, Foulkes WD, Daly M, Wagner-Costalas J, Godwin A, Olopade OI, Moslehi R, Liede A, Futreal PA, Weber BL, Lenoir GM, Lynch HT, Narod SA. Effect of smoking on breast cancer in carriers of mutant BRCA1 or BRCA2 genes. *J Natl Cancer Inst* 1998; **90**: 761-766 [PMID: 9605646]

19 **Qin Y**, Xu J, Aysola K, Oprea G, Reddy A, Matthews R, Okoli J, Cantor A, Grizzle WE, Partridge EE, Reddy ES, Landen C, Rao VN. BRCA1 proteins regulate growth of ovarian cancer cells by tethering Ubc9. *Am J Cancer Res* 2012; **2**: 540-548 [PMID: 22957306]

20 **Walsh MD**, Buchanan DD, Cummings MC, Pearson SA, Arnold ST, Clendenning M, Walters R, McKeone DM, Spurdle AB, Hopper JL, Jenkins MA, Phillips KD, Suthers GK,

George J, Goldblatt J, Muir A, Tucker K, Pelzer E, Gattas MR, Woodall S, Parry S, Macrae FA, Haile RW, Baron JA, Potter JD, Le Marchand L, Bapat B, Thibodeau SN, Lindor NM, McGuckin MA, Young JP. Lynch syndrome-associated breast cancers: clinicopathologic characteristics of a case series from the colon cancer family registry. *Clin Cancer Res* 2010; **16**: 2214-2224 [PMID: 20215533 DOI: 10.1158/1078-0432.CCR-09-3058]

21 **Jones S**, Wang TL, Shih IeM, Mao TL, Nakayama K, Roden R, Glas R, Slamon D, Diaz LA Jr, Vogelstein B, Kinzler KW, Velculescu VE, Papadopoulos N. Frequent mutations of chromatin remodeling gene ARID1A in ovarian clear cell carcinoma. *Science* 2010; **330**: 228-231 [PMID: 20826764 DOI: 10.1126/science.1196333]

22 **Baylin SB**, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. *Trends Genet* 2000; **16**: 168-174 [PMID: 10729832 DOI: 10.1016/S0168-9525(99)01971-X]

23 **Aunoble B**, Sanches R, Didier E, Bignon YJ. Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer (review). *Int J Oncol* 2000; **16**: 567-576 [PMID: 10675491]

24 **Verri E**, Guglielmini P, Puntoni M, Perdelli L, Papadia A, Lorenzi P, Rubagotti A, Ragni N, Boccardo F. HER2/neu oncoprotein overexpression in epithelial ovarian cancer: evaluation of its prevalence and prognostic significance. Clinical study. *Oncology* 2005; **68**: 154-161 [PMID: 16020953 DOI: 10.1159/000086958]

25 **Havrilesky L**, Darcy kM, Hamdan H, Priore RL, Leon J, Bell J, Berchuck A. Prognostic significance of p53 mutation and p53 overexpression in advanced epithelial ovarian cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2003; **21**: 3814-3825 [PMID: 14551300 DOI: 10.1200/JCO.2003.11.052]

26 **Risch HA**. Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J Natl Cancer Inst* 1998; **90**: 1774-1786 [PMID: 9839517 DOI: 10.1093/jnci/90.23.1774]

27 **Banks E**, Beral V, Reeve G. The epidemiology of epithelial ovarian cancer: a review. *Int J Gynecol Cancer* 1997; **7**: 425 [DOI: 10.1046/j.1525-1438.1997.09756.x]

28 **Casagrande JT**, Louie EW, Pike MC, Roy S, Ross RK, Henderson BE. "Incessant ovulation" and ovarian cancer. *Lancet* 1979; **2**: 170-173 [PMID: 89281]

29 **Fathalla MF**. Incessant ovulation--a factor in ovarian neoplasia? *Lancet* 1971; **2**: 163 [PMID: 4104488 DOI: 10.1016/S0140-6736(71)92335-X]

30 **Cramer DW**, Welch WR. Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. *J Natl Cancer Inst* 1983; **71**: 717-721 [PMID: 6578367]

31 **Cramer DW**, Barbieri RL, Fraer AR, Harlow BL. Determinants of early follicular phase gonadotrophin and estradiol concentrations in women of late reproductive age. *Hum Reprod* 2002; **17**: 221-227 [PMID: 11756392 DOI: 10.1093/humrep/17.1.221]

32 **Serov SF**, Scully RE, Sobin IH. International histological classification of tumors no. 9. Histological typing of ovarian tumours. World Health Organization, Geneva, 1973

33 **Malpica A**, Deavers MT, Lu K, Bodurka DC, Atkinson EN, Gershenson DM, Silva EG. Grading ovarian serous carcinoma using a two-tier system. *Am J Surg Pathol* 2004; **28**: 496-504 [PMID: 15087669 DOI: 10.1097/00000478-200404000-00009]

34 **Gershenson DM**, Sun CC, Bodurka D, Coleman RL, Lu KH, Sood AK, Deavers M, Malpica AL, Kavanagh JJ. Recurrent low-grade serous ovarian carcinoma is relatively chemoresistant. *Gynecol Oncol* 2009; **114**: 48-52 [PMID: 19361839 DOI: 10.1016/j.ygyno.2009.03.001]

35 **Meinhold-Heerlein I**, Bauerschlag D, Hilpert F, Dimitrov P, Sapinoso LM, Orlowska-Volk M, Bauknecht T, Park TW, Jonat W, Jacobsen A, Sehouli J, Luttges J, Krajewski M, Krajewski S, Reed JC, Arnold N, Hampton GM. Molecular and prognostic distinction between serous ovarian carcinomas of varying grade and malignant potential. *Oncogene* 2005; **24**: 1053-1065 [PMID: 15558012 DOI: 10.1038/sj.onc.1208298]

36 **Seidman JD**, Ronnett BM, Kurman RJ. Pathology of borderline (low malignant potential) ovarian tumours. *Best Pract Res Clin Obstet Gynaecol* 2002; **16**: 499-512 [PMID: 12413931 DOI: 10.1053/beog.2002.0300]

37 **Storey DJ**, Rush R, Stewart M, Rye T, Al-Nafussi A, Williams AR, Smyth JF, Gabra H. Endometrioid epithelial ovarian cancer : 20 years of prospectively collected data from a single center. *Cancer* 2008; **112**: 2211-2220 [PMID: 18344211 DOI: 10.1002/cncr.23438]

38 **Duska LR**, Garrett L, Henretta M, Ferriss JS, Lee L, Horowitz N. When 'never-events' occur despite adherence to clinical guidelines: the case of venous thromboembolism in clear cell cancer of the ovary compared with other epithelial histologic subtypes. *Gynecol Oncol* 2010; **116**: 374-377 [PMID: 19922988 DOI: 10.1016/j.ygyno.2009.10.069]

39 **Bristow RE**, Tomacruz RS, Armstrong DK, Trimble EL, Montz FJ. Survival effect of maximal cytoreductive surgery for advanced ovarian carcinoma during the platinum era: a meta-analysis. *J Clin Oncol* 2002; **20**: 1248-1259 [PMID: 11870167 DOI: 10.1200/JCO.20.5.1248]

40 **Trimbos JB**, Parmar M, Vergote I, Guthrie D, Bolis G, Colombo N, Vermorken JB, Torri V, Mangioni C, Pecorelli S, Lissoni A, Swart AM. International Collaborative Ovarian Neoplasm trial 1 and Adjuvant ChemoTherapy In Ovarian Neoplasm trial: two parallel randomized phase III trials of adjuvant chemotherapy in patients with early-stage ovarian carcinoma. *J Natl Cancer Inst* 2003; **95**: 105-112 [PMID: 12529343 DOI: 10.1093/jnci/95.2.113]

41 **Colombo N**, Guthrie D, Chiari S, Parmar M, Qian W, Swart AM, Torri V, Williams C, Lissoni A, Bonazzi C. International Collaborative Ovarian Neoplasm trial 1: a randomized trial of adjuvant chemotherapy in women with early-stage ovarian cancer. *J Natl Cancer Inst* 2003; **95**: 125-132 [PMID: 12529345 DOI: 10.1093/jnci/95.2.125]

42 **Hoskins WJ**, McGuire WP, Brady MF, Homesley HD, Creasman WT, Berman M, Ball H, Berek JS. The effect of diameter of largest residual disease on survival after primary cytoreductive surgery in patients with suboptimal residual epithelial ovarian carcinoma. *Am J Obstet Gynecol* 1994; **170**: 974-979; discussion 979-980; [PMID: 8166218 DOI: 10.1016/S0002-9378(94)70090-7]

43 **Hoskins WJ**. Surgical staging and cytoreductive surgery of epithelial ovarian cancer. *Cancer* 1993; **71**: 1534-1540 [PMID: 8431891 DOI: 10.1002/cncr.2820710420]

44 **Armstrong DK**, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL, Burger RA. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. *N Engl J Med* 2006; **354**: 34-43 [PMID: 16394300 DOI: 10.1056/NEJMoa052985]

45 **Howell SB**, Zimm S, Markman M, Abramson IS, Cleary S, Lucas WE, Weiss RJ. Long-term survival of advanced refractory ovarian carcinoma patients with small-volume disease treated with intraperitoneal chemotherapy. *J Clin Oncol* 1987; **5**: 1607-1612 [PMID: 2443622]

46 **van der Burg ME**, van Lent M, Buyse M, Kobierska A, Colombo N, Favalli G, Lacave AJ, Nardi M, Renard J, Pecorelli S. The effect of debulking surgery after induction chemotherapy on the prognosis in advanced epithelial ovarian cancer. Gynecological Cancer Cooperative Group of the European Organization for Research and Treatment of Cancer. *N Engl J Med* 1995; **332**: 629-634 [PMID: 7845426 DOI: 10.1056/NEJM199503093321002]

47 **Goodman HM**, Harlow BL, Sheets EE, Muto MG, Brooks S, Steller M, Knapp RC, Berkowitz RS. The role of cytoreductive surgery in the management of stage IV epithelial ovarian carcinoma. *Gynecol Oncol* 1992; **46**: 367-371 [PMID: 1526516 DOI: 10.1016/0090-8258(92)90234-A]

48 **Copeland LJ**, Bookman M, Trimble E. Clinical trials of newer regimens for treating ovarian cancer: the rationale for Gyne-

cologic Oncology Group Protocol GOG 182-ICON5. *Gynecol Oncol* 2003; **90**: S1-S7 [PMID: 12927999 DOI: 10.1016/S0090-8258(03)00337-8]

49 **Vasey PA**, Jayson GC, Gordon A, Gabra H, Coleman R, Atkinson R, Parkin D, Paul J, Hay A, Kaye SB. Phase III randomized trial of docetaxel-carboplatin versus paclitaxel-carboplatin as first-line chemotherapy for ovarian carcinoma. *J Natl Cancer Inst* 2004; **96**: 1682-1691 [PMID: 15547181 DOI: 10.1093/jnci/djh323]

50 **Burger RA**, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, Mannel RS, Homesley HD, Fowler J, Greer BE, Boente M, Birrer MJ, Liang SX. Incorporation of bevacizumab in the primary treatment of ovarian cancer. *N Engl J Med* 2011; **365**: 2473-2483 [PMID: 22204724 DOI: 10.1056/NEJMoa1104390]

51 **Perren TJ**, Swart AM, Pfisterer J, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Kurzeder C, du Bois A, Sehouli J, Kimmig R, Stähle A, Collinson F, Essapen S, Gourley C, Lortholary A, Selle F, Mirza MR, Leminen A, Plante M, Stark D, Qian W, Parmar MK, Oza AM. A phase 3 trial of bevacizumab in ovarian cancer. *N Engl J Med* 2011; **365**: 2484-2496 [PMID: 22204725 DOI: 10.1056/NEJMoa1103799]

52 **Eskander RN**, Ansaloni L, Bristow RE, Coccolini F. Cytoreductive surgery and hyperthermic intraperitoneal chemotherapy in epithelial ovarian cancer: State of the art. *World J Obstet Gynecol* 2013; **2:** 94-100 [DOI: 10.5317/wjog.v2.i4.94]

53 **Aghajanian C**, Blank SV, Goff BA, Judson PL, Teneriello MG, Husain A, Sovak MA, Yi J, Nycum LR. OCEANS: a randomized, double-blind, placebo-controlled phase III trial of chemotherapy with or without bevacizumab in patients with platinum-sensitive recurrent epithelial ovarian, primary peritoneal, or fallopian tube cancer. *J Clin Oncol* 2012; **30**: 2039-2045 [PMID: 22529265 DOI: 10.1200/JCO.2012.42.0505]

54 **Pujade-Lauraine E**, Hilpert F, Weber B, Reuss A, Poveda A, Kristensen G, Sorio R, Vergote I, Witteveen P, Bamias A, Pereira D, Wimberger P, Oaknin A, Mirza MR, Follana P, Bollag D, Ray-Coquard I. AURELIA-the first randomized phase III trial of bevacizumab with standard chemotherapy in platinum-resistant ovarian cancer. ASCO, 2012

55 **Katsumata N**. Dose-dense therapy is of benefit in primary treatment of ovarian cancer? In favor. *Ann Oncol* 2011; **22** Suppl 8: viii29-viii32 [PMID: 22180396]

56 **Cancer Genome Atlas Network**. Comprehensive molecular portraits of human breast tumours. *Nature* 2012; **490**: 61-70 [PMID: 23000897]

57 **Karihtala P**, Soini Y, Vaskivuo L, Bloigu R, Puistola U. DNA adduct 8-hydroxydeoxyguanosine, a novel putative marker of prognostic significance in ovarian carcinoma. *Int J Gynecol Cancer* 2009; **19**: 1047-1051 [PMID: 19820366 DOI: 10.1111/IGC.0b013e3181ad0f0d]

58 **Martinez-Outschoorn UE**, Balliet RM, Lin Z, Whitaker-Menezes D, Howell A, Sotgia F, Lisanti MP. Hereditary ovarian cancer and two-compartment tumor metabolism: epithelial loss of BRCA1 induces hydrogen peroxide production, driving oxidative stress and NFκB activation in the tumor stroma. *Cell Cycle* 2012; **11**: 4152-4166 [PMID: 23047606 DOI: 10.4161/cc.22226]

59 **Kandalaft LE**, Powell DJ Jr, Chiang CL, Tanyi J, Kim S, Bosch M, Montone K, Mick R, Levine BL, Torigian DA, June CH, Coukos G. Autologous lysate-pulsed dendritic cell vaccination followed by adoptive transfer of vaccine-primed ex vivo co-stimulated T cells in recurrent ovarian cancer. *Oncoimmunolgy* 2013; **2**: e22664 [PMID: 23482679 DOI: 10.4161/onci.22664]

60 **Bryant HE**, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005; **434**: 913-917 [PMID: 15829966 DOI: 10.1038/nature03443]

61 **Farmer H**, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005; **434**: 917-921 [PMID: 15829967 DOI: 10.1038/nature03445]

62 **Marchetti C**, Imperiale L, Gasparri ML, Palaia I, Pignata S, Boni T, Bellati F, Benedetti Panici P. Olaparib, PARP1 inhibitor in ovarian cancer. *Expert Opin Investig Drugs* 2012; **21**: 1575-1584 [PMID: 22788971 DOI: 10.1517/13543784.2012.707189]

63 **Menon U**, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, Lewis S, Davies S, Philpott S, Lopes A, Godfrey K, Oram D, Herod J, Williamson K, Seif MW, Scott I, Mould T, Woolas R, Murdoch J, Dobbs S, Amso NN, Leeson S, Cruickshank D, McGuire A, Campbell S, Fallowfield L, Singh N, Dawnay A, Skates SJ, Parmar M, Jacobs I. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). *Lancet Oncol* 2009; **10**: 327-340 [PMID: 19282241 DOI: 10.1016/S1470-2045(09)70026-9]

P- Reviewers: Coccolini F, Schulten HJ, Sonoda K, Yokoyama Y
S- Editor: Song XX **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Translational Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5528/wjtm.v3.i1.9

World J Transl Med 2014 April 12; 3(1): 9-16
ISSN 2220-6132 (online)

REVIEW

Neurological and behavioral manifestations of cerebral malaria: An update

Marta Chagas Monteiro, Fabio Rodrigues Oliveira, Gedeão Batista Oliveira, Pedro Roosevelt Torres Romão, Cristiane Socorro Ferraz Maia

Marta Chagas Monteiro, Cristiane Socorro Ferraz Maia, Programa de Pós-Graduação em Ciências Farmacêuticas, Instituto de Ciências da Saúde, Universidade Federal do Pará, Rua Augusto Corrêa, Campus Universitário do Guamá, Belém, PA 66075900, Brazil
Marta Chagas Monteiro, Fabio Rodrigues Oliveira, Gedeão Batista Oliveira, Cristiane Socorro Ferraz Maia, Program in Pharmaceutical Sciences, Faculty of Pharmacy, Laboratory of inflammation and behavior, Federal University of Pará/UFPA, Belém, PA 66075900, Brazil
Pedro Roosevelt Torres Romão, Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, RS 90050170, Brazil
Author contributions: All authors contributed equally to this work.
Correspondence to: Cristiane Socorro Ferraz Maia, PhD, Program in Pharmaceutical Sciences, Faculty of Pharmacy, Laboratory of inflammation and behavior, Federal University of Pará/UFPA, Rua Augusto Correia SN, Guamá, Belém, PA 66075900, Brazil. crismaia@ufpa.br
Telephone: +55-91-32018826 Fax: +55-91-32017201
Received: December 28, 2013 Revised: March 6, 2014
Accepted: March 13, 2014
Published online: April 12, 2014

Abstract

Neglected tropical diseases are a group of tropical diseases endemic in poor countries even though medical treatment and cures are available. They are considered a global health problem due to the severity of the physiological changes they induce in their hosts. Malaria is a disease caused by *Plasmodium* sp. that in its cerebral form may lead to acute or long-term neurological deficits, even with effective antimalarial therapy, causing vascular obstruction, reduced cerebral blood flow and many other changes. However, *Plasmodium falciparum* infection can also develop into a cerebral malaria (CM) disease that can produce neurological damage. This review will discuss the mechanisms involved in the neuropathology caused by CM, focusing on alterations in cognitive, behavior and neurological functions in human and experimental models.

Key words: Malaria; Cerebral malaria; Neuropathology; *Plasmodium* sp; Plasmodium falciparum

Core tip: This review attempts to compile the limited current knowledge on the behavioral and cognitive effects of cerebral malaria (CM) and the possible pathological mechanisms related to neurobehavioral manifestations. CM induces acute/chronic neurological damage, affecting several Central Nervous System regions responsible for behavioral, neurological and cognitive functions which may result in motor deficits, epilepsy, blindness, speech/hearing and memory/attention disorders, hyperactivity, anxiety-like behavior, neuropsychiatric manifestations of post malaria neurological syndrome, both in humans and animal models. The action mechanisms involved in the alterations are not yet clearly defined; however proinflammatory mediators have been described with consequent axonal damage and demyelination.

Monteiro MC, Oliveira FR, Oliveira GB, Romão PRT, Maia CSF. Neurological and behavioral manifestations of cerebral malaria: An update. *World J Transl Med* 2014; 3(1): 9-16 Available from: URL: http://www.wjgnet.com/2220-6132/full/v3/i1/9.htm DOI: http://dx.doi.org/10.5528/wjtm.v3.i1.9

INTRODUCTION

Malaria, leishmaniasis and tuberculosis together with other neglected tropical diseases (NTDs) cause 32% of

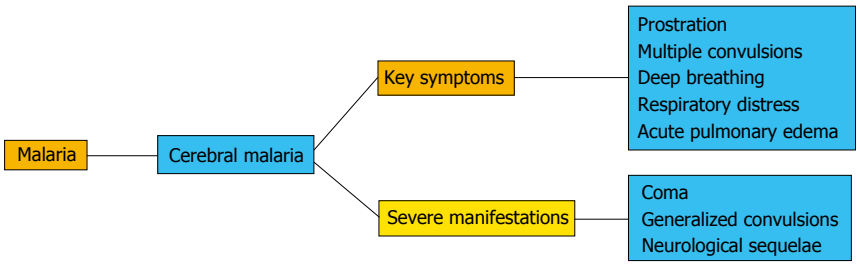

Figure 1 Clinical manifestations of cerebral malaria.

the burden of ill health in Africa and seriously impact on health outcomes in many regions of the world. NTDs share common features such as high endemicity in rural and impoverished urban areas of low-income countries. Some NTDs are disfiguring and stigmatizing, being considered poverty-promoting conditions, particularly in Africa, Asia, and the tropical regions of the Americas[1,2].

Among various NTDs, malaria is one of the most life-threatening diseases, provided that the currently recommended interventions are not adequately implemented[2]. In 2011, the World Health Organization (WHO) estimated that 3.3 billion people were at risk of malaria. More than 274 million clinical cases and 1.1 million deaths occurred between 2001 and 2010 worldwide, with approximately 80% of cases and 90% of deaths estimated to occur in the African Region, mostly in children under five years of age and in pregnant women[2,3]. Kiszewski *et al*[4] estimated that Global resource requirements for malaria control totaling USD 38-45 billion will be spent from 2006 to 2015 for the diagnosis and treatment of malaria, mainly in countries and populations at risk of epidemic, such as sub-Saharan Africa.

Human malaria is caused by five species of obligate intraerythrocytic protozoa of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*[2], and is transmitted by the bite of an female anopheles mosquito. At least three-dozen different species of *Anopheles* mosquitoes can transmit malaria worldwide[5]. However, infections can also occur through exposure to infected blood products (transfusion malaria) and *via* congenital transmission[6].

Of these, *P. falciparum* is the organism primarily responsible for severe malaria, although *P. vivax*[3] and *P. Knowle*[7,8] can also cause severe disease. According to WHO's criteria[9], severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction and/or high parasite burden; this high parasitemia can be a risk factor for death from *P. falciparum* malaria[9].

Overall, clinical features of severe malaria include cerebral malaria (CM) with impaired consciousness (including coma), prostration, multiple convulsions, deep breathing and respiratory distress (metabolic acidosis), acute pulmonary edema and acute respiratory distress syndrome, circulatory collapse or shock and acute kidney injury[9,10]. However, severe malaria is a complex multisystem disorder that can mimic many other diseases that are also common in malaria-endemic countries, such as central nervous system (CNS) infections, sepsis, severe pneumonia and typhoid fever[9].

In this review, we described neurocognitive and behavioral outcomes of CM in humans and animals so as to facilitate further understanding of the disease's pathogenesis in the CNS.

CM

CM is one of the most severe and rapidly fatal neurological complications caused by *Plasmodium* species, mainly *P. falciparum*, with around one million deaths per year in children from sub-Saharan Africa[11,12]. The first manifestations of CM are non-specific fever, chills, irritability, agitation or psychotic behavior, vomiting and cough. In adults, complications are severe jaundice, respiratory distress syndrome, and severe intravascular hemolysis leading to hemoglobinuria and anemia, which further contributes to renal failure (Figure 1). The most severe manifestations are impaired consciousness with coma, generalized convulsions and neurological sequelae. Pregnant women are also vulnerable and develop anemia, hypoglycemia, coma and pulmonary edema. In children, the main symptoms are severe anemia, metabolic acidosis, hypoglycemia, coma and gastrointestinal symptoms[13-15], as shown in Figure 1.

CM may result in acute or long-term neurological deficits, even with effective antimalarial therapy[16,17]. CM is a neurological complication that occurs in approximately 1% of infections caused by *P. falciparum*[18,19]; however, a high mortality rate follows[14,20].

PATHOLOGICAL MECHANISMS

The pathological mechanisms that lead to neurological complications and mortality are not yet clearly defined. It is believed that in infected erythrocytes, platelets, and activated leukocytes inflammatory events occur owing to increased levels of adhesion molecules on the inflamed endothelium, leading to a reduction in microvascular blood flow, decreased delivery of nutrients to affected brain tissue and vessel walls, followed by hemorrhage and neuronal alterations[21-23].

The blood-brain barrier (BBB) acts as a physical barrier that limits the trafficking of substances *via* transcellular transport and is responsible for regulating ion and nutrient transport into the brain, a feature that restricts the free flow of physiological molecules between the bloodstream and brain parenchyma[18,24].

Conversely, perturbations to the BBB can lead to deregulation in any of the neurovascular components,

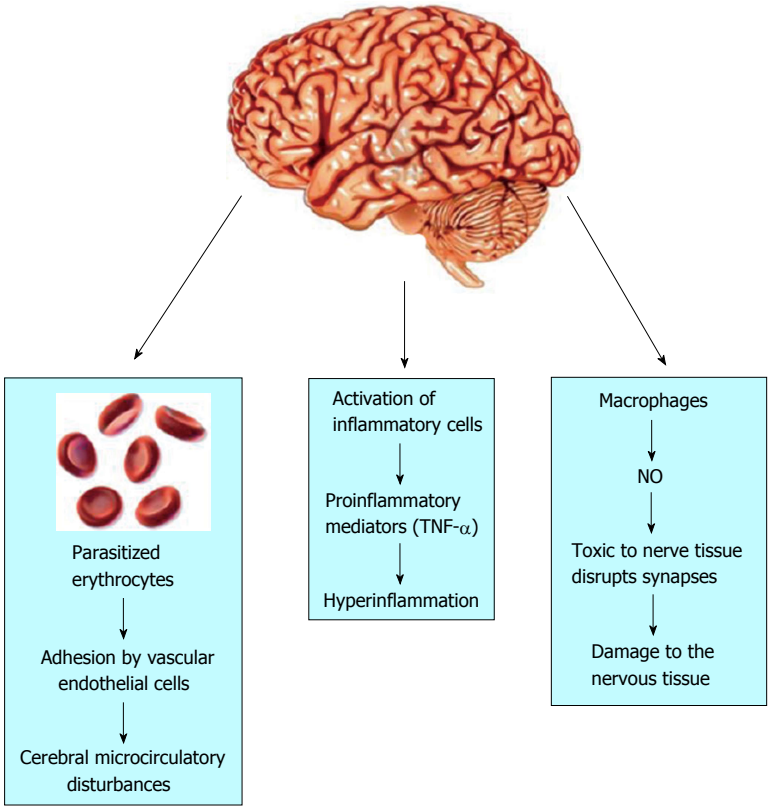

Figure 2 The pathological mechanisms that lead to neurological complications and mortality in patients. NO: Nitric oxide; TNF-α: Tumor necrosis factor-alpha.

which in turn can alter the brain's homeostasis leading to a multitude of neural dysfunctions and inappropriate BBB activation as observed in multiple sclerosis, Alzheimer's disease, stroke, certain depression disorders and parasitic infections, among others[25-29]. Vascular dysfunction with subsequent BBB damage has been observed both in human CM and in animal models[18,19,30].

The pathogenesis of CM is associated with cerebral microcirculatory disturbances resulting from the adhesion to and sequestration of parasitized erythrocytes, immune cells and platelets by vascular endothelial cells that line the small blood vessels of the brain, leading to their blockage[30], as shown in Figure 2. In this regard, several studies provide evidence that in the erythrocytic phase the merozoites modify the surface of erythrocytes, inducing the expression of a surface protein-*Plasmodium falciparum* erythrocyte membrane protein 1-that has a strong affinity for adhesion molecules expressed on the surface of vascular endothelium, such as intracellular adhesion molecule 1, vascular cell adhesion molecule 1, and platelet endothelial cell adhesion molecule 1, among others[31].

In sequestration, *P. falciparum*-infected erythrocytes adhere to the brain endothelium through binding to PfEMP1[32]. There is no evidence to date of infected erythrocyte entry into brain parenchyma, suggesting that these cells remain in the vascular space where they are sequestered. This sequestration of parasitized erythrocytes leads to multiple vascular effects, including the formation of clusters of agglomerated platelets and leukocytes, increased vasoconstriction, as well as the agglutination of erythrocytes not parasitized by generating so-called rosettes, which significantly reduce cerebral blood flow in the capillaries and cause vascular obstruction, leading to hypoxia, brain parenchymal hemorrhage[22] and disruption of BBB integrity[11,33].

Moreover, hyperinflammation in the brain has also been related to CM and is another mechanism responsible for the vasculopathy observed during infection (Figure 2). Some studies report that during the inflammatory response, activation of inflammatory cells may occur accompanied by an overproduction of type-1 proinflammatory mediators, especially tumor necrosis factor-alpha (TNF-α), which is produced by microglia, astrocytes, monocytes and cerebral vascular endothelium[34]. In humans, this cytokine induces the upregulation of adhesion molecules on endothelial cell surfaces, which contributes to the increased capture of erythrocytes in the cerebral capillaries and other organs[25,35]. Furthermore, inflammation enhances nitric oxide (NO) production by macrophages, which seems linked to the pathogenesis of the disease, considering that it is extremely toxic to nerve tissue and disrupts synapses, contributing to the damage to the nervous tissue[36]. In addition, inflammation can lead to micro- and ring hemorrhages and necrosis of surrounding tissues and cerebral edema, resulting in significant compression of cerebral arteries that can lead to death, as well as the various symptoms of CM, such as confusion or stupor of obtundation, or deep coma with long-term neurological deficits such as cortical blindness[25,37].

Postmortem analyses of children who died with CM revealed that the axonal and myelin damage was associated with ring hemorrhages and vascular thrombosis in the cerebral and cerebellar white matter and brainstem. Disruption of the BBB and accumulation of monocytes

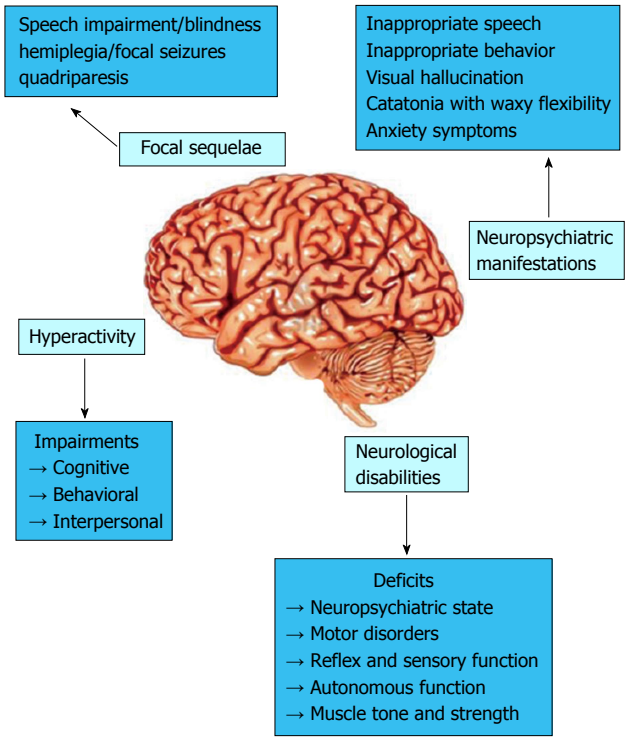

Figure 3 Neurological features of cerebral malaria.

with phagocytosed hemozoin within microvessels containing infected erythrocytes was found, suggesting a link between infected erythrocyte sequestration and intravascular/perivascular pathology in fatal pediatric CM[38]. In animal models, the presence of apoptosis was also observed initially in endothelial cells and later in neurons and glia[39], and may be associated with persistent cognitive impairment[17]. These disturbances in the homeostasis of the cerebral microcirculation play an important role in the pathogenesis of CM, generating vascular obstructions, reduced cerebral blood flow and BBB disruption associated with high cerebral vasoconstriction[24,40]. In addition, in the presence of seizures and/or fever, the metabolic demand increases with consequent risk of neural injury[41], as shown in Figure 2.

NEUROLOGICAL FEATURES

Unfortunately, severe brain injury occurs after CM and 25% of pediatric cases result in epilepsy or long-term neurological and cognitive deficits[42-44]. According to the time of symptom onset, CM may be classified into two patterns of neurological sequelae[45], as shown in Figure 3. The first is immediate and characterized by coma and status epilepticus during the acute illness, resulting in focal sequelae such as hemiplegia and focal seizures, or multifocal sequelae with spastic quadriparesis, motor disorders, cognitive and behavioral impairment, blindness, speech or hearing impairment. The second pattern develops within months or years after CM, and behavioral deficits and/or epilepsy may occur.

Among gross motor deficits, hemiplegia, diplegia, quadriparesis or quadriplegia may be observed after CM[46]. Disorders in movement and gait can be noted, including ataxia, choreoathetosis, dystonia and poor neck control, as well as feeding difficulties[46]. Dai *et al*[47] demonstrated that motor coordination impairment was associated with dysregulation of Akt and GSK3β signaling in a murine model of CM. The inhibition of the Akt pathway results in modifications in neuronal integrity, since it is a protein kinase playing a key role in the insulin signaling pathway and an important regulator of apoptosis, being consequently important for cell viability, although *via* an GSK3β-dependent pathway. In addition, the intracranial hypertension may contribute to the motor sequelae, given that it reduces the cerebral perfusion pressure, nutrient and oxygen delivery and, where death does not occur, subsequent global ischemic injury and brainstem compression can lead to cerebral atrophy, which may result in motor and cognitive impairment[48].

Convulsions in CM are common and inflammatory products such as quinolinic acid contribute to the neuropathology, considering that this metabolite from the kynurenine pathway is a N-methyl-D-aspartate agonist that causes neuroinflammation, convulsions, and cell death[49-51]. Dobbie *et al*[52] demonstrated that quinolinic acid provokes seizures in animals, possibly, altering the neurotransmission excitatory and triggering long-term deleterious effects on cognitive function and/or behavior. Sokol *et al*[53] demonstrated irreversible neuron damage after long-term seizure activity, followed by gliosis and focal atrophy, resulting in more seizures and brain damage. The epilepsy (recurrence of seizures without apparent cause) occurs in approximately 10% of pediatric cases and may be occasioned by focal or global hypoxia or ischemia[54,55]. The epileptogenesis mechanisms are unclear. Structural brain damage and the presence of Durck's malarial granuloma may contribute to the epileptogenesis mechanisms[56]; however, other factors should also be considered, like genetic propensity[57].

It has been observed that speech and language were the most common neurocognitive impairments found in Kenyan children who survived severe malaria[58]. The authors suggested that language impairment may be part of a broad impairment that is most noted in the patterns of language, which probably contributes to deficits on verbal components of other cognitive assessments. On the other hand, Dugbartey[59] reported that children affected by CM develop impairments in bimanual tactile discrimination, accuracy of visual scanning, visual memory, perceptual abstraction and rule learning skills, right ear auditory information processing, and dominant-hand motor speed. Other studies revealed deficits in spatial memory, mental processing, sequential processing, and attention tasks[58,60,61]. Indeed, other kinds of memory, such as episodic memory, also seem to be affected by CM[62].

Dai *et al*[47,63] demonstrated that memory deficits either during or after successful treatment were associated with reduced Akt expression and dysregulation of Akt/GSK3β signaling in a murine CM model. GSK3β

plays a key role in the process of neurodevelopment and the transcription of brain derived neurotrophic factor, affecting long-term memory and synaptic plasticity[64]. In this context, it has been associated with hyperphosphorylation of tau protein[65], which is the major component in neurodegenerative disorders like Alzheimer's disease[66]. Abnormal tau levels in the cerebral spinal fluid in CM survivors[47,67] lead to long-term deficits in cognitive areas like memory, learning, language and psychiatric disorders[17,42,68,69]. Specific damage in neuronal areas such as the hippocampus and sub-cortical white matter may lead to impairments in learning, memory and language function[70-72].

Hyperactivity, impulsiveness and inattentiveness have also been observed in CM survivors[45], similar to what occurs in attention deficit hyperactivity disorder (ADHD), which produces impairments in the cognitive, behavioral, and interpersonal domains[73]. Dysregulated reward processing in the frontostriatal system has been proposed as a central mechanism in prevailing theoretical models of ADHD[74,75], and altered dopamine signaling underlies a number of ADHD symptoms[75]. Several anatomical changes in the brain are related to ADHD, including in the caudate nucleus, prefrontal cortex white matter, corpus callosum, cerebellar vermis[76] and globus pallidus[77], which are all areas that contain high densities of dopamine receptors. Most probably, damage occasioned by CM in the frontostriatal and cerebellar areas by a decrease in local blood flow or neuronal loss may produces impairments in dopamine signaling and consequently ADHD[78].

Animal model parameters may reproduce some symptoms related to ADHD and stroke. In this regard, a murine study demonstrated a lower level of general activity associated with reduced response to touch escape and absent vocalization correlated with large areas of hemorrhage in animals with CM[39]. These findings suggest an important influence of parenchymal hemorrhage distribution on the severity of neurological deficits in the late stage of the illness[46].

An inflammatory cytokine profile has been associated with CNS dysfunction found in human and experimental CM. In the course of experimental CM induced by *Plasmodium berghei* (strain ANKA), leukocyte migration into the brain, as well as the production of TNF-α and chemokines (CCL2, CCL3, CCL5 and CXCL9) preceded neurological changes including in the neuropsychiatric state, motor behavior, autonomic function, muscle tone and strength, suggesting that the inflammatory changes may be involved in the neurological impairment[79]. In this context, de Miranda *et al*[80] demonstrated an anxiety-like behavior in C57BL/6 mice infected with *P. berghei* using the elevated plus maze test. The anxiety symptoms were correlated with histopathological alterations in the brainstem, cerebrum and hippocampus and increased cerebral levels of interleukin-1 beta and TNF-α. In humans, Dugbartey *et al*[81] described anxiety disorders in a CM patient' s recovery, suggesting that *falciparum* malaria is associated with enduring, albeit subclinical, anxiety and depressive symptoms.

Some authors have reported correlations between neurological disabilities and glutamate levels and their contribution to the pathogenesis of these deficits, showing increased glutamate levels in cerebral spinal fluid and cerebrocortical synaptosomes from CM animals associated with alterations in neuropsychiatric state, motor behavior, reflex and sensory function, autonomous function, muscle tone and strength[82]. Glutamate is the principal excitatory neurotransmitter in the mammalian CNS, participating in several cognitive and neurological functions under physiological conditions[83]. Therefore, large amounts of glutamate release trigger neurotoxicity and neuronal cell death, being involved in neurodegenerative disorders[84]. Thus, the imbalance in the neurotransmitter glutamate may be important in the establishing the pathogenesis mechanism of CM[82].

The neuropsychiatric manifestations of post malaria neurological syndrome (PMNS) are highly variable and include an acute confusional state or acute psychosis with one or more of the following symptoms: inappropriate speech or behavior, visual hallucination, catatonia with waxy flexibility, generalized convulsion, fine postural tremor, clouding of consciousness and decreased muscle tone. It may occur within 2 mo after acute MC, with either neurologic or psychiatric symptoms[85]. A case report on a Taiwan CM patient reported severe headache, dizziness, delirium and polyneuropathy within 2 mo after recovery[86]; even psychotic symptoms with both visual and auditory hallucinations, aggressiveness, and inability to communicate have been related[87,88] that can last for 12 d. The symptoms observed may not be attributed only to CM, since other factors could be responsible; however, the neurologic and psychiatric presentations were compatible with PMNS and the mechanisms are the same as those related to other neurologic CM deficits, including cerebral hypoperfusion and immunologic mechanism, which prompts psychosis in a small minority[88].

CONCLUSION

Malaria is a parasitic disease that can affect the CNS, altering cognitive and behavioral functions. Neurological and behavioral changes described in the course of experimental or human CM are mainly a consequence of brain hyperinflammation, vascular obstruction, reduced cerebral blood flow, and disruption of the BBB associated with high levels of cerebral vasoconstriction, thrombus, ring hemorrhage, ruptured capillaries, and cerebral blood vessels filled with infected erythrocytes, with consequent axonal damage and demyelination. Additionally, neurologic alterations have been observed as motor deficits, seizures and epilepsy; neurocognitive impairment in language, speech, learning, and memory; and behavioral damage with hyperactivity, anxiety, PMNS and psychosis.

ACKNOWLEDGMENTS

We are grateful to the Conselho Nacional de Desenvolvi-

mento Científico e Tecnológico (CNPq), FAPESPA, Federal University of Pará and the Federal University of Health Sciences of Porto Alegre for granting financial support for this work. P.R.T. Romão and M.C. Monteiro were recipients of fellowships from CNPq.

REFERENCES

1 **World Health Organization**. Neglected tropical diseases. The 17 neglected tropical diseases. access in 2013. Available from: URL: http: //www.who.int/neglected_diseases/diseases/en/
2 **World Health Organization**. World Malaria Report 2012. Available from: URL: http: //www.who.int/malaria/publications/world_malaria_report_2012/report/en/
3 **World Health Organization**. Global Health Observatory (GHO). Number of malaria deaths. Access in 2013. Available from: URL: http: //www.who.int/gho/malaria/epidemic/deaths/en/
4 **Kiszewski A**, Johns B, Schapira A, Delacollette C, Crowell V, Tan-Torres T, Ameneshewa B, Teklehaimanot A, Nafo-Traoré F. Estimated global resources needed to attain international malaria control goals. *Bull World Health Organ* 2007; **85**: 623-630 [PMID: 17768521 DOI: 10.1590/S0042-96862007000800015]
5 **malERA Consultative Group on Vector Control**. A research agenda for malaria eradication: vector control. *PLoS Med* 2011; **8**: e1000401 [PMID: 21311587 DOI: 10.1371/journal.pmed.1000401]
6 **Trampuz A**, Jereb M, Muzlovic I, Prabhu RM. Clinical review: Severe malaria. *Crit Care* 2003; **7**: 315-323 [PMID: 12930555 DOI: 10.1186/cc2183]
7 **Cox-Singh J**, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, Rahman HA, Conway DJ, Singh B. Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis* 2008; **46**: 165-171 [PMID: 18171245 DOI: 10.1086/524888]
8 **Kantele A**, Jokiranta TS. Review of cases with the emerging fifth human malaria parasite, Plasmodium knowlesi. *Clin Infect Dis* 2011; **52**: 1356-1362 [PMID: 21596677 DOI: 10.1093/cid/cir180]
9 **World Health Organization**. Management of severe malaria: a practical handbook-3rd ed. Geneva: World Health Organization, 2012
10 **Idro R**, Jenkins NE, Newton CR. Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *Lancet Neurol* 2005; **4**: 827-840 [PMID: 16297841 DOI: 10.1016/S1474-4422(05)70247-7]
11 **Desruisseaux MS**, Machado FS, Weiss LM, Tanowitz HB, Golightly LM. Cerebral malaria: a vasculopathy. *Am J Pathol* 2010; **176**: 1075-1078 [PMID: 20093501 DOI: 10.2353/ajpath.2010.091090]
12 **Waknine-Grinberg JH**, McQuillan JA, Hunt N, Ginsburg H, Golenser J. Modulation of cerebral malaria by fasudil and other immune-modifying compounds. *Exp Parasitol* 2010; **125**: 141-146 [PMID: 20093114 DOI: 10.1016/j.exppara.2010.01.005]
13 **Dondorp AM**. Pathophysiology, clinical presentation and treatment of cerebral malaria. *Neurology Asia* 2005; **10**: 67-77
14 **Idro R**, Marsh K, John CC, Newton CR. Cerebral malaria: mechanisms of brain injury and strategies for improved neurocognitive outcome. *Pediatr Res* 2010; **68**: 267-274 [PMID: 20606600 DOI: 10.1203/00006450-201011001-00524]
15 **Jain K**, Sood S, Gowthamarajan K. Modulation of cerebral malaria by curcumin as an adjunctive therapy. *Braz J Infect Dis* 2013; **17**: 579-591 [PMID: 23906771 DOI: 10.1016/j.bjid.2013.03.004]
16 **Desruisseaux MS**, Gulinello M, Smith DN, Lee SC, Tsuji M, Weiss LM, Spray DC, Tanowitz HB. Cognitive dysfunction in mice infected with Plasmodium berghei strain ANKA. *J Infect Dis* 2008; **197**: 1621-1627 [PMID: 18419550 DOI: 10.1086/587908]
17 **Kihara M**, Carter JA, Newton CR. The effect of *Plasmodium falciparum* on cognition: a systematic review. *Trop Med Int Health* 2006; **11**: 386-397 [PMID: 16553922 DOI: 10.1111/j.1365-3156.2006.01579.x]
18 **Shikani HJ**, Freeman BD, Lisanti MP, Weiss LM, Tanowitz HB, Desruisseaux MS. Cerebral malaria: we have come a long way. *Am J Pathol* 2012; **181**: 1484-1492 [PMID: 23021981 DOI: 10.1016/j.ajpath.2012.08.010]
19 **Nacer A**, Movila A, Baer K, Mikolajczak SA, Kappe SH, Frevert U. Neuroimmunological blood brain barrier opening in experimental cerebral malaria. *PLoS Pathog* 2012; **8**: e1002982 [PMID: 23133375 DOI: 10.1371/journal.ppat.1002982]
20 **Claessens A**, Adams Y, Ghumra A, Lindergard G, Buchan CC, Andisi C, Bull PC, Mok S, Gupta AP, Wang CW, Turner L, Arman M, Raza A, Bozdech Z, Rowe JA. A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells. *Proc Natl Acad Sci USA* 2012; **109**: E1772-E1781 [PMID: 22619330 DOI: 10.1073/pnas.1120461109]
21 **Newton CR**, Taylor TE, Whitten RO. Pathophysiology of fatal falciparum malaria in African children. *Am J Trop Med Hyg* 1998; **58**: 673-683 [PMID: 9598460]
22 **Faille D**, El-Assaad F, Alessi MC, Fusai T, Combes V, Grau GE. Platelet-endothelial cell interactions in cerebral malaria: the end of a cordial understanding. *Thromb Haemost* 2009; **102**: 1093-1102 [PMID: 19967139 DOI: 10.1160/TH09-05-0337]
23 **Cox D**, McConkey S. The role of platelets in the pathogenesis of cerebral malaria. *Cell Mol Life Sci* 2010; **67**: 557-568 [PMID: 20091081 DOI: 10.1007/s00018-009-0211-3]
24 **Abbott NJ**, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 2006; **7**: 41-53 [PMID: 16371949 DOI: 10.1038/nrn1824]
25 **Combes V**, Coltel N, Faille D, Wassmer SC, Grau GE. Cerebral malaria: role of microparticles and platelets in alterations of the blood-brain barrier. *Int J Parasitol* 2006; **36**: 541-546 [PMID: 16600245 DOI: 10.1016/j.ijpara.2006.02.005]
26 **Reale M**, Iarlori C, Thomas A, Gambi D, Perfetti B, Di Nicola M, Onofrj M. Peripheral cytokines profile in Parkinson's disease. *Brain Behav Immun* 2009; **23**: 55-63 [PMID: 18678243 DOI: 10.1016/j.bbi.2008.07.003]
27 **Tung JN**, Tsao TY, Chen SL, Tai CJ, Shen SC, Cheng YW, Jiang MC. Presence of secretory cellular apoptosis susceptibility protein in cerebrospinal fluids of patients with intracerebral hemorrhage caused by stroke and neurotrauma. *Neuro Endocrinol Lett* 2010; **31**: 390-398 [PMID: 20588233]
28 **Holman DW**, Klein RS, Ransohoff RM. The blood-brain barrier, chemokines and multiple sclerosis. *Biochim Biophys Acta* 2011; **1812**: 220-230 [PMID: 20692338 DOI: 10.1016/j.bbadis.2010.07.019]
29 **Zlokovic BV**. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 2008; **57**: 178-201 [PMID: 18215617 DOI: 10.1016/j.neuron.2008.01.003]
30 **Coltel N**, Combes V, Hunt NH, Grau GE. Cerebral malaria-a neurovascular pathology with many riddles still to be solved. *Curr Neurovasc Res* 2004; **1**: 91-110 [PMID: 16185187 DOI: 10.2174/1567202043480116]
31 **Rowe JA**, Claessens A, Corrigan RA, Arman M. Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. *Expert Rev Mol Med* 2009; **11**: e16 [PMID: 19467172 DOI: 10.1017/S1462399409001082]
32 **Milner DA**. Rethinking cerebral malaria pathology. *Curr Opin Infect Dis* 2010; **23**: 456-463 [PMID: 20613511 DOI: 10.1097/QCO.0b013e32833c3dbe]
33 **Hunt NH**, Grau GE. Cytokines: accelerators and brakes in the pathogenesis of cerebral malaria. *Trends Immunol* 2003; **24**: 491-499 [PMID: 12967673 DOI: 10.1016/S1471-4906(03)00229-1]

34 **Medana IM**, Hunt NH, Chaudhri G. Tumor necrosis factor-alpha expression in the brain during fatal murine cerebral malaria: evidence for production by microglia and astrocytes. *Am J Pathol* 1997; **150**: 1473-1486 [PMID: 9095002]

35 **Schofield L**, Grau GE. Immunological processes in malaria pathogenesis. *Nat Rev Immunol* 2005; **5**: 722-735 [PMID: 16138104 DOI: 10.1038/nri1686]

36 **Zanini GM**, Cabrales P, Barkho W, Frangos JA, Carvalho LJ. Exogenous nitric oxide decreases brain vascular inflammation, leakage and venular resistance during Plasmodium berghei ANKA infection in mice. *J Neuroinflammation* 2011; **8**: 66 [PMID: 21649904 DOI: 10.1186/1742-2094-8-66]

37 **Medana IM**, Turner GD. Human cerebral malaria and the blood-brain barrier. *Int J Parasitol* 2006; **36**: 555-568 [PMID: 16616145 DOI: 10.1016/j.ijpara.2006.02.004]

38 **Dorovini-Zis K**, Schmidt K, Huynh H, Fu W, Whitten RO, Milner D, Kamiza S, Molyneux M, Taylor TE. The neuropathology of fatal cerebral malaria in malawian children. *Am J Pathol* 2011; **178**: 2146-2158 [PMID: 21514429 DOI: 10.1016/j.ajpath.2011.01.016]

39 **Lackner P**, Beer R, Heussler V, Goebel G, Rudzki D, Helbok R, Tannich E, Schmutzhard E. Behavioural and histopathological alterations in mice with cerebral malaria. *Neuropathol Appl Neurobiol* 2006; **32**: 177-188 [PMID: 16599946 DOI: 10.1111/j.1365-2990.2006.00706.x]

40 **Kennan RP**, Machado FS, Lee SC, Desruisseaux MS, Wittner M, Tsuji M, Tanowitz HB. Reduced cerebral blood flow and N-acetyl aspartate in a murine model of cerebral malaria. *Parasitol Res* 2005; **96**: 302-307 [PMID: 15918069 DOI: 10.1007/s00436-005-1349-z]

41 **Idro R**, Carter JA, Fegan G, Neville BG, Newton CR. Risk factors for persisting neurological and cognitive impairments following cerebral malaria. *Arch Dis Child* 2006; **91**: 142-148 [PMID: 16326798 DOI: 10.1136/adc.2005.077784]

42 **Carter JA**, Lees JA, Gona JK, Murira G, Rimba K, Neville BG, Newton CR. Severe falciparum malaria and acquired childhood language disorder. *Dev Med Child Neurol* 2006; **48**: 51-57 [PMID: 16359594 DOI: 10.1017/S0012162206000107]

43 **Ngoungou EB**, Dulac O, Poudiougou B, Druet-Cabanac M, Dicko A, Mamadou Traore A, Coulibaly D, Farnarier G, Tuillas M, Keita MM, Kombila M, Doumbo OK, Preux PM. Epilepsy as a consequence of cerebral malaria in area in which malaria is endemic in Mali, West Africa. *Epilepsia* 2006; **47**: 873-879 [PMID: 16686652 DOI: 10.1111/j.1528-1167.2006.00558.x]

44 **John CC**, Bangirana P, Byarugaba J, Opoka RO, Idro R, Jurek AM, Wu B, Boivin MJ. Cerebral malaria in children is associated with long-term cognitive impairment. *Pediatrics* 2008; **122**: e92-e99 [PMID: 18541616 DOI: 10.1542/peds.2007-3709]

45 **Idro R**, Kakooza-Mwesige A, Balyejjussa S, Mirembe G, Mugasha C, Tugumisirize J, Byarugaba J. Severe neurological sequelae and behaviour problems after cerebral malaria in Ugandan children. *BMC Res Notes* 2010; **3**: 104 [PMID: 20398391 DOI: 10.1186/1756-0500-3-104]

46 **Daroff RB**. Cerebral malaria. *J Neurol Neurosurg Psychiatry* 2001; **70**: 817-818 [PMID: 11430300 DOI: 10.1136/jnnp.70.6.817]

47 **Dai M**, Freeman B, Shikani HJ, Bruno FP, Collado JE, Macias R, Reznik SE, Davies P, Spray DC, Tanowitz HB, Weiss LM, Desruisseaux MS. Altered regulation of Akt signaling with murine cerebral malaria, effects on long-term neurocognitive function, restoration with lithium treatment. *PLoS One* 2012; **7**: e44117 [PMID: 23082110 DOI: 10.1371/journal.pone.0044117]

48 **Newton CR**, Crawley J, Sowumni A, Waruiru C, Mwangi I, English M, Murphy S, Winstanley PA, Marsh K, Kirkham FJ. Intracranial hypertension in Africans with cerebral malaria. *Arch Dis Child* 1997; **76**: 219-226 [PMID: 9135262 DOI: 10.1136/adc.76.3.219]

49 **Stone TW**, Connick JH, Winn P, Hastings MH, English M. Endogenous excitotoxic agents. *Ciba Found Symp* 1987; **126**: 204-220 [PMID: 2884085]

50 **Heyes MP**, Saito K, Major EO, Milstien S, Markey SP, Vickers JH. A mechanism of quinolinic acid formation by brain in inflammatory neurological disease. Attenuation of synthesis from L-tryptophan by 6-chlorotryptophan and 4-chloro-3-hydroxyanthranilate. *Brain* 1993; **116** (Pt 6): 1425-1450 [PMID: 8293279]

51 **Nakamura TA**, Yamada K, Hasegawa T, Nabeshima T. Possible involvement of nitric oxide in quinolinic acid-induced convulsion in mice. *Pharmacol Biochem Behav* 1995; **51**: 309-312 [PMID: 7667345 DOI: 10.1016/0091-3057(94)00385-V]

52 **Dobbie M**, Crawley J, Waruiru C, Marsh K, Surtees R. Cerebrospinal fluid studies in children with cerebral malaria: an excitotoxic mechanism? *Am J Trop Med Hyg* 2000; **62**: 284-290 [PMID: 10813486]

53 **Sokol DK**, Demyer WE, Edwards-Brown M, Sanders S, Garg B. From swelling to sclerosis: acute change in mesial hippocampus after prolonged febrile seizure. *Seizure* 2003; **12**: 237-240 [PMID: 12763472 DOI: 10.1016/S1059-1311(02)00195-4]

54 **Crawley J**, Smith S, Kirkham F, Muthinji P, Waruiru C, Marsh K. Seizures and status epilepticus in childhood cerebral malaria. *QJM* 1996; **89**: 591-597 [PMID: 8935480 DOI: 10.1093/qjmed/89.8.591]

55 **Walker O**, Salako LA, Sowunmi A, Thomas JO, Sodeine O, Bondi FS. Prognostic risk factors and post mortem findings in cerebral malaria in children. *Trans R Soc Trop Med Hyg* 1992; **86**: 491-493 [PMID: 1475813]

56 **Aleem MA**. Epilepsy in malaria. *Epilepsia* 2005; **46**: 601 [DOI: 10.1111/j.0013-9580.2005.t01-3-64204_5.x]

57 **Versteeg AC**, Carter JA, Dzombo J, Neville BG, Newton CR. Seizure disorders among relatives of Kenyan children with severe falciparum malaria. *Trop Med Int Health* 2003; **8**: 12-16 [PMID: 12535243 DOI: 10.1046/j.1365-3156.2003.00965.x]

58 **Carter JA**, Ross AJ, Neville BG, Obiero E, Katana K, Mung'ala-Odera V, Lees JA, Newton CR. Developmental impairments following severe falciparum malaria in children. *Trop Med Int Health* 2005; **10**: 3-10 [PMID: 15655008 DOI: 10.1111/j.1365-3156.2004.01345.x]

59 **Dugbartey AT**. The neuropsychology of cerebral malaria. PhD Dissertation, University of Victoria, Canada, 1995

60 **Boivin MJ**. Effects of early cerebral malaria on cognitive ability in Senegalese children. *J Dev Behav Pediatr* 2002; **23**: 353-364 [PMID: 12394524]

61 **Carter JA**, Mung'ala-Odera V, Neville BG, Murira G, Mturi N, Musumba C, Newton CR. Persistent neurocognitive impairments associated with severe falciparum malaria in Kenyan children. *J Neurol Neurosurg Psychiatry* 2005; **76**: 476-481 [PMID: 15774431 DOI: 10.1136/jnnp.2004.043893]

62 **Vargha-Khadem F**, Gadian DG, Watkins KE, Connelly A, Van Paesschen W, Mishkin M. Differential effects of early hippocampal pathology on episodic and semantic memory. *Science* 1997; **277**: 376-380 [PMID: 9219696 DOI: 10.1126/science.277.5324.376]

63 **Dai M**, Reznik SE, Spray DC, Weiss LM, Tanowitz HB, Gulinello M, Desruisseaux MS. Persistent cognitive and motor deficits after successful antimalarial treatment in murine cerebral malaria. *Microbes Infect* 2010; **12**: 1198-1207 [PMID: 20800692 DOI: 10.1016/j.micinf.2010.08.006]

64 **Salas TR**, Reddy SA, Clifford JL, Davis RJ, Kikuchi A, Lippman SM, Menter DG. Alleviating the suppression of glycogen synthase kinase-3beta by Akt leads to the phosphorylation of cAMP-response element-binding protein and its transactivation in intact cell nuclei. *J Biol Chem* 2003; **278**: 41338-41346 [PMID: 12900420 DOI: 10.1074/jbc.M302972200]

65 **Wang JZ**, Liu F. Microtubule-associated protein tau in development, degeneration and protection of neurons. *Prog Neurobiol* 2008; **85**: 148-175 [PMID: 18448228 DOI: 10.1016/j.pneurobio.2008.03.002]

66 **Iqbal K**, Alonso Adel C, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H,

Grundke-Iqbal I. Tau pathology in Alzheimer disease and other tauopathies. *Biochim Biophys Acta* 2005; **1739**: 198-210 [PMID: 15615638 DOI: 10.1016/j.bbadis.2004.09.008]
67 **Medana IM**, Idro R, Newton CR. Axonal and astrocyte injury markers in the cerebrospinal fluid of Kenyan children with severe malaria. *J Neurol Sci* 2007; **258**: 93-98 [PMID: 17459417 DOI: 10.1016/j.jns.2007.03.005]
68 **Boivin MJ**, Bangirana P, Byarugaba J, Opoka RO, Idro R, Jurek AM, John CC. Cognitive impairment after cerebral malaria in children: a prospective study. *Pediatrics* 2007; **119**: e360-e366 [PMID: 17224457 DOI: 10.1542/peds.2006-2027]
69 **Holding PA**, Stevenson J, Peshu N, Marsh K. Cognitive sequelae of severe malaria with impaired consciousness. *Trans R Soc Trop Med Hyg* 1999; **93**: 529-534 [PMID: 10696414]
70 **Richardson ED**, Spring JA, Varney NR, Struchen MA, Roberts RJ. Dichotic listening in the clinic: new neuropsychological applications. *Clinical Neuropsychiatry* 1994; **8**: 416-428 [DOI: 10.1080/13854049408402044]
71 **Grote CL**, Pierre-Louis SJ, Durward WF. Deficits in delayed memory following cerebral malaria: a case study. *Cortex* 1997; **33**: 385-388 [PMID: 9220268]
72 **Varney NR**, Roberts RJ, Springer JA, Connell SK, Wood PS. Neuropsychiatric sequelae of cerebral malaria in Vietnam veterans. *J Nerv Ment Dis* 1997; **185**: 695-703 [PMID: 9368547]
73 American Psychiatric Association: Diagnostic and statistical manual of mental disorders, 4th edition. Washington: DSM-IV, 1994
74 **Tripp G**, Wickens JR. Research review: dopamine transfer deficit: a neurobiological theory of altered reinforcement mechanisms in ADHD. *J Child Psychol Psychiatry* 2008; **49**: 691-704 [PMID: 18081766 DOI: 10.1111/j.1469-7610.2007.01851.x]
75 **Tripp G**, Wickens JR. Neurobiology of ADHD. *Neuropharmacology* 2009; **57**: 579-589 [PMID: 19627998 DOI: 10.1016/j.neuropharm.2009.07.026]
76 **Valera EM**, Faraone SV, Murray KE, Seidman LJ. Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2007; **61**: 1361-1369 [PMID: 16950217 DOI: 10.1016/j.biopsych.2006.06.011]
77 **Swanson JM**, Kinsbourne M, Nigg J, Lanphear B, Stefanatos GA, Volkow N, Taylor E, Casey BJ, Castellanos FX, Wadhwa PD. Etiologic subtypes of attention-deficit/hyperactivity disorder: brain imaging, molecular genetic and environmental factors and the dopamine hypothesis. *Neuropsychol Rev* 2007; **17**: 39-59 [PMID: 17318414 DOI: 10.1007/s11065-007-9019-9]
78 **Lou HC**, Henriksen L, Bruhn P, Børner H, Nielsen JB. Striatal dysfunction in attention deficit and hyperkinetic disorder. *Arch Neurol* 1989; **46**: 48-52 [PMID: 2783366 DOI: 10.1001/archneur.1989.00520370050018]
79 **Lacerda-Queiroz N**, Rodrigues DH, Vilela MC, Miranda AS, Amaral DC, Camargos ER, Carvalho LJ, Howe CL, Teixeira MM, Teixeira AL. Inflammatory changes in the central nervous system are associated with behavioral impairment in Plasmodium berghei (strain ANKA)-infected mice. *Exp Parasitol* 2010; **125**: 271-278 [PMID: 20138873 DOI: 10.1016/j.exppara.2010.02.002]
80 **de Miranda AS**, Lacerda-Queiroz N, de Carvalho Vilela M, Rodrigues DH, Rachid MA, Quevedo J, Teixeira AL. Anxiety-like behavior and proinflammatory cytokine levels in the brain of C57BL/6 mice infected with Plasmodium berghei (strain ANKA). *Neurosci Lett* 2011; **491**: 202-206 [PMID: 21256928 DOI: 10.1016/j.neulet.2011.01.038]
81 **Dugbartey AT**, Dugbartey MT, Apedo MY. Delayed neuropsychiatric effects of malaria in Ghana. *J Nerv Ment Dis* 1998; **186**: 183-186 [PMID: 9521354]
82 **Miranda AS**, Vieira LB, Lacerda-Queiroz N, Souza AH, Rodrigues DH, Vilela MC, Gomez MV, Machado FS, Rachid MA, Teixeira AL. Increased levels of glutamate in the central nervous system are associated with behavioral symptoms in experimental malaria. *Braz J Med Biol Res* 2010; **43**: 1173-1177 [PMID: 21085889 DOI: 10.1590/S0100-879X2010007500130]
83 **Meldrum BS**. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr* 2000; **130**: 1007S-1015S [PMID: 10736372]
84 **Mehta A**, Prabhakar M, Kumar P, Deshmukh R, Sharma PL. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol* 2013; **698**: 6-18 [PMID: 23123057 DOI: 10.1016/j.ejphar.2012.10.032]
85 **Nguyen TH**, Day NP, Ly VC, Waller D, Mai NT, Bethell DB, Tran TH, White NJ. Post-malaria neurological syndrome. *Lancet* 1996; **348**: 917-921 [PMID: 8843810 DOI: 10.1016/S0140-6736(96)01409-2]
86 **Hsieh CF**, Shih PY, Lin RT. Postmalaria neurologic syndrome: a case report. *Kaohsiung J Med Sci* 2006; **22**: 630-635 [PMID: 17116625 DOI: 10.1016/S1607-551X(09)70364-X]
87 **Falchook GS**, Malone CM, Upton S, Shandera WX. Postmalaria neurological syndrome after treatment of *Plasmodium falciparum* malaria in the United States. *Clin Infect Dis* 2003; **37**: e22-e24 [PMID: 12856230 DOI: 10.1086/375269]
88 **Brewster DR**, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria in children. *Lancet* 1990; **336**: 1039-1043 [PMID: 1977027 DOI: 10.1016/0140-6736(90)92498-7]

P- Reviewer: Zhou M **S- Editor**: Song XX
L- Editor: A **E- Editor**: Liu SQ

World Journal of Translational Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5528/wjtm.v3.i1.17

World J Transl Med 2014 April 12; 3(1): 17-30
ISSN 2220-6132 (online)

REVIEW

Physics and mathematics of magnetic resonance imaging for nanomedicine: An overview

Odey Samuel Onwu, Oluwaseun Michael Dada, Omotayo Bamidele Awojoyogbe

Odey Samuel Onwu, Oluwaseun Michael Dada, Omotayo Bamidele Awojoyogbe, Department of Physics, Federal University of Technology, Minna, Niger State 920001, Nigeria
Author contributions: Onwu OS, Dada OM and Awojoyogbe OB contributed equally to the completion of the manuscript.
Correspondence to: Oluwaseun Michael Dada, Department of Physics, Federal University of Technology, P.M.B. 65, Minna, Niger State 920001, Nigeria. das_niger@yahoo.co.uk
Telephone: +234-813-8065478 Fax: +234-813-8065478
Received: October 7, 2013 Revised: February 16, 2014
Accepted: March 3, 2014
Published online: April 12, 2014

Abstract

Magnetic resonance imaging (MRI), magnetic resonance angiography (MRA) and magnetic resonance spectroscopy (MRS) are fundamental concepts used in modern medicine to improve health care. These concepts are based on the principle of nuclear magnetic resonance (NMR). Over the years, various laboratories around the world have applied different numerical techniques based on the Bloch NMR equations to solve specific problems in physics, biology, chemistry, engineering and medicine. The ultimate goal of any physician is to obtain maximum physical, biophysical, chemical and biological information on any tissue or cell under examination. This goal can be achieved by solving the Bloch NMR flow equations analytically. In this review, we present the basic principle of NMR/MRI in a way that can be easily understood by any researcher who needs an NMR concept to solve a specific medical problems. After a very brief history of the subject, a second order, non homogeneous, time-dependent differential equation derived from the Bloch NMR equation is presented. This equation has the basic intrinsic properties of MRI, MRA and MRS that can be extracted by means of classical and quantum mechanics for possible application in nanomedicine.

Key words: Bloch flow equations; Rotational diffusion; Molecular dynamics of biological fluids; Nuclear magnetic resonance diffusion equation; Rotational correlation time; Spherical harmonics; Molecular flow

Core tip: Magnetic resonance imaging is one of the most powerful methods for investigating structural and dynamics of biological matter. Based on quantum mechanical principles applied to Bloch nuclear magnetic resonance (NMR) flow equations, we aimed to apply the analytical solutions obtained from the Bloch NMR flow equations to nanomedicine. This may trigger research towards the design of nano devices that capable of delivering drugs directly to specifically targeted cells, with the possibility of very early diagnosis of diseases and treating them with powerful drugs at the pathological site alone, reducing any harmful side effects.

Onwu OS, Dada OM, Awojoyogbe OB. Physics and mathematics of magnetic resonance imaging for nanomedicine: An overview. *World J Transl Med* 2014; 3(1): 17-30 Available from: URL: http://www.wjgnet.com/2220-6132/full/v3/i1/17.htm DOI: http://dx.doi.org/10.5528/wjtm.v3.i1.17

INTRODUCTION

Nuclear magnetic resonance (NMR) is a very important analytical and experimental tool for physical, chemical and structural analysis of certain organic materials. Magnetic resonance is a branch of spectroscopy that detects the quantum-mechanical transitions induced by electromagnetic (EM) radiation in a system of discrete energy levels of electrons or nuclei placed in a static magnetic field[1,2]. NMR employs EM waves in the radio-frequency range between 900 MHz and 2 KHz. Some nuclei experience nuclear resonance, while others do not. Exhibition

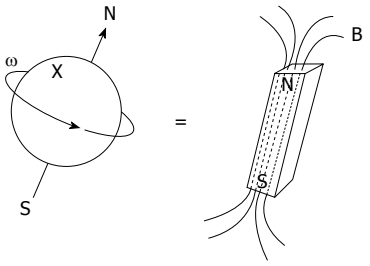

Figure 1 The charged nucleus (for example, ^{1}H) rotating with angular frequency $\omega = 2\pi\nu$ creates a magnetic field B and is equivalent to a small bar magnet whose axis is coincident with the spin rotation axis[4].

of this phenomenon is dependent on whether they possess a property called "spin"[2].

NMR is one of the most powerful methods for investigating the structure and dynamics of matter in different states of aggregation. This is due to the following features: (1) the interactions of nuclear magnetic moments are very weak compared with the thermal energy; therefore, we are dealing with para-magnetism. Moreover, the energy delivered by the radio-frequency generator are much larger compared with the strength of these internuclear couplings. That leads to the possibility of manipulating these interactions in a specific way and simplifying the spectral response; (2) the radio-frequency photons have much lower energy compared with the energy of chemical bonds. Therefore, the interaction of EM radiation with matter, particularly biomolecules, is non-ionizing; and (3) the number of radio-frequency photons with a specific frequency is very large. Hence, the phase of the associated EM wave is very well defined. The high degree of coherence of radio-frequency radiation is essential to implement NMR experiments, including magnetic resonance imaging (MRI)[3].

SPIN

Spin is a fundamental property of nature, like electrical charge or mass. Spin comes in multiples of 1/2 and can be positive (+) or negative (-). Protons, electrons and neutrons possess spins. Individual unpaired electrons, protons and neutrons each possess a spin of 1/2. In the deuterium atom (^{2}H), for example, with one unpaired electron, one unpaired proton and one unpaired neutron, the total electronic spin is equal to 1/2 and the total nuclear spin is equal to 1. Two or more particles with spins having opposite signs can pair up to eliminate the observable manifestations of spin. An example is helium, (^4_2He). In NMR, it is the unpaired nuclear spins that are important. When placed in a magnetic field of strength B, a particle with a net spin can absorb a photon, of frequency ω. The frequency of ω depends on the gyromagnetic ratio γ, of the particle [as shown in equation (1)], given by the expression:

$$\omega = \gamma B \quad (1)$$

For hydrogen nuclei, the gyromagnetic ratio γ = 42.58 MHz/T[4]. Nuclei are composed of positively charged protons and uncharged neutrons held together by nuclear forces[4,5], as shown in Figure 1.

Table 1 Properties of nuclei most useful for biological studies[5]

Nucleus	Spin quantum number (I)	Natural abundance (%)	Gyromagnetic ratio γ (10^{-7} rad/T sec)	Sensitivity[1] (% *vs* ^{1}H)	Electric quadruple moment (Q) (e.10^{24} cm^2)
^{1}H	1/2	99.9844	26.7520	100.000	-
^{2}H	1/1	0.0156	4.1067	0.965	0.00277
^{13}C	1/2	1.1080	6.7265	1.590	-
^{15}N	1/2	0.3650	-2.7108	0.104	-
^{19}F	1/2	100.0000	25.167	83.300	-
^{31}P	1/2	100.0000	10.829	6.630	-

[1]Relative sensitivity for equal number of nuclei at constant magnetic field strength.

The shell model for the nucleus tells us that nucleons, just like electrons, fill orbitals. When the number of protons or neutrons equals 2, 8, 20, 28, 50, 82 and 126, the orbitals are filled, because nucleons have spin, just like electrons do, and their spins can pair up when the orbitals are being filled and cancel out. Almost every element in the periodic table has an isotope with a non-zero nuclear spin[4,5]. NMR can only be performed on isotopes whose natural abundance is high enough to be detected; some of the nuclei that are of interest in NMR/MRI are listed in Table 1.

We have seen that $\omega = \gamma B$ and hence the energy of the radio waves needed to cause a transition between the two spin states is given by equation (2):

$$E = \eta\gamma B \quad (2)$$

When the energy of the photon matches the energy difference between the two spin states, absorption of energy occurs. In an NMR experiment, the frequency of the photon is in the radio frequency (RF) range. In NMR spectroscopy, ω is between 600 and 800 MHz for hydrogen nuclei. However, in clinical MRI, ω is typically between 15 and 80 MHz for hydrogen imaging[6] (Table 2).

To get a better understanding of how particles with spin behave under a magnetic field, we consider a proton that has a spin property. If we imagine the spin of this proton as a magnetic moment vector, causing the proton to behave like a tiny magnet with a North and South Poles. When the proton is placed in an external magnetic field, the spin vector of the particle aligns itself with the external field, just like a magnet would. There is a low energy configuration or state where the poles are aligned N-S-N-S and a high energy state N-N-S-S.

This particle can undergo a transition between the two energy states by the absorption of a photon. A particle in the lower energy state absorbs a photon and ends up in the higher energy state. The energy of this photon must exactly match the energy difference between the two states. The energy E, of a photon is related to its frequency ω, by Planck's constant ($\eta = h/2\pi$, h = 6.626 × 10^{-34} Js).

Table 2 Nuclear Spin values and gyromagnetic ratios of some nuclei[5]

Nuclei	Unpaired protons	Unpaired neutrons	Net spin	γ (MHz/T)
^{1}H	1	0	1/2	42.58
^{2}H	1	1	1/1	6.54
^{31}P	1	0	1/2	17.25
^{23}Na	1	2	3/2	11.27
^{14}N	1	1	1/1	3.08
^{13}C	0	1	1/2	10.71
^{19}F	1	0	1/2	40.08

$$E = \eta\omega \quad (3)$$

In NMR and MRI, the quantity ω is called the resonance frequency or the Larmor Frequency[6].

MRI

MRI is an imaging technique used primarily in medical settings to produce high quality images of the inside of the human body. MRI is based on the principles of NMR, a spectroscopic technique used by scientists to obtain microscopic chemical and physical information about molecules. The technique was called MRI rather than nuclear MRI because of the negative connotations associated with the word nuclear in the late 1970's. MRI started as a tomographic imaging technique; that is, it produced an image of the NMR signal in a thin slice through the human body. MRI has advanced from a tomographic imaging technique to a volume imaging technique. MRI is based on the absorption and emission of energy in the RF range of the EM spectrum[3,7].

In the past, many scientists were taught that one cannot obtain an image smaller than the wavelength of the energy being used to image it. MRI gets around this limitation by producing images based on spatial variations in the phase and frequency of the RF energy being absorbed and emitted by the imaged object.

Clinical MRI uses the magnetic properties of hydrogen and its interaction with both a large external magnetic field and radio waves to produce highly detailed images of the human body. Hydrogen has a significant magnetic moment and is the most abundant nucleus in the human body. For these reasons, we use only the hydrogen proton in routine clinical imaging[7].

To perform MRI, we first need a strong magnetic field. The field strength of the magnets used for MR is measured in units of Tesla. One (1) Tesla is equal to 10000 Gauss. The magnetic field of the earth is approximately 0.5 Gauss. Given that relationship, a 1.0 T magnet has a magnetic field approximately 20000 times stronger than that of the earth. The type of magnets used for MRI usually belongs to one of three types; permanent, resistive, and superconductive.

A permanent magnet is sometimes referred to as a vertical field magnet. These magnets are constructed of two magnets (one at each pole). The patient lies on a scanning table between these two plates[8].

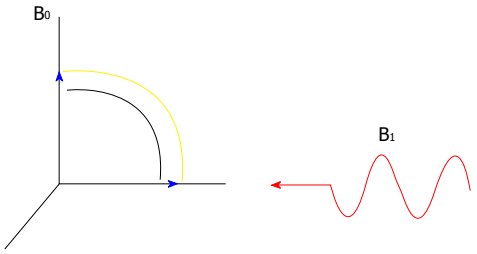

Figure 2 A 90-degree flip of the net magnetization.

The advantages of these systems are: relatively low cost, no electricity or cryogenic liquids are needed to maintain the magnetic field, their more open design may help alleviate some patient anxiety and their nearly non-existent fringe field. It should be noted that not all vertical field magnets are permanent magnets.

Resistive magnets are constructed from a coil of wire. The more turns to the coil, and the more current in the coil, the higher the magnetic field. These types of magnets are most often designed to produce a horizontal field because of their solenoid design. As previously mentioned, some vertical field systems are based on resistive magnets. The main advantages of these types of magnets are: no liquid cryogen, the ability to "turn off" the magnetic field and their relatively small fringe field.

Superconducting magnets are the most common. They are made from coils of wire (as are resistive magnets) and thus produce a horizontal field. They use liquid helium to keep the magnet wire at 4 degrees Kelvin where there is no resistance. The current flows through the wire without having to be connected to an external power source. The main advantage of superconducting magnets is their ability to attain field strengths of up to 3 Tesla for clinical imagers and up to 10 Tesla or more for small bore spectroscopy magnets[9].

CREATION OF AN MR SIGNAL

A radio wave is actually an oscillating EM field. The RF field is also referred to as the B_1 field. It is oriented perpendicular to the main magnetic field (B_0). If we apply a pulse of RF energy into the tissue at the Larmor frequency, we first find the individual spins begin to precess in phase, as will the net magnetization vector. As the RF pulse continues, some of the spins in the lower energy state absorb energy from the RF field and make a transition into the higher energy state. This has the effect of "tipping" the net magnetization toward the transverse plane. This phenomenon is illustrated in Figure 2. For the purpose of this explanation, we will assume sufficient energy is applied to produce a 90-degree flip of the net magnetization. In such an example, it is said that a 90-degree flip angle or a 90-degree pulse has been applied[10].

Oriented perpendicular to B_0 is a receiver coil. As the magnetization (now referred to as transverse magnetization, or M_{xy}) precesses through the receiver coil, a current or signal is induced in the coil. The principle behind

this signal induction is Faraday's Law of Induction. This states that if a magnetic field is moved through a conductor, a current will be produced in the conductor. If we increase the size of the magnetic field, or increase the speed with which it moves, we will increase the size of the signal (current) induced in the conductor.

To detect the signal produced in the coil, the transmitter must be turned off. When the RF pulse is discontinued, the signal in the coil begins at given amplitude (determined by the amount of magnetization precesssing in the transverse plane (Figure 2) and the precessional frequency) and fades rapidly away. This initial signal is referred to as the Free Induction Decay (FID). The signal fades as the individual spins contributing to the net magnetization lose their phase coherence, making the vector sum equal to zero. At the same time, but independently, some of the spins that had moved into the higher energy state give off their energy to their lattice and return to the lower energy state, causing the net magnetization to re-grow along the z axis. This re-growth occurs at a rate given by the tissue relaxation parameter, known as T_1[9,10].

DEFINITION OF TERMS IN NMR/MRI

Spin packets

A spin packet is a group of spins experiencing the same magnetic field strength. At any instant in time, the magnetic field caused by the spins in each spin packet can be represented by a magnetization vector; $\vec{M}$. The vector sum of the magnetization vectors from all the spin packets is the net magnetization. Adapting the conventional NMR coordinate system, the external magnetic field and the net magnetization vector at equilibrium are both along the Z axis.

T_1 relaxation time

The time constant that describes how M_z returns to its equilibrium value is called the spin lattice relaxation time (T_1). The equation governing this behavior as a function of time t after its displacement is:

$$M_z = M_o (1 - e^{-t/T_1}) \quad (4)$$

At equilibrium, the net magnetization vector lies along the direction of the applied magnetic field B_o and is called the equilibrium magnetization M_o. In this configuration, the Z component of magnetization M_z equals M_o. M_z is referred to as the longitudinal magnetization. There is no transverse (M_x or M_y) magnetization here.

Larmor frequency

The resonant frequency of a nucleus is determined by a combination of nuclear characteristics and the strength of the magnetic field. The specific relationship between resonant frequency and the field strength is an inherent characteristic of each nuclide and is generally designated as gyromagnetic ratio γ. The resonant frequency is also known as the Larmor frequency.

T_2 relaxation time

The time constant that describes the return to equilibrium of the transverse magnetization, M_{xy}, is called the spin-spin relaxation time, T_2.

$$M_{xy} = M_{xyo}\, e^{-t/T_2} \quad (5)$$

T_2 is always less than or equal to T_1. The net magnetization in the XY plane goes to zero and then the longitudinal magnetization grows in until we have M_o along Z.

Excitation

If a pulse of RF energy with a frequency corresponding to the nuclear precession rate is applied to a material, some of the energy will be absorbed by the individual nuclei. The absorption of energy by a nucleus flips its alignment away from the direction of the magnetic field. This increased energy places the nucleus in an excited state. In this excited state, the precession is now transformed into a spinning motion of the nucleus around the axis of the magnetic field[1-14].

BRIEF HISTORY ON THE DEVELOPMENT OF NMR AND MRI

The history of the development of the concept of NMR started with Felix Bloch at Harvard, and Edward Purcell at Stanford, both of whom were awarded the Nobel Prize in 1952, discovered the magnetic resonance phenomenon independently in 1946, using different instrumentation. In the period between 1950 and 1970, NMR was developed and used for chemical and physical molecular analysis. In 1971 Raymond Damadian (an Armenian-American medical practitioner and inventor of the first MR Scanning Machine) showed that the nuclear magnetic relaxation times of tissues and tumors differed, thus motivating scientists to consider magnetic resonance for the detection of disease. In 1973 the X-ray-based computerized tomography (CT) was introduced by Hounsfield. MRI was first demonstrated on small test tube samples that same year by Paul Lauterbur. He used a technique similar to that used in CT. In 1975 Richard Ernst, a Swiss physical chemist, proposed MRI using phase and frequency encoding, and the Fourier Transform. This technique is the basis of current MRI techniques. A few years later, in 1977, Raymond Damadian demonstrated MRI called field-focusing NMR. In this same year, Peter Mansfield developed the echo-planar imaging (EPI) technique. This technique was later developed to produce images at video rates (30 ms/image). Edelstein and coworkers demonstrated imaging of the body using Ernst's technique in 1980. A single image could be acquired in approximately five minutes by this technique. By 1986, imaging time was reduced to about five seconds, without sacrificing significant image quality. In the same year, the NMR microscope was developed, which allowed approximately 10m resolution on approximately one cm samples. In 1987 EPI was used to perform real-time moving imaging of a single cardiac cycle. In this same year, Charles Dumoulin perfected magnetic resonance angiography (MRA), which allowed imaging of flowing blood without the use of contrast agents.

In 1991, Richard Ernst was rewarded for his achievements in pulsed Fourier Transform NMR and MRI with the Nobel Prize in Chemistry. In 1992 functional MRI (fMRI) was developed. This technique allows the mapping of the functions of the various regions of the human brain. Five years earlier, many clinicians thought EPI's primary application was to be in real-time cardiac imaging. The development of fMRI opened up a new application for EPI in mapping the regions of the brain responsible for thought and motor control. In 1994, researchers at the State University of New York at Stony Brook and Princeton University demonstrated the imaging of hyperpolarized ^{129}Xe gas for respiration studies.

In 2003, Paul C Lauterbur of the University of Illinois and Sir Peter Mansfield of the University of Nottingham were awarded the Nobel Prize in Medicine for their discoveries concerning MRI. MRI is clearly a young, but growing science[1,3,5-16].

THE THEORY OF NMR

The appearance of NMR spectra, and consequently the molecular structure they are able to provide, arises from the discrete nature of the energy levels pertaining to a nuclear spin system. The energy levels are mainly a result of Zeeman interaction, $-\vec{\mu}\vec{B}_0$ between the static magnetic field of induction $\vec{B}_0$ and nuclear magnetic moment $\vec{\mu}$. The quantum-mechanical quantity called spin momentum, $\vec{I}$ is related to magnetic moment by $\vec{\mu} = \gamma\eta\vec{I}$, where γ is the gyromagnetic ratio and η is the Planck's constant divided by 2π.

In the absence of the magnetic nuclear, the spin states are generated. The application of a static magnetic field $\vec{B}_0$ which induces a magnetic interaction, is described by Zeeman Hamiltonian $H = -\vec{\mu}\vec{B}_0$. Taking the magnetic field orientation to be along the z-direction we get:

$$H = -\gamma\eta B_0 I_z \quad (6)$$

The Eigen values E_m of this Hamiltonian can be evaluated from the Schrodinger equation

$$H|m\rangle = -\gamma\eta B_0 m|m\rangle \quad (7)$$

where $|m\rangle$ is the Eigen state corresponding to the Eigen value $E_m = -\gamma\eta B_0 m$. The magnetic quantum number is m, where $m = l, l\text{-}1, \ldots, -l$. Therefore, the equidistant energy differences are for the single-quantum transitions $m = \pm 1$ given by[1,4,5,7]

$$\Delta E = \eta\omega_0 \quad (8)$$

where the Larmor frequency is defined as[1,4,5,7]

$$\nu_0 = \nu_L = \omega_0/2\pi$$

Another important ingredient for a magnetic resonance experiment is represented by the presence of the RF field. Only the magnetic component of the EM field, *i.e.*, $\vec{B}_1(t) = \vec{B}_{10}\cos(2\pi\nu t)$ interacts with the magnetic moment of the nuclei. The amplitude of the RF field is $\vec{B}_{10}$ and ν is the carrier frequency. This field is produced by an RF coil and leads to a perturbation Hamiltonian:

$$\vec{H}_p = -\gamma\eta\vec{B}_{10}\vec{I}\cos(2\pi\nu t) \quad (9)$$

From the time-dependent perturbation theory of quantum mechanics, it can be stated that a transition between two states $|\psi\rangle$ and $|\phi\rangle$ is allowed, provided that $\langle\psi|H_p|\phi\rangle \neq 0$. This takes place if $\nu \approx \nu_0$ (*i.e.*, the resonance condition) and the alternative magnetic field $\vec{B}_0$ is polarized perpendicular to the static magnetic field $\vec{B}_{10}$[11].

In general, NMR experiments are performed at high temperatures, employing a large number of spins. These features lead to the possibility to treat classically some aspects of the experiments. The excess of spins oriented along the static magnetic field $\vec{B}_0$ with respect to those oriented in the opposite direction results in a macroscopic nuclear magnetization $\vec{M}$, aligned along the static magnetic field, which is called the equilibrium magnetization. It can be displaced from this equilibrium by an appropriate perturbation, for instance, by an RF excitation. It is then subject to a precessional motion around $\vec{B}_0$ with the Larmor frequency ν_L. The EM perturbation that brings $\vec{M}$ into a plane perpendicular to $\vec{B}_0$ allows the observation of the Larmor precession through an electromotive force that occurs in a coil whose axis is contained in that plane. This can be done by rotation of the magnetization using a resonant 90° RF pulse. The nuclear magnetization $\vec{M}$ can be oriented antiparallel to $\vec{B}_0$ by the action of a 180° pulse. The majority of NMR experiments used pulse sequences composed of 90° and 180° RF pulses[9-18].

PULSED NMR SPECTROSCOPY

A coil of wire placed around the x-axis will provide a magnetic field along the x-axis when a direct current is passed through the coil. An alternating current will produce a magnetic field that alternates in direction. In a frame of reference rotating about the z-axis at a frequency equal to that of the alternating current, the magnetic field along the x'-axis will be constant, just as in the direct current case in the laboratory frame. This is the same as moving the coil about the rotating frame coordinate system at the Larmor Frequency. In magnetic resonance, the magnetic field created by the coil passing an alternating current at the Larmor frequency is called the B_1 magnetic field. When the alternating current through the coil is turned on and off it creates a pulsed B_1 magnetic field along the x'-axis. The spins respond to this pulse in such a way as to cause the net magnetization vector to rotate about the direction of the applied B_1 field. The rotation angle depends on the length of time τ for which the field is switched on and its magnitude, B_1[2,6,7,9].

A 90° pulse is one that rotates the magnetization vector clockwise by 90° about the x'-axis and rotates the equilibrium magnetization down to the y'-axis. In the laboratory frame, the equilibrium magnetization spirals down around the z-axis to the xy-plane. One can now see why the rotating frame of reference is helpful in describing the behavior of magnetization in response to a pulsed magnetic field. A 180° pulse will rotate the magnetization vector by 180° and rotates the equilibrium magnetization

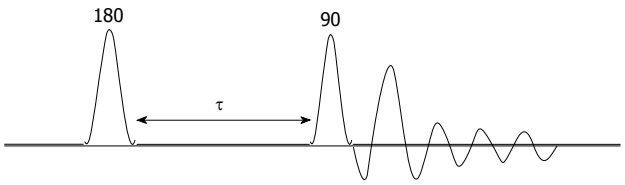

Figure 3 A series of spectra recorded with different values of τ to map out the recovery of the magnetization.

down along the z-axis.

The net magnetization at any orientation will behave according to the rotation equation. For example, a net magnetization vector along the y'-axis will end up along the y'-axis when acted upon by a 180° pulse of B_1 along the x'-axis. A net magnetization vector between x'- and y'- will end up between x' and y' after the application of 180° pulse of B_1 applied along the x'-axis[9].

Longitudinal magnetization is aligned along the field axis B_0 (that is, the z-axis) and has a finite equilibrium value of M_{0z}. M_{0z} represents the equilibrium magnetization at the start of any NMR experiment.

Transverse magnetization is perpendicular to B_0, precesses around the z-axis at the Larmor frequency and has an equilibrium value of zero[13].

$$\omega = 2\pi\nu = -\gamma B_0$$

A simple 1D NMR spectrum is obtained by a 90° pulse, followed by detection of the FID and Fourier transformation of the FID. More complicated pulse sequences allow measurement of specific parameters, 2D and multi-dimensional data.

MEASUREMENT OF T_1 RELAXATION TIMES

The inversion-recovery (IR) pulse sequence can be used to measure the T_1 relaxation times of all the resonances in a spectrum. IR pulse sequence is pictorially represented in Figure 3.

Alternatively, the zero crossing point for each peak can be used to estimate the T_1. Using the time τ_0 at which the signal I is zero:

$$\tau_0 = T_1 \ln 2 = 0.693\ T_1 \quad (10)$$

This provides a very useful spot check for the value of T_1 in any sample. We must note that the relaxation delay between subsequent scans must be set to at least 5 T_1 for experiments for good estimates of T_1.

The saturation-recovery pulse sequence starts from perfectly equal populations of α and β spin states (M_z = 0, achievable by saturation). During a subsequent waiting period t, the z-magnetization reappears in an exponential recovery until it has assumed equilibrium value M_{z0} for a very long time t:

$$M_z = M_{z0}\ (1 - e^{-t/T_1}) \quad (11)$$

An experiment of this nature does not require long recovery delays between scans; however, saturation of the spin states is not always easy[10].

PRACTICAL IMPORTANCE OF T_1 RELAXATION

Recovery delays

Any pulsed NMR experiment is repeated many times, and the scans added together to improve signal-to-noise (S/N) ratio. If the signals are not fully relaxed before each pulse/acquisition, then the signal in each scan will progressively decrease.

A recovery delay of about 5 × T_1 between subsequent scans almost completely restores M_{z0}, yielding reliable integrals. Recovery delays of about 1.4 T_1 maximizes S/N ratio, but the integrals of slowly relaxing signals will be smaller than those of the rapidly relaxing signals.

Solvent saturation

If a signal, or a whole spectrum, is irradiated continuously with RF, then the populations N_α and N_β will equalize and no signal can be observed. This can be used for selective removal of a peak, *e.g.*, a solvent peak from a spectrum. For example, in biological samples, H_2O would yield a huge signal without solvent suppression. A simple way of solvent suppression is presented by selective saturation by pre-irradiation = weak irradiation of the solvent signal during the recovery delay before the 90° pulse[10,18].

Weak interactions between the small magnetic moments of nuclear spins with the environment result in slow relaxation rates and allow the design of long and complicated pulse sequences. In contrast to optical spectroscopy, nuclear spins can access only a finite number of energy levels. This allows the accurate calculation of the outcome of pulse sequences. Transverse magnetization can be destroyed by a "pulsed field gradient": an electric current is sent through a gradient coil for a few milliseconds. This results in a wide distribution of Larmor frequencies during the gradient and, hence, averaging of the transverse magnetization vectors to zero[18].

NUCLEAR ENERGIES IN A MAGNETIC FIELD

The energy of the magnetic moment of a nuclear spin in a magnetic field is given by

$$E = -\mu B \quad (12)$$

where μ is the spin angular momentum and B is the magnetic field (in general B is a vector quantity). To conduct an NMR experiment, a sample is first placed in a static field. By convention, the direction of the static magnetic field is along the z-axis, and the magnitude of the magnetic field is given by B_0 (no longer a vector because it points only along the z-axis). In this case, the energy of a nuclear spin in an NMR magnet becomes

$$E = -\mu_z B_0 = -\gamma I_z B_0 \quad (13)$$

where γ is the gyromagnetic ratio (sometimes called the magnetogyric ratio) and I_z is the spin quantum number in the z-axis direction. The energy associated with a particu-

lar quantum number m is given as:

$$Em = -m\eta\gamma B_o \quad (14)$$

NMR transition energies are very small. These small energies translate into low sensitivity. When samples are placed into a magnetic field, a small excess of nuclei fall into the α state. This excess of spins in the α over the β states accounts for the entire net magnetization that is used in the NMR experiment. The ratio of the number of spins in the β state to those in the α state is given by a Boltzmann distribution:

$$N_\alpha/N_\beta = e^{\Delta E/(k_B T)} \quad (15)$$

where ΔE is the difference of energies of the α and β states, k_B is Boltzmann constant, and T is the absolute temperature. Higher magnetic fields produce correspondingly larger differences in spin states, leading to greater sensitivity[12,14,15].

BULK MAGNETIZATION IN AN ELECTRIC FIELD

If the bulk magnetization is along the field direction, as it is at equilibrium, then there is no torque and hence no motion. As expected, at equilibrium the system is stationary. If the system is away from equilibrium and the bulk magnetization vector is oriented other than along the z-axis, then the magnetization precesses (rotates) about the z-axis with an angular velocity given by the energy separation of the two states (γB_o). This torque will not change the length of the magnetization vector; it only varies its orientation. This orientation cannot be the only motion, since the system would never return to equilibrium. Therefore, along with the rotation, there is a relaxation of the vector to bring it back along the z-axis. Therefore the x- and y-components of the nuclear magnetization decay towards zero, and the z-component decays towards the equilibrium value (M_o)[4,10,14].

Considering bulk magnetization M that arises from all the magnetic moments in a sample, M experiences a torque when placed in a magnetic field, according to the expression given below:

$$dJ(t)/dt = M(t) \times B(t) \quad (16)$$

where $J(t)$ is the bulk spin angular momentum. The vector quantities in equation (16) are time dependent. The time-dependence of the magnetic field comes about when we apply RF pulses along the x- or y-axis. Equation (16) is essentially identical to an equation that describes the motion of a gyroscope[18-20]:

$$dL(t)/dt = r \times mg \quad (17)$$

where $L(t)$ is the gyroscope's angular momentum, r the radius from the fixed point of rotation, m is the mass, and g is gravity. Thus, a nuclear spin in a magnetic field will behave much like a gyroscope in a gravitational field. To make equation (16) useful, we use the relationship for the z-component of the magnetic moment:

$$\mu_z = \gamma I_z = \gamma\eta m \quad (18)$$

Then multiply each side by γ to yield

$$dM(t)/dt = M(t) \times \gamma B(t) \quad (19)$$

Equation (19) is the basis of the Bloch equations[18-20].

BLOCH EQUATIONS

In 1946 Felix Bloch formulated a set of equations that describe the behavior of nuclear spin in a magnetic field under the influence of RF pulses. He modified equation (19), given above, to account for the observation that nuclear spins "relax" to equilibrium values following the application of RF pulses. Bloch assumed they relax along the z-axis and in the x-y plane at different rates, but following first order kinetics. These rates are designated $1/T_1$ and $1/T_2$ for the z-axis and x-y plane, respectively. T_1 is called spin-lattice relaxation and T_2 the spin-spin relaxation. With the addition of relaxation, equation (19) becomes:

$$dM(t)/dt = M(t) \times \gamma B(t) - R\,[M(t) - M_o] \quad (20)$$

where R is the "relaxation matrix". Equation (20) can best be explained by considering each of its components:

$$dM_z(t)/dt = \gamma[M_x(t)\,B_y(t) \times M_y(t)\,B_x(t)] - [M_z(t) - M_o]/T_1$$
$$dM_x(t)/dt = \gamma[M_y(t)\,B_z(t) \times M_z(t)\,B_y(t)] - M_x(t)/T_2$$
$$dM_y(t)/dt = \gamma[M_z(t)\,B_x(t) \times M_x(t)\,B_z(t)] - M_y(t)/T_2 \quad (21)$$

The terms in equation (21) that do not involve either T_1 or T_2 are the result of the cross product in equation (20). Equation (21) describes the motion of magnetization in the "laboratory frame", an ordinary coordinate is stationary. Mathematically, the laboratory frame is not the simplest coordinate system, because the magnetization is moving at a frequency $\omega_0 = \gamma B_o$ in the x-y (transverse) plane. A simpler coordinate system is the "rotating frame", in which the x-y plane rotates around the z-axis at a frequency $\Omega = -\gamma B_o$. In the rotating frame, magnetization "on resonance" does not precess in the transverse plane. The transformation of equation (21) to the rotating frame is achieved by replacing each B_z (defined as B_o) by Ω/λ:

$$dM_z(t)/dt = \gamma\,[M_x(t)\,B_y^r(t) - M_y(t)\,B_x^r(t)] - [M_z(t) - M_o]/T_1$$
$$dM_x(t)/dt = -\Omega\,M_y(t) - \gamma\,M_z(t)\,B_y^r(t) - M_x(t)/T_2$$
$$dM_y(t)/dt = \gamma\,M_z(t)\,B_x^r(t) + \Omega\,M_x(t) - M_y(t)/T_2 \quad (22)$$

In equation (22), the components of B have been written with r superscripts to denote that it is a rotating frame[17-23].

PHYSICAL INTERPRETATION OF BLOCH EQUATIONS

We shall examine the behavior of equation (22) under two different limiting conditions, the effect of a short *RF* pulse and free precession. The RF pulse will be assumed to be very short compared to either relaxation times T_1 and T_2, as well as the angular frequency Ω. This assumption is valid for many typical pulsed NMR experiments, in which the pulse lengths can be as short as 5 μs. If the

RF pulse is applied along the x-axis, these conditions will allow us to neglect terms in equation (22) that contain T_1, T_2, Ω, and B_y.

$$dM_z/dt = -M_y(t)\,\gamma\,B_x^r(t)$$
$$dM_x(t)/dt = 0$$
$$dM_y(t)/dt = M_z(t)\,\gamma\,B_x^r(t) \quad (23)$$

Before solving equation (23), we need to discuss the meaning of $B_x(t)$ and $B_y(t)$. We can recall that B_o is the static magnetic field strength oriented along the z-axis. $B_x(t)$ and $B_y(t)$ are magnetic fields oriented along the x- and y-axes that are generated by rf pulses. By analogy to $\omega_0 = \gamma B_o$ defining the frequency of the NMR transitions in the static magnetic field, we can see that the terms $\gamma B_x(t)$ and $\gamma B_y(t)$ are frequencies of the magnetization rotating around the x- or y-axis. Thus, applying these frequencies for different periods of time will allow for different degrees of rotation around the x- or y-axis. If we introduce a frequency of rotation about the x-axis as $\omega_x = \gamma B_x(t)$, solutions to equation (23) can now be given as:

$$M_z(t) = M_o\cos(\omega_x t)$$
$$M_x(t) = 0$$
$$M_y(t) = M_o\sin(\omega_x t) \quad (24)$$

Finally, if we let $\theta = \omega_x t$ be the pulse angle, equation (24) shows that application of a magnetic field (RF pulse) along the x-axis causes the magnetization that was originally along the z-axis to rotate toward the y-axis by an angle θ. Note that when $\theta = 0$, $M_z(t) = M_o$ and $M_Y(t) = 0$ (all the magnetization is still pointing along the z-axis). When $\theta = 90°$, $M_Y(t) = M_o$ and $M_z(t) = 0$ (all the magnetization is still pointing along the y-axis). These have described the effects of a simple RF pulse. The second limiting condition for equation (22) is free precession in the absence of any applied pulse. In that case, B_x and B_y are both equal to zero, and equation (22) becomes:

$$dM_z(t)/\mathrm{d}t = -[M_z(t) - M_o]/T_1$$
$$dM_x(t)/\mathrm{d}t = -\Omega\,M_y(t) - M_x(t)/T_2$$
$$dM_y(t)/\mathrm{d}t = -\Omega M_x(t) - M_y(t)/T_2 \quad (25)$$

The solutions to equation (25) can be given as:

$$M_z(t) = M_o\,(1 - e^{-t/T_1})$$
$$M_x(t) = M_o\cos(\Omega\,t)\,e^{-t/T_2}$$
$$M_y(t) = M_o\sin(\Omega\,t)\,e^{t/T_2} \quad (26)$$

Equation (26) describes magnetization precessing in the x-y plane at a frequency Ω, while it is relaxing along the z-axis at a rate of $1/T_1$ and relaxing in the x-y plane at a rate $1/T_2$[17-19,21,24].

THE GENERAL BLOCH NMR FLOW EQUATION

The Bloch NMR flow equations can be written as[25,26]:

$$\partial M_x/\partial t + v\,\partial M_x/\partial x = -M_x/T_2 \quad (27)$$
$$\partial M_y/\partial t + v\,\partial M_y/\partial x = \gamma M_z B_1(x) - M_y/T_2 \quad (28)$$
$$\partial M_z/\partial t + v\,\partial M_z/\partial x = -\gamma\,M_z\,B_1(x) + (M_o - M_z)/T_1 \quad (29)$$

From equation (28 and 29), we have

$$v^2\frac{\partial M_y}{\partial x^2} + 2v\frac{\partial^2 M_y}{\partial x\partial t} + v\left(\frac{1}{T_1}+\frac{1}{T_2}\right)\frac{\partial M_y}{\partial x} + \left(\frac{1}{T_1}+\frac{1}{T_2}\right)\frac{\partial M_y}{\partial t}$$
$$+\frac{\partial^2 M_y}{\partial t^2} + \left[\frac{1}{T_1T_1} + \gamma^2 B_1^2(x,t)\right]M_y = \frac{\gamma\,B_1(x,t)\,M_o}{T_1} \quad (30)$$

Equation (30) is a general second order, non-homogeneous, time dependent differential equation that can be applied to any fluid flow problem. At any given time t, we can obtain information about the system, provided that appropriate boundary conditions are applied. From equation (30), we can obtain the diffusion equation, the wave equation, telephone and telegraph equations, Schrödinger's equation, Legendre's equation, *etc.*, and solve them in terms of NMR parameters by the application of appropriate initial or boundary conditions. Hence, we can obtain very important information about the dynamics of the system. It should be noted, however, that the term $F_o\gamma B_1(x, t)$ is the forcing function ($F_o = M_o/T_1$). If the function is zero, we have a freely vibrating system; otherwise, the system is undergoing a forced vibration.

NMR DIFFUSION EQUATION

A diffusion equation can easily be obtained from equation (30) if we assume that the NMR wave is a plane wave such that:

$$M_y(x, t) = A\,e^{\mu x + \eta t} \quad (31)$$

subject to the following MRI experimental conditions:

$$\gamma^2 B_1^2(x, t) << 1/(T_1\,T_2) \quad (32)$$

where μ and η are dependent on the NMR parameters. Taking

$$\eta^2 = T_g \text{ and } 2\eta = T_0 \quad (33)$$

Equation (30) becomes

$$v^2\,\partial^2 M_y/\partial x^2 + T_0\,\partial M_y/\partial t = F_o\gamma\,B_1(x, t) \quad (34)$$

If we write

$$D = -v^2/T_0$$
$$v = (-D\,T_0)^{1/2} \quad (35)$$

Then equation (34) becomes

$$\partial M_y/\partial t = D\,\partial^2 M_y/\partial x^2 + F_o/T_0\,\gamma B_1(x, t) \quad (36a)$$

Equation (36a) can be written in generalized co-ordinates as:

$$\partial M_y/\partial t = D\nabla^2 M_y + F_o/T_0\,\gamma B_1(t) \quad (36b)$$

If D represents the diffusion coefficient, then Equation (36) is the equation of diffusion of magnetization as the nuclear spins move. The function $F_o/T_0\gamma B_1(x, t)$ is the forcing function, which shows that application of the RF B_1 field has an influence on the diffusion of magnetization within a voxel. It is interesting to note that the dimension of equation (35) exactly matches that of the diffusion coefficient.

Equation (36) is only applicable when D is non-directional. That is, we have a constant diffusion coefficient (isotropic medium). Equation (36) can be considered for

restricted diffusion in various geometries[25,26]. This model would work quite well for molecules that move very short distances over a very considerable amount of time; where

$$\Omega = T_g + \gamma^2 B_1^2(x, t);$$
$$F_o = M_o/T_1;$$
$$T_g = 1/(T_1 T_2)$$
$$\text{and } T_0 = 1/T_1 + 1/T_2 \quad (37)$$

where γ is the gyromagnetic ratio, D is the diffusion coefficient, v is the fluid velocity, T_1 is the spin lattice relaxation time, T_2 is the spin relaxation time, M_o is the equilibrium magnetization, $B_1(x, t)$ is the applied magnetic field and M_y is the transverse magnetization. Solutions to equation (36) have been discussed by applying a number of analytical methods[26], and for the present purpose it is sufficient to design the NMR system in such a way that the transverse magnetization M_y, takes the form of a plane wave.

MATHEMATICAL CONCEPT OF ROTATIONAL DIFFUSION MRI AND MOLECULAR DYNAMICS OF BIOLOGICAL FLUIDS

The random re-orientation of molecules (or larger systems) is an important process for many biophysical probes. By the equipartition theorem, larger molecules re-orient more slowly than do smaller objects and, hence, measurements of the rotational diffusion constants can give insight into the overall mass and its distribution within an object. In this study, the mathematical concept of rotational diffusion MRI and molecular dynamics of biological fluids is presented. This approach ensures the analytical solution of the Bloch NMR flow equation, which enables us to obtain the NMR transverse magnetization in terms of spherical harmonic functions and NMR relaxation parameters for measuring rotational diffusion at the molecular level.

Theoretical and experimental studies to determine rotational diffusion coefficients using Fluorescence Correlation Spectroscopy, fluorescence anisotropy, flow birefringence, dielectric spectroscopy, NMR relaxation and other biophysical methods that are sensitive to picosecond or slower rotational processes have been published earlier studies[1-12].

In this study, we have presented a new method based on the Bloch NMR flow equation to measure rotational diffusion of biological fluids. The approach ensures that analytical solutions to the Bloch NMR flow equation yield the NMR transverse magnetization in terms of spherical harmonic functions and NMR relaxation parameters. The NMR/MRI technique can generate exquisite images of the soft tissue anatomy of the human body; therefore, this method is expected to become an efficient and reliable technique for measuring rotational diffusion at the molecular level for application in nanomedicine.

We consider a tumbling molecule that can be completely described by a rotational diffusion equation where the radius is fixed ($r = R$). It would be very important to derive the diffusion system directly from equations (36). Equations (36) within a spherical cavity is given by

$$\frac{\partial M_y}{\partial t} = D[\frac{1}{r^2}\frac{\partial}{\partial r}(r^2\frac{\partial M_y}{\partial r}) + \frac{1}{r^2\sin^2\theta}\frac{\partial^2 M_y}{\partial \phi^2} + \frac{1}{r^2\sin\theta}\frac{\partial}{\partial\theta}(\sin\theta\frac{\partial M_y}{\partial\theta})] \quad (38)$$

A tumbling molecule exhibits rotational diffusion that describes the tumbling motion of the molecules. In this case, the radius is fixed ($r = R$) and equation (38) becomes the rotational diffusion equation (the radial differential terms disappear):

$$\frac{\partial M_y}{\partial t} = D_r[\frac{1}{\sin^2\theta}\frac{\partial^2 M_y}{\partial \phi^2} + \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}(\sin\theta\frac{\partial M_y}{\partial\theta})] \quad (38)$$

where $D_r = D/R^2$ is the rotational diffusion coefficient and R is the fixed radius of a sphere. The parameter D is called translational diffusion coefficient. The NMR transverse magnetization M_y of the diffusing molecule which is making a tumbling motion is the general solution of equation (39) obtained by the method of separation of variables:

$$M_y(\phi, \theta, t) = A\, e^{-D_r l(l+1)t}\, Y_l^m(\phi, \theta) \quad (40)$$

Equation (40) can be related to the correlation time constant τ:

$$1/\tau_1 = D/R^2\, l(l+1) = D_r\, l(l+1) \quad (41)$$

Equation (40) becomes:

$$M_y(\phi, \theta, t) = A\, e^{-t/\tau_l}\, Y_l^m(\phi, \theta) \quad (42)$$

MOLECULAR HYDRODYNAMICS IN NMR OF PROTEINS

If we sum over all possible values of m and l, equation (42) gives

$$M_y(\phi, \theta, t) = \sum_{l,m} A\, e^{-t/\tau_l}\, Y_l^m(\phi, \theta) \quad (43)$$

The rotational correlation time τ_l, is the characteristic time constant associated with the Brownian rotation diffusion of a particle in a solution. This is the time it takes the particle to rotate by one radian and it is a function of the particle size. For globular proteins, a spherical approximation can be used and the rotational correlation time is given by equation (44)[21]

$$\tau_l = 4\pi\,\eta R^3/(3kT) \quad (44)$$

where η is the viscosity of the solvent, R is the effective hydrodynamic radius of the protein molecule, k is the Boltzmann constant and T is the temperature. From the molecular weight (MW) of the protein (M), the hydrodynamic radius can be calculated as follows:

$$R = [3M/(4\pi\,\rho\, N_a)]^{1/3} + R_w \quad (45)$$

where ρ is the average density for proteins (1.37 g/cm^3), N_a is the Avogadro's number and r_w is the hydration ra-

Table 3 Nuclear magnetic resonance determined rotational correlation time values for known monomeric NESG targets[19]

NESG target (isotope labeling)	MW (kDa)	^{15}N T_1 (ms)	^{15}N T_2 (ms)	$I = T_1/T_2$	τ_I (ns)
PsR76A (NC5)	7.2	478	128	3.734375	5.1
VfR117 (NC)	11.2	605	119	5.084034	6.3
SyR11 (NC5)	12.4	630	104	6.057692	7.1
ER541-37-162 (NC5)	15.8	729	66.5	10.96241	10.0
ER540 (NC5)	18.8	909	66.5	13.66917	11.3
SoR190 (NC)	13.8	697.5	100.9	6.912785	7.7
TR80 (NC5)	10.5	612.8	102.9	5.955296	7.0
Ubiquitin (NC)	9	441.8	144.6	3.055325	4.4
HR2873B (NC)	10.7	492	115	4.278261	5.7
B-domain (NC)	7.2	423.5	153.3	2.762557	4.05
BcR97A (NC)	13.1	705.8	80.6	8.756824	8.8
PfR193A (NC)	13.6	733.9	80.9	9.071693	9.0
MvR76 (NC)	20.2	1015	64.5	15.73643	12.2
DvR115G (NC)	10.9	608.7	115.6	5.265571	6.5
MrR110B (NC5)	11.8	707	99.2	7.127016	7.8
VpR247 (NC5)	12.5	661.2	88.3	7.488109	8.05
BcR147A (NC)	11.9	645	104	6.201923	7.2
WR73 (NC5)	21.9	1261	41.3	30.53269	13.0
NsR431C (NC5)	16.8	855.5	71.2	12.01545	10.6
StR82 (NC)	9.2	537.3	100.4	5.351594	6.6

MW: Molecular weight.

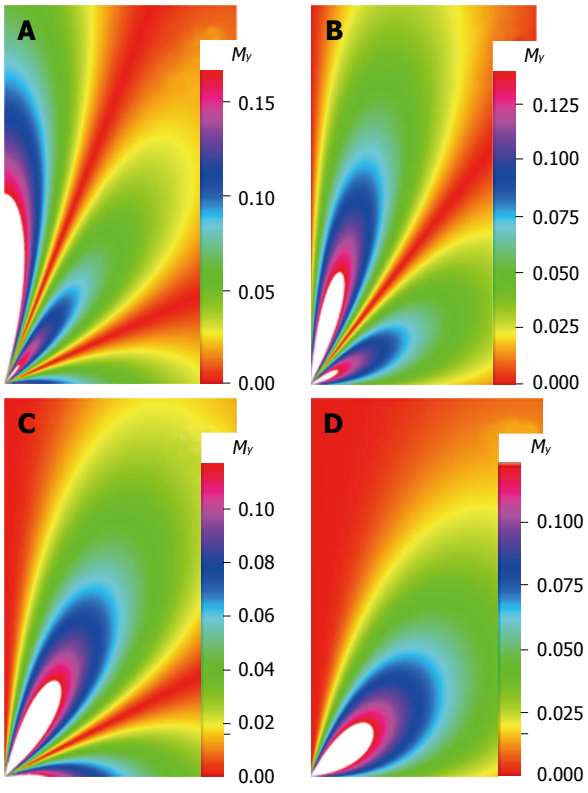

Figure 4 Image from the transverse magnetization as it varies with time, *t* = 3 ns, and the relaxation parameters τ_I = 5.1 ns, *I* = 3.734375 for (A) m = 0; (B) m = 1; (C) m = 2; (D) m = 3.

dius (1.6Å to 3.2Å). For rigid protein molecules, in the limit of slow molecular motion ($\tau_I >> 0.5$ ns) and high magnetic field (500 MHz or greater), a closed-form solution for τ_I as a function of the ratio of the longitudinal (T_1) and transverse (T_2) ^{15}N relaxation times is

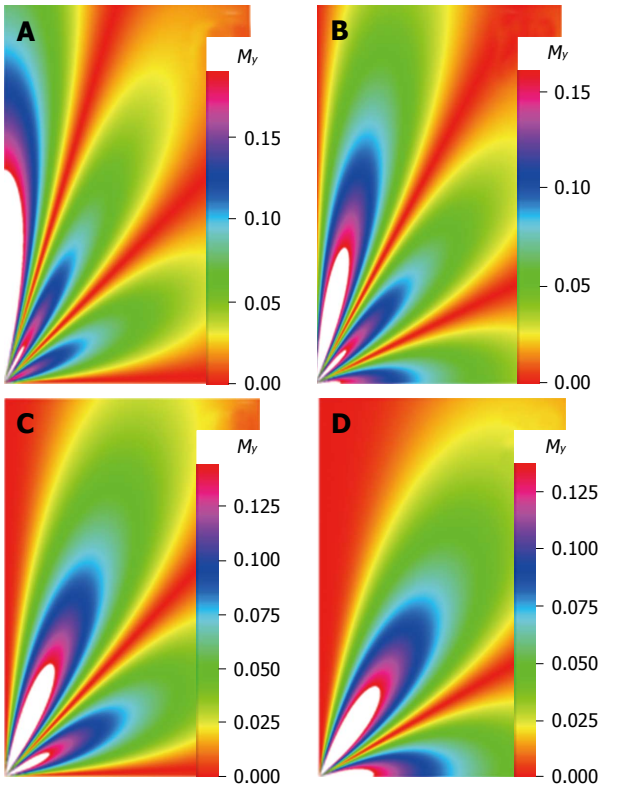

Figure 5 Image from the transverse magnetization as it varies with time, *t* = 3 ns, and the relaxation parameters τ_I = 6.3 ns, *I* = 5.084034 for (A) m = 0; (B) m = 1; (C) m = 2; (D) m = 3.

$$\tau_I = 1/(4\pi\,\omega_N)\,(6T_1/T_2 - 7)^{1/2} \qquad (46a)$$

where ω_N is the ^{15}N resonance frequency (Hz). Average ^{15}N T_1 and T_2 relaxation times for a given protein can be measured using 1D ^{15}N-edited relaxation experiments. To minimize contributions from unfolded segments, each 1D spectrum is integrated over the downfield backbone amide ^{1}H region (typically 10.5 to 8.5 ppm) and the results are used to fit an exponential decay as a function of delay time. One then computes the correlation time and compares it to a standard curve of τ_I *vs* protein MW obtained at the same temperature on a series of known monomeric proteins of varying size. The T_1/T_2 method is suitable for proteins with MW of up to MW $\approx$ 25 kDa. Accurate measurement of the diminishing ^{15}N T_2 becomes difficult for larger proteins and cross-correlated relaxation rates are measured instead[21].

The parameter I is a dimensionless constant; therefore, it may be appropriate in this study to define I as

$$I = T_1/T_2 \qquad (46b)$$

Values of rotational correlated time for some monomeric NESG (North East Structural Genomics Consortium) targets are shown in Table 1.

The density images below are obtained for the first three isotopes of Table 3, for M_0 = 1, m = 0, 1, 2, 3, and a time of 3 ns. The plots shown in Figures 4-7 are made with the assumption that the spins move across rigid spheres whose radii (= R) are in the range $\{0, 8^{1/2}\}$. Figures 4-7 give the density mapping of the transverse magnetization for specific correlation times (*i.e.*, for selected

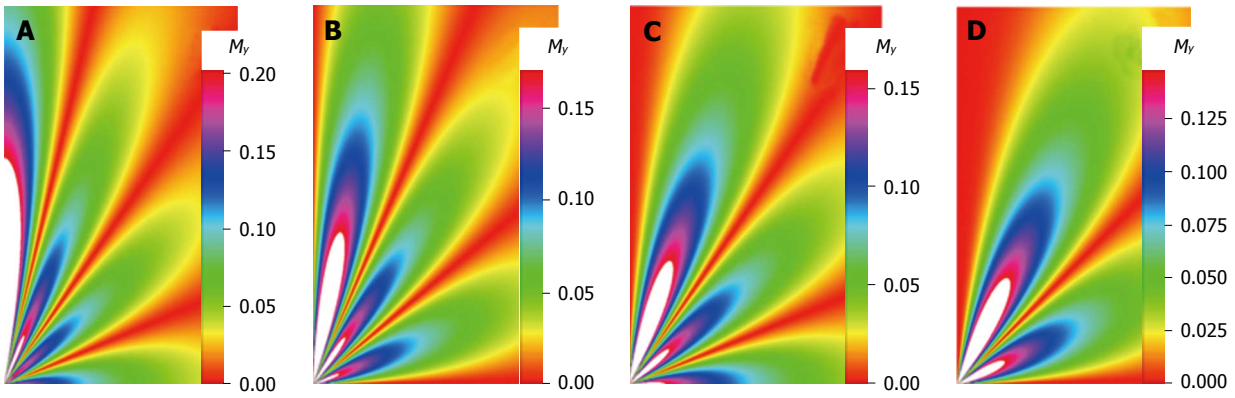

Figure 6 Image from the transverse magnetization as it varies with time, t = 3 ns, and the relaxation parameters τ_l = 7.1 ns, l = 6.057692 for (A) m = 0; (B) m = 1; (C) m = 2; (D) m = 3.

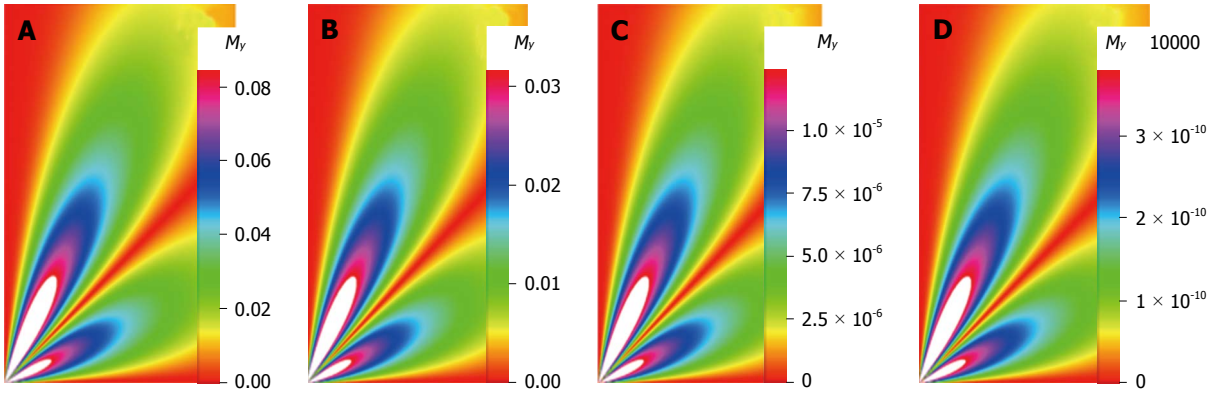

Figure 7 Image from the transverse magnetization as it varies with m = 3, and the relaxation parameters τ_l = 7.1 ns, l = 6.057692 for (A) t = 5 ns; (B) t = 10 ns; (C) t = 50 ns; (D) t = 150 ns.

NESG targets) and NMR relaxation parameters.

ROTATIONAL FRICTIONAL COEFFICIENT AND MOLECULAR NMR

When a particle moves in a fluid, either under the influence of an applied force or torque, or due to Brownian motion, it experiences frictional resistance. The proportionality between particle velocity and frictional resistance is the frictional coefficient.

It may be significant to note that the rotational diffusion coefficient D_r, can be defined from equation (39) as

$$D_r = D/R^2 = k_B T/(R^2 f_r) \quad (47)$$

where k_B is the Boltzmann's constant, f_r is the rotational frictional coefficient, R is the hydrodynamic radius of the molecule being observed and T is the absolute temperature. Equation (47) becomes discretized when the time constant is introduced:

$$1/\tau_l = k_B T/(R^2 f_r)\ l\,(l+1) \quad (48)$$

$$f_r = k_B T\,\tau_l/R^2\ l\,(l+1) \quad (49)$$

Therefore, for the NESG target PsR76A (NC5)[21], the rotational friction coefficient can easily be calculated:

$$f_r = 90.16765 \times 10^{-9}\ k_B T/R^2$$

It may be very important to note from equations (35, 39) that

$$D = -v^2/T_0 = D_r R^2 \quad (50a)$$

And

$$D_r = -v^2/(T_0 R^2) = -\omega^2/T_0 \quad (50b)$$

where

$$\omega = v/R$$

is the angular velocity. Hence, we have:

$$f_r = k_B T\,T_0/v^2 \quad (50c)$$

The angular drift velocity can be defined as

$$\Omega_d = d\omega/dT_0 = F_\omega/f_r \quad (51)$$

Equation (50b) defines the angular deviation in terms of the T_1 and T_2 relaxation parameters for rotational diffusion about a single axis

$$\omega^2 = D_r T_0 \quad (52)$$

Equation (52) describes the response of the angular drift velocity to an external torque F_ω assuming that the flow stays non-turbulent and that inertial effects can be neglected.

RELAXATION STUDIES OF DIATOMIC MOLECULES IN ROTATIONAL DIFFUSION

Rotational diffusion is a process by which the equilib-

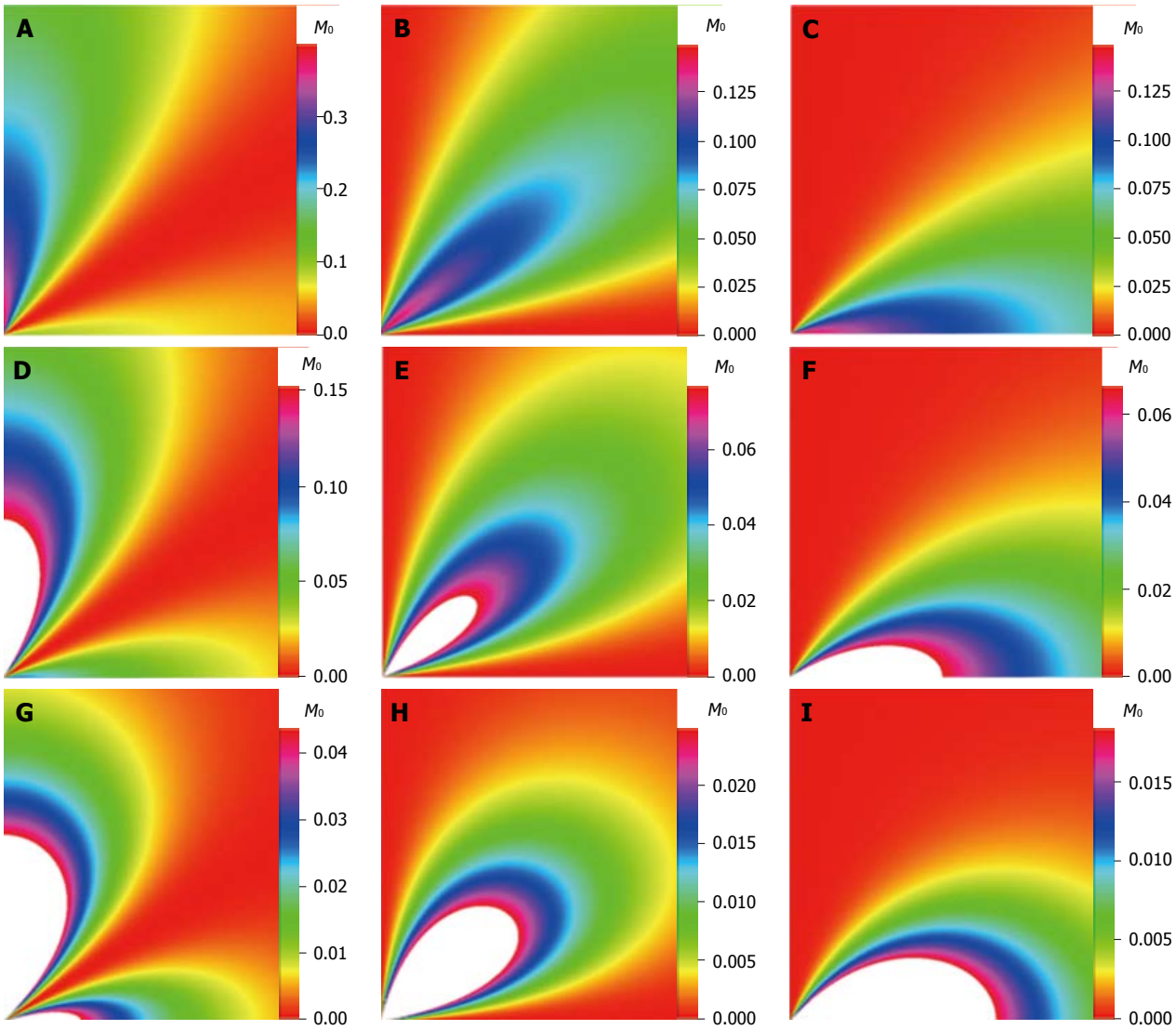

Figure 8 Density maps of M_0 using Equation (8) for l = 2 and (A) m = 0, $0 \leqslant R \leqslant 2^{1/2}$; (B) m = 1, $0 \leqslant R \leqslant 2^{1/2}$; (C) m = 2, $0 \leqslant R \leqslant 2^{1/2}$; (D) m = 0, $0 \leqslant R \leqslant 8^{1/2}$; (E) m = 1, $0 \leqslant R \leqslant 8^{1/2}$; (F) m = 2, $0 \leqslant R \leqslant 8^{1/2}$; (G) m = 0, $0 \leqslant R \leqslant 32^{1/2}$; (H) m = 1, $0 \leqslant R \leqslant 32^{1/2}$; (I) m = 2, $0 \leqslant R \leqslant 32^{1/2}$.

rium statistical distribution of the overall orientation of molecules is maintained or restored. The random re-orientation of molecules is an important process for many biophysical probes. The rotational diffusion of molecules in the presence of static magnetic and RF fields can be described by the NMR diffusion equation. The NMR diffusion equation within a spherical cavity has been described in equation (42).

The radial parameter R is constant; therefore, we may assume that $A = M_0 e^{-R}$. If we sum over all m and l, we have:

$$M_y(\phi, \theta, t) = \sum_{l,m} M_0 e^{-R_m^l} e^{-t/\tau_l} Y_l^m(\phi, \theta) \quad (53)$$

If at $t = 0$, $(\phi, \theta) = (\phi_0, \theta_0)$, we write:

$$M_y(\phi, \theta, 0) = \sum_{l,m} M_0 e^{-R_m^l} Y_l^m(\phi, \theta) = \delta[(\phi, \theta) - (\phi_0, \theta_0)] \quad (54)$$

Then, the delta function may be expanded such that:

$$\delta[(\phi, \theta) - (\phi_0, \theta_0)] = \sum_{l,m} Y_l^{m*}(\phi_0, \theta_0) Y_l^m(\phi, \theta);$$

$$M_0 e^{-R_m^l} = Y_l^{m*}(\phi_0, \theta_0) \quad (55)$$

$$M_y(\phi, \theta, t) = \sum_{l,m} Y_l^{m*}(\phi_0, \theta_0)\ Y_l^m(\phi, \theta) e^{-t/\tau_l} \quad (56)$$

For this system, the autocorrelation function may be given as:

$$G(t) = 12\pi/20\ [\mu_0/(4\pi)]^2\ \eta^2 \gamma^4/r^6$$
$$[Y_2^{m*}(\phi, \theta)\ Y_2^m(\phi, \theta) e^{-t/\tau_l}] \quad (57)$$

The angle bracket is the average over the transverse magnetization of the rotating molecules. This average is given as:

$$1/4 \int d(\phi, \theta) \int d(\phi_0, \theta_0) \sum_{l,m} Y_l^{m*}(\phi_0, \theta_0)\ Y_l^m(\phi, \theta)$$
$$Y_2^m(\phi_0, \theta_0)\ Y_2^{m*}(\phi, \theta) e^{-t/\tau_l} \quad (58)$$

If we perform the integral, we obtain[27-31]:

$$G(t) = K/3\ e^{-t/\tau_l} \quad (59)$$

where $\tau_l = R^2/(6D) = 1/(6Dr)$, and $K = 9/20\ [\mu_0/(4\pi)]^2\ \eta^2 \gamma^4/r^6$ is the second moment of interaction and r is the separation between two nuclear spins. The spectral density function is the Fourier transformation[27-31] of equation (53):

$$J(\omega) = 2/3\ [K\tau_l/(1 + \omega^2\tau_l^2)] \quad (60)$$

$$\frac{1}{T_1} = K\tau_l\left(\frac{2/3}{1 + \omega^2\tau_l^2} + \frac{8/3}{1 + 4\omega^2\tau_l^2}\right)$$

$$\frac{1}{T_2} = K\tau_l\left(1 + \frac{5/3}{1 + \omega^2\tau_l^2} + \frac{2/3}{1 + 4\omega^2\tau_l^2}\right) \quad (61)$$

MAPPING OF EQUILIBRIUM MAGNETIZATION

From equation (55), we can map M_o as a function of the radius of the rigid rotator R (which is also dependent on θ_0 and ϕ_0). At the point when RF B_1 field is just removed, M_o starts building up from its lowest value. For multi-voxel imaging, R may be changing with different tissue conditions. This may have very important influence on the changes in M_o. Figure 8 show the changes in M_o with assumed ranges for R.

CONCLUSION

We have presented the basic principle of NMR/MRI in a way that can be easily understood and that may fascinate researchers into the field of NMR/MRI. After a very brief history of the subject, a second order non-homogeneous, time dependent differential equation derived from the Bloch NMR equation was presented. Note that equation (30) uniquely assembles all the NMR, MRI, MRA and magnetic resonance spectroscopy parameters in an exciting way ready to be explored. The NMR signals as represented by equations (40-42) and Figures 4-7 are greatly influenced by the T_1 and T_2 relation times and the NMR parameter m. As l increases the motion gets faster and as m is increased, the particle's motion moves closer to orbiting the equator. This can greatly motivate further research into the use of rotational motion of nanoparticles to perform medical procedures inside the human body, noninvasively. Equations (40-42) are also the solutions for a rigidly rotating diatomic molecule. They are the angular parts of the hydrogen atom wave functions. These functions are important in many theoretical and practical applications, particularly in the computation of atomic orbital electron configurations, representation of gravitational fields, geoids, and the magnetic fields of planetary bodies and stars, and characterization of the cosmic microwave background radiation. In 3D computer graphics, spherical harmonics play a special role in a wide variety of topics including indirect lighting (ambient occlusion, global illumination, pre computed radiance transfer, *etc.*) and recognition of 3D shapes. The concept presented in this study can also be used to analyze the Earth's magnetic resonance. Application of this concept to nanomedicine will be the focus of our next investigation. Towards this goal, we derived the standard parameters of NMR relaxometry of diatomic molecules directly from the NMR diffusion equation. The advantage of this is that we are able to obtain the autocorrelation function and the spectral density function without the use of the rigorous method of probability distribution function.

REFERENCES

1 **Clare S**. Functional MRI: Methods and Applications. PhD thesis, University of Nottingham, 1997: 1-12
2 **Hornak JP**. The Basics of NMR. Available from: URL: http: //www.cis.rit.edu/htbooks/nmr/nmr-main.htm
3 **Lerner L**, Horita DA. Teaching high-resolution nuclear magnetic resonance to graduate students in biophysics. *Biophys J* 1993; **65**: 2692-2697 [PMID: 8312503 DOI: 10.1016/S0006-3495(93)81323-7]
4 **James TL**. Fundamentals of NMR. Online Textbook: Department of Pharmaceutical Chemistry, University of California, San Francisco, 1998: 1-31. Available from: URL: http: //www.biophysics.org/Portals/1/PDFs/ProfessionalDevelopment/James.T.pdf
5 **Haacke ME**, Brown RW, Thompson MR, Venkatesan R. Magnetic resonance imaging: Physical principles and sequence design. New York: John Wiley and Sons, 1999: 3-13
6 **Legault P**. Classical Description of NMR Parameters: The Bloch Equations. Online Lectures: Department de Biochimie Universite de Montreal, Montreal, 2008: 1-16. Available from: URL: http://esilrch1.esi.umontreal.ca/~syguschj/cours/BCM6200/bcm6200_NMR%20Parameters_21oct08.pdf
7 **Brix G**, Kolem H, Nitz WR, Bock M, Huppertz A, Zech CJ, Dietrich O. Basics of Magnetic Resonance Imaging and Magnetic Resonance Spectroscopy. Heidelberg: Springer Berlin, 2008: 3-167
8 **Posadas AND**, Tannus A, Panepucci CH, Crestana S. Magnetic Resonance Imaging as a non–invasive technique for investigating 3-D preferential flow occurring within stratified soil samples. *Computers and Electronics in Agriculture* 1996; **14**: 255-267 [DOI: 10.1016/0168-1699(95)00032-1]
9 **Schumacher RA**. Nuclear Magnetic Resonance. Online Publications: Carnegie Mellon University, Pittsburgh, 2009: 1-40. Available from: URL: http://www-meg.phys.cmu.edu/physics_33340/experiments/mpl_nmr.pdf
10 **Chrysikopoulos HS**. Clinical MR Imaging and Physics: A tutorial. Berlin: Springer Berlin Heidelberg, 2009: 1-31 [DOI: 10.1007/978-3-540-78023-6]
11 **Shankar R**. Principles of Quantum Mechanics. New York: Plenum Press, 1994: 265-266
12 **Slichter CP**. Principles of Magnetic Resonance. New York: Harper and Row, 1963: 1-28
13 **Abragam A**. The Principles of Nuclear Magnetism. Oxford: Clarendon Press, 1961: 1-15
14 **Ernst RR**, Bodenhausen G, Wokaun A. Principles of Nuclear Magnetic Resonance in one and two Dimensions. Oxford: Oxford University, 1990: 152-162
15 **Edison AS**. Theory and Applications of NMR Spectroscopy. Online Lecture Notes: University of Florida, Florida, 2000: 5-6. Available from: URL: http://nmrc.wsu.edu/files/ pdf/theory.pdf
16 **Cowan BP**. Nuclear Magnetic Resonance and Relaxation (First Edition). Cambridge: Cambridge University Press, 1997: 1-6
17 **Lent AH**, Hinshaw WS. An Introduction to NMR Imaging: From the Bloch Equation to the Imaging Equation. *Proc of the IEEE* 1983; **71**: 338-350 [DOI: 10.1109/PROC.1983.12592]
18 **Walker R**. Mathematics of Nuclear Magnetic Resonance Imaging. Online Publications, 2011-03-08, cited 2013-09-01; 1-8 screens. Available from: URL: http: //www.ms.uky.edu/~rwalker/research/NMR_GSC.pdf
19 **Wolff-Reichert B**. A Conceptual Tour of Pulsed NMR. Online Lecture Notes: Department of Physics and Astronomy, University of Hawaii, Hawaii, 2008: 1-8. Available from: URL: http://www.phys.hawaii.edu/~teb/phys481l/PS2A6_ConceptualTour.pdf
20 **Awojoyogbe OB**, Dada M. Mathematical Design of a Magnetic Resonance Imaging Sequence based on Bloch NMR

Flow Equations and Bessel Functions. *Chinese Journal of Magn Reson Imaging* 2013; **5**: 373-381 [DOI: 10.3969/j.issn.1674-8034.2013.05.011]
21 NMR determined Rotational correlation time. University of Buffalo Online Publication, 2011-10-13, cited 2013-09-01. Available from: URL: http: //www.nmr2.buffalo.edu/nesg.wiki/NMR_determined_Rotational_correlation_time
22 **Wang Y**. Physics of MRI. Online Lecture Notes: Polytechnic University, Brooklyn, New York, 2006: 15-25. Available from: URL: http://eeweb.poly.edu/~yao/EL5823/MRI_physics_ch12.pdf
23 **Peleg Y**, Pnini R, Zaarur E. Schaum's Outline of Theory and Problems of Quantum Mechanics. New York: McGraw-Hill Professional, 1998: 123-124
24 **Dada OM**, Awojoyogbe OB, Adesola OA, Boubaker K. Magnetic Resonance Imaging-derived Flow Parameters for the Analysis of Cardiovascular Diseases and Drug Development. *Magnetic Resonance Insights* 2013; **6**: 83-93 [DOI: 10.4137/MRI.S12195]
25 **Awojoyogbe OB**. A quantum mechanical model of the Bloch NMR flow equations for electron dynamics in fluids at the molecular level. *Phys Scr* 2007; **75**: 788 [DOI: 10.1088/0031-8949/75/6/008]
26 **Awojoyogbe OB**, Dada OM, Faromika OP, Dada OE. Mathematical Concept of the Bloch Flow Equations for General Magnetic Resonance Imaging: A Review. *Concep in Magn Reson Part A* 2011; **38A**: 85-101 [DOI: 10.1002/cmr.a.20210]
27 **Bottomley PA**, Foster TH, Argersinger RE, Pfeifer LM. A review of normal tissue hydrogen NMR relaxation times and relaxation mechanisms from 1-100 MHz: dependence on tissue type, NMR frequency, temperature, species, excision, and age. *Med Phys* 1984; **11**: 425-448 [PMID: 6482839 DOI: 10.1118/1.595535]
28 **Carneiro AAO**, Vilela GR, de Araujo DB, Baffa O. MRI relaxometry: methods and applications. *Braz J Phys* 2006; **36**: 9-15 [DOI: 10.1590/S0103-97332006000100005]
29 **Quigley MF**, Iskandar B, Quigley ME, Nicosia M, Haughton V. Cerebrospinal fluid flow in foramen magnum: temporal and spatial patterns at MR imaging in volunteers and in patients with Chiari I malformation. *Radiology* 2004; **232**: 229-236 [PMID: 15155896 DOI: 10.1148/radiol.2321030666]
30 **Compston A**, Coles A. Multiple sclerosis. *Lancet* 2002; **359**: 1221-1231 [PMID: 11955556 DOI: 10.1016/S0140-6736(02)08220-X]
31 **Miller SP**, Vigneron DB, Henry RG, Bohland MA, Ceppi-Cozzio C, Hoffman C, Newton N, Partridge JC, Ferriero DM, Barkovich AJ. Serial quantitative diffusion tensor MRI of the premature brain: development in newborns with and without injury. *J Magn Reson Imaging* 2002; **16**: 621-632 [PMID: 12451575 DOI: 10.1002/jmri.10205]

P- Reviewer: Sijens PE **S- Editor**: Song XX
L- Editor: Stewart G **E- Editor**: Liu SQ

World Journal of Translational Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
doi:10.5528/wjtm.v3.i1.31

World J Transl Med 2014 April 12; 3(1): 31-36
ISSN 2220-6132 (online)

MINIREVIEWS

Translational medical mycology guides clinical and laboratory practice on fungal diseases

Jebina Lama, Xin Ran, Yu-Ping Ran

Jebina Lama, Yu-Ping Ran, Department of Dermatovenerology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Xin Ran, Department of Dermatology and Rheumatology, The Second Affiliated Hospital of Kunming Medical University, Kunming 650101, Yunnan Province, China
Author contributions: Ran YP substantially contributed to the conception and design of the work; Lama J contributed to data acquisition, analysis and interpretation; Ran X contributed to data interpretation and revised the manuscript critically for important intellectual content.
Correspondence to: Yu-Ping Ran, MD, PhD, Professor, Department of Dermatovenerology, West China Hospital of Sichuan University, No. 37 of Guo Xue Xiang, Wuhou District, Chengdu 610041, Sichuan Province, China. ranyuping@gmail.com
Telephone: +86-28-85422114
Received: November 4, 2013 Revised: December 18, 2013
Accepted: January 17, 2014
Published online: April 12, 2014

Abstract

Patients with fungal infection having skin lesions may consult a dermatologist, which is a diagnostic and therapeutic challenge. Dermatologists take samples from the lesion to check the fungal elements under a microscope by KOH preparation and then treat the patient. This model has advanced from bedside to bench and from bench to bedside (B to B to B), which is defined as Translational Medical Mycology. Dermatologists have an advantageous position in finding, isolating and identifying the pathogenic fungi and treating the patient with antifungal drugs. Samples should be cultured in different media with or without chloramphenicol and cycloheximide and incubated at room temperature or 37 ℃. Non-culture techniques such as polymerase chain reaction based molecular identification, transmission electron microscopy, scanning electron microscopy, biochemistry tests and histopathology are also necessary to confirm the identification of the species, especially when the routine culture is negative. We start treatment upon obtaining evidence of fungal infection, *i.e.*, positive KOH examination. Antifungal drugs such as itraconazole, fluconazole, terbinafine and amphotericin B can be used alone or in combination based on the fungal species and the location of the lesion. Practice on fungal infection includes screening of the patient, merging all of the laboratory techniques and methods from the microbiologists, pathologists, molecular researchers, identification of the pathogen and determination of the optimum antifungal drug.

Key words: Translational; Mycology; Bedside; Bench; Fungal infections

Core tip: "Translational medical mycology" has transformed vastly in recent years, which is aimed to solve clinical problems. This transformation is a dynamic, multi-level, multi-directional and continuously improving process. There are different processes in translational medicine, with the main objective being translation journey from bench to bedside to the community. We can solve a clinical problem by using a number of new technologies with the help of advanced laboratories and professionals, which will eventually promote the overall progress of medical mycology and ultimately provide an access to the effective treatment and prevention of the fungal disease.

Lama J, Ran X, Ran YP. Translational medical mycology guides clinical and laboratory practice on fungal diseases. *World J Transl Med* 2014; 3(1): 31-36 Available from: URL: http://www.wjgnet.com/2220-6132/full/v3/i1/31.htm DOI: http://dx.doi.org/10.5528/wjtm.v3.i1.31

INTRODUCTION

As modern science continuously spawns even more dra-

matic discoveries about human health, more research with the help of new technology and advanced laboratories is increasingly encouraged to find the practical application that can improve human lives. The problems encountered in clinical situation undergo laboratory studies through the problem-solving process (such as finding etiology and pathogenesis to find a key factor against the development of the cause or drugs) and then return to clinical guided treatment, and this process is summarized as from the bedside to the laboratory and vice-versa (Bench to Bedside and Bedside to Bench, *i.e.*, B to B to B, defined as Translational Medicine). Furthermore there are different processes in translational medicine, with the main objective being translation journey from bench to bedside to community. As the medicine enters the era of translational medicine, medical mycology as an important part of medicine has become translational medical mycology[1,2].

Although in translational medical mycology the patient's history and clinical manifestations are important clues to clinical diagnosis, sample collection from lesion sites is a crucial step in order to identify patients with fungal infection specimens (scales, pus, sputum, blood, cerebrospinal fluid, *etc.*). Obtaining high quality specimens to meet the laboratory requirements for etiological diagnosis is critical, including professional training in mycology, biologically secure laboratory conditions, preparation of different media, culture incubator (24 ℃-26 ℃ and 37 ℃), and most importantly avoidance of contamination during the procedure. Microscopic computer image acquisition and processing system is the basic premise of fungal microscopic examination, specimens are processed (KOH preparation) to observe fungi (hyphae/spores, different shapes and size), and for further inquiry there is also molecular identification. It is estimated that there are more than 1.5 million kinds of fungi on the Earth. So far more than 100000 kinds have been described, but only just over 100 kinds are known as common pathogenic fungi, suggesting that there is a great development space for medical mycology.

The rapid developed molecular biology techniques are used for molecular identification of not only fungi isolated in culture but also those from non-culture specimens, like direct DNA extraction using universal fungal primers by polymerase chain reaction (PCR) amplification/sequencing; further pathological examination (Periodic acid-Schiff or Methenamine-silver staining) can be used to identify the fungus and its special structures. Electron microscopy is used to observe the ultra-structure and fungal pathogens for species identification and study of pathogenesis. For translational medical mycology, molecular biology and individualized treatment should include both the host and the fungus. To perform host susceptibility analysis to the pathogenic species and the virulence factors, molecular biology techniques are required to analyze the host susceptibility genes, environmental and exposure factors. DNA from the fungal specimens (isolated colonies in culture, non-cultured clinical specimens or pathological paraffin block specimens) for molecular amplification and sequencing of pathogenic fungi is equally important. For identification, common fungal universal primers to amplify the internal transcribed spacer 1/4 region and D1/D2 domain are used, but species-specific primers can also be designed.

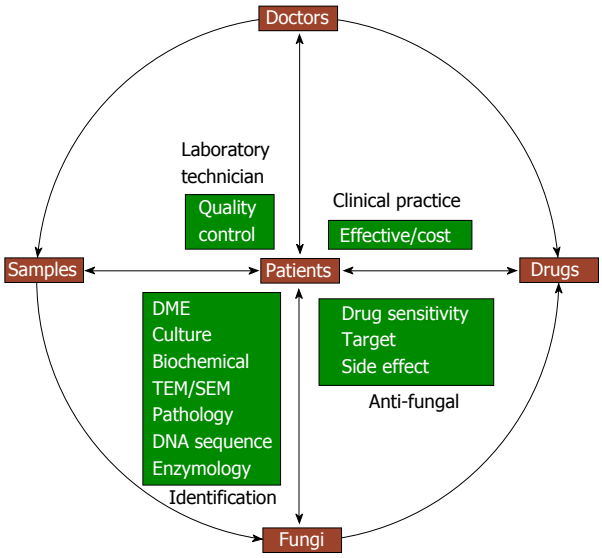

Figure 1 Essential factors for translational medical mycology. DME: Direct microscopic examination; TEM: Transmission electron microscopy; SEM: Scanning electron microscopy.

Various comprehensive detection techniques are designed to quickly identify pathogenic fungi for administration of antifungal therapy on time. Antifungal drug sensitivity test is a superior choice to select drugs to which the particular strain is highly sensitive, but at the same time, drug interactions, safety and drug prices should be taken into consideration. Each link has a puzzle challenge with a variety of problems and each clinical problem is transformed into medical mycology research topic. The essential factors for translational medical mycology and their relationship are summarized in Figure 1.

With the improvement in patient acceptance and the rate of positive fungal results[3], we can improve the traditional methods for sample collection. In one patient with Candidosis intertrigo in the groin, specimens were collected using a cotton swab, tape and blunt scraping of scales, respectively, for direct DNA extraction by PCR/sequencing. The results confirmed that the three different methods all identified the pathogenic fungi as *Candida albicans*, and the results were verified by cultured colony identification. The new method for sample collection by using a cotton swab, tape and using blunt scraping of scales for direct DNA extraction by PCR/sequencing does not only solve the problem of sample collection from sensitive parts, but also can be used for early identification of pathologic fungi and treatment accordingly, which in turn saves time[4]. Direct DNA extraction from the clinical samples (hair or nail) for identification of the pathogenic strains has also been successfully developed to distinguish between tinea capitis[5] or onychomycosis. In one case, hair samples collected from a 6-year-old girl

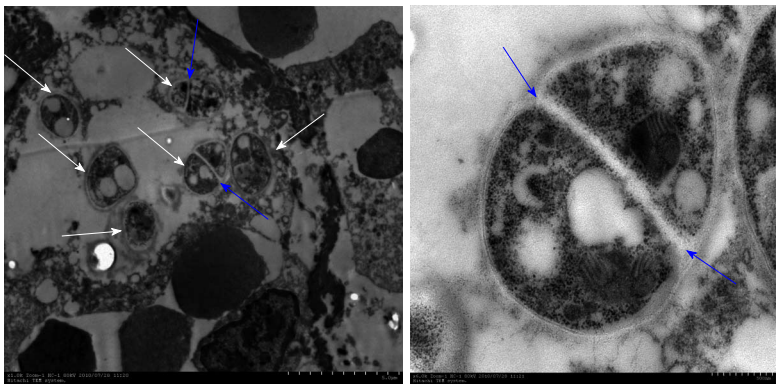

Figure 2 In transmission electron microscopic examination, there are 6 organisms with cell wall (white arrows), of which 2 have septated wall (blue arrows) within a macrophage. This specific structure of *Penicillium marneffei* helped to clarify the pathogen as *Penicillium marneffei*.

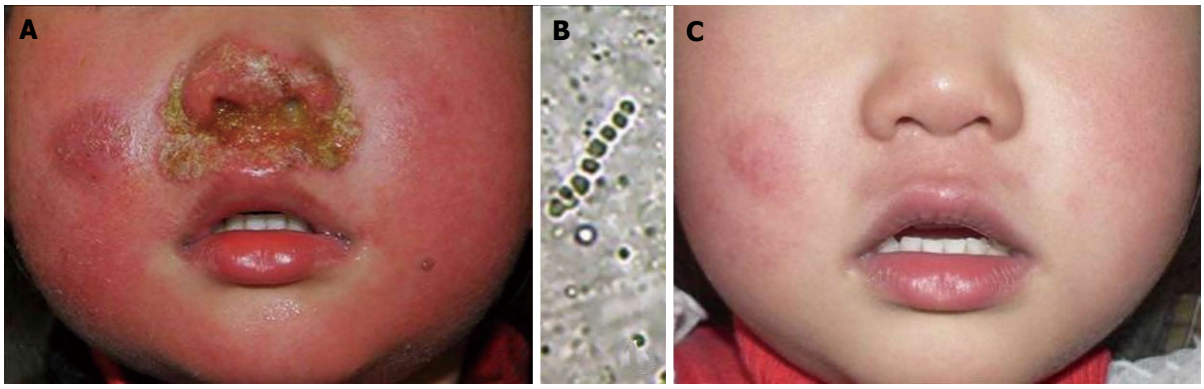

Figure 3 A 3-year-old girl was consulted with impetigo-like lesions around the nostrils. A: Pustules and erythema around nostrils; B: Hyphae and clustered spores in the crust but the culture was negative. The pathogenic agent was identified as *Arthroderma vanbreuseghemii* (a teleomorph of *Trichophyton interdigitale*) using polymerase chain reaction-based sequencing of the crusts; C: The pustules and erythema disappeared after 35 d of antifungal treatment.

who suffered from kerion were divided into two parts for culture dependent and independent methods, respectively. Since the patient was already on terbinafine, the culture grew slowly. Two sequences from the hair and the culture were both identified as *Arthroderma vanbreuseghemii*. Hence, PCR based molecular biological validation of DNA directly from the sample can rapidly identify the pathogenic fungus, especially after the use of antifungal drugs. Pathological examination is important in the field of medical mycology, but it has its own limitations. It is important to verify the results since specific fungal strains cannot be determined by pathological reports. In a patient with oral ulcers, histopathology reported a diagnosis of "Leishmaniasis", but the review of the pathological section found that "*Leishmania*" is more like *Penicillium marneffei* yeast cells. The oral specimens were negative for fungal culture. To rule out the "Leishmaniasis" and to establish a new diagnosis as "Penicilliosis marneffei", paraffin-embedded tissue was used to extract DNA using universal primers for PCR, and the obtained sequence was compared with the sequences in database by Blast and found to share a 99% similarity to the *Penicillium marneffei*. In addition, the paraffin-embedded tissue was prepared for transmission electron microscopy observation, which showed the septated wall, the specific structure for *Penicillium marneffei* yeast in tissue (Figure 2). The diagnosis was confirmed as a case of HIV-negative primary, localized, penicilliosis marneffei by the non-culture method[6].

The fungal culture result can be influenced by various factors like culture media, incubation conditions, the volume and quality of inoculation, previous history of use of antifungal drugs and the technique of the inoculation, which lead to unsuccessful attempt to isolate the pathogenic fungi. Direct extraction of DNA is a useful method for guiding treatment in these cases where microscopic tests are positive and cultures are negative. We have encountered some cases in which there was no growth in fungal culture but a significant treatment effect was achieved with antifungal agents[7]. A 3-year-old girl was consulted with impetigo-like lesions around the nostrils. KOH examination showed hyphae and clustered spores in the crust but the culture was negative. The pathogenic agent was identified as *Arthroderma vanbreuseghemii*, a teleomorph of *Trichophyton interdigitale* using PCR-based sequencing of the crusts taken from the lesion. She recovered from the infection after oral and topical antifungal agents (Figure 3).

Regarding the treatment, whenever the direct microscopic examination with KOH is positive, the antifungal treatment should be started immediately without waiting for the fungus to be isolated or the species be identified. As this might take weeks and the patient's

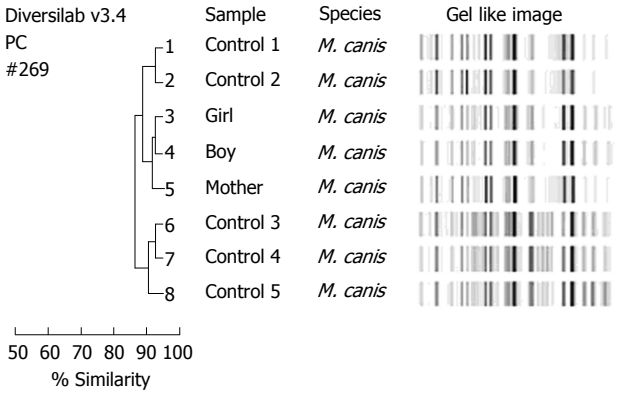

Figure 4 Molecular typing of eight *Microsporum canis* isolates showed distinct and similar fingerprint patterns visualized by the gel-like image and as indicated by a > 90% similarity coefficient in the dendrogram. The three isolates from patients of a same family (number 3, 4, 5) were grouped together with a ⩾ 98% similarity compared with the control isolates of *M. canis*. *M. canis*: *Microsporum canis*.

condition might deteriorate. More importantly, sample collection from the lesions for culture and pathology should be conducted just before starting the antifungal agents. As the treatment progresses, a fungal culture (the conventional method and subsequent identification and susceptibility testing) and direct extraction of DNA for molecular identification should be performed in addition to the weekly follow-up. Samples should be collected and examined until the culture becomes negative before stopping the antifungal treatment[8].

Antifungal drugs are the key factor in the treatment of fungal infection. Commonly used topical antifungal drugs consist of allylamines (such as terbinafine, naftifine, butenafine), imidazoles (such as ketoconazole and bifonazole) or a mixture of the two (naftifine with ketoconazole), and some are even mixed with steroids (triamcinolone acetonide with econazole or miconazole nitrate). Topical drugs are made by mixing various concentrations of antifungal agents with matrices composed of different vehicles and formula. The antifungal spectrum and activity of this mixture (final product) are determined by applying a fixed volume directly to the pathogenic fungus, and then observing and comparing the size of the inhibition zone around the well containing the tested cream. This modified agar diffusion method is a practical agar-based method, which enables determination of the activity of various antifungal drugs against an array of fungal genera and species. It is useful to assess antifungal activity and spectrum against fungal skin infection and to determine the *in vitro* spectrum and activity of different topical antifungal creams by studying their direct inhibition activity against clinical isolates. We can select the product with the best effect/cost potency ratio from the commercial antifungal creams based on the comparison of the inhibition zone in the same condition to the same species of pathogenic fungus[9,10].

Sometimes in clinical practice we might encounter multiple mixed fungal infections, which will require different media. The samples should be incubated at different temperatures to distinguish them and we must not jump into a premature conclusion that there is a "contaminated fungus". Generally, the fungus which invades the skin during trauma at the initial stage is considered contaminated. When the local condition becomes suitable for its growth, this "contaminated fungus" could become as the pathogenic fungus (opportunistic fungal infection). This idea leads us to find out the uncommon pathogen and the cure to the difficult cases[11,12].

It is important to use techniques of molecular biology and electron microscopy to identify fungi from all the lesions, particularly in cases of rare fungi, where there is not much of a morphological characteristic. In one case, several samples were collected from patient's face, neck, back, buttocks, foot as well as toenail lesions and all were isolated as *Trichophyton rubrum*. By using *Trichophyton rubrum* specific random amplified polymorphic DNA (RAPD) and tandem repeat subunit Trs-1/Trs-2 PCR product analysis, we found that the patient was infected with six different strains at various sites of the infection, proving that various strains did not occur by autoinoculation[13]. RAPD and Trs-1/Trs-2 analysis of 8 family members showed that although *Trichophyton rubrum* can infect one member of the family to another (2 families), but more cases showed that the infections are beyond the families[14]. The members from one family had kerion (son) and tinea corporis (father and mother), and *Arthroderma vanbreuseghemii* was isolated from their lesions. By using the morphology, rapid urease test, hair perforation experiment, random primers polymorphism and zymogram analysis, it was proved that the pathogen was the same and it was transmitted from the neighbor's rabbit to the son and later to his parents[15]. In another case, two children (tinea capitis) and mother (tinea corporis) were diagnosed based on the positive KOH examination. Morphological characteristics and sequencing of the internal transcribed spacers 1 and 2 amplified from primary culture isolates confirmed that their infections were caused by the zoophilic *Microsporum canis*. Repetitive sequence-based molecular typing using the DiversiLab system, secreted enzymatic activity analysis and antifungal susceptibility indicated that these isolates might share the same source (Figure 4)[16]. Therefore, molecular identification can give us clues about the source of infection whether the infection is caused by autoinoculation, by some external sources or by close contact within the family members.

In recent years we have seen several cases of common fungi causing debilitating rare diseases, which give us a new thought about how people with normal immune system can get infected with common pathogens. In one case, a boy had scalp laceration from a road accident, and the treatment included debridement and scalp transplantation in orthopedic surgery. After the treatment, the scalp had honeycomb nodules and abscesses. Culture, morphology and molecular identification showed that it was *Microsporum gypseum* (*M. gypseum*) infection. *M. gypseum* is a geophilic fungus commonly found in soil, and it may have lurked in the scalp when the wound came in contact

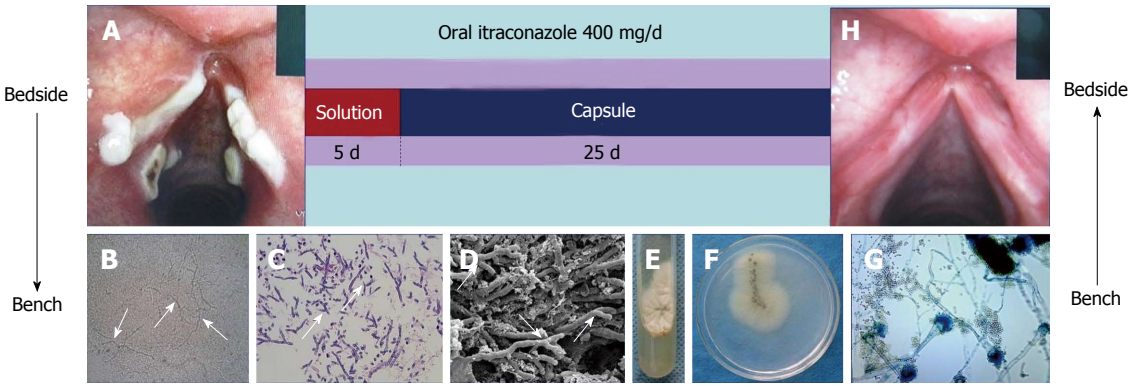

Figure 5 Treatment was successful with oral itraconazole solution the first five days followed by capsules, which completely cured the patient in 30 d. A: A laryngoscopy was performed and revealed obvious white plaques on the swollen vocal cords and laryngeal ventricle. Samples were taken from the vocal cords; B: Numerous hyphae branching at 45° angles were detected by light microscopy; C: Pathology; D: Scanning electron microscopy; E and F: A velvety and powdery colony developed on Sabouraud Dextrose Agar at 28 ℃; G: Microscopic examination of a slide culture revealed the morphologic features consistent with those of Aspergillus fumigatus. The patient was treated with oral itraconazole solution followed by capsules for 30 d; H: Laryngoscopy showed that the vocal cords were smooth without any white plaques.

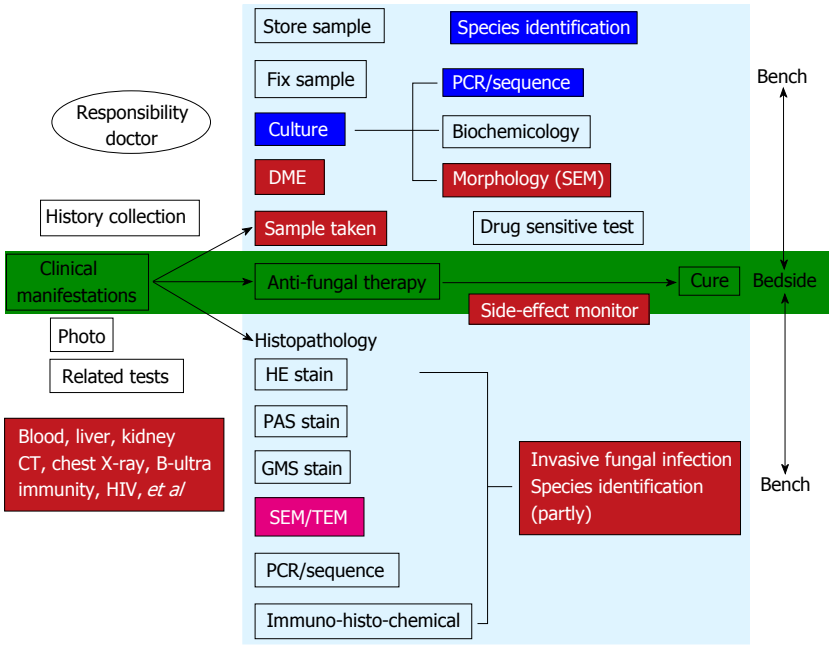

Figure 6 Translational medical mycology diagnosis and treatment pathway-from bedside to bench to bedside. CT: Computed tomography; DME: Direct microscopic examination; TEM: Transmission electron microscopy; SEM: Scanning electron microscopy; HE stain: Haematoxylin and eosin stain; GMS stain: Gomort methenamine silver stain; PAS stain: Periodic Acid Schiff stain; PCR: Polymerase chain reaction.

with soil during the accident and caused secondary infection after incomplete debridement[17]. A patient with severe facial seborrheic dermatitis had a fever of unknown origin. The direct microscopic examination of his face scales showed a large number of *Malassezia* yeast cells. Therefore, we treated him with itraconazole, and one day after the administration of antifungal drug his temperature dropped down and became normal, and seborrheic dermatitis was significantly improved during three weeks of antifungal therapy. This gives us a picture that systemic fungal infection can be the cause of the fever, though routine fungal culture of blood and even cerebrospinal fluid was negative. Since *Malassezia* is a lipophilic fungus that needs oil in the medium for the growth, but usually the general laboratory does not provide this type of medium[18]. A woman with severe hoarseness in whom antibiotic treatment did not improve the symptoms received fibrolaryngoscopic examination, and it was discovered that there were plaques on the vocal cord. By using microscopic examination, culture and molecular identification, the results showed *Aspergillus fumigatus* (*A. fumigatus*) and later she was cured with itraconazole[19]. In another a woman who had hoarseness because of a lump on her vocal cord, examination of tissues extracted for scanning electron microscopy revealed that they were destroyed by the hyphae of *A. fumigatus*. In addition, we also discovered several kinds of exocrine protease activities in the specimen[20]. The third female patient with a similar pathogen had severe hoarseness and a medical history of oral sex. The pathogen was identified by morphology and molecular identification. Treatment was successful by oral itraconazole solution for the first five days and then it was followed by capsules, which completely cured the patient in 30 d (Figure 5). This case gives us a new thought about how people with normal immune system can be infected with common airborne pathogenic *A. fumigates*.

The vocal mucous membrane barrier which was damaged by oral sex made it easy for the colonization of the spore which later invades the tissue[21].

Over the past one century medical mycology has had a typical "transformation" characteristic: we used to take samples from the lesion of a patient to check the fungal elements under the microscope to make the diagnosis and then treat the patient. Translational Medical Mycology is not just an empty theory and slogan, it should be in the classic "transformation" based on constantly enhanced (electron microscopy, *etc.*) and enriched contents (molecular biology, *etc.*) and individual medicine. In summary, in "translational medicine mycology" the starting point and end point are the clinical and laboratory studies, which are aimed to solve clinical problems (Figure 6). Patient is the "center" and problems that are encountered during the diagnosis and treatment are transforming medical mycology. For specific diseases, we might need to integrate microbiologists, pathologists, molecular biologists and other laboratory experts with all techniques and methods to determine the pathogenic fungi, help with susceptibility testing, remote consultation, so that we can start the treatment as soon as possible to save patient lives. Transformation is a dynamic, multi-level, multi-directional and continuously improving process. We can solve a clinical problem by using a number of new technologies, new methods and training, and then there will be an improvement in the treatment of fungal diseases. It will eventually promote the overall progress of medical mycology and ultimately provide an access to the effective treatment of the disease as well as prevention.

REFERENCES

1 **Wu S**, Guo R. Translational Medical Mycology can service mankind in a better way. *Zhongguo Zhenjunxue Zazhi* 2011; **6**: 321-323

2 **Liao W**, Zhang C. Can the idea of translational medicine make medical mycology developing? *Yixue Zhengming* 2012; **3**: 4-7

3 **Ran Y**. Introduction of sample taking and culture methods of Malassezia furfur. *Zhongguo Pifu Xingbingxue Zazhi* 1999; **13**: 181-182

4 **Kang D**, Ran Y, Dai Y, Lama J. Direct extraction of DNA from positive transparent dressing and swab to identify Candida albicans infection in intertriginous Candidiasis in the inguinal region. *Mycopathologia* 2012; **174**: 519-521 [PMID: 22772509 DOI: 10.1007/s11046-012-9564-5]

5 **Kang D**, Ran Y, Dai YL. Direct extraction of DNA from hair to diagnose and treat kerion caused by Arthroderma vanbreuseghemii. *Linchuang Pifu Ke Zazhi* 2013; **42**: 348-350

6 **Hua X**, Zhang RF, Yang H, Lei S, Zhang Y, Ran Y. Primary oral Penicillium marneffei infection diagnosed by PCR amplification and transmission electron microscopy from formalin-fixed, paraffin-embedded tissues. *Med Mycol Case Reports* 2012; **45**: 727-730 [DOI: 10.1016/j.bbr.2011.03.03]

7 **Kang D**, Ran Y, Li C, Dai Y, Lama J. Impetigo-like tinea faciei around the nostrils caused by Arthroderma vanbreuseghemii identified using polymerase chain reaction-based sequencing of crusts. *Pediatr Dermatol* 2013; **30**: e136-e137 [PMID: 23278484 DOI: 10.1111/pde.12044]

8 **Kang D**, Ran Y, Yin B, Dai Y. A model of translational medical mycology-A case of facial tinea incognito identified by direct DNA extraction from scales and cured by anti-fungal cream under the guidance of drug susceptibility test in vitro. *Zhongguo Zhenjunxue Zazhi* 2011; **6**: 324-327

9 **Zhang R**, Ran Y, Wang P, Dai Y. In vitro antifungal spectrum and activity of 1% naftifine-0.25% ketoconazole cream compared with 2% ketoconazole cream and 1% terbinafine cream. *Zhongguo Xun Zheng Yixue Zazhi* 2011; **11**: 508-514

10 **Yin B**, Zhuang K, Kang D, Ran Y, Dai Y. Antifungal spectrum and activity of butenafine and mometasone cream. *Pifu Bing Yu Xing Bing* 2012; **34**: 311-315

11 **Zhang H**, Ran Y, Li D, Liu Y, Xiang Y, Zhang R, Dai Y. Clavispora lusitaniae and Chaetomium atrobrunneum as rare agents of cutaneous infection. *Mycopathologia* 2010; **169**: 373-380 [PMID: 20020214 DOI: 10.1007/s11046-009-9266-9]

12 **Lu W**, Lu J, Ran Y, Lin ZC, Wan HY, Cui f, Cao LD, Pan N, Song X, Chen J, Wang YW, Yu M. Cutaneous and subcutaneous coinfection by Lichtheimia corymbifera and Candida parapsilosis: a case report. *Zhongguo Pifuke Zazhi* 2012; **45**: 727-730

13 **Qing C**, Ran Y, Li F, Dai Y, Zhao GQ, Liu SL, He XD, Zhou GP. Molecular identification of Trichophyton rubrum strains from different parts of a patient. *Zhonghua Pifuke Zazh*i 2008; **41**: 552-553

14 **He X**, Ran Y, Dai Y, Qing C, Zhang H, Xiang Y. Variability analysis of Trichophyton rubrum isolates in members of the same family. *Zhongguo Zhenjunxue Zazhi* 2010; **5**: 5-8

15 **Zhang H**, Ran Y, Liu Y, Zhang R, Lin X, Yan W, Dai Y. Arthroderma vanbreuseghemii infection in three family members with kerion and tinea corporis. *Med Mycol* 2009; **47**: 539-544 [PMID: 19115135 DOI: 10.1080/13693780802644627]

16 **Yin B**, Xiao Y, Ran Y, Kang D, Dai Y, Lama J. Microsporum canis infection in three familial cases with tinea capitis and tinea corporis. *Mycopathologia* 2013; **176**: 259-265 [PMID: 23918090 DOI: 10.1007/s11046-013-9685-5]

17 **Zhang R**, Ran Y, Dai Y, Zhang H, Lu Y. A case of kerion celsi caused by Microsporum gypseum in a boy following dermatoplasty for a scalp wound from a road accident. *Med Mycol* 2011; **49**: 90-93 [PMID: 20662636 DOI: 10.3109/13693786.2010.503196]

18 **Ran Y**, He X, Zhang H, Dai Y, Li L, Bulmer GS. Seborrheic dermatitis flare in a Dutch male due to commensal Malassezia furfur overgrowth. *Med Mycol* 2008; **46**: 611-614 [PMID: 18608906 DOI: 10.1080/13693780802140931]

19 **Ran Y**, Yang B, Liu S, Dai Y, Pang Z, Fan J, Bai H, Liu S. Primary vocal cord aspergillosis caused by Aspergillus fumigatus and molecular identification of the isolate. *Med Mycol* 2008; **46**: 475-479 [PMID: 18636395 DOI: 10.1080/13693780701851703]

20 **Ran Y**, Li L, Cao L, Dai Y, Wei B, Zhao Y, Liu Y, Bai H, Zhang C. Primary vocal cord aspergillosis and scanning electron microscopical observation of the focus of infection. *Mycoses* 2011; **54**: e634-e637 [PMID: 21535453 DOI: 10.1111/j.1439-0507.2010.01954.x]

21 **Ran Y**, Lu Y, Cao L, Li C, Dai Y, Yang H, Liu Y, Bai H, Zhang C. Primary laryngeal aspergillosis related to oral sex? A case report and review of the literature. *Med Mycol Case Rep* 2013; **2**: 1-3 [DOI: 10.1016/j.bbr.2011.03.031]

P- Reviewers: Carter WG, Sadighha A **S- Editor**: Song XX
L- Editor: Wang TQ **E- Editor**: Liu SQ

World Journal of Translational Medicine

Online Submissions: http://www.wjgnet.com/esps/
bpgoffice@wjgnet.com
www.wjgnet.com

World J Transl Med 2014 April 12; 3(1): I-V
ISSN 2220-6132 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

World Journal of Translational Medicine (*World J Transl Med*, *WJTM*, online ISSN 2220-6132, DOI: 10.5528) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

Aim and scope

WJTM publishes articles that report the results of translational medicine-related applied and basic research in fields such as immunology, physiopathology, cell biology, pharmacology, medical genetics, and pharmacology of Chinese herbs. The current columns of *WJTM* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of translational medicine diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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

WJTM is edited and published by Baishideng Publishing Group (BPG). BPG has a strong professional editorial team composed of science editors, language editors and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial borad members or peer reivewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJTM* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in translational medicine; (12) Brief Articles: To briefly report the novel and innovative findings in translational medicine; (13) Meta-Analysis: To evaluate the clinical effectiveness in translational medicine by using data from two or more randomised control trials; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJTM*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of translational medicine; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal

World Journal of Translational Medicine

ISSN

ISSN 2220-6132 (online)

Launch date

June 12, 2012

Frequency

Four-monthly

Editorial office
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Translational Medicine
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Publisher
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Telephone: +852-58042046
Fax: +852-31158812
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Production center
Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

Representative office
USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/2220-6132/g_info_20100722180909.htm.

Indexed and Abstracted in
Digital Object Identifier.

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc.* The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJTM* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For

the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

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

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/2220-6132/g_info_20100722180909.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to bpgoffice@wjgnet.com, or by telephone: +86-10-85381892. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

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

Title page

Title: Title should be less than 12 words.

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

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

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

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

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

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

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, *e.g.*, Telephone: +86-10-85381892 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, *e.g.*, 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

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

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into

the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

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

Acknowledgments

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

REFERENCES

Coding system

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

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

PMID and DOI

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

Style for journal references

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

Style for book references

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

Format

Journals

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

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

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

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

In press

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

Organization as author

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

Both personal authors and an organization as author

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

No author given

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

Volume with supplement

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

Issue with no volume

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

No volume or issue

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

Books

Personal author(s)

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

Chapter in a book (list all authors)

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

Author(s) and editor(s)

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

Conference proceedings

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

Conference paper

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

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05;

1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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

Units

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

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2220-6132/g_info_20100725073806.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade A certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

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

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/2220-6132/g_info_20100725073726.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/2220-6132/g_info_20100725073445.htm.

Proof of financial support

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

STATEMENT ABOUT ANONYMOUS PUBLICATION OF THE PEER REVIEWERS' COMMENTS

In order to increase the quality of peer review, push authors to carefully revise their manuscripts based on the peer reviewers' comments, and promote academic interactions among peer reviewers, authors and readers, we decide to anonymously publish the reviewers' comments and author's responses at the same time the manuscript is published online.

PUBLICATION FEE

WJTM is an international, peer-reviewed, OA online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium and format, provided the original work is properly cited. The use is non-commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 698 USD per article. All invited articles are published free of charge.

百世登

Baishideng®

Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza, 315-321 Lockhart Road,
Wan Chai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com