ISSN 2222-0682 (online)

World Journal of *Methodology*

World J Methodol 2015 December 26; 5(4): 175-254

Published by Baishideng Publishing Group Inc

World Journal of Methodology

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

Editorial Board

2011-2015

The *World Journal of Methodology* Editorial Board consists of 322 members, representing a team of worldwide experts in methodology. They are from 45 countries, including Argentina (4), Australia (11), Austria (3), Belgium (3), Bosnia and Herzegovina (1), Brazil (4), Canada (12), China (39), Croatia (1), Cuba (1), Czech Republic (4), Denmark (2), Egypt (1), France (8), Germany (5), Greece (6), Hungary (3), India (9), Iran (3), Israel (1), Italy (25), Japan (14), Lithuania (1), Malaysia (1), Mexico (4), Netherlands (3), New Zealand (1), Norway (2), Pakistan (2), Poland (2), Portugal (3), Romania (5), Russia (2), Senegal (1), Singapore (1), South Africa (1), South Korea (4), Spain (18), Sweden (2), Thailand (3), Turkey (4), United Arab Emirates (1), United Kingdom (14), United States (86), and Uruguay (1).

Tong-Wen Xu, *Hefei*
Shi-Ying Xuan, *Qingdao*
Xi-Lin Yang, *Hong Kong*
Bang-Ce Ye, *Shanghai*
Wen-Wu Yu, *Nanjing*
Yue-Hong Zhang, *Hangzhou*
Zhong-Ying Zhao, *Hong Kong*
Chun-Fu Zheng, *Wuhan*
Ma Zheng, *Beijing*
Jun-Jie Zhu, *Nanjing*

Croatia

Marijeta Kralj, *Zagreb*

Cuba

Mariano R Ricard, *Habana*

Czech Republic

Kamil Kuca, *Hradec Kralove*
Bozena Novotna, *Prague*
Jiri Sedy, *Prague*
Miroslav Sip, *Ceske Budejovice*

Denmark

Morten Mørup, *Lyngby*
Hans Sanderson, *Roskilde*

Egypt

Nervana S Bayoumi, *Cairo*

France

Marc Y Bardou, *Dijon*
Mohammed M Bettahar, *Nancy*
Olivier David, *Grenoble*
Guido Kroemer, *Paris*
Florian Lesage, *Sophia Antipolis*
Patrick Maison, *Creteil*
Sandrine Marquet, *Marseille*
Jean Yves Rotge, *Bordeaux*

Germany

Harald Hampel, *Frankfurt*
Frank Peinemann, *Cologne*
M Lienhard Schmitz, *Giessen*
Alfons Schnitzler, *Duesseldorf*
Frank Werner, *Magdeburg*

Greece

Konstantinos P Economopoulos, *Athens*
Georgios A Koumantakis, *Aegion*
Michael Koutsilieris, *Athens*
Demosthenes Panagiotakos, *Athens*
Issidora Papassideri, *Athens*
Falaras Polycarpos, *Pallini Attikis*

Hungary

Péter Halász, *Budapest*
András Komócsi, *Pécs*
László Vécsei, *Szeged*

India

Dipshikha Chakravortty, *Bangalore*
DK Dhawan, *Chandigarh*
R Jayakumar, *Cochin*
Abdul Viqar Khan, *Aligarh*
Geetha Manivasagam, *Vellore*
Jacob Peedicayil, *Vellore*
YS Prabhakar, *Lucknow*
Debasish Sarkar, *Orissa*
Rakesh Kumar Sinha, *Ranchi*

Iran

Mehran Javanbakht, *Tehran*
Enayat Kalantar, *Sanandaj*
Shekoufeh Nikfar, *Tehran*

Israel

Dan Frenkel, *Tel Aviv*

Italy

Giuseppe Biondi-Zoccai, *Latina*
Carlo Bonanno, *Vicenza*
Paolo Borrione, *Turin*
Filippo Cademartiri, *Monastier di Treviso*
Alberto Chiesa, *Bologna*
Annamaria Cimini, *L'Aquila*
Giovanni Di Leo, *San Donato Milanese*
Rosario Donato, *Via del Giochetto*
Alfio Ferlito, *Udine*
Giovanna Ferraioli, *Milan*
Irene Floriani, *Milan*
Landoni Giovanni, *Milano*
Stefano Girotti, *Bologna*
Paola Irato, *Padova*
Giovanni Martinotti, *Rome*
Mario Mascalchi, *Florence*
Patrizia Mecocci, *Perugia*
Germano Orrù, *Cagliari*
Maurizio Pompili, *Rome*
Carlo Riccardi, *Perugia*
Domenico Rubello, *Rovigo*
Gianfranco Spalletta, *Rome*
Gambardella Stefano, *Rome*
Mauro Valtieri, *Rome*

Japan

Kohei Akazawa, *Niigata*
Subash CB Gopinath, *Tsukuba*
Masafumi Goto, *Miyagi*
Koichi Hattori, *Tokyo*
Satoshi Hirohata, *Okayama*
Yukihiro Ikeda, *Osaka-sayama*
Masahiro Kohzuki, *Sendai*
Yoshinori Marunaka, *Kyoto*
Kenji Miura, *Tokorozawa*
Ryuichi Morishita, *Suita*
Mitsuhiko Noda, *Tokyo*
Yurai Okaji, *Tokyo*
Hirosato Seki, *Osaka*
Hisanori Umehara, *Kahoku-gun*

Lithuania

Giedrius Barauskas, *Kaunas*

Malaysia

Iis Sopyan, *Kuala Lumpur*

Mexico

Javier Camacho, *Mexico City*
Mejía Aranguré Juan Manuel, *Col Doctores*
Martha Rodríguez-Moran, *Durango*
Julio Sotelo, *Mexico City*

Netherlands

Kristien Hens, *Maastricht*
Bart J Polder, *Emmeloord*
Frank Twisk, *Limmen*

New Zealand

Valery Feigin, *Auckland*

Norway

David F Mota, *Oslo*
Tore Syversen, *Trondheim*

Pakistan

Muhammad A Noor, *Islamabad*
Yasir Waheed, *Islamabad*

Poland

Piotr Dziegiel, *Wroclaw*
Tadeusz Robak, *Lodz*

Portugal

Nuno Lunet, *Porto*
Hugo Sousa, *Porto*

Romania

Elena Moldoveanu, *Bucharest*
Monica Neagu, *Bucharest*
Florin-Dan Popescu, *Bucharest*

Eugen Rusu, *Galati*

Russia

Galina B Bolshakova, *Moscow*
Sergey V Dorozhkin, *Moscow*

Senegal

Badara Cissé, *Dakar*

Singapore

Zhang Yong, *Singapore*

South Africa

Robin Alexander Emsley, *Cape Town*

South Korea

Sang Soo Hah, *Seoul*
Chang-Yong Lee, *Kongju*
Kwan Sik Lee, *Seoul*

Spain

Salvador F Aliño, *Valencia*
Mohamed Farouk Allam, *Cordoba*
Alejandro Cifuentes, *Madrid*
Miren Lopez de Alda, *Barcelona*
Joaquin de Haro, *Madrid*
M de la Guardia, *Valencia*
Emma Garcia-Meca, *Cartagena*
Mónica H Giménez, *Zaragoza*
Josep M Guerrero, *Barcelona*
Fernando Marin, *Madrid*
José A Orosa, *A Coruña*
Jesús Osada, *Zaragoza*
Soledad Rubio, *Córdoba*
Helmut Schröder, *Barcelona*
Jesus Simal-Gandara, *Ourense*
Bahi Takkouche, *Santiago de Compostela*
Gabriela Topa, *Madrid*
Miguel A Vallejo, *Madrid*

Sweden

Stefan Karlsson, *Lund*
Jenny Selander, *Stockholm*

Thailand

Amporn Jariyapongskul, *Bangkok*
Apiwat Mutirangura, *Bangkok*
Bungorn Sripanidkulchai, *Khon Kaen*

Turkey

Mehmet Doğan, *Çağış-Balikesir*
Ferda E Percin, *Ankara*
Ahmet Yildirim, *Bornova-Izmir*
Aysegul Yildiz, *Izmir*

United Arab Emirates

Hassib Narchi, *Al Ain*

United Kingdom

Richard H Barton, *London*
Paul Evans, *London*
Giuseppe Garcea, *Leicester*
Marta I Garrido, *London*
Sinead Keeney, *Belfast*
Maurice J O'Kane, *Londonderry*
Abdullah Pandor, *Sheffield*
Susan Pang, *Teddington*
Pankaj Sharma, *London*
Andrew Harvey Sims, *Edinburgh*
David E Whitworth, *Aberystwyth*
Sorrel E Wolowacz, *Manchester*
Feng Wu, *Headington*
Shangming Zhou, *Swansea*

United States

Nasar U Ahmed, *Miami*
Mike Allen, *Milwaukee*
Srinivas Ayyadevara, *Little Rock*
Charles F Babbs, *West Lafayette*
Janet Barletta, *Baltimore*
Lawrence T Bish, *Philadelphia*
Richard W Bohannon, *Storrs*
M Ahmad Chaudhry, *Burlington*
Pei Chen, *Beltsville*
Tao Chen, *Jefferson*
Yong Q Chen, *Winston-Salem*
Machado Christian, *Southfield*
Patricia Ann D'Amore, *Boston*
Undurti N Das, *Shaker Heights*
Feng Ding, *Chapel Hill*
Mary E Edgerton, *Houston*
D Mark Estes, *Athens*
Bingliang Fang, *Houston*
Ronnie Fass, *Tucson*
Vesna D Garovic, *Rochester*
Alexandros Georgakilas, *Greenville*
Ronald Gillam, *Logan*
Shannon S Glaser, *Temple*
Ga Nagana Gowda, *West Lafayette*
Anton B Guliaev, *San Francisco*
Zong Sheng Guo, *Pittsburgh*
James P Hardwick, *Rootstown*
Diane M Harper, *Kansas*
Odette A Harris, *Stanford*
Rod Havriluk, *Tallahassee*
Moonseong Heo, *Bronx*
Guoyuan Huang, *Evansville*
Michael Huncharek, *Columbia*
Reinhold J Hutz, *Milwaukee*
Bankole A Johnson, *Charlottesville*
Joseph M Kaminski, *Silver Spring*
Yong S Kim, *Bethesda*
Mark S Kindy, *Charleston*
Jennifer Kisamore, *Tulsa*
Georgios D Kitsios, *Boston*
Ronald Klein, *Shreveport*
Heidemarie Kremer, *Miami*
S Lakshmivarahan, *Norman*
Dawei Li, *New Haven*
Kenneth Maiese, *Newark*
Sameer Malhotra, *New York*
JL Mehta, *Little Rock*
Ray M Merrill, *Provo*
M Mimeault, *Nebraska*
Ron B Mitchell, *St Louis*
Anirban P Mitra, *Los Angeles*
Walter P Murphy, *Evanston*
Marja Tuuli Nevalainen, *Philadelphia*
Yan Peng, *Dallas*
George Perry, *San Antonio*
Ilona Petrikovics, *Huntsville*
Shengping Qin, *Davis*
Peter J Quesenberry, *Providence*
P Hemachandra Reddy, *Beaverton*
James V Rogers, *Columbus*
Troy Rohn, *Boise*
Paul R Sanberg, *Tampa*
Tor C Savidge, *Galveston*
Dong-Chul Seo, *Bloomington*
Igor Sevostianov, *Las Cruces*
Judy Y Tan, *Hillside*
Weihong Tan, *Gainesville*
Guangwen Tang, *Boston*
Paul D Terry, *Knoxville*
Guochuan Emil Tsai, *Torrance*
Catherine E Ulbricht, *Somerville*
Thomas TH Wan, *Orlando*
Xiao-Jing Wang, *Aurora*
Jang-Yen Wu, *Boca Raton*
Qing Wu, *Scottsdale*
Eleftherios S Xenos, *Lexington*
Lijun Xia, *Oklahoma City*
Xiong Xu, *New Orleans*
Li-Jun Yang, *Gainesville*
Wancai Yang, *Chicago*
Xinan Yang, *Chicago*
Fahd A Zarrouf, *Anderson*
Henry Zeringue, *Pittsburgh*
Jingbo Zhang, *New York*

Uruguay

Matias Victoria, *Salto*

World Journal of Methodology

Contents

Quarterly Volume 5 Number 4 December 26, 2015

World Journal of Methodology
Volume 5 Number 4 December 26, 2015

Contents

NAME OF JOURNAL
World Journal of Methodology

ISSN
ISSN 2222-0682 (online)

LAUNCH DATE
September 26, 2011

FREQUENCY
Quarterly

EDITOR-IN-CHIEF
Yicheng Ni, MD, PhD, Professor, Department of Radiology, University Hospitals, KU, Leuven, Herestraat 49, B-3000, Leuven, Belgium

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director

World Journal of Methodology
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: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

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

PUBLICATION DATE
December 26, 2015

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2222-0682/g_info_20100722180909.htm

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

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.175

World J Methodol 2015 December 26; 5(4): 175-178
ISSN 2222-0682 (online)

EDITORIAL

Conflicts of interest in nutritional sciences: The forgotten bias in meta-analysis

Michel Lucas

Michel Lucas, Department of Social and Preventive Medicine, Université Laval, Population Health and Optimal Health Practices Research Unit, Centre Hospitalier Universitaire de Québec, Québec G1V 2M2, Canada

Michel Lucas, Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA 02115, United States

Author contributions: Lucas M conceived the idea of the manuscript, interpreted the data and wrote the manuscript.

Conflict-of-interest statement: (1) Dr. Lucas is Assistant Professor at Université Laval since July 2012, and Visiting Scientist at the Harvard T.H. Chan School of Public Health. Full salary of Dr. Lucas comes from Université Laval and CHU de Québec, and is cover by a salary award from the Fonds de recherche du Québec - Santé (FRQS); (2) Between 1999 and 2012, while he was a student, Dr. Lucas often spoke at conferences (mainly on omega-3 fatty acids). His honoraria and expenses were covered by private industries. He has never received research funding from private industries; (3) Dr. Lucas has no relationships with entities that might have an interest in the submitted work; (4) Dr. Lucas' spouse, children and partners have no financial relationships with the submitted work; and (5) Dr. Lucas does not have any non-financial interests in the submitted work.

Correspondence to: Dr. Michel Lucas, Assistant Professor, Department of Social and Preventive Medicine, Université Laval, Population Health and Optimal Health Practices Research Unit, Centre Hospitalier Universitaire de Québec, 2875 Laurier Blvd., Delta 2 Building, Suite 600, Québec G1V 2M2, Canada. michel.lucas@crchuq.ulaval.ca
Telephone: +1-418-5254444
Fax: +1-418-6542726

Received: May 22, 2015
Peer-review started: May 22, 2015
First decision: August 19, 2015
Revised: September 14, 2015
Accepted: September 29, 2015
Article in press: September 30, 2015
Published online: December 26, 2015

Abstract

Awareness of conflicts of interest (COI) in medicine began in the 1980s. More recently, the problem has gained notoriety in nutritional sciences. COI with industry could bias study conclusions in the context of research activities and scientific publications on nutritional sciences. The issue of COI in nutritional sciences deserves more attention and requires careful analyses as biased information can negatively impact the development of dietary guidelines and, ultimately, population health. Decision-making is generally based on available, published evidence, but when the results are ambivalent, it is easier to opt for the status quo and ask for more studies. Readers might wonder if research is subsidized by industry as a counterbalancing strategy based on levels of evidence-only to slow down eminent positions and/or legislation on the food sector? How can this problem be overcome without producing paranoia and McCarthyism while trying to be as methodological as possible?

Key words: Conflicts of interest; Nutrition; Nutritional sciences; Bias; Systematic reviews; Meta-analysis

Core tip: Decision-making in the field of nutrition is based on published evidence, but when results are ambivalent, it is easier to opt for the status quo and ask for more studies. Because conflicts of interest (COI) in nutritional sciences can bias conclusions and negatively impact dietary recommendations and population health,

it deserves more attention and requires careful analyses. To regard evidence properly and in a rigorous manner, COI in systematic reviews and meta-analyses must be evaluated systematically to guarantee the trustworthiness of nutrition-related studies, and must therefore be obligatory sub-analyses to reduce the risk of bias in data interpretation.

Lucas M. Conflicts of interest in nutritional sciences: The forgotten bias in meta-analysis. *World J Methodol* 2015; 5(4): 175-178 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/175.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.175

INTRODUCTION

Thompson[1] defined conflicts of interest (COI) as "a set of conditions in which professional judgment concerning a primary interest (such as a patient's welfare or the validity of research) tends to be unduly influenced by a secondary interest (such as financial gain)". To researchers, financial incentive is not necessarily the only influent interest. The desire for recognition, academic advancement, success in publication and funding are other powerful stimulants[2]. Scientists delight in believing they are immune to and very mindful of COI. According to Young[3]: "We are not always aware of our own biases. The idea that scientists are objective seekers of truth is a pleasing fiction, but counterproductive in so far as it can lessen vigilance against bias." The purpose of this editorial is to discuss justifiable concern about the COI problem in nutritional sciences and the need to seriously take it into account with critical reading of scientific journals and inclusion of its analysis in systematic reviews (SR) as well as meta-analyses (MA). The nature of COI and human behavior relevant to COI are beyond the scope of this editorial, as they have already been well-described by other authors[3,4].

Even if we cannot accurately ascertain the beginnings of COI investigation in medicine, its consideration intensified in the 1980's[5,6] and it still continues[7]. In 2009, the Institute of Medicine dedicated a full report to COI, indicating that its concerns are justifiable[8]. Corruption of healthcare by Big Pharma is a long-standing debate, but the one on Big Food is much more recent[9]. Indeed, *PloS Med* and *BMJ* recently published a complete series on the food and beverage industries, their influence and COI[10,11]. To Loder[10], the industry-researcher relationship "is not evidence of research malpractice. It does, however, contribute to perceptions that nutrition science might be for sale." However, nutritional sciences are not the exclusive domain of COI. Such biases are well-known in the tobacco[12] and pharmaceutical industries[2], and parallels also apply to the food industry[13].

Although most scientific journals instruct authors to report all COI, not all published studies declare them. Lesser *et al*[14] noted that 54% of scientific articles - relating to drinks (beverages, juice, and milk) and published between 1999 and 2003 - named their financial sponsorships. They assessed the influence of funding bias by determining the relationship between industry (sponsorship) funding (yes, no, mixed support) and the conclusions of scientific articles (favorable, unfavorable or neutral). They reported an odds ratio of 7.61 (95%CI: 1.27-45.73) for favorable *vs* unfavorable conclusions in all industry-funded articles compared to those without industry funding. They concluded that "industry funding of nutrition-related scientific articles may bias conclusions in favor of sponsors products, with potentially significant implications for public health". Diels *et al*[15] scrutinized the relationship between COI and study outcomes (favorable/unfavorable) in the realm of genetically-modified food products, using similar methodology. They found that financial COI were not associated with the results, but discerned strong linkage between professional COI (author affiliation with industry) and study outcomes. Bes-Rastrollo *et al*[16] examined relationships between COI and food companies, conclusions on sugar-sweetened beverage (SSB) consumption, and weight gain in published SR. Among the 6 SR that identified "COI with food companies", 83.3% (n = 5) reported no positive linkage between SSB intake and weight gain, whereas among the 12 SR that found "no COI with food companies", 83.3% ascertained positive associations. They noted that studies with "COI with food companies" were 5 times (relative risk = 5.0; 95%CI: 1.3-19.3) more likely to present no positive association between SSB consumption and weight gain than those without COI. These contradictory findings do not, however, establish which SR is right, but they clearly indicate discrepancies, depending on whether or not COI exist.

Drug studies have advantages over those in nutrition-the results are first submitted to government agencies for scrutiny before drug approval. Therefore, data from unpublished drug research are available, but this is not the case in nutrition. Moreover, by accessing trials registered with the United States Food and Drug Administration[17], the European Medicines Agency[18] and other government bodies, it can be determined if they have been published or not, if more negative studies are unpublished, if published results agree or conflict with agency decisions, and if there is risk of publication bias. Although not perfect, different methods detect and correct for publication bias[19,20]. However, unless bias is severe, these tests have low power and high false-positive rates in perceiving significant asymmetry[19]. Therefore, no statistical methods are superior to any others in assessing publication bias and they should be viewed as exploratory analyses. Publication bias in SR and MA is related not only to published and unpublished studies, but also to factors which influence published studies (*e.g.*, statistical significance, study size and quality, type of study design, *etc.*)[21]. COI are often forgotten factors.

To help in the critical analysis of published SR and MA,

guidelines such as PRISMA[22] (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) should be followed. The publication of protocols for SR and MA in the Cochrane Library (http://community.cochrane.org/) or Prospero (http://www.crd.york.ac.uk/prospero/)[23] could ensure rigorous methodology. Although these tools are driven by methodological markers, they do not constitute an analysis of quality in themselves. Peer review committees and scientific panels do not always guarantee the quality of published studies. If included studies are of poor methodological quality, their results are likely to be biased. Therefore, the outcomes of SR and MA could be linked with high risk of bias, even if the methodology is implemented with great rigor. The Cochrane Statistical Methods Group and the Cochrane Bias Methods Group developed a risk of bias tool that focuses on 6 domains: Selection, performance, detection, attrition, reporting and other biases[24]. However, this risk of bias tool seems insufficient to capture biases related to study funding sources[25]. A Cochrane Review found that the risk of bias between drug industry and non-drug industry-funded studies was similar, but observed that drug industry-funded studies reported more "favorable" results than non-drug industry-funded studies[26].

As mentioned earlier, the problem of COI is complicated by the fact that not all studies sufficiently declare sponsorships and the financial affiliations of authors. Indeed, in the 2010 Cochrane Database of Systematic Reviews, a very low proportion of published drug studies reported funding sources (30%, 46/151 reviews), author-industry financial ties or employment (11%, 16/151 reviews)[27]. These findings are overwhelming, considering that Methodological Expectations of Cochrane Intervention Reviews Item No. C44 (Describing Studies) is mandatory[28]. Funding sources and COI declarations by primary researchers should be collected in this process and appear in the table on "Characteristics of included studies".

As suggested by Bero[25]: "The impact of the bias can be assessed descriptively or by using subgroup analysis, comparing industry-funded to non-industry-funded studies, as is commonly done in Cochrane Reviews. A bias should not be ignored even if we do not fully understand its mechanism, just as we should not ignore harms of interventions if we don't understand how they arose, or ignore the harm of smoking because we don't know how smoking causes cancer. Therefore, a study's funding source should be evaluated as an independent risk of bias." As pointed out by Rothman[29], there is a risk of McCarthyism with labeling of scientists as having COI. He recommended "that a work should be judged solely on its merits" and "We can halt this new McCarthyism in science and get back to focusing on the work of a scientist rather than on his or her life story".

To regard evidence properly and in a rigorous manner, COI in SR and MA must be evaluated systematically to guarantee the trustworthiness of nutrition-related studies, and must therefore be obligatory sub-analyses to reduce the risk of bias in data interpretation. COI may influence the results not only by showing statistically significant associations between exposure and disease, but also by demonstrating lack of associations, especially among groups that protect interests by inducing doubts and claiming unproven causation. Surprisingly and unfortunately, none of the MA in nutritional sciences assessed COI - to the best of the author's knowledge while writing this editorial! Therefore, SR and MA must include sub-analyses that try to examine if studies with COI: (1) industry-sponsored; (2) authors-industry-affiliated; and (3) sponsorship or author affiliation - have more favorable outcomes (results, conclusions) than other investigations. The methodology employed by Diels *et al*[15] for SR and by Lundh *et al*[26] for MA could establish definitions, such as: (1) Sponsorships: industry-funded, non-industry-funded, and unknown/unclear sponsorship; (2) Authors' affiliations: Industry-affiliated, non-industry-affiliated, and unknown/unclear; and (3) Classifications that combine sponsorship and author affiliations: COI (sponsorship or author affiliations), no COI, and unknown/unclear.

Nutrition is one of the most vital health determinants of society, not only in regard to the etiology of chronic diseases, but also because it is an important target for public health interventions. Investment in epidemiological approaches - allowing rigorous study into the roles of both individual and overall diets in disease risk - is undoubtedly a key to success. However, to continue to attract interest and trustworthiness, nutritional sciences must be faultless.

REFERENCES

1 **Thompson DF**. Understanding financial conflicts of interest. *N Engl J Med* 1993; **329**: 573-576 [PMID: 8336759 DOI: 10.1056/NEJM199308193290812]

2 **Bekelman JE**, Li Y, Gross CP. Scope and impact of financial conflicts of interest in biomedical research: a systematic review. *JAMA* 2003; **289**: 454-465 [PMID: 12533125 DOI: 10.1001/jama.289.4.454]

3 **Young SN**. Bias in the research literature and conflict of interest: an issue for publishers, editors, reviewers and authors, and it is not just about the money. *J Psychiatry Neurosci* 2009; **34**: 412-417 [PMID: 19949717]

4 **Levinsky NG**. Nonfinancial conflicts of interest in research. *N Engl J Med* 2002; **347**: 759-761 [PMID: 12213950 DOI: 10.1056/NEJMsb020853]

5 **Relman AS**. Dealing with conflicts of interest. *N Engl J Med* 1985; **313**: 749-751 [PMID: 4033698 DOI: 10.1056/NEJM198405033101809]

6 **Relman AS**. The new medical-industrial complex. *N Engl J Med* 1980; **303**: 963-970 [PMID: 7412851 DOI: 10.1056/NEJM198010233031703]

7 **Rosenbaum L**. Understanding bias--the case for careful study. *N Engl J Med* 2015; **372**: 1959-1963 [PMID: 25970055 DOI: 10.1056/NEJMms1502497]

8 **Lo B**, Baldwin W, Bellini L. for the Institute of Medicine. Conflict of interest in medical research, education, and practice [consensus report]. In: Lo B, Field MJ, editors. National Academy of Sciences. Available from: URL: http://www.iom.edu/Reports/2009/Conflict-of-Interest-in-Medical-Research-Education-and-Practice.aspx

9 **Nestle M**. Food company sponsorship of nutrition research and professional activities: a conflict of interest? *Public Health Nutr* 2001; **4**: 1015-1022 [PMID: 11784415 DOI: 10.1079/PHN2001253]

10 **Loder E**. Big food, big pharma: is science for sale? *BMJ* 2015; **350**: h795 [DOI: 10.1136/bmj.h795]
11 **Barbour V**, Clark J, Simpson P, Veitch E. PLoS Medicine series on Big Food: the food industry is ripe for scrutiny. *PLoS Med* 2012; **9**: e1001246 [PMID: 22723749 DOI: 10.1371/journal.pmed.1001246]
12 **Barnes DE**, Hanauer P, Slade J, Bero LA, Glantz SA. Environmental tobacco smoke. The Brown and Williamson documents. *JAMA* 1995; **274**: 248-253 [PMID: 7609234 DOI: 10.1001/jama.1995.03530030068036]
13 **Brownell KD**, Warner KE. The perils of ignoring history: Big Tobacco played dirty and millions died. How similar is Big Food? *Milbank Q* 2009; **87**: 259-294 [PMID: 19298423 DOI: 10.1111/j.1468-0009.2009.00555.x]
14 **Lesser LI**, Ebbeling CB, Goozner M, Wypij D, Ludwig DS. Relationship between funding source and conclusion among nutrition-related scientific articles. *PLoS Med* 2007; **4**: e5 [PMID: 17214504 DOI: 10.1371/journal.pmed.0040005]
15 **Diels J**, Cunha M, Manaia C, Sabugosa-Madeira B, Silva M. Association of financial or professional conflict of interest to research outcomes on health risks or nutritional assessment studies of genetically modified products. *Food Policy* 2011; **36**: 197-203 [DOI: 10.1016/j.foodpol.2010.11.016]
16 **Bes-Rastrollo M**, Schulze MB, Ruiz-Canela M, Martinez-Gonzalez MA. Financial conflicts of interest and reporting bias regarding the association between sugar-sweetened beverages and weight gain: a systematic review of systematic reviews. *PLoS Med* 2013; **10**: e1001578; dicsussion e1001578 [PMID: 24391479 DOI: 10.1371/journal.pmed.1001578]
17 **Turner EH**, Matthews AM, Linardatos E, Tell RA, Rosenthal R. Selective publication of antidepressant trials and its influence on apparent efficacy. *N Engl J Med* 2008; **358**: 252-260 [PMID: 18199864 DOI: 10.1056/NEJMsa065779]
18 **Taylor D**, Sparshatt A, Varma S, Olofinjana O. Antidepressant efficacy of agomelatine: meta-analysis of published and unpublished studies. *BMJ* 2014; **348**: g1888 [PMID: 24647162 DOI: 10.1136/bmj.g1888]
19 **Song F**, Khan KS, Dinnes J, Sutton AJ. Asymmetric funnel plots and publication bias in meta-analyses of diagnostic accuracy. *Int J Epidemiol* 2002; **31**: 88-95 [PMID: 11914301 DOI: 10.1093/ije/31.1.88]
20 **Thornton A**, Lee P. Publication bias in meta-analysis: its causes and consequences. *J Clin Epidemiol* 2000; **53**: 207-216 [PMID: 10729693 DOI: 10.S0895-4356(99)00161-4]
21 **Delgado-Rodríguez M**, Llorca J. Bias. *J Epidemiol Community Health* 2004; **58**: 635-641 [PMID: 15252064 DOI: 10.1136/jech.2003.008466]
22 **Moher D**, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009; **339**: b2535 [PMID: 19622551 DOI: 10.1136/bmj.b2535]
23 **Booth A**, Clarke M, Dooley G, Ghersi D, Moher D, Petticrew M, Stewart L. The nuts and bolts of PROSPERO: an international prospective register of systematic reviews. *Syst Rev* 2012; **1**: 2 [PMID: 22587842 DOI: 10.1186/2046-4053-1-2]
24 **Higgins JPT**, Altman DG, Sterne JACe. Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration. Available from: URL: http://www.cochrane-handbook.org
25 **Sterne JA**. Why the Cochrane risk of bias tool should not include funding source as a standard item. *Cochrane Database Syst Rev* 2013; **12**: ED000076 [PMID: 24575440 DOI: 10.1002/14651858.ed000076]
26 **Lundh A**, Sismondo S, Lexchin J, Busuioc OA, Bero L. Industry sponsorship and research outcome. *Cochrane Database Syst Rev* 2012; **12**: MR000033 [PMID: 23235689 DOI: 10.1002/14651858.MR000033.pub2]
27 **Roseman M**, Turner EH, Lexchin J, Coyne JC, Bero LA, Thombs BD. Reporting of conflicts of interest from drug trials in Cochrane reviews: cross sectional study. *BMJ* 2012; **345**: e5155 [PMID: 22906823 DOI: 10.1136/bmj.e5155]
28 **Chandler J**, Churchill R, Higgins J, Lasserson T, Tovey D. Methodological Expectations of Cochrane Intervention Reviews (MECIR). Methodological standards for the conduct of new Cochrane Intervention Reviews. Version 2.3. Available from: URL: http://www.editorial-unit.cochrane.org/mecir
29 **Rothman KJ**. Conflict of interest. The new McCarthyism in science. *JAMA* 1993; **269**: 2782-2784 [PMID: 8192721 DOI: 10.1001/jama.1993.03500210082037]

P- Reviewer: Costa RF, Gonzalez-Reimers E, Zhang Q
S- Editor: Qiu S **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.179

World J Methodol 2015 December 26; 5(4): 179-184
ISSN 2222-0682 (online)

EDITORIAL

Toward phase 4 trials in heart failure: A social and corporate responsibility of the medical profession

Pupalan Iyngkaran, Glen S Beneby

Pupalan Iyngkaran, NT Medical School, Research Fellow and Senior Lecturer Flinders University, Darwin NT 0815, Australia

Glen S Beneby, Commonwealth of the Bahamas, 3rd and West Centerville, Nassau 999154, Bahamas

Author contributions: Iyngkaran P wrote the manuscript; Beneby GS edited and added in additional relevant points.

Conflict-of-interest statement: All co-authors have won independent and governmental research funding. Several members provide counsel to pharmaceuticals. None pose a conflict of interest for this short paper.

Correspondence to: Dr. Pupalan Iyngkaran, MBBS, FRACP, Consultant Cardiologist, NT Medical School, Research Fellow and Senior Lecturer Flinders University, PO Box 41326, Casuarina, Darwin NT 0815, Australia. gbeneby@batelnet.bs
Telephone: +61-88-9467488
Fax: +61-88-9467599

Received: December 10, 2014
Peer-review started: December 12, 2014
First decision: January 8, 2015
Revised: October 20, 2015
Accepted: October 23, 2015
Article in press: October 27, 2015
Published online: December 26, 2015

Abstract

Congestive heart failure (CHF) is a chronic condition, requiring polypharmacy, allied health supports and regular monitoring. All these factors are needed to ensure compliance and to deliver the positive outcomes demonstrated from randomized controlled trials. Unfortunately many centers around the world are unable to match trial level support. The outcomes for many communities are thus unclear. Research design factors in post-marketing surveillance to address this issue. Phase 4 studies is the name given to trials designed to obtain such community level data and thus address issues of external validity. CHF phase 4 studies are relatively underutilized. We feel the onus for this research lies with the health profession. In this commentary we provide arguments as to why phase 4 studies should be viewed as a social and corporate responsibility of health professional that care for clients with CHF.

Key words: Clinical trial; Corporate responsibility; Health system; Congestive heart failure; Phase 4

Core tip: This commentary brings needed and timely attention to phase 4 or postmarketing surveillance. Only a handful of congestive heart failure (CHF) therapies have actually been studied in the community after the randomized controlled trial. In this millennium it is important we not only innovate and support trials of new therapies, but also ensure the therapies we are already using are effective for all patients. As drug discovery and randomised controlled trial evidence is often done by private sector pharmaceuticals, we thus feel the need to bring attention on treating health care teams to regularly generate efficacy and effectiveness data for the CHF treatments they prescribe.

Iyngkaran P, Beneby GS. Toward phase 4 trials in heart failure: A social and corporate responsibility of the medical profession. *World J Methodol* 2015; 5(4): 179-184 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/179.htm DOI:

http://dx.doi.org/10.5662/wjm.v5.i4.179

INTRODUCTION

"…'medical Profession' consists of the sum of the identities of its membership … if the 'profession' is held responsible for something, each of its members is responsible, in some way, for it". -French PA

It is the collective responsibility of the medical profession to seek and administer processes to account for past practices and improve future health practices. The practice of medicine, today, is a complex process that has to factor many considerations. An important consideration is the evidence based practice. This evolution initially saw prescribing that was based on uncontrolled observations of physiology in individuals, to controlled observations on groups. The pursuit of this new goal has become quite complex and corporatized, such that we often forget some of the basics that has safely steered the profession. All systems also have to factor the social and ethical contracts between governments, and its citizens, demanding equitable health services, or risk community wrath at the ballot box. The prescription of pharmaceuticals is one arm of this complex process. Many pharmaceutical manufacturers operate from the private sector. A financial investment is made in developing a drug, where there is always the risk that it may not provide the necessary benefits, hence unmarketable. To standardize this competition with accountability, the randomised controlled trial (RCT) has been used to generate the evidence base. Should there be positive findings, the company that has made that investment now has the legitimacy to market the product. There are however limitations with this process. In this editorial we discuss the importance of continuing governance once the drug is approved for community use. In this, all health professionals have to ask the hard questions and truly understand the entirety of their responsibilities not only in the delivery but the governance of clinical as well as the corporate issues. Let us look at examples for this from several vantage points that are timeless.

ETHICS

Whenever a doctor cannot do good, he must be kept from doing harm. -Hippocrates

Safety, the pillar of medical practice, ingrained at the start of training and reminded at graduation with the "Hippocratic Oath", is a social contract we as health care workers (HCW) have with our clients. Before a HCW does anything we should firstly do no harm. As soon as a HCW starts their duties whether it is consulting, diagnosing, prescribing, dispensing, delivering, promoting or preventing, they run the risk of doing harm. There is no way to determine risk-benefits ratios of any intervention without adequate checks and balances. Medication errors are the eighth leading cause of death in the United States. Cardiovascular medications account for a large proportion of these errors, predominately as inpatients in the emergency department and acute hospital settings. Errors include omissions, incorrect dosage, under prescribing, and failure to consider adverse interactions. Errors are more likely to occur when clinical workload is heavy; there is language, communication, cultural barriers; although, generally the majority of these errors occur from a lack of intention[1]. Geographical distances and unavailability of services are not factored much in guidelines to achieve simplicity of the therapeutic regime. Universal and standardized reporting of errors and adverse side effects has been in play from many health bodies and centralised to government bodies to ensure accountability. Standardization of knowledge and training at undergraduate and continuity thereafter are also important measures to reduce this risk[2,3]. System wide the monitoring of this, however, remains inadequate.

STANDARDS

"…The art of medicine was to be properly learned only from its practice and its exercise…" -Thomas Sydenham

Health regulators have ensured that delivery of health care has a minimum basic standard through law and enforcement by regulatory bodies. Such examples include the medical universities, training colleges, therapeutic goods admirations, medical councils and overseas trained doctor regulators. At the core of this is the curriculum. As health information and technologies are evolving so must HCW and systems. Continuous medical education (CME) is now required by medical bodies to maintain up-to-date knowledge, although barriers remain. Among a small number of general practitioners, barriers were identified in many dimension of care[4]. With system wide barriers it will be difficult for regulators to introduce standards for improvement at the level of the health clusters. In addition there are silos between the administrators, HCW and clients. This often makes it difficult for HCW to practice in-sync with advancements, while using their local experience. This in fact is a translational block that occurs far too often. In-fact these local experiences in the practice of medicine are not given any emphasis. No doubt HCW may use this in their practice, perhaps unregulated, without the knowledge of how it is translating. As an example, Joynt *et al*[5] highlighted the differences in mortality outcomes between physicians managing a high *vs* low volume of congestive heart failure (CHF) cases. These benefits were noted regardless of age, sex, race and comorbidities. Such care was also more intense, and with greater use of skilled nurse and rehabilitation. However, readmission rates were higher[5]. Identifying priorities is one reason. The experience of HCW also appears vital for improved outcomes. In the real world

there remain many clusters that never achieve this high volume status, and where admitting patients more frequently are not possible. How we learn from these positive examples, how we disseminate that knowledge and how we use technology to share workloads to achieve the adequate standards and outcomes are issues health systems must address. To truly factor in experiences, guidelines need to achieve consensus and standardize sections in it that reflects on the benefits of regional variations in practice. Creating options of how such variations can be created, while ensuring all these deviations are audited is an important standard to set in shaping CHF guidelines of the future. Another issue of concern when dealing with fixed guidelines is its generalizability.

GENERALIZABILITY

"We should be concerned not only about the health of individual patients, but also the health of our entire society". -Benjamin Carson

It is important we pay attention to the generalizability of standards and guidelines. Rothwell, highlighted two vital questions when evidence is gathered through RCTs: firstly, are the results valid for patients other than from the trials; and are the results generalizable to similar patients but in a different treatment setting[6]. Due to the way trials are set up, it will be impossible to test every conceivable permutation (scenario) while controlling biases. Cultural sensitivities, an example of one such, are important areas to negotiate. Often HCW exercise judgement which on occasion could flirt the boundaries of such guidelines or the skills they are thought[7]. This art of medicine touches greatly on subjectivity and relies on the HCW intuition or perhaps experience. In a study of general practitioners if was found that prescribing closely of HF guidelines varied inversely with age[7]. While the younger group could relate to familiarity with guidelines, the importance of age and experience, perhaps related more to on the ground realities in different communities, and may have influenced these differences. There are unfortunately no universal ways to standardize this, but we can still account for this. As highlighted earlier experience can be a factor that affects outcomes. From this HCW may start being creative in their administration of health services. It is not only important that there be accountability but also sharing of this experience to reflect in the published literature.

EXPERIENCE

"It is much more important to know what sort of a patient has a disease than what sort of a disease a patient has". -William Osler

No two health care workers will be identical in the way they practice medicine. Experiences of both client and physicians are important as they tailor their individual views on health and illness. The undisputed common outcome is perhaps the ability to "…live a long, productive and quality life…". Again studies have shown that the higher the volume of HF clients seen by physicians, outcomes tended to be better, suggesting the importance of clinical experience[5]. While we allow doctors to practice in this fashion, we find it difficult to find guidelines that describe medical care in this fashion. Similarly client experiences are important. The clients view on how they are treated in the medical system will reinforce their attitudes to health. These views will have cultural and socioeconomic slants, factors that are not often factored into RCT or guidelines. It may not be as simple in all cases to tell a patient that the medicine you are giving them is the best and they ought to comply regardless of how they feel about it[8,9]. In this sense two powerful qualities are relationships which is vital to develop a good understanding, and choice, which is needed to provide for a holistic HCW-patient experience.

RELATIONSHIPS

"The good physician treats the disease; the great physician treats the patient who has the disease". -William Osler

It is universally accepted that most clients and systems rate HCWs who provide a holistic approach to medical care. There is a spectrum of what is considered holistic including partnerships and seeking of expertise when unavailable. It is also imperative that HCW look out for and form partnerships with other specialists in their own right. Knowledge is not the right of one person and no one group has all the knowledge. To provide a comprehensive client experience all HCW at some point must seek the assistance of their colleague. This area becomes more complex when skills are outside the health cluster. Forming relationships with centres of excellence will help. Technology can be used to bridge such gaps. Translational blocks for these are administrative, requiring a new mind set from all parties. Similarly health services with a greater density of HF specialist were associated with improved outcomes[10,11]. These are other reasons for such partnerships. Good client HCW relationship is an independent marker of positive outcomes[12]. The National institute of health which advocates for improved evidence translation discusses the continuation of "Bench to Bedside" research. In the second arm, "Bedside to Bench", clinical and basic research are equally important in the delivery. It is the obligation of the HCW to seek these out, to improve translation of evidence or to generate greater evidence should it be required. Healthy clinical and scientist HCW relationships are an obligation in pursuit of the optimal client services, where there is also adequate choice.

CHOICE

"Never go to a doctor whose office plants have died". -Erma Bombeck

HCW and clients alike need choices, and the freedom to make informed choices. This includes the

choice of doctor, therapies and the manner in which all aspects of their care are delivered. Guideline based care and the Western paradigm of ambulatory chronic condition care has subverted this process, subtlety such that both HCW and clients deliberate from a small basket of choices[12,13]. We are acknowledged that "a one shoe fits all" approach does not work. Having choices is not an easy process as it requires extension of evidence beyond a Bench to Bedside approach. In the vast majority of cases a guideline based approach is sufficient. In perhaps one in ten cases there is a need for a more creative approach. This may involve using a medication with improved activity for a comorbidity, that is easier to use, that has potentially less side effects. Drug companies that develop the evidence will often stop after the RCT is concluded. In the conclusion of the presented findings it is often written to imply a wide generalizability when in actual fact the results apply to the chosen population treated. It is often left to the HCW to generate this evidence. This involves a competitive process from formulation of the research question to grant funding applications for investigator initiated research. Again this process could be simplified where regulatory authorities directly approve the investigator initiated research and provide the formulary medication. Clearly health economics is a factor and this needs to be discussed. A new approach is also encouraged. Understanding how clients and HCW interact within clusters could be important in reducing this block.

COST

"A hospital bed is a parked taxi with the meter running". -Groucho Marx

"There are no free lunches", "the age of entitlement is over", are choruses that can be heard loudly at political rallies. Escalating health care costs are a concern. The increase in pharmaceuticals prescribed will see funds diverted from other essential services. The easiest way to save cost is to keep the client healthy, prevent future illnesses and reduce tertiary hospital utilization. CHF alone utilizes close to 2% of health care costs, in the United States estimated at close to $35 billion dollars. Among 1054 CHF clients, when this cost is broken down, after a mean follow-up of 4.6 years 73% had died. The estimated lifetime costs were close to $110000 where more than three fourths the costs are accumulated from hospitalizations. The majority of HF patients will suffer at least one other chronic comorbidity[14,15]. As there are overlaps with care within the chronic ambulatory care model ways can be found to minimize this overlap and reduce duplication. This is one simple way. Other options include preventing the treatment of one illness affecting the outcome of another. In these cases we have previously discussed how extra-class effects of drugs, their variable pharmacological properties could be better suited some clients. It is important we generate this evidence at the community level.

MORAL RESPONSIBILITY

"Modern medicine is a negation of health. It isn't organized to serve human health, but only itself, as an institution. It makes more people sick than it heals". -Ivan Illich

Do medical professionals have responsibility beyond the description of their respective professions? There have been in our time physicians who have stood out more than others. Many of these, some included in the above quotes, point to an unwritten, subjective obligation that comes closer to the spiritual than the scientific domain. Regardless of our view points on this we have to accept that the practice of medicine is an inexact science. We are there for obligated to keep an open mind and to continuously strive for improvement in our clients welfare. This can be defined as common sense by pragmatist; of interest by health systems focused on cost and a moral imperative by others. CME is one arm of this where HCW increase their armament for service delivery. Prospective clinical audits are another arm where the focus is on the health system. They should be run by centralised committees within local health clusters controlling the key performance indicators. Six domains from preadmission, emergency, admission, discharge, community and the dimensions of care in each of these domains should be negotiated. An agreed framework for data mining will allow for better and quicker access to information. Health systems in the future should work on standardising the entry, sharing of data and allow for easier access to prospective health data so that local situations can be addressed quicker. We must always find way to provide a frame for the picture to the painted. The barriers here are often administrative and jurisdiction. It is a moral imperative we balance our views on this, prevent silos and look for commonalities. From this picture we can execute our social responsibility as citizens. Thus HCW have responsibilities "…Beyond their call for Duty…".

SOCIAL AND CORPORATE RESPONSIBILITY

"The physician's highest calling, his only calling, is to make sick people healthy-to heal, as it is termed". -Samuel Hahnemann

It is important that systems ask socially responsible questions, *e.g.*, should we provide health services to a community for a decade what is the expected outcome at the end of that time? Is there anything we should do differently? Do we have the adequate skills? Phase 4 studies are a continuation of research once evidence is generated from a RCT. They seek to advance the translational factors such as access, organizational and client factors that could hinder delivery of best practice. Such research could understand both clinical and economic

issues relevant to health clusters and health systems. It provides both information and hypotheses generating questions and should be part of continuous quality improvement[16-18]. It is our collective responsibility to ask these post marketing questions such as: What are the strains on the health systems? What can I or we as a group do to help? When and where to seek assistance? When and where to draw attention? It is thus a social and corporate responsibility for HCW to continue to audit their work to look for better and cheaper ways to provide health services moving into the future.

In summary, to provide medical services and reinforce the RCT findings is a complex process with many factors at play. Health systems have to factor all these. It may seem difficult as such it may be easy to merely do the same. In fact with very simple measures we could achieve an improved standard of medical care. We feel that this standard involves a dedicated emphasis by HCW and systems for post marketing surveillance to address issues within health clusters[19-25]. There are technological advancements now to ensure that this process need not be as laborious as it previously was. Data storage, data mining and standardization of key performance indicators in HF suggest that a subtle shift in thinking and an investment in technology could prove useful. The future must encompass a dedication to regular audits to inform a dynamic CME education curriculum, by breaking down of silos, and embracing technology[26-30]. More powers be given and greater accountability requested from heath clusters by preventing translational blocks. Phase 4 trials should not be viewed as a rigorous process, a vindictive process where some are rewarded and other punished. It should be viewed as a process to generate evidence, improve service delivery, understands subtle local variations, inform the health cluster and add to the global pool of knowledge. Doing so will not only reduce costs but uphold the social contract between providers and recipients of health care.

REFERENCES

1 **Michaels AD**, Spinler SA, Leeper B, Ohman EM, Alexander KP, Newby LK, Ay H, Gibler WB. Medication errors in acute cardiovascular and stroke patients: a scientific statement from the American Heart Association. *Circulation* 2010; **121**: 1664-1682 [PMID: 20308619 DOI: 10.1161/CIR.0b013e3181d4b43e]

2 **Yancy CW**, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation* 2013; **128**: e240-e327 [PMID: 23741058 DOI: 10.1016/j.jacc.2013.05.019]

3 **Heart Foundation of Australia**. Living well with chronic heart failure. Available from: URL: http://www.heartfoundation.org.au/SiteCollectionDocuments/Living-well-with-chronic-heart-failure.pdf

4 **Peters-Klimm F**, Natanzon I, Müller-Tasch T, Ludt S, Nikendei C, Lossnitzer N, Szecsenyi J, Herzog W, Jünger J. Barriers to guideline implementation and educational needs of general practitioners regarding heart failure: a qualitative study. *GMS Z Med Ausbild* 2012; **29**: Doc46 [PMID: 22737201 DOI: 10.3205/zma000816]

5 **Joynt KE**, Orav EJ, Jha AK. Physician volume, specialty, and outcomes of care for patients with heart failure. *Circ Heart Fail* 2013; **6**: 890-897 [PMID: 23926203 DOI: 10.1161/CIRCHEARTFAILURE.112.000064]

6 **Rothwell PM**. Commentary: External validity of results of randomized trials: disentangling a complex concept. *Int J Epidemiol* 2010; **39**: 94-96 [PMID: 19776246 DOI: 10.1093/ije/dyp305]

7 **Parnicka A**, Wizner B, Fedyk-Łukasik M, Windak A, Grodzicki T. Knowledge about heart failure in primary care: need for strengthening of continuing medical education. *Cardiol J* 2013; **20**: 356-363 [PMID: 23913453 DOI: 10.5603/CJ.2013.0093]

8 **Sacco SJ**, Park CL, Suresh DP, Bliss D. Living with heart failure: psychosocial resources, meaning, gratitude and well-being. *Heart Lung* 2014; **43**: 213-218 [PMID: 24661743 DOI: 10.1016/j.hrtlng.2014.01.012]

9 **Browne S**, Macdonald S, May CR, Macleod U, Mair FS. Patient, carer and professional perspectives on barriers and facilitators to quality care in advanced heart failure. *PLoS One* 2014; **9**: e93288 [PMID: 24676421 DOI: 10.1371/journal.pone.0093288]

10 **Kulkarni VT**, Ross JS, Wang Y, Nallamothu BK, Spertus JA, Normand SL, Masoudi FA, Krumholz HM. Regional density of cardiologists and rates of mortality for acute myocardial infarction and heart failure. *Circ Cardiovasc Qual Outcomes* 2013; **6**: 352-359 [PMID: 23680965 DOI: 10.1161/CIRCOUTCOMES.113.000214]

11 **Goodrich K**, Krumholz HM, Conway PH, Lindenauer P, Auerbach AD. Hospitalist utilization and hospital performance on 6 publicly reported patient outcomes. *J Hosp Med* 2012; **7**: 482-488 [PMID: 22689448 DOI: 10.1002/jhm.1943]

12 **Olshansky B**. Placebo and nocebo in cardiovascular health: implications for healthcare, research, and the doctor-patient relationship. *J Am Coll Cardiol* 2007; **49**: 415-421 [PMID: 17258086 DOI: 10.1016/j.jacc.2006.09.036]

13 **Sanders T**, Harrison S, Checkland K. Evidence-based medicine and patient choice: the case of heart failure care. *J Health Serv Res Policy* 2008; **13**: 103-108 [PMID: 18416916 DOI: 10.1258/jhsrp.2008.007130]

14 **Dunlay SM**, Shah ND, Shi Q, Morlan B, VanHouten H, Long KH, Roger VL. Lifetime costs of medical care after heart failure diagnosis. *Circ Cardiovasc Qual Outcomes* 2011; **4**: 68-75 [PMID: 21139091 DOI: 10.1161/CIRCOUTCOMES.110.957225]

15 **Smith DH**, Johnson ES, Blough DK, Thorp ML, Yang X, Petrik AF, Crispell KA. Predicting costs of care in heart failure patients. *BMC Health Serv Res* 2012; **12**: 434 [PMID: 23194470 DOI: 10.1186/1472-6963-12-434]

16 **Strachan PH**, Joy C, Costigan J, Carter N. Development of a practice tool for community-based nurses: the Heart Failure Palliative Approach to Care (HeFPAC). *Eur J Cardiovasc Nurs* 2014; **13**: 134-141 [PMID: 24434049 DOI: 10.1177/1474515113519522]

17 **Ralph AP**, Fittock M, Schultz R, Thompson D, Dowden M, Clemens T, Parnaby MG, Clark M, McDonald MI, Edwards KN, Carapetis JR, Bailie RS. Improvement in rheumatic fever and rheumatic heart disease management and prevention using a health centre-based continuous quality improvement approach. *BMC Health Serv Res* 2013; **13**: 525 [PMID: 24350582 DOI: 10.1186/1472-6963-13-525]

18 **Brush JE**, Balakrishnan SA, Brough J, Hartman C, Hines G, Liverman DP, Parker JP, Rich J, Tindall N. Implementation of a continuous quality improvement program for percutaneous coronary intervention and cardiac surgery at a large community hospital. *Am Heart J* 2006; **152**: 379-385 [PMID: 16875926 DOI: 10.1016/j.ahj.2005.12.014]

19 **Bergman SM**, Vernillo AT. The ethics of corporate social responsibility. *J Am Coll Dent* 2014; **81**: 41-45 [PMID: 25080670]

20 **Sass HM**. The clinic as a good corporate neighbor. *Croat Med J*

2013; **54**: 78-82 [PMID: 23444251 DOI: 10.3325/cmj.2013.54.78]
21 **Bassett S**, Westmore K. How nurse leaders can foster a climate of good governance. *Nurs Manag* (Harrow) 2012; **19**: 22-24 [PMID: 23008901]
22 **Brandão C**, Rego G, Duarte I, Nunes R. Social responsibility: a new paradigm of hospital governance? *Health Care Anal* 2013; **21**: 390-402 [PMID: 22481565 DOI: 10.1007/s10728-012-0206-3]
23 **Haddad AM**. The nurse's role and responsibility in corporate-level planning. *Nurs Adm Q* 1981; **5**: 1-6 [PMID: 6907745 DOI: 10.1097/00006216-198100520-00003]
24 **French PA**. Collective responsibility and the practice of medicine. *J Med Philos* 1982; **7**: 65-85 [PMID: 7086316 DOI: 10.1093/jmp/7.1.65]
25 **Foreman S**. Social responsibility and the academic medical center: building community-based systems for the nation's health. *Acad Med* 1994; **69**: 97-102 [PMID: 8311894 DOI: 10.1097/00001888-199402000-00002]
26 **Krumholz HM**, Currie PM, Riegel B, Phillips CO, Peterson ED, Smith R, Yancy CW, Faxon DP. A taxonomy for disease management: a scientific statement from the American Heart Association Disease Management Taxonomy Writing Group. *Circulation* 2006; **114**: 1432-1445 [PMID: 16952985 DOI: 10.1161/CIRCULATIONAHA.106.177322]
27 **Bonow RO**, Bennett S, Casey DE, Ganiats TG, Hlatky MA, Konstam MA, Lambrew CT, Normand SL, Pina IL, Radford MJ, Smith AL, Stevenson LW, Burke G, Eagle KA, Krumholz HM, Linderbaum J, Masoudi FA, Ritchie JL, Rumsfeld JS, Spertus JA. ACC/AHA Clinical Performance Measures for Adults with Chronic Heart Failure: a report of the American College of Cardiology/American Heart Association Task Force on Performance Measures (Writing Committee to Develop Heart Failure Clinical Performance Measures): endorsed by the Heart Failure Society of America. *Circulation* 2005; **112**: 1853-1887 [PMID: 16160201 DOI: 10.1161/CIRCULATIONAHA.105.170072]
28 **Spertus JA**, Bonow RO, Chan P, Diamond GA, Drozda JP, Kaul S, Krumholz HM, Masoudi FA, Normand SL, Peterson ED, Radford MJ, Rumsfeld JS. ACCF/AHA new insights into the methodology of performance measurement: a report of the American College of Cardiology Foundation/American Heart Association Task Force on performance measures. *Circulation* 2010; **122**: 2091-2106 [PMID: 21060078 DOI: 10.1161/CIR.0b013e3181f7d78c]
29 **Riegel B**, Lee CS, Sochalski J. Developing an instrument to measure heart failure disease management program intensity and complexity. *Circ Cardiovasc Qual Outcomes* 2010; **3**: 324-330 [PMID: 20484202 DOI: 10.1161/CIRCOUTCOMES.109.877324]
30 **Iyngkaran P**, Tinsley J, Smith D, Haste M, Nadarajan K, Ilton M, Battersby M, Stewart S, Brown A. Northern Territory Heart Failure Initiative-Clinical Audit (NTHFI-CA)-a prospective database on the quality of care and outcomes for acute decompensated heart failure admission in the Northern Territory: study design and rationale. *BMJ Open* 2014; **4**: e004137 [PMID: 24477314 DOI: 10.1136/bmjopen-2013-004137]

P- Reviewer: Chawla M, Sugawara A, Simkhovich BZ
S- Editor: Gong XM **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.185

World J Methodol 2015 December 26; 5(4): 185-195
ISSN 2222-0682 (online)

REVIEW

Immunodiagnosis of human hydatid disease: Where do we stand?

Bahador Sarkari, Zahra Rezaei

Bahador Sarkari, Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz 71345-1735, Iran

Zahra Rezaei, Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz 71345-1735, Iran

Author contributions: Sarkari B performed the literature search, wrote the first draft of the manuscript, edited and approved the final version; Rezaei Z performed the literature and approved the final version of the manuscript.

Conflict-of-interest statement: The authors declare that there are no conflicts of interest.

Correspondence to: Bahador Sarkari, PhD, Professor of Immunoparasitology, Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Zand Street, Building No. 3, Shiraz 71345-1735, Iran. sarkarib@sums.ac.ir
Telephone: +98-71-32305291
Fax: +98-71-32305291

Received: August 4, 2015
Peer-review started: August 6, 2015
First decision: September 22, 2015
Revised: October 26, 2015
Accepted: November 13, 2015
Article in press: November 17, 2015
Published online: December 26, 2015

Abstract

Cystic echinococcosis (CE) is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus*. Diagnosis of CE mainly relies on a combination of serological testing along with imaging approaches. A variety of serological methods, mainly based on hydatid cyst fluid, antigen B (AgB) and antigen 5, have been developed and used for immunodiagnosis of CE, yet their performances are not satisfactory. Although utilizing of recombinant or synthetic antigens, improved the performance of serological tests, it has not applicably overcome the problem of low sensitivity and cross reactivity, seen in the diagnosis of CE. Performances of immunodiagnostic tests based on AgB subunits are promising. The 8 kDa subunit of AgB is the most studied antigen in native, synthetic or recombinant form for diagnosis of CE. From the 5 subunits of AgB, antigen B8/1 and B8/2 provided the highest diagnostic sensitivity and specificity. Moreover, detecting of specific antibodies of IgG subclasses has improved the efficacy of immunodiagnostic tests. Among the IgG subclasses, both IgG2 and IgG4 are considered as good markers for diagnosis and IgG4 as a suitable marker for follow up of the patients. In this review an overview of immunodiagnostic methods, related antigens and their performances in the diagnosis of CE are given. The paper highlights pitfall and challenges in the serological diagnosis of CE. Moreover, limitation of currently available immunodiagnostic tests and the most recent development in the designing and application of serological assays for diagnosis of CE in human are addressed.

Key words: Immunodiagnosis; Cystic echinococcosis; Hydatid cyst

Core tip: Cystic echinococcosis (CE) (hydatid cyst) is one of the most important parasitic diseases, causing tremendous morbidity and mortality for the human patients. Diagnosis of CE mainly relies on ultrasound images of the cyst along with serological testing. So far, there is no highly specific and sensitive immunodiagnostic test for diagnosis of CE

and performances of the currently available tests are not satisfactory. Different antigenic sources including hydatid cyst fluid, antigen B and 5, excretory-secretory antigens of larval stage or adult worm have widely been used for development of serological assays for diagnosis of CE. Utilizing of antigen B subunits in immunodiagnostic tests and detection of IgG subclasses, as a good marker, opened a promising perspective in diagnosis of this debilitating disease.

Sarkari B, Rezaei Z. Immunodiagnosis of human hydatid disease: Where do we stand? *World J Methodol* 2015; 5(4): 185-195 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/185.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.185

INTRODUCTION

Cystic echinococcosis (CE), known as hydatid cyst or hydatid disease, is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus* (*E. granulosus*). Dogs and other canids harbor the adults tape worm and herbivores acts as intermediate host and become infected through ingestion of parasite's eggs. Human acquire the infection by accidental ingestion of *E. granulosus* eggs.

CE with its significant economic and medical impact constitutes an important public health problem in many developing countries[1-3]. An estimated 1.2 million people worldwide are affected by CE and the disease accounts for annual estimate of 3.6 million DALYs (disability adjusted life years) through the world[4]. Early and proper diagnosis of CE can provide appropriate management and suitable treatment of the disease[5].

Diagnosis of CE is mainly confirmed through a combination of relevant history, serological testing, along with imaging approaches. A variety of serological methods have been developed and used for immunodiagnosis of CE in recent years, including indirect hemagglutination (IHA), immunoblotting, enzyme-linked immunosorbent assay (ELISA), indirect fluorescent-antibody (IFA), latex agglutination test, and immunochromatography test[1,6-11]. For the development of these assays different antigens from adult worm, protoscolices, worm eggs or hydatid cyst fluid have been defined, purified and evaluated in the aforementioned serological tests.

Diagnosis of CE has drastically improved during the last two decades. Progress in methods for antigen purification, cloning expression and purification of *E. granulosus* recombinant antigens, and defining and synthesis of immunodominant peptides contributed to this development. Nevertheless, immunodiagnosis of CE is still problematic. Commercially available serological tests show unsatisfactory performance. The lack of standardization of immunodiagnostic assays and also antigen preparation contribute to discrepancy in results reported in different laboratories. Cyst size, stage and location as well as patients characteristics may be accounted for the discrepancy of the same test performance in different clinical diagnostic laboratories.

Hence, serological assays still have a complementary role to imaging in the diagnosis of CE. Low sensitivity (up to 30% of false negativity) and also low specificity (up to 25% of false positivity) make serological results difficult to interpret[12-17].

Pitfalls and challenges in the diagnosis of CE

In spite of the development of a variety of immunodiagnostic test, following diagnostic pitfalls and challenges still exist in the diagnosis of CE.

Available immunodiagnostic tests give a relatively high rate of false-negativity. False negative results in immunodiagnostic tests for CE may be seen in patients with small cysts, intact cysts, cysts in extrahepatic locations, heavily calcified cysts (*e.g.,* non-viable), or cyst in privilege sites (brain or eye). Akbulut *et al*[18] reported that 15 out of 40 patients with pancreatic echinococcosis, found in the literature have had negative serological testing for CE. Among 65 CE patients in Germany, false negative serological results were reported in 18% by IHA and in 15% by ELISA[19]. In a study by Akcam *et al*[20] more than 20% of patients with extra-hepatic cysts were reported to be negative by IHA test. Using WB, 10 cases of IHA-negative were found to be positive. In a study by Wuestenberg *et al*[21], CE was confirmed in 9 cases of IHA-negative by clinical findings and imaging (US). Cardiac hydatid cyst, with 54 mm × 45 mm size, was serologically negative in Canpolat *et al*[22] report. Karakasli *et al*[23] reported a case of large spinal-para spinal hydatid cyst with negative ELISA and WB testing. They suggested that clinical and neuro-radiological findings should be considered in such cases. Review of 100 case of pulmonary hydatidosis by Zapatero *et al*[24] revealed that positive serological test have been present with ruptured cyst (positive IHA in all of ruptured cyst) while the test detected only 80% of patients with unruptured cysts. Serological test for CE have been negative in human immunodeficiency virus (HIV)-positive cases[25].

Currently available tests give rather high rate of false-positive reaction in patient infected with other parasites (notably cestodes) or even in healthy subjects. False positive results are related to cross reactant antibodies.

Differentiation of past (cured or calcified cyst) from present (active or progressive) hydatid infection is difficult by existing antibody detection assays. Antibody titer may remain for years, even after surgical removal of the cyst or proper drug treatment[26,27]. Therefore a positive serological test may not necessarily imply the presence of active cyst or even the reactivation of CE.

Hydatid cysts in unusual locations may complicate its diagnosis. Congenital, choledochal and pancreatic pseudocysts along with lipoma, ovarian intra-abdominal cystadenoma and intra-hepatic haematoma may be misdiagnosed as hydatid cyst by ultrasonography/computerized tomography (US/CT)[13]. In all of these

conditions an appropriate serological test would be quite helpful with negative results.

Performance of serological tests varies in different pathological stage of CE according to WHO classification[28].

A single defined molecule may not be sufficient for diagnosis of CE. Recent immunoproteome analysis of hydatid cyst fluid (HCF), in different stages of cyst (based on WHO classification), revealed that specific immunodominant epitopes changes from[29] one stage to another stage. This indicates that more than one defined immunodominant antigen may be needed to diagnosis CE in different status of the cyst.

Antigenic sources for immunodiagnosis of CE

Antigenic sources which have widely been used for immunodiagnosis of CE are HCF, component of HCF, ES of protoscolices or adult worm, and also extract of adult worm or larval stage. Antigen for immunodiagnosis of CE has been comprehensively reviewed by Carmena *et al*[12]. The main antigens for diagnosis of CE and their performance in diagnosis of CE are discussed below.

HCF: HCF is the most common antigenic source which has been used for diagnosis of CE. HCF is a mixture of host (albumin, globulins) and parasite components[30-32]. Sensitivities of serological tests based on HCF are high but their specificities are far from satisfactory (30%-90%)[8,10,12,28,31,33-35]. Many of available commercial kits are using HCF in ELISA system for diagnosis of CE. Table 1 summarizes the performance of HCF in diagnosis of CE in different serological assays.

Using HCF as a source of antigen, Sedaghat *et al*[35] evaluated the performance of a simple dot ELISA and CCIEP (counter current immunoelectrophoresis) for diagnosis of human hydatidosis and found a sensitivity and specificity of 100% and 89.1% for Dot-ELISA and 80% and 62% for CCIEP. Dot ELISA had a better performance in comparison with CCIEP. Using HCF, EL-Shazly *et al*[8] reported a sensitivity of 96.7% and specificity of 97.5% for ELISA and 86.7% and 95% for IHA. Al-Sherbiny *et al*[6] applied the camel HCF in a dipstick assay and reported a high diagnostic sensitivity (100%) and specificity (91.4%).

CCIEP is a relatively sensitive, but not specific method for diagnosis of CE. In a retrospective study conducted by Sadjjadi *et al*[15] hospital records of 1227 surgically proven CE cases were examined and found that only 62% of cases had a CCIEP positive test in comparison with 96.3% of positive findings by US and pathology.

Antigen 5: Antigen 5 (Ag5) is one of the most immunogenic and abundant part of HCF. It composed of 57 and 67 kDa components and dissociate into 38 and 22-24 kDa subunits under reducing conditions[36]. Ag5, after AgB, is one of the most studied antigens in the serodiagnosis of CE. Numerous studies pointed out that Ag5 has a high rate of cross-reactivity with sera of healthy controls or other non-CE patients[12,17,37]. Its performances in native, recombinant or synthetic forms have not been satisfactory due to either low sensitivity (50-54), or specificity because of cross reactivity with sera of the patients with other cestoda, trematoda or even nematoda. In an Ag5-based ELISA, Khabiri *et al*[38] reported that IgE and IgG4 are the most important antibodies, with low cross reactivity with sera of healthy control and non-CE cases.

Contrary to these reports, a recent study by Pagnozzi *et al*[39] demonstrated that highly enriched Ag5, by chromatographic method, attained highly specific and unambiguous results, in Western blotting and ELISA system in diagnosis of CE. The authors indicated that low performance of this antigen in previous studies is related to non-properly purified antigen which have been used and considered that highly purified Ag5 is a promising antigen in diagnosis of CE. Having said that, the low number of sera tested in their study does not allow drawing a decisive conclusion. Table 2 shows the performance of Ag5 in diagnosis of CE in different serological assays.

Antigen B: Antigen B (AgB) is a thermostable polymeric lipoprotein of 120-160 kDa, composed of 8 kDa subunits which dissociates into 8/12, 16 and 24 kDa subunits, under reducing condition in SDS-PAGE[36]. AgB is considered as the main antigen of HCF with high specificity and sensitivity in serological diagnosis of CE[9,10,31,40-42].

AgB is highly immunogenic, a feature that makes this antigen a suitable candidate for immunodiagnosis of CE. The smallest subunit, 8 kDa, considered as the most appropriate antigen in diagnosis of CE. Not surprisingly, the 8 kDa subunit of AgB is the most studied antigen in native, synthetic or recombinant form for diagnosis of CE. Sarkari *et al*[42] obtained diagnostic sensitivity and specificity of 100% and 80% when AgB was evaluated in an immunoblotting system. In their study from 40 sera of hydatidosis patients, 32 cases (80%) detected the 8 kDa subunit, 29 cases (72.5%) recognized the 16 kDa component and 29 cases (72.5%) detected the 24 kDa subunit of antigen B. In continuation of their study, when the AgB was used in an ELISA system, sensitivity of the system was determined to be 92.5% and the specificity was found to be 97.3%[10].

Recombinant AgB are not doing much better in diagnosis of CE when compared with native homologues antigens. The performance of rAgB subunits for diagnosis of CE was evaluated by Jiang *et al*[40] where they reported performance order of AgB1 > AgB4 > AgB2 > AgB5 > AgB3. It was found that in some cases antibodies against subunits of AgB was not produced. In another study, Jiang *et al*[43] reported that AgB1 has higher diagnostic sensitivity in comparison with AgB2 and AgB4. However, in Virginio *et al*[44] study, antigen B8/2 provided the highest diagnostic sensitivity (93.1%) and specificity (99.5%) in ELISA system. In Leggatt *et al*[45] study, a sensitivity of 90.9% was reported for the 12 kDa subunit of AgB (corresponding to the smallest

Table 1 Performance of hydatid cyst fluid in diagnosis of cystic echinococcosis in different serological assays

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reactions	Year	Ref.
	CE patients	Other disease	Healthy control						
SHCF	78	24	15	IgG ELISA	72.4		NR	2001	[71]
BHCF	129	65	203	IgG ELISA	77.6	96.6	Cysts, Toxoc.	2003	[44]
CHCF	26	35	10	Dipstick assay	100	91.4	Cysts, AE, Trichinosis, Schist., Fascio	2004	[6]
CHCF	26	35	10	EITB	100	91.4	Cysts, AE, trichinosis, Schist, Fascio	2004	[6]
CHCF	26	30	10	IgG ELISA	96.2	100	None	2004	[6]
SHCF	102	68	95	IgG ELISA	88.2	80.9	AE, Cysts, Schist, Fascio, Taeniasis, Dirofilariasis	2008	[72]
SHCF	120			Casoni's skin test	88.2	80.9	NR	2005	[73]
SHCF	120			Casoni's skin test	70	87	NR	2005	[73]
SHCF	120			Casoni's skin test	62	85	NR	2005	[73]
SHCF	120			IHA	56	84	NR	2005	[73]
SHCF	25	15	25	ELISA on serum	72	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	25	15	25	ELISA on urine	84	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	25	15	25	ELISA on saliva	56	76	Cysts, Ascaris, Ambs liver abscess	2007	[74]
SHCF	40	40	70	CCIEP	97.5	58.1	Fascio, Toxoc, Taenia, Malignancies	2007	[10]
SHFF	204	53	90	IEP	31	100	None	2000	[75]
SHFF	204	53	90	IHA	54	100	None	2000	[75]
SHFF	204	53	90	IB	80	96	Cysts, Serous cysts	2000	[75]
SHCF	35	12	25	Dot-ELISA	100	89.1	Ascaris, Taenia, Strogyl	2010	[1]
SHCF	35	12	25	CCIEP	80	62	Ascaris, Strogyl, Toxop	2010	[1]
SHCF	59	60	39	IgG ELISA	91.5	96	Clonorchiasis	2013	[76]
hHCF	50	15	20	IB	83	98	None	2014	[28]
hHCF	50	15	20	IgG IB	83	98	None	2014	[28]
SHCF	50	40	20	IgG ELISA	92	85	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgM ELISA	70	93.33	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgE ELISA	86	96.66	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG1 ELISA	82	98.33	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG2 ELISA	74	95	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG3 ELISA	52	36	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
SHCF	50	40	20	IgG4 ELISA	86	28	Ascaris, Ambs, Malignancy, Toxop	2014	[67]
Psx Ag	113	112	121	DIGFA	87.6	90.90	Hd, Cysts, HCC, HH	2015	[48]
CPsx extract	147	88	60	IgG ELISA	90	57	AE, Trypanosomiasis	2002	[76]
Emwl Ag	50	154		WB IgG	98		NCC	2000	[14]
Emwl Ag	50	154		IHA > 80	94.3		NR	2000	[14]
Emwl Ag	50	154		IHA > 320	80		NR	2000	[14]
Emwl Ag	50	154		IgG ELISA	79.4		NR	2000	[14]

EITB: Enzyme linked immunoelectrotransfer blot; IHA: Immune hemagglutination assay; CHCF: Camel hydatid cyst fluid; SHCF: Sheep hydatid cyst fluid; hHCF: Human hydatid cyst fluid; SHFF: Sheep hydatid fluid fraction; Hd.: Hepatic distomiasis; Emwl Ag: Whole larval antigen from *Echinococcus multilocularis*; CPsx extract: Crude protoescolex extract; Psx Ag: Protoscoleces antigen; BHCF: Bovine hydatid cyst fluid; Ascaris: Ascariasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; Ambs: Amebiasis; Toxoc: Toxocariasis; Schist: Schistosomiasis; AE: Alveolar echinococcosis; Strongyl: Strongyloidiasis; HH: Hepatic hemangioma; NR: Not reported; CE: Cystic echinococcosis; ELISA: Enzyme-linked immunosorbent assay.

subunit of AgB) in a blotting system. More than 5% (5.5%) of cysticercosis patients reacted with this subunit.

The 12 kDa subunit of AgB, was cloned and expressed by Abdi *et al*[46]. The antigen was comparatively evaluated for diagnosis of CE, with native AgB and HCF. The sensitivity and specificity of rAgB, in ELISA system was similar to HCF (96% and 97%), and lower than native AgB (98.6% and 100%).

A recombinant antigen of B8/1 (rAgB), showed a high sensitivity (94.6%) and specificity (93.9%) for diagnosis of CE, using serum samples from Iran, China and Japan, in comparison with HCF, native AgB, prepared from sheep HCF, either from Iran of Japan[31].

Mamuti *et al*[41] cloned and produced recombinants of EmAgB8/1 from *E. multilocularis* and EgAgB/1 from

Table 2 Performances of antigen 5 for immunodiagnosis of cystic echinococcosis in different serological assays

	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reaction	Year	Ref.
	CE patient	Other disease	Healthy control						
Ag5	39	51	29	IgG ELISA	54	89	AE	2000	[9]
Ag5	58	36	40	IgG ELISA	100	70.17	Leish, Toxop, Fascio	2006	[39]
Ag5	58	36	40	IgG1 ELISA	100	70.17	Leish, Toxop, Fascio	2006	[38]
Ag5	58	36	40	IgG4 ELISA	75.8	93.02	Toxop, Fascio	2006	[38]
Ag5	58	36	40	IgE ELISA	70.1	100	None	2006	[38]
rAg5	34	36	18	IgG ELISA	65	89	AE, Cysts	2005	[77]
rAg5-38s	34	36	18	IgG ELISA	21	97	AE	2005	[77]

Leish: Leishmaniasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; AE: Alveolar echinococcosis.

E. granulosus and evaluated their antigenic reactivity in Western Blotting and ELISA in comparison with that of counterpart, an 8 kDa subunit of AgB. WB showed reactivity with 81.3% of sera from CE patients and 40.6% of sera from alveolar echinococcosis (AE) patients, while EgAgB8/1 showed reactivity with 86% of CE and 42% of AE patients. Both EmAgB/1 and EgAgB/1 showed similar reactivity with 37.8% of sera from AE and 88% of sera from CE patients.

A synthetic P176 peptide related to N-terminal extreme of AgB/1 subunit yielded a sensitivity and specificity of 78.69 and 96.88 for pulmonary hydatid cyst[47].

Application of antigen B in a dot immunogold filtration assay increased the test specificity (98.3%) but in turn decreased the sensitivity (77.9%) of the assay, compared to native antigen[48].

Source of antigen B is an important factor which affects the performance of the test for diagnosis of CE. In agreement with this, Rahimi *et al*[49] showed that AgB isolated from human and sheep liver cyst have the best performance in diagnosis of CE when compared with those antigen obtained from liver or lungs cyst of goat, cattle or camel.

Combination of antigen B and antigen 5 may increase the sensitivity of the test as currently used in a commercially available test. The commercially available Rapid Immunochromatography test VIRapid® HYDATIDOSIS test (Vircell, Spain) using antigen 5/B was evaluated by Tamer *et al*[50] for diagnosis of CE where they reported a sensitivity of 96.8% and specificity of 87.5%. In their study, the antigen cross reacted with sera from taeniasis and leishmaniasis patients and also a few (4%) of healthy controls.

Nature and quality of antigen B, isolated from HCF, may be variable based on the host species, cyst location, cyst status and also parasite strain. This is one of the reasons that different laboratories attain different results using AgB in serodiagnosis of CE. In view of this point, discrepancies in results of serodiagnosis of CE, using antigen B might be related to, method of antigen preparation, variation in host and strain of parasite, differences in antigen B, site of the cyst, clinical status and type of the cyst. Table 3 shows the performances of antigen B in diagnosis of CE in different serological assays.

Protoscolices antigens: Native metacestode-derived antigens show substantial (mainly more than 90%) sensitivities in diagnosis of CE[51]. However cross-reactivity with other parasitic diseases (fascioliasis, schistosomiasis, amebiasis, taeniasis, cysticercosis and filariasis) is the main drawback of using such antigens for serodiagnosis of CE. The best performance for serological tests of ELISA, IHA and IFA, was achieved for ELISA (87.5% sensitivity and 100% specificity), using metacestode antigen[52].

Detection of IgG subclasses: Detecting of specific antibodies of IgG subclasses may improve the diagnostic performance of immunodiagnostic tests. Xu *et al*[53] examined the seroreactivity of 42 IgG negative (total IgG) with IgM, IgE, IgA, and IgG subclasses and found that 32 cases were positive with either one or combined of two of other antibodies. The best seropositivity (42.95%) was reported with either IgG1 alone or a combination of IgG1 + IgA + IgM. IgG subclasses is usually linked to the status of cyst development. Findings of Daeki *et al*[54] demonstrated that IgG antibody response is associated with the growth and development of cyst, while IgG1, 2 and 3 responses are predominantly related to involutive phase in CE cysts. Patients with relapsing disease have a high level of IgG4 titer.

Lawn *et al*[55] demonstrated that concentration of CE-specific IgG subclasses (IgG1-4), are much correlated with disease activity than total IgG. Among the IgG subclasses, IgG2 provided the best correlation with clinical outcome. In a lateral fellow dipstick test, a sensitivity of 95% and specificity of 100% was reported for detection of IgG4, in comparison with IgG dipstick with 87.5% specificity[56]. Detection of antibodies mainly IgG subclasses (IgG1, 4) in urine of CE patients provide a similar result in comparison to serum sample in Chirag study[57].

Antigen detection for immunodiagnosis of CE

Antigen detection has been used for diagnosis of a few of parasitic diseases with satisfactory results[58-61]. Antigen detection might be useful for detection of current infection and also post treatment follow up of CE patients. However results with detection of hydatid cyst antigen

Table 3 Performances of antigen B in diagnosis of cystic echinococcosis

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reactions	Year	Ref.
	CE patients	Other disease	Healthy control						
nAg B	204	21	90	IB	66	100	None	2000	[75]
nAg B	59	55	15	IgG ELISA	80	77	AE, NCC	2005	[77]
nAg B	90	86	27	IgG ELISA	77	85	AE, RA	2000	[9]
nAg B	204	21	90	IgG ELISA	74	100	None	2000	[75]
nAg B	31	87	29	IgG ELISA	77.41	81.9	AE, Ev, Schist, Toxoc	2000	[78]
nAg B	78	24	15	IgG ELISA	93.5	89.7	Distomatosis, Schist	2001	[71]
nAg B	129	65	203	IgG ELISA	60.3	92.6	Cysts,Toxoc	2003	[44]
nAg B	22	12	4	WB	77	100	Toxoc, Other cestodes	2010	[30]
nAg B	40	40	70	IgG ELISA	92.5	97.3	Fascio	2007	[10]
nAg B	40	40	70	CCIEP	97.5	58.2	Fascio, Toxoc, Taenia, Malignancy	2007	[10]
nAg B	204	53	90	IB	66	100	None	2000	[75]
nAg B	204	53	90	IgG ELISA	74	100	None	2000	[75]
nAg B	35	29	25	IgG ELISA	94.2	81.6	NR	2009	[16]
nAg B	55	72	50	IgG ELISA	96.4	97.2	None	2014	[68]
nAg B	113	112	121	DIGFA	92.9	81	HD, Cysts, HCC, HH	2015	[48]
Goat liver Ag B	47	30	40	IgG ELISA	91.4	92.8	NR	2011	[49]
Human liver Ag B	47	30	40	IgG ELISA	97.8	97.1	NR	2011	[49]
Bovine lung Ag B	47	30	40	IgG ELISA	78.7	85.7	NR	2011	[49]
Sheep lung Ag B	47	30	40	IgG ELISA	93.6	88.5	NR	2011	[49]
Camel lung Ag B	47	30	40	IgG ELISA	93.6	90	NR	2011	[49]
Sheep liver Ag B	47	30	40	IgG ELISA	95.7	92.8	NR	201	[49]
rAgB	204	21	90	IB	72	100	None	2000	[75]
rAgB	113	112	121	DIGFA	77.9	98.3	None	2015	[48]
rAgB8/1	31	87	29	IgG ELISA	54.84	80.17	AE, Schist, Toxoc	2000	[78]
rAgB8/1	129	65	203	IgG4 ELISA	91.4	91.7	Cysts	2003	[44]
rAgB8/1	59	55	15	IgG ELISA	68	88	AE, NCC	2005	[80]
rAgB8/2	31	87	29	IgG ELISA	83.87	98.28	Schist, Toxoc	2000	[78]
rAgB8/2	129	65	203	IgG ELISA	93.1	99.5	Cysts, Toxoc	2003	[45]
rAgB8/2	129	65	203	IgG4 ELISA	69	87.5	Cysts	2003	[45]
rAgB8/2	59	55	15	IgG ELISA	45	86	AE, NCC	2005	[77]
rAgB8/1	129	65	203	IgG4 ELISA	91.4	91.7	Cysts	2003	[44]
rAgB8/1	59	55	15	IgG ELISA	68	88	AE, NCC	2005	[77]
rAgB8/2	31	87	29	IgG ELISA	83.87	98.28	Schist, Toxoc	2000	[78]
rAgB8/2	129	65	203	IgG ELISA	93.1	99.5	Cysts, Toxoc	2003	[44]
rAgB8/2	129	65	203	IgG4 ELISA	69	87.5	Cysts	2003	[44]
rAgB8/2	59	55	15	IgG ELISA	45	86	AE, NCC	2005	[77]
B1t	102	68	95	IgG ELISA	83.3	87.5	AE, Schist, Cysts, Fascio,	2008	[9]
B2t	102	68	95	IgG ELISA	91.2	93	Cysts, Schist, Fascio	2008	[9]
2B2t	186	174	110	IgG ELISA	87.6	99.1	AE, NCC, Hepatitis	2012	[9]
rEgAFFPt	129	65	203	IgG ELISA	58.6	95.6	Cysts, Toxoc	2003	[44]
rEgCaBP2	129	65	203	IgG ELISA	84.5	96.6	Cysts, Toxoc	2003	[44]
rEgcMDH	129	65	203	IgG ELISA	89.7	95.1	Cysts	2003	[44]
rEgAFFPf	129	65	203	IgG ELISA	69	89.7	Cysts, Toxoc	2003	[44]
rEpC1-GST	324	502	70	IgG IB	92.2	95.6	AE, NCC, Schist, Liver cancer	2003	[79]
rTPxEg	100	218	20	IgG IB	39	69.3	AE, NCC	2004	[78]
rEgG5	23	138	20	IgG IB	61	70	AE, Cysts	2004	[79]
E14t	102	68	95	IgG ELISA	35.3	91.7	Schist	2008	[9]
C317	102	68	95	IgG ELISA	58.8	80.9	AE, Cysts, Taeniasis, Schist,	2008	[9]
p65	90	86	27	IgG ELISA	44	96	AE, Schist, Toxoc	2000	[9]
p175	90	86	27	IgG ELISA	49	94	AE, Schist, Toxoc	2000	[9]
p176	90	86	27	IgG ELISA	80	93	AE, Schist, Toxoc, Syph, Chagas	2000	[9]
p177	90	86	27	IgG ELISA	38	92	AE, Toxoc, Syph, Chagas	2000	[9]
pGu4	90	86	27	IgG ELISA	18	98	AE	2000	[9]

Leish: Leishmaniasis; Toxop: Toxoplasmosis; Fascio: Fascioliasis; Cysts: Cysticercosis; Ascaris: Ascariasis; Syph: Syphilis; Ambs: Amebiasis; Toxoc: Toxocariasis; Schist: Schistosomiasis; NCC: Neurocysticercosis; AE: Alveolar echinococcosis; nAg B: Native antigen B; rAgB: Recombinant antigen B; Ev: Ev: Polycistic hydatid disease (*E. vogeli*); RA: Rheumatoid arthritis; HCC: Hepatocellular carcinoma; NR: Not reported; CE: Cystic echinococcosis; ELISA: Enzyme-linked immunosorbent assay.

Table 4 Performances of antigen detection assays in immunodiagnosis of cystic echinococcosis

Antigen	No. of subjects			Test	Sensitivity (%)	Specificity (%)	Cross reaction (%)	Year	Ref.
	CE patient	Other disease	Healthy control						
Urinary antigen	40	24	25	Co-A	50	89.09	12.5	2000	[62]
Serum antigen	40	24	25	Co-A	73.08	94.23	12.5	2000	[62]
Serum antigen	35	29	25	IgG ELISA	25.7	98	3.4	2009	[16]
Serum antigen	141	25	25	LAT	72	98	4	2003	[7]
Serum antigen	40	24	25	CIEP	45	100	None	1997	[80]
Urinary antigen (ucon)	40	24	25	CIEP	22.5	95.91	8.33	1997	[80]
Urinary antigen (con)	40	24	25	CIEP	47.5	95.91	None	1997	[80]

ucon: Unconcentrated; con: Concentrated; CE: Cystic echinococcosis; LAT: Latex agglutination test; Co-A: Coagglutination; CIEP: Countercurrent immunoelectrophoresis; ELISA: Enzyme-linked immunosorbent assay.

for detection of CE are far from satisfaction[7,11,62-64].

Antigen detection in CE is much less sensitive than antibody detection and the later remains the most commonly used approach for diagnosis of this disease. Antigen can be detected in sera of 35%-85% of CE patients depends on the status and location of the cyst[7,16,63]. In some cases of CE circulating antigen has been detected in sera of patients who had not shown anti-hydatid antibodies in their serum. Swarna *et al*[11] reported a sensitivity of only 53.33% and specificity of 96.66% in a Dot-ELISA system for detection of hydatid cyst antigen in urine samples. Lower sensitivity (29.68) was obtained when CCIEP was used for detection of hydatid urinary antigen[62].

Using coagglutination test, a sensitivity of 47.5% was achieved for detection of hydatid antigen in urine[63]. Several interfering factors have been proposed to explain the poor performance of antigen-detection assays in diagnosis of CE. Among them are formation of immune complexes and low availability of free antigen, sequestration of antigen due to cyst layers, especially in intact cyst, and presence of interfering component in serum or urine, as demonstrated in other studies[58]. Cysts in privileged sites (*e.g.,* eye and brain) do not release enough antigens to be detected by serological assays.

Location of the cyst is an important issue in diagnosis of CE as one study pointed out that CE antigen can be detected in 46% of patients with liver cyst but not in any of patients with lung hydatid cyst[16]. In an attempt to develop an antigen detection assay for diagnosis of CE, Sadjjadi *et al*[16] evaluated an ELISA system for detection of circulation antigens in serum of CE patients. In their study, antigen was detected in only 9 out of 35 (25.7%) of cases. Table 4 summarizes the performance of antigen detection assays in diagnosis of CE.

Post treatment follow up: CE patients need to be followed up after treatment, to make sure about the risk of recurrence. Anti-CE antibodies may persist for several years after treatment[55]. Although antigen detection might be a useful approach in post-treatment follow-up, however its low sensitivity hampered its use for patients' follow-up. Different serological assays have been used for monitoring of surgically or chemically treated CE patients[26,64-66].

In a recent cohort study, CE patients were followed for a mean of 6 years and the level and isotypes of antibodies were evaluated before and after surgical or anti-helminthic drugs treatment. Results demonstrated that IgE, IgG1 and IgG4 are the most important antibodies for serological diagnosis of active CE. During post-operation, IgM, IgE, IgG1, IgG2 and IgG4 were the best correlative with disease activities[67]. Reiterová *et al*[68] reported that antibodies to AgB was not detectable three months after treatment but antibodies to HCF were remained detectable.

It has been reported that subclasses of IgG have different performance in diagnosis of primary in comparison to relapse cases of CE. One study suggested IgG2 as a good marker for primary infection and total IgG for detection of relapse cases[69].

Recombinant P29 protein of *E. granulosus* was synthesized by Ben Nouir *et al*[64] and evaluated for post-surgical follow-up of CE patients, in an ELISA and WB systems. Results indicated that, using P29-ELISA, all of initially seropositive cases of CE seroconverted to negative within three years after treatments, while HCF-ELISA remained positive in 90% of cases. Western Blotting, using P29, remained positive in only 10% of cases after 3 years while HCF-WB remained positive in more than 25% of cases after 3 years of follow-up. However the performance of P29 in initial diagnosis of CE has not been satisfactory.

In another study by this group, somatic protoscolex antigens of *E. granulosus* have been assessed for follow-up of surgically treated CE patients and found that only 29% of treated patients reaching seronegativity after 5 years of follow up. The conventional HCF-ELISA becoming negative in 15% of cases at the end of the follow up period[65].

A double 27 and 28 kDa antigen, in WB, was also reported as useful antigen for the follow up of CE

patients. However such bands were only detectable in 75% of the patients before treatment[65]. The prognostic value of AgB subunits was evaluated by Ben Nouir *et al*[64] in ELISA and WB systems. Patients were grouped into either cured or non-cured CE patient. Findings of the study showed that ELISA remained positive 4-5 years after treatment in 57.1% of cured and 100% of non-cured patients. Immunoblotting, based on AgB subunits (8 and 16 kDa), revealed 14.3% of seropositivity after 4 years, with no reactivity to the components after 5 years of follow up. Interestingly, WB remained positive in 100% of non-cured patients up to 5 years (end of follow-up period). Serum antibodies to a certain bands (24 and 39) of HCF in Western blotting decreased in post-surgical monitoring of CE patients[70].

CONCLUSION

The performances of currently available immunodiagnostic test in diagnosis of CE are not satisfactory and the best serological test for diagnosis of CE is still the subject of debate. Over the time, particularly during the last two decades, several immunodiagnostic tests have been developed, mainly based on HCF, AgB and Ag5, yet their performance in diagnosis of human hydatidosis are unsatisfactory.

The most widely used antigens for serological diagnosis of CE are AgB and Ag5. Yet new antigens are being constantly evaluated and new serological assays are being developed to improve the performance of serological diagnostic tests.

Utilizing of recombinant or synthetic antigen although improved the performance, but has not overcome the problem of low sensitivity or even cross reactivity with other antigen in diagnosis of CE and these problems still remained. Considerable variation in performance of serological test for diagnosis of CE between different laboratories is mainly related to lack of standardization of antigen preparation, inadequate sensitivities and specificity, and also strain of the parasite that antigens have been purified from its content.

Immunodiagnostic tests based on recombinant antigen has drawn the attention of many researchers and the outcomes of such studies are promising. These antigens, especially based on AgB subunits, showed not all the times, but in most cases, satisfactory performance in comparison to their homologues antigens.

New interesting perspective in the development of serological assays for diagnosis of CE might be derive from recent observation that IgG subclasses are good markers for diagnosis and also follow up of CE patients. Moreover, the evaluation of highly purified Ag5 for immunodiagnosis of CE seems to be a promising task ahead which must be undertaken in the future. And finally immunodiagnosis assays may well be improved through combining of several well-defined antigens, notably immunodominant antigen in different stages of the cyst development.

ACKNOWLEDGMENTS

Technical assistance of Reza Shahriari Rad is appreciated.

REFERENCES

1 **Sarkari B**, Sadjjadi SM, Beheshtian MM, Aghaee M, Sedaghat F. Human cystic echinococcosis in Yasuj District in Southwest of Iran: an epidemiological study of seroprevalence and surgical cases over a ten-year period. *Zoonoses Public Health* 2010; **57**: 146-150 [PMID: 19175567 DOI: 10.1111/j.1863-2378.2008.01200.x]

2 **Sadjjadi SM**. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006; **55** Suppl: S197-S202 [PMID: 16337429 DOI: 10.1016/j.parint.2005.11.030]

3 **Eckert J**, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004; **17**: 107-135 [PMID: 14726458]

4 **Budke CM**, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. *Emerg Infect Dis* 2006; **12**: 296-303 [PMID: 16494758 DOI: 10.3201/eid1202.050499]

5 **Zhang W**, Wen H, Li J, Lin R, McManus DP. Immunology and immunodiagnosis of cystic echinococcosis: an update. *Clin Dev Immunol* 2012; **2012**: 101895 [PMID: 22235225 DOI: 10.1155/2012/101895]

6 **Al-Sherbiny MM**, Farrag AA, Fayad MH, Makled MK, Tawfeek GM, Ali NM. Application and assessment of a dipstick assay in the diagnosis of hydatidosis and trichinosis. *Parasitol Res* 2004; **93**: 87-95 [PMID: 15103552]

7 **Devi CS**, Parija SC. A new serum hydatid antigen detection test for diagnosis of cystic echinococcosis. *Am J Trop Med Hyg* 2003; **69**: 525-528 [PMID: 14695090]

8 **El-Shazly AM**, Saad RM, Belal US, Sakr T, Zakae HA. Evaluation of ELISA and IHAT in serological diagnosis of proven cases of human hydatidosis. *J Egypt Soc Parasitol* 2010; **40**: 531-538 [PMID: 21246959]

9 **González-Sapienza G**, Lorenzo C, Nieto A. Improved immunodiagnosis of cystic hydatid disease by using a synthetic peptide with higher diagnostic value than that of its parent protein, Echinococcus granulosus antigen B. *J Clin Microbiol* 2000; **38**: 3979-3983 [PMID: 11060055]

10 **Sadjjadi SM**, Abidi H, Sarkari B, Izadpanah A, Kazemian S. Evaluation of enzyme linked immunosorbent assay, utilizing native antigen B for serodiagnosis of human hydatidosis. *Iran J Immunol* 2007; **4**: 167-172 [PMID: 17767016]

11 **Swarna SR**, Parija SC. Evaluation of Dot-ELISA and enzyme-linked immuno-electrotransfer blot assays for detection of a urinary hydatid antigen in the diagnosis of cystic echinococcosis. *Trop Parasitol* 2012; **2**: 38-44 [PMID: 23508649 DOI: 10.4103/2229-5070.97238]

12 **Carmena D**, Benito A, Eraso E. Antigens for the immunodiagnosis of Echinococcus granulosus infection: An update. *Acta Trop* 2006; **98**: 74-86 [PMID: 16527225 DOI: 10.1016/j.actatropica.2006.02.002]

13 **Hira PR**, Shweiki HM, Francis I. Cystic hydatid disease: pitfalls in diagnosis in the Middle East endemic area. *J Trop Med Hyg* 1993; **96**: 363-369 [PMID: 8254715]

14 **Liance M**, Janin V, Bresson-Hadni S, Vuitton DA, Houin R, Piarroux R. Immunodiagnosis of Echinococcus infections: confirmatory testing and species differentiation by a new commercial Western Blot. *J Clin Microbiol* 2000; **38**: 3718-3721 [PMID: 11015390]

15 **Sadjjadi SM**, Ardehali S, Noman-Pour B, Kumar V, Izadpanah A. Diagnosis of cystic echinococcosis: ultrasound imaging or countercurrent immunoelectrophoresis? *East Mediterr Health J* 2001; **7**: 907-911 [PMID: 15332731]

16 **Sadjjadi SM**, Sedaghat F, Hosseini SV, Sarkari B. Serum antigen and antibody detection in echinococcosis: application in serodiagnosis of human hydatidosis. *Korean J Parasitol* 2009; **47**: 153-157 [PMID: 19488422 DOI: 10.3347/kjp.2009.47.2.153]

17 **Siracusano A**, Bruschi F. Cystic echinococcosis: progress and limits in epidemiology and immunodiagnosis. *Parassitologia* 2006; **48**: 65-66 [PMID: 16881399]

18 **Akbulut S**, Yavuz R, Sogutcu N, Kaya B, Hatipoglu S, Senol A, Demircan F. Hydatid cyst of the pancreas: Report of an undiagnosed case of pancreatic hydatid cyst and brief literature review. *World J Gastrointest Surg* 2014; **6**: 190-200 [PMID: 25346801 DOI: 10.4240/wjgs.v6.i10.190]

19 **Orhun A**, Müller-Stöver I, Holtfreter MC, Dedelen H, Häussinger D, Richter J. [Epidemiological and clinical characteristics of patients with echinococcosis - management in an infectiological service in Germany]. *Dtsch Med Wochenschr* 2012; **137**: 1039-1044 [PMID: 22570097 DOI: 10.1055/s-0032-1304951]

20 **Akcam AT**, Ulku A, Koltas IS, Izol V, Bicer OS, Kilicbagir E, Sakman G, Poyrazoglu H, Erman T, Aridogan IA, Parsak CK, Inal M, Iskit S. Clinical characterization of unusual cystic echinococcosis in southern part of Turkey. *Ann Saudi Med* 2014; **34**: 508-516 [PMID: 25971825 DOI: 10.5144/0256-4947.2014.508]

21 **Wuestenberg J**, Gruener B, Oeztuerk S, Mason RA, Haenle MM, Graeter T, Akinli AS, Kern P, Kratzer W. Diagnostics in cystic echinococcosis: serology versus ultrasonography. *Turk J Gastroenterol* 2014; **25**: 398-404 [PMID: 25254522 DOI: 10.5152/tjg.2014.7112]

22 **Canpolat U**, Yorgun H, Sunman H, Aytemir K. Cardiac hydatid cyst mimicking left ventricular aneurysm and diagnosed by magnetic resonance imaging. *Turk Kardiyol Dern Ars* 2011; **39**: 47-51 [PMID: 21358231]

23 **Karakasli A**, Yilmaz M, Mucuoglu AO, Yurt A. A large primary dumbbell hydatid cyst causing neural foraminal widening of the thoracic spine: A case report and literature review. *Int J Surg Case Rep* 2015; **8**C: 55-58 [PMID: 25625491 DOI: 10.1016/j.ijscr.2014.12.036]

24 **Zapatero J**, Madrigal L, Lago J, Baschwitz B, Pérez E, Candelas J. Surgical treatment of thoracic hydatidosis. A review of 100 cases. *Eur J Cardiothorac Surg* 1989; **3**: 436-440 [PMID: 2635924]

25 **Coupland U**, Dobosz S, Zawadka K, Marczyńska M. Cystic echinococcosis in a child infected with HIV. *Ann Parasitol* 2012; **58**: 101-103 [PMID: 25165762]

26 **Nouir NB**, Nuñez S, Frei E, Gorcii M, Müller N, Gianinazzi C, Mekki M, Nouri A, Babba H, Gottstein B. Post-surgical follow-up (by ELISA and immunoblotting) of cured versus non-cured cystic echinococcosis in young patients. *Parasitology* 2008; **135**: 105-114 [PMID: 17767795 DOI: 10.1017/s0031182007003502]

27 **Piccoli L**, Tamarozzi F, Cattaneo F, Mariconti M, Filice C, Bruno A, Brunetti E. Long-term sonographic and serological follow-up of inactive echinococcal cysts of the liver: hints for a "watch-and-wait" approach. *PLoS Negl Trop Dis* 2014; **8**: e3057 [PMID: 25122222 DOI: 10.1371/journal.pntd.0003057]

28 **Mariconti M**, Bazzocchi C, Tamarozzi F, Meroni V, Genco F, Maserati R, Brunetti E. Immunoblotting with human native antigen shows stage-related sensitivity in the serodiagnosis of hepatic cystic echinococcosis. *Am J Trop Med Hyg* 2014; **90**: 75-79 [PMID: 24297816 DOI: 10.4269/ajtmh.13-0341]

29 **Ahn CS**, Han X, Bae YA, Ma X, Kim JT, Cai H, Yang HJ, Kang I, Wang H, Kong Y. Alteration of immunoproteome profile of Echinococcus granulosus hydatid fluid with progression of cystic echinococcosis. *Parasit Vectors* 2015; **8**: 10 [PMID: 25566682 DOI: 10.1186/s13071-014-0610-7]

30 **Haghpanah B**, Mosavat B, Ghayour Z, Oreizi F. Diagnostic value of hydatid cyst antigens using western blotting method. *Jundishapur J Microbiol* 2010; **3**: 175-185

31 **Mohammadzadeh T**, Sako Y, Sadjjadi SM, Sarkari B, Ito A. Comparison of the usefulness of hydatid cyst fluid, native antigen B and recombinant antigen B8/1 for serological diagnosis of cystic echinococcosis. *Trans R Soc Trop Med Hyg* 2012; **106**: 371-375 [PMID: 22472966 DOI: 10.1016/j.trstmh.2012.01.012]

32 **Tawfeek GM**, Elwakil HS, El-Hoseiny L, Thabet HS, Sarhan RM, Awad NS, Anwar WA. Comparative analysis of the diagnostic performance of crude sheep hydatid cyst fluid, purified antigen B and its subunit (12 Kda), assessed by ELISA, in the diagnosis of human cystic echinococcosis. *Parasitol Res* 2011; **108**: 371-376 [PMID: 20922427]

33 **Jin Y**, Anvarov K, Khajibaev A, Hong S, Hong ST. Serodiagnosis of echinococcosis by ELISA using cystic fluid from Uzbekistan sheep. *Korean J Parasitol* 2013; **51**: 313-317 [PMID: 23864742 DOI: 10.3347/kjp.2013.51.3.313]

34 **List C**, Qi W, Maag E, Gottstein B, Müller N, Felger I. Serodiagnosis of Echinococcus spp. infection: explorative selection of diagnostic antigens by peptide microarray. *PLoS Negl Trop Dis* 2010; **4**: e771 [PMID: 20689813 DOI: 10.1371/journal.pntd.0000771]

35 **Sedaghat F**, Sadjjadi SM, Hosseini SV, Kazemian S, Sarkari B. Evaluation of a simple Dot-ELISA in comparison with countercurrent immunoelectrophoresis for diagnosis of human hydatidosis. *Clin Lab* 2011; **57**: 201-205 [PMID: 21500727]

36 **Lightowlers MW**, Liu DY, Haralambous A, Rickard MD. Subunit composition and specificity of the major cyst fluid antigens of Echinococcus granulosus. *Mol Biochem Parasitol* 1989; **37**: 171-182 [PMID: 2481826]

37 **Di Felice G**, Pini C, Afferni C, Vicari G. Purification and partial characterization of the major antigen of Echinococcus granulosus (antigen 5) with monoclonal antibodies. *Mol Biochem Parasitol* 1986; **20**: 133-142 [PMID: 3092046]

38 **Khabiri AR**, Bagheri F, Assmar M, Siavashi MR. Analysis of specific IgE and IgG subclass antibodies for diagnosis of Echinococcus granulosus. *Parasite Immunol* 2006; **28**: 357-362 [PMID: 16879307]

39 **Pagnozzi D**, Biosa G, Addis MF, Mastrandrea S, Masala G, Uzzau S. An easy and efficient method for native and immunoreactive Echinococcus granulosus antigen 5 enrichment from hydatid cyst fluid. *PLoS One* 2014; **9**: e104962 [PMID: 25119821 DOI: 10.1371/journal.pone.0104962]

40 **Jiang L**, Zhang YG, Liu MX, Feng Z. Analysis on the reactivity of five subunits of antigen B family in serodiagnosis of echinococcosis. *Exp Parasitol* 2012; **131**: 85-91 [PMID: 22446351 DOI: 10.1016/j.exppara.2012.03.009]

41 **Mamuti W**, Yamasaki H, Sako Y, Nakao M, Xiao N, Nakaya K, Sato N, Vuitton DA, Piarroux R, Lightowlers MW, Craig PS, Ito A. Molecular cloning, expression, and serological evaluation of an 8-kilodalton subunit of antigen B from Echinococcus multilocularis. *J Clin Microbiol* 2004; **42**: 1082-1088 [PMID: 15004057]

42 **Sarkari B**, Sadjjadi S, Abidi H, Izadpanah A, Kazemian S, Rafati A. Application of western blotting using native antigen B for serodiagnosis of human cystic echinococcosis. *Iran J Parasitol* 2007; **2**: 7-12

43 **Jiang L**, Feng Z, Zhang YG, Wang ZY. [Serodiagnosis of the recombinant multi-epitope antigens from antigen B subunits of Echinococcus granulosus]. *Zhongguo Jishengchongxue Yu Jishengchongbing Zazhi* 2013; **31**: 438-442 [PMID: 24818408]

44 **Virginio VG**, Hernández A, Rott MB, Monteiro KM, Zandonai AF, Nieto A, Zaha A, Ferreira HB. A set of recombinant antigens from Echinococcus granulosus with potential for use in the immunodiagnosis of human cystic hydatid disease. *Clin Exp Immunol* 2003; **132**: 309-315 [PMID: 12699422]

45 **Leggatt GR**, Yang W, McManus DP. Serological evaluation of the 12 kDa subunit of antigen B in Echinococcus granulosus cyst fluid by immunoblot analysis. *Trans R Soc Trop Med Hyg* 1992; **86**: 189-192 [PMID: 1440787]

46 **Abdi J**, Kazemi B, Mohebali M, Bandehpour M, Rahimi MT, Rokni MB. Gene cloning, expression and serological evaluation of the 12-kDa antigen-B subunit from Echinococcus granulosus. *Ann Trop Med Parasitol* 2010; **104**: 399-407 [PMID: 20819308 DOI: 10.1179/136485910X12743554760261]

47 **Santivañez SJ**, Arias P, Portocarrero M, Rodriguez S, Gonzalez AE, Gilman RH, Gavidia CM, Garcia HH. Serological diagnosis of lung cystic hydatid disease using the synthetic p176 peptide. *Clin Vaccine Immunol* 2012; **19**: 944-947 [PMID: 22518012 DOI: 10.1128/cvi.05540-11]

48 **Chen X**, Chen X, Lu X, Feng X, Wen H. The production and comparative evaluation of native and recombinant antigens for the

fast serodiagnosis of cystic echinococcosis with dot immunogold filtration assay. *Parasite Immunol* 2015; **37**: 10-15 [PMID: 25313824 DOI: 10.1111/pim.12151]

49 **Rahimi H**, Sadjjadi S, Sarkari B. Performance of antigen B isolated from different hosts and cyst locations in diagnosis of cystic echinococcosis. *Iran J Parasitol* 2011; **6**: 12-19 [PMID: 22347269]

50 **Tamer GS**, Dündar D, Uzuner H, Baydemir C. Evaluation of immunochromatographic test for the detection of antibodies against Echinococcosis granulosus. *Med Sci Monit* 2015; **21**: 1219-1222 [PMID: 25921809 DOI: 10.12659/msm.893155]

51 **Schweiger A**, Grimm F, Tanner I, Müllhaupt B, Bertogg K, Müller N, Deplazes P. Serological diagnosis of echinococcosis: the diagnostic potential of native antigens. *Infection* 2012; **40**: 139-152 [PMID: 22076692 DOI: 10.1007/s15010-011-0205-6]

52 **Sari C**, Ertuğ S, Karadam SY, Ozgün H, Karaoğlu AO, Ertabaklar H. [The comparative evaluation of Enzyme Linked Immunosorbent Assay (ELISA), Indirect Hemagglutination Test (IHA) and Indirect Fluorescent Antibody Test (IFAT) in the diagnosis of cystic echinococcosis]. *Turkiye Parazitol Derg* 2009; **33**: 73-76 [PMID: 19367551]

53 **Xu MQ**, Zhu B, Xue HC, Li X, Guo XR, Zhang YH, Li H. [Reexamination of specific antibodies in sera of cystic echinococcosis patients with IgG negative seroresponse]. *Zhongguo Jishengchongxue Yu Jishengchongbing Zazhi* 2002; **20**: 148-151 [PMID: 12567989]

54 **Daeki AO**, Craig PS, Shambesh MK. IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. *Ann Trop Med Parasitol* 2000; **94**: 319-328 [PMID: 10945041]

55 **Lawn SD**, Bligh J, Craig PS, Chiodini PL. Human cystic echinococcosis: evaluation of post-treatment serologic follow-up by IgG subclass antibody detection. *Am J Trop Med Hyg* 2004; **70**: 329-335 [PMID: 15031526]

56 **Khalilpour A**, Sadjjadi SM, Moghadam ZK, Yunus MH, Zakaria ND, Osman S, Noordin R. Lateral flow test using Echinococcus granulosus native antigen B and comparison of IgG and IgG4 dipsticks for detection of human cystic echinococcosis. *Am J Trop Med Hyg* 2014; **91**: 994-999 [PMID: 25200268 DOI: 10.4269/ajtmh.14-0170]

57 **Chirag S**, Fomda BA, Khan A, Malik AA, Lone GN, Khan BA, Zahoor D. Detection of hydatid-specific antibodies in the serum and urine for the diagnosis of cystic echinococcosis in patients from the Kashmir Valley, India. *J Helminthol* 2015; **89**: 232-237 [PMID: 24429044 DOI: 10.1017/s0022149x13000837]

58 **Sarkari B**, Chance M, Hommel M. Antigenuria in visceral leishmaniasis: detection and partial characterisation of a carbohydrate antigen. *Acta Trop* 2002; **82**: 339-348 [PMID: 12039673]

59 **Sarkari B**, Hatam GR, Mikaeili F, Sadeghi H, Ebrahimi S. A comparative study of antigen and antibody detection in visceral leishmaniasis using serum and urine-based ELISA. *Trop Biomed* 2008; **25**: 96-99 [PMID: 18948879]

60 **Ghatei MA**, Hatam GR, Hossini MH, Sarkari B. Performance of latex agglutination test (KAtex) in diagnosis of visceral leishmaniasis in Iran. *Iran J Immunol* 2009; **6**: 202-207 [PMID: 20054108]

61 **van Dam GJ**, de Dood CJ, Lewis M, Deelder AM, van Lieshout L, Tanke HJ, van Rooyen LH, Corstjens PL. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. *Exp Parasitol* 2013; **135**: 274-282 [PMID: 23850995 DOI: 10.1016/j.exppara.2013.06.017]

62 **Parija SC**. Urinary antigen detection for diagnosis of parasitic infections. *Parasitol Today* 1998; **14**: 5-6 [PMID: 17040680]

63 **Ravinder PT**, Parija SC, Rao KS. Urinary hydatid antigen detection by coagglutination, a cost-effective and rapid test for diagnosis of cystic echinococcosis in a rural or field setting. *J Clin Microbiol* 2000; **38**: 2972-2974 [PMID: 10921961]

64 **Ben Nouir N**, Gianinazzi C, Gorcii M, Müller N, Nouri A, Babba H, Gottstein B. Isolation and molecular characterization of recombinant Echinococcus granulosus P29 protein (recP29) and its assessment for the post-surgical serological follow-up of human cystic echinococcosis in young patients. *Trans R Soc Trop Med Hyg* 2009; **103**: 355-364 [PMID: 19027129 DOI: 10.1016/j.trstmh.2008.09.020]

65 **Ben Nouir N**, Nuñez S, Gianinazzi C, Gorcii M, Müller N, Nouri A, Babba H, Gottstein B. Assessment of Echinococcus granulosus somatic protoscolex antigens for serological follow-up of young patients surgically treated for cystic echinococcosis. *J Clin Microbiol* 2008; **46**: 1631-1640 [PMID: 18367566 DOI: 10.1128/jcm.01689-07]

66 **Ciobotaru MD**, Luca M, Cobzaru RG. Surgical management of pulmonary hydatidosis in children. *Rev Med Chir Soc Med Nat Iasi* 2014; **118**: 753-758 [PMID: 25341297]

67 **Tenguria RK**, Naik MI. Evaluation of human cystic echinococcosis before and after surgery and chemotherapy by demonstration of antibodies in serum. *Ann Parasitol* 2014; **60**: 297-303 [PMID: 25706429]

68 **Reiterová K**, Auer H, Altintaś N, Yolasigmaz A. Evaluation of purified antigen fraction in the immunodiagnosis of cystic echinococcosis. *Parasitol Res* 2014; **113**: 2861-2867 [PMID: 24828349 DOI: 10.1007/s00436-014-3947-0]

69 **Benabid M**, Galai Y, Nouira R, Bouchoucha S, Bouratbine A, Aoun K. Contribution of specific anti-hydatid IgG subclasses in the diagnosis of echinococcosis primary infection and relapses. *Clin Lab* 2013; **59**: 293-298 [PMID: 23724617]

70 **Kumar Tenguria R**, Naik MI, Fomda B. Application of Western Blotting for the post-treatment monitoring of human cystic echinococcosis. *Iran J Public Health* 2013; **42**: 826-832 [PMID: 26056636]

71 **Sbihi Y**, Rmiqui A, Rodriguez-Cabezas MN, Orduña A, Rodriguez-Torres A, Osuna A. Comparative sensitivity of six serological tests and diagnostic value of ELISA using purified antigen in hydatidosis. *J Clin Lab Anal* 2001; **15**: 14-18 [PMID: 11170228]

72 **Hernández-González A**, Muro A, Barrera I, Ramos G, Orduña A, Siles-Lucas M. Usefulness of four different Echinococcus granulosus recombinant antigens for serodiagnosis of unilocular hydatid disease (UHD) and postsurgical follow-up of patients treated for UHD. *Clin Vaccine Immunol* 2008; **15**: 147-153 [PMID: 17989342 DOI: 10.1128/CVI.00363-07]

73 **Gonlugur U**, Ozcelik S, Gonlugur TE, Celiksoz A. The role of Casoni's skin test and indirect haemagglutination test in the diagnosis of hydatid disease. *Parasitol Res* 2005; **97**: 395-398 [PMID: 16151737 DOI: 10.1007/s00436-005-1473-9]

74 **Sunita T**, Dubey ML, Khurana S, Malla N. Specific antibody detection in serum, urine and saliva samples for the diagnosis of cystic echinococcosis. *Acta Trop* 2007; **101**: 187-191 [PMID: 17335765 DOI: 10.1016/j.actatropica.2006.07.014]

75 **Ortona E**, Riganò R, Margutti P, Notargiacomo S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profumo E, Teggi A, Siracusano A. Native and recombinant antigens in the immunodiagnosis of human cystic echinococcosis. *Parasite Immunol* 2000; **22**: 553-559 [PMID: 11116435 DOI: 10.1046/j.1365-3024.2000.00336.x]

76 **Rafiei A**, Craig PS. The immunodiagnostic potential of protoscolex antigens in human cystic echinococcosis and the possible influence of parasite strain. *Ann Trop Med Parasitol* 2002; **96**: 383-389 [PMID: 12171619 DOI: 10.1179/000349802125001195]

77 **Lorenzo C**, Ferreira HB, Monteiro KM, Rosenzvit M, Kamenetzky L, García HH, Vasquez Y, Naquira C, Sánchez E, Lorca M, Contreras M, Last JA, González-Sapienza GG. Comparative analysis of the diagnostic performance of six major Echinococcus granulosus antigens assessed in a double-blind, randomized multicenter study. *J Clin Microbiol* 2005; **43**: 2764-2770 [PMID: 15956395 DOI: 10.1128/JCM.43.6.2764-2770.2005]

78 **Rott MB**, Fernández V, Farias S, Ceni J, Ferreira HB, Haag KL, Zaha A. Comparative analysis of two different subunits of antigen B from Echinococcus granulosus: gene sequences, expression in Escherichia coli and serological evaluation. *Acta Trop* 2000; **75**: 331-340 [PMID: 10838217 DOI: 10.1016/S0001-706X(00)00069-3]

79 **Li J**, Zhang WB, Wilson M, Ito A, McManus DP. A novel

recombinant antigen for immunodiagnosis of human cystic echinococcosis. *J Infect Dis* 2003; **188**: 1951-1960 [PMID: 14673776 DOI: 10.1086/379976]

80 **Parija SC**, Ravinder PT, Rao KS. Detection of hydatid antigen in urine by countercurrent immunoelectrophoresis. *J Clin Microbiol* 1997; **35**: 1571-1574 [PMID: 9163484]

P- Reviewer: Jose R, Ni Y **S- Editor**: Qiu S **L- Editor**: A
E- Editor: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.196

World J Methodol 2015 December 26; 5(4): 196-202
ISSN 2222-0682 (online)

MINIREVIEWS

Selecting the best strategy of treatment in newly diagnosed advanced-stage ovarian cancer patients

Lucas Minig, Cristina Zorrero, Pablo Padilla Iserte, Andres Poveda

Lucas Minig, Cristina Zorrero, Pablo Padilla Iserte, Gynecology Department, Clinical Area of Gynecologic Oncology, Valencian Institue of Oncology, 46009 Valencia, Spain

Andres Poveda, Medical Oncology Department, Clinical Area of Gynecologic Oncology, Valencian Institue of Oncology, 46009 Valencia, Spain

Author contributions: All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict-of-interest statement: No potential conflicts of interest. No financial support.

Correspondence to: Lucas Minig, MD, PhD, MBA, Gynecology Department, Clinical Area of Gynecologic Oncology, Valencian Institue of Oncology, C/del Profesor Beltran Baguena 8, 46009 Valencia, Spain. miniglucas@gmail.com
Telephone: +34-96-1114024
Fax: +34-96-1114024

Received: July 28, 2015
Peer-review started: July 31, 2015
First decision: August 14, 2015
Revised: August 27, 2015
Accepted: October 12, 2015
Article in press: October 13, 2015
Published online: December 26, 2015

Abstract

Although it is assumed that the combination of chemotherapy and radical surgery should be indicated in all newly diagnosed advanced-stage ovarian cancer patients, one of the main raised questions is how to select the best strategy of initial treatment in this group of patients, neoadjuvant chemotherapy followed by interval debulking surgery or primary debulking surgery followed by adjuvant chemotherapy. The selection criteria to offer one strategy over the other as well as a stepwise patient selection for initial treatment are described. Selecting the best strategy of treatment in newly diagnosed advanced stage ovarian cancer patients is a multifactorial and multidisciplinary decision. Several factors should be taken into consideration: (1) the disease factor, related to the extension and localization of the disease as well as tumor biology; (2) the patient factor, associated with patient age, poor performance status, and co-morbidities; and (3) institutional infrastructure factor, related to the lack of prolonged operative time, an appropriate surgical armamentarium, as well as well-equipped intensive care units with well-trained personnel.

Key words: Ovarian cancer; Advanced stage; Primary debulking surgery; Neoadjuvant chemotherapy; Patients' selection

Core tip: Selecting the best strategy of treatment in newly diagnosed advanced-stage ovarian cancer patients is a multifactorial and multidisciplinary decision. Surgeries performed by gynecologic oncologists with formal training in cytoreductive techniques at referral centers are crucial factors in obtaining better oncologic outcomes. However, other factors such as clinical status of the patients, the hospital's infrastructure and equipment, as well as the tumor biology of each individual patient should also be taken into account before deciding on an initial strategy of treatment in women with advanced-stage ovarian cancer.

Minig L, Zorrero C, Iserte PP, Poveda A. Selecting the best

strategy of treatment in newly diagnosed advanced-stage ovarian cancer patients. *World J Methodol* 2015; 5(4): 196-202 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/196.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.196

INTRODUCTION

It is estimated that over 80% of women with ovarian cancer are diagnosed at advanced stages, when the disease is already extended in the abdominal cavity or beyond. Primary complete debulking surgery (PDS) followed by adjuvant chemotherapy is associated with the best oncological outcome and is considered, therefore, the standard of care[1]. Limitations, however, have been postulated with respect to this treatment strategy. First, patients with postoperative residual disease have no meaningful impact on overall survival (OS)[2-4]. Second, only in few cases is the complete primary cytoreduction rate acceptable, and only when the procedure is performed by experienced surgeons with extended formal training in cytoreductive techniques. Third, PDS is associated with a high incidence of postoperative complications[5,6].

Consequently, an alternative strategy based on neoadjuvant chemotherapy followed by interval debulking surgery (NACT-IDS) has been proposed[7]. Patients receive three to four courses of platinum-taxanes NACT and then, in the absence of progression disease, IDS is performed. The proposed advantages include a reduced risk of peri-operative morbidity, a higher rate of complete tumor resection, and a contention that deferring the initial attempt at surgical debulking does not compromise survival[8].

Nevertheless, there currently exist several controversies regarding the best strategy of treatment[9,10]. Although it is assumed that the combination of chemotherapy and radical surgery should be indicated in all newly diagnosed advanced-stage ovarian cancer patients, one of the main questions raised is how to select the best strategy for initial treatment in this group of patients, a topic that will be the focus of this review.

STEPWISE PATIENT SELECTION FOR NACT OR PDS

An algorithm of management for newly suspected or diagnosed advanced-stage ovarian cancer patients has been developed at the Instituto Valenciano de Oncologia (IVO), Valencia, Spain. Hence, patients are initially evaluated with computer tomography (CT) of thorax-abdomen and pelvis, plus tumor markers including CA-125, CA-19.9, and CEA.

Pre-operative tumor markers can also provide additional information to allow discrimination between an ovarian or extra-ovarian origin of peritoneal carcinomatosis. In this case, if the CA-125 (UI/mL)/CEA (ng/mL) ratio is < 25, mammography and endoscopy are mandatory to exclude primary breast, gastric, or colon cancer[11] (Figure 1). The main aim of the pre-operative CT scan is to localize intra-abdominal disease at non-resectable structures such as liver hilium, celiac trunk, superior mesenteric artery, supra-renal lymph node metastases, and intrahepatic metastases; as well as to identify extra-abdominal disease.

In cases of non-resectable disease at CT scan or in patients older than 75, with poor status or surgical contraindications, a core biopsy or a diagnostic laparoscopy with biopsy is performed to obtain a tissue sample. If the final diagnosis confirms an epithelial ovarian carcinoma, NACT is indicated. According to our series, a total of 30% of patients would receive NACT at this point. In the absence of the previously mentioned criteria, a mini-laparotomy is performed to rule out any extended small bowel carcinomatosis and mesentery roof retraction. These findings are present in 10% of cases, a tumor biopsy is performed and NACT is started in these patients a few days later (Figure 1). The remaining 90% of our patients undergo complete PDS, following the surgical step-wise description detailed elsewhere[12].

Patients undergoing NACT receive 3 courses of carboplatin/paclitaxel intravenously every 3 wk, and are then evaluated by using clinical examination, CA-125 and CT scan. Women with a partial response undergo IDS in an attempt to complete tumor resection. Second-line chemotherapy or inclusion in clinical trials is proposed to women with stable disease or progression to NACT.

DISCUSSION

Role of primary complete cytoreduction

Complete resection of all macroscopic disease at primary debulking surgery is the single most important independent prognostic factor in women with advanced ovarian cancer[2,4-6,13-15]. The definition of "optimal" cytoreduction has been the subject of debate for decades. Therefore, optimal residual disease, such as that measuring 1-2 cm in diameter, has been traditionally considered[16]. However, a significant improvement in survival after complete tumor resection at the time of primary surgical cytoreduction has been observed[2-4]. Thus, according to the last Gynecological Cancer Inter Group (GCIG) consensus conference, cytoreduction should be classified as "complete", without residual disease"; or "incomplete", if residual disease is left at the end of the surgery. In addition, the consensus established that the aim of surgical debulking should be to obtain a complete tumor resection[1]. The final decision as to whether or not to perform a tumor debulking depends on the surgeon's training and confidence in the majority of her or his operations on patients[17]. A great body of evidence suggests that patients operated on by gynecologic oncologists with formal training in cytoreductive techniques are more likely to undergo a complete cytoreduction in comparison to those treated by general gynecologists or general surgeons, with significantly better oncologic outcome[18,19]. Therefore, the main worldwide

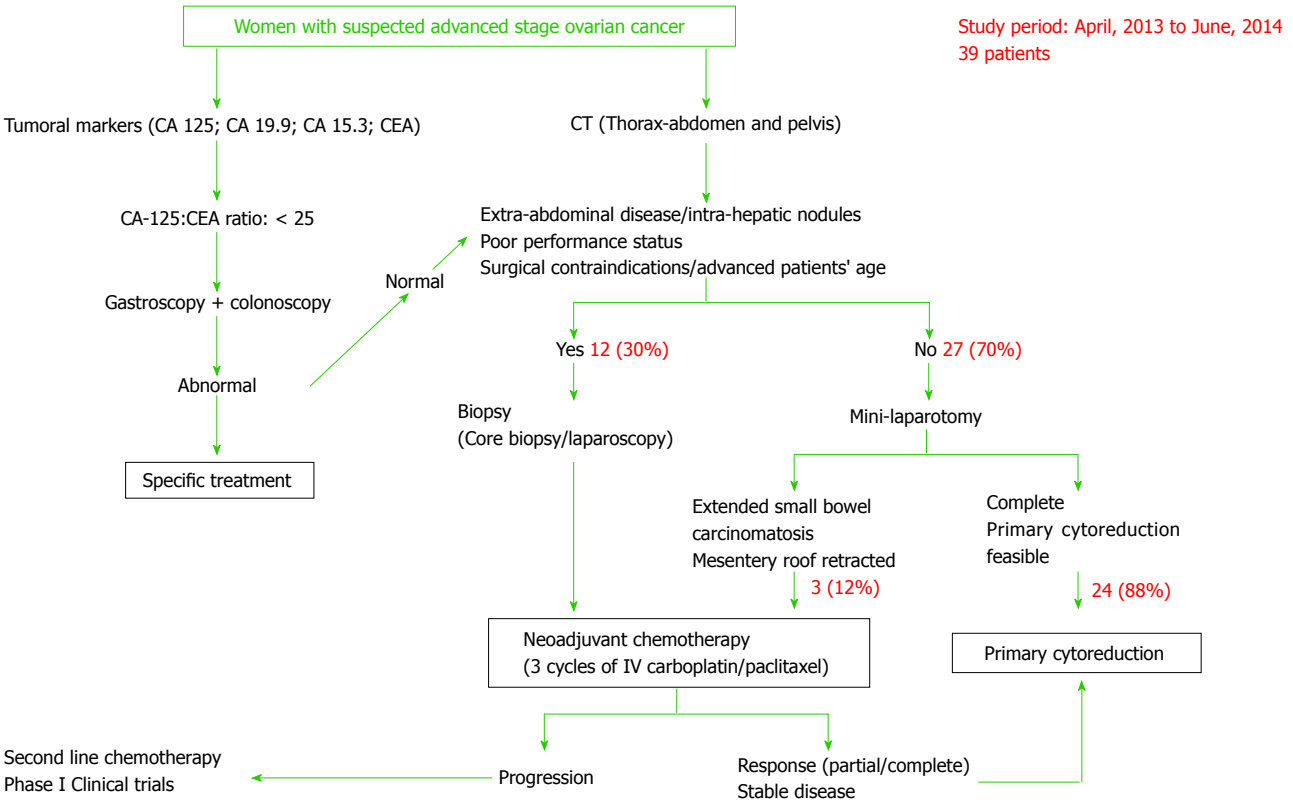

Figure 1 Stepwise management of women with suspected advanced-stage ovarian cancer. CT: Computer tomography.

consensus[1,12] states that gynecologic oncologists should make the decision regarding whether to start treatment with PDS or NACT in patients with advanced-stage ovarian cancer.

The decision regarding the initial strategy of treatment, based on NACT or PDS, in women with advanced ovarian cancer has been largely debated. A large meta-analysis involving 6885 patients in 53 studies after PDS demonstrated that each 10% increase in cytoreduction correlated with a 5.5% increase in median survival time. Patients with 75% or greater maximal cytoreductive efforts had a median survival of 37 mo compared with a 23 mo for patients with 25% or less maximal effort[14]. On the other hand, Bristow *et al*[20] studied 835 patients in 22 cohorts with advanced ovarian cancer who received NACT. The study showed a median OS of 24.5 mo, range 10-42 mo. Despite the fact that this rate was shorter than what was obtained after PDS, this comparison should be taken with caution given that a bias upon the selection of patients to receive NACT might exist. On the basis regarding the extension of the disease or performance status, within patients who underwent NACT might have a worse prognosis.

The results of the first randomized controlled trial (RCT) in patients with ovarian cancer FIGO stage ⅢC-Ⅳ of the European Organization for Treatment and Research (EORTC) and the National Cancer Institute of Canada, comparing PDS *vs* NACT-IDS, were published in 2010[11]. The authors randomized 718 patients with stage ⅢC-Ⅳ ovarian cancer, excluding ⅢC by node metastases only. Surgical time was 180 min in both arms and the median OS and progression-free survival was 30 mo and 12 mo, respectively, in the two arms. One of the main criticisms of the EORTC trial was, however, that NACT was compared to a weak PDS arm. The study was conducted in non-selected centers, achieving a median OS of 30 mo, with a complete cytoreduction rate in the PDS arm of 21%. A similar RCT performed in 87 hospitals in the United Kingdom and New Zealand found the same results[21]. It is interesting to note that these rates are markedly inferior to the outcomes reported by other international multicenter studies[2-4]. When surgery is performed at referral oncologic centers by well-trained surgeons, the complete primary cytoreduction rate can be over 40%-50%, with a median 5-year OS of 50-60 mo[2,5,6].

Despite the fact that the radicalness of the surgery is the most important factor to obtain a better oncologic outcome, other issues should also be taken into account. These factors include: (1) the time since the first visit of the patient to the commencement of the treatment; (2) the time from the hospital discharge after primary or interval debulking surgery to the initiation of adjuvant chemotherapy. Median time should not exceed 40 d, a longer period of time is related with a high incidence of postoperative complications; (3) the number of cycles in relation to neoadjuvant chemotherapy, should not be more than four; and (4) the time from the end of neoadjuvant chemotherapy to interval debulking surgery.

Table 1 Factors associated with cytoreduction rate

Factor	Characteristic
Surgeon	Adequate skills and training in cytoreductive techniques
Disease	Extension and localization of the disease Tumor biology
Patient	Advanced age Comorbidities Poor performance status
Institutional infrastructure	Ovarian cancer multidisciplinary surgical team Availability of prolonged operative time Appropriate surgical armamentarium Well-trained ICU personnel Well-equipped ICU capability

ICU: Intensive care unit.

Whether tumor biology or maximal up-front cytoreduction surgery is the most important determinant for better outcomes is being largely debated. At same time that some studies found cytoreduction removal of visible disease had a more significant impact on survival than the extent of the disease before surgery[22], other studies observed opposite results[23]. Thus, other factors should be taken into consideration in an attempt to classify ovarian tumors as with "bad" or "good" prognosis. Recent molecular studies, using microarray analysis, have associated overall survival with gene expression profiles in ovarian cancer patients after up-front surgical treatment[24]. Although future large analysis should confirm these findings, it should be expected that molecular studies using genes and proteomic pattern might represent the tools to select patients for the best individual treatment rather than to generalize one strategy over the other for all women with ovarian cancer.

However, beyond the surgeon factor previously described, the cytoreduction rate is also associated to other variables such as: (1) the disease factor, related to the extension and localization of the disease as well as tumor biology; (2) the patient factor, associated with patient age, poor performance status, and co-morbidities; and (3) institutional infrastructure factor, related to the lack of prolonged operative time, an appropriate surgical armamentarium, as well as well-equipped Intensive Care Units with well-trained personnel[12,25,26] (Table 1). It is crucial, moreover, to establish an adequate ovarian cancer multidisciplinary surgical team that includes other specialists such as general surgeons, anesthesiologists, infectologists, *etc*. (Figure 2).

Pre-operative evaluation in women with suspected advanced stage ovarian cancer

CT scan is recommended as the most appropriate imaging test prior to treatment planning in women with a suspected advanced stage ovarian cancer[27]. However, limitations with CT scan have been associated with its inability to accurately predict extensive serosal and mesenteric disease[28], and as it was previously described, these anatomical localizations are major limits to obtaining a complete cytoreduction. In fact, several models were developed to predict suboptimal cytoreduction by using CT scan parameters, but with very poor outcome[28-30].

Clinical studies have also evaluated the role of positron emission tomography (PET) and PET/CT as part of pre-operative evaluation in women with advanced-stage ovarian cancer[31]. However, based on the available literature, there is still no evidence that PET or PET/CT works better than CT in detecting the extension of primary ovarian cancer[31].

Diffusion-weighted magnetic resonance imaging (DW-MRI) is another tool under investigation used to predict resectability in women with advanced-stage ovarian cancer. The evidence for using the DW-MRI in improving detection of the true extent of the disease seems promising. The utility of DW-MRI in predicting intra-abdominal spreading in women with ovarian cancer has recently been evaluated in some investigations[32-34]. A recent study of 32 patients with ovarian cancer found the main gains of using DW-MRI were the detection of bowel serosal and mesenteric disease, with an accuracy for detection of peritoneal disease of 91% on DW-MRI compared with 75% on CT and 71% on FDG-PET/CT[34]. The results of this technique do appear to be promising for improving the detection of small volume-diffuse peritoneal disease. This encouraged data from a small number of studies; however, it should be prospectively evaluated and validated with a larger sample of patients to establish stronger conclusions in this regard.

Additional clinical factors can help surgeons to identify high-risk patients with postoperative complications and mortality after primary cytoreductive surgery. Two studies tried to correlate clinical factors with increased risk for postoperative morbidity after primary cytoreduction[35,36]. The studies observed for those aged over 75 together with either FIGO stage Ⅲ or Ⅳ and coincident comorbidity[35]; or aged over 75 combined with serum albumin < 3 g/dL or ASA score of at least 3 and high initial tumor burden (FIGO IV or high volume FIGO ⅢC)[36] identifies a subgroup of 7%-12% of patients with advanced ovarian cancer where upfront debulking surgery is associated with unacceptably high rates of morbidity and peri-operative mortality.

Laparoscopy vs laparotomy to evaluate the intra-abdominal extension of the disease

The majority of women receive either NACT or PDS based upon tumor extension and on estimated tumor resectability[17]. As has been previously detailed, there is no current imaging tool that can predict complete cytoreduction in women with advanced-stage ovarian cancer. Therefore, a direct laparoscopic or laparotomic assessment of the abdominal cavity is sometimes needed.

A pre-treatment laparoscopic score to predict tumor

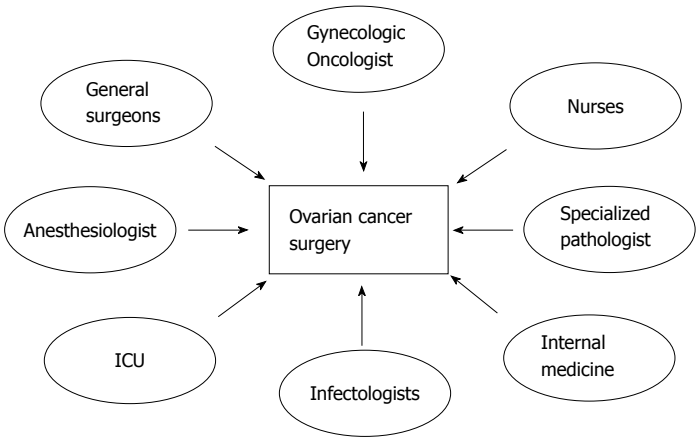

Figure 2 Multidisciplinary surgical team for treating women with advanced-stage ovarian cancer. ICU: Intensive care unit.

resectability was developed at a referral Italian cancer center[37]. This model established a predictive index value (PIV) with punctuation between 0-2 if tumors were present or not in specific areas of the pelvis and the abdomen. A score of 2 corresponded when the parameters were present, and score of 0 when they were absent. The study found that a predictive index value $\geqslant$ 8 resulted in a predictive probability of cytoreduction to less than 1 cm of zero (specificity of 100%), thus, avoiding unnecessary laparotomies. The PIV of the laparoscopic evaluation was then validated at 4 Italian Satellite Centers[38] and, more recently, the prognostic value of the laparoscopy-based-score was also established[39]. However, despite the fact that this strategy seems to be promising, some open questions still need to be clarified before its implementation into clinical practice: (1) the definition of each item is subjective, including terms such as "Unresectable massive peritoneal involvement plus milliary pattern of distribution" or "Obvious neoplastic involvement of the gastric wall"; (2) the oncologic impact of the missed assessment of the retroperitoneum is unknown; and (3) the model does not take into consideration clinical factors such as age, performance status or comorbidity. There are currently three ongoing trials which will probably answer some of these questions[40].

By using our algorithm, the evaluation of complete resectability is performed by a periumbilical longitudinal 10-cm mini-laparotomy instead of laparoscopy. By this approach, a surgeon's hand can be introduced into the abdominal cavity to carefully determine the extension of the disease on the liver surface, abdominal wall, hilium of the spleen and pancreatic tail, as well as the anterior stomach surface. In addition, this maneuver allows palpation of the most critical area of unresectability, such as liver hilium, celiac trunk, the mesenterium and the small bowel surface. This is a 40-min intervention with very low morbidity, allowing patients to start NACT 10-15 d later.

Surgical steps to obtain complete tumor resection

At our center, if complete tumor resectability is possible at the time of mini-laparotomy, patients undergo an immediate xipho-pubic midline incision with full exposure of the abomino-pelvic organs in order to establish the true extent of the disease. In this sense, before starting the removal of the disease, a stepwise systematic evaluation of the abdominal cavity is performed in order to avoid the so-called "point of no return" with unnecessary patient morbidity[12]. This standardized strategy has been well described previously[12], and includes two points of stop-or-go decisions. Initially, the *ligamentum falciforme* is resected, and the peritoneum of the paracolic gutters and the omentum are dissected from the transverse colon. Then, the lesser sac is opened allowing the evaluation of the pancreas, the *truncus coeliacus*, the liver, and the hepatoduodenal ligament with portal vein, hepatic artery and *ductus choledochus*. If a non-resectable tumor is present, surgery is stopped. If not, the second point of decision is the evaluation of the *radix mesenterii* and the small bowel surface by dissecting the adhesions and separating the small bowel from the colon and the greater omentum[12].

CENTRALIZATION OF CARE

Surgical training plays a crucial role in treating women with advanced-stage ovarian cancer[18]. Given the complexity of surgical procedures in obtaining a complete primary cytoreduction, as well as its positive impact on OS, not surprisingly, many studies from several countries have shown better OS when ovarian cancer patients were initially operated by a gynecologic oncologist rather than general gynecologist[41-43] or general surgeon[44].

Several authors have proposed the centralization of care of ovarian cancer[14,18,42,45] as an approach for improving the quality of care and outcomes. The main demonstrated benefits include better optimal cytoreduction rate[42,45], better chemotherapeutic administration rate and schemes[44,45], and better overall quality of treatment; therefore, improving the patient's quality of life. Thus, in comparison with unspecialized hospitals, patients who receive care at specialized centers may prolong their OS by almost a year[19,45]. Nevertheless, despite these clear advantages and according to population-based studies, fewer than 40% of patients with ovarian cancer have access to a specialized center in developed countries[43,44]. More recently, a study-population performed in California,

United States, demonstrated that only 4% of women with advanced-stage ovarian cancer were operated on by high-volume physicians at high-volume teaching hospitals[19].

CONCLUSION

Selecting the best strategy for treatment in newly diagnosed advanced-stage ovarian cancer patients is a multifactorial and multidisciplinary decision. Surgeries performed by gynecologic oncologists with formal training in cytoreductive techniques at referral centers are crucial factors in obtaining better oncologic outcomes. However, other factors such as clinical status of the patients, hospital infrastructure and equipment, as well as tumor biology of each individual patient should also be taken into account before deciding on an initial strategy of treatment in women with advanced-stage ovarian cancer.

REFERENCES

1 **Stuart GC**, Kitchener H, Bacon M, duBois A, Friedlander M, Ledermann J, Marth C, Thigpen T, Trimble E; participants of 4th Ovarian Cancer Consensus Conference (OCCC); Gynecologic Cancer Intergroup. 2010 Gynecologic Cancer InterGroup (GCIG) consensus statement on clinical trials in ovarian cancer: report from the Fourth Ovarian Cancer Consensus Conference. *Int J Gynecol Cancer* 2011; **21**: 750-755 [PMID: 21543936 DOI: 10.1097/IGC.0b013e31821b2568]

2 **du Bois A**, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). *Cancer* 2009; **115**: 1234-1244 [PMID: 19189349 DOI: 10.1002/cncr.24149]

3 **Bookman MA**, Brady MF, McGuire WP, Harper PG, Alberts DS, Friedlander M, Colombo N, Fowler JM, Argenta PA, De Geest K, Mutch DG, Burger RA, Swart AM, Trimble EL, Accario-Winslow C, Roth LM. Evaluation of new platinum-based treatment regimens in advanced-stage ovarian cancer: a Phase III Trial of the Gynecologic Cancer Intergroup. *J Clin Oncol* 2009; **27**: 1419-1425 [PMID: 19224846 DOI: 10.1200/JCO.2008.19.1684]

4 **Winter WE**, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, Markman M, Armstrong DK, Muggia F, McGuire WP. Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. *J Clin Oncol* 2007; **25**: 3621-3627 [PMID: 17704411 DOI: 10.1200/JCO.2006.10.2517]

5 **Chi DS**, Eisenhauer EL, Lang J, Huh J, Haddad L, Abu-Rustum NR, Sonoda Y, Levine DA, Hensley M, Barakat RR. What is the optimal goal of primary cytoreductive surgery for bulky stage IIIC epithelial ovarian carcinoma (EOC)? *Gynecol Oncol* 2006; **103**: 559-564 [PMID: 16714056 DOI: 10.1016/j.ygyno.2006.03.051]

6 **Aletti GD**, Dowdy SC, Podratz KC, Cliby WA. Surgical treatment of diaphragm disease correlates with improved survival in optimally debulked advanced stage ovarian cancer. *Gynecol Oncol* 2006; **100**: 283-287 [PMID: 16182350 DOI: 10.1016/j.ygyno.2005.08.027]

7 **Vergote I**, De Wever I, Tjalma W, Van Gramberen M, Decloedt J, van Dam P. Neoadjuvant chemotherapy or primary debulking surgery in advanced ovarian carcinoma: a retrospective analysis of 285 patients. *Gynecol Oncol* 1998; **71**: 431-436 [PMID: 9887245 DOI: 10.1006/gyno.1998.5213]

8 **Huober J**, Meyer A, Wagner U, Wallwiener D. The role of neoadjuvant chemotherapy and interval laparotomy in advanced ovarian cancer. *J Cancer Res Clin Oncol* 2002; **128**: 153-160 [PMID: 11935302 DOI: 10.1007/s00432-001-0312-3]

9 **Vergote I**, Tropé CG, Amant F, Ehlen T, Reed NS, Casado A. Neoadjuvant chemotherapy is the better treatment option in some patients with stage IIIc to IV ovarian cancer. *J Clin Oncol* 2011; **29**: 4076-4078 [PMID: 21931032 DOI: 10.1200/JCO.2011.36.9785]

10 **du Bois A**, Marth C, Pfisterer J, Harter P, Hilpert F, Zeimet AG, Sehouli J. Neoadjuvant chemotherapy cannot be regarded as adequate routine therapy strategy of advanced ovarian cancer. *Int J Gynecol Cancer* 2012; **22**: 182-185 [PMID: 22274314 DOI: 10.1097/IGC.0b013e31821d419a]

11 **Vergote I**, Tropé CG, Amant F, Kristensen GB, Ehlen T, Johnson N, Verheijen RH, van der Burg ME, Lacave AJ, Panici PB, Kenter GG, Casado A, Mendiola C, Coens C, Verleye L, Stuart GC, Pecorelli S, Reed NS. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV ovarian cancer. *N Engl J Med* 2010; **363**: 943-953 [PMID: 20818904 DOI: 10.1056/NEJMoa0908806]

12 **Vergote I**, du Bois A, Amant F, Heitz F, Leunen K, Harter P. Neoadjuvant chemotherapy in advanced ovarian cancer: On what do we agree and disagree? *Gynecol Oncol* 2013; **128**: 6-11 [PMID: 23006973 DOI: 10.1016/j.ygyno.2012.09.013]

13 **Zivanovic O**, Eisenhauer EL, Zhou Q, Iasonos A, Sabbatini P, Sonoda Y, Abu-Rustum NR, Barakat RR, Chi DS. The impact of bulky upper abdominal disease cephalad to the greater omentum on surgical outcome for stage IIIC epithelial ovarian, fallopian tube, and primary peritoneal cancer. *Gynecol Oncol* 2008; **108**: 287-292 [PMID: 17996927 DOI: 10.1016/j.ygyno.2007.10.001]

14 **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]

15 **Polterauer S**, Vergote I, Concin N, Braicu I, Chekerov R, Mahner S, Woelber L, Cadron I, Van Gorp T, Zeillinger R, Castillo-Tong DC, Sehouli J. Prognostic value of residual tumor size in patients with epithelial ovarian cancer FIGO stages IIA-IV: analysis of the OVCAD data. *Int J Gynecol Cancer* 2012; **22**: 380-385 [PMID: 22266934 DOI: 10.1097/IGC.0b013e31823de6ae]

16 **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]

17 **Chi DS**, Schwartz PE. Cytoreduction vs. neoadjuvant chemotherapy for ovarian cancer. *Gynecol Oncol* 2008; **111**: 391-399 [PMID: 19041036 DOI: 10.1016/j.ygyno.2008.07.058]

18 **Vernooij F**, Heintz P, Witteveen E, van der Graaf Y. The outcomes of ovarian cancer treatment are better when provided by gynecologic oncologists and in specialized hospitals: a systematic review. *Gynecol Oncol* 2007; **105**: 801-812 [PMID: 17433422 DOI: 10.1016/j.ygyno.2007.02.030]

19 **Bristow RE**, Chang J, Ziogas A, Randall LM, Anton-Culver H. High-volume ovarian cancer care: survival impact and disparities in access for advanced-stage disease. *Gynecol Oncol* 2014; **132**: 403-410 [PMID: 24361578 DOI: 10.1016/j.ygyno.2013.12.017]

20 **Bristow RE**, Chi DS. Platinum-based neoadjuvant chemotherapy and interval surgical cytoreduction for advanced ovarian cancer: a meta-analysis. *Gynecol Oncol* 2006; **103**: 1070-1076 [PMID: 16875720 DOI: 10.1016/j.ygyno.2006.06.025]

21 **Kehoe S**, Hook J, Nankivell M, Jayson GC, Kitchener H, Lopes T, Luesley D, Perren T, Bannoo S, Mascarenhas M, Dobbs S, Essapen S, Twigg J, Herod J, McCluggage G, Parmar M, Swart AM. Primary chemotherapy versus primary surgery for newly diagnosed advanced ovarian cancer (CHORUS): an open-label, randomised, controlled, non-inferiority trial. *Lancet* 2015; **386**: 249-257 [PMID: 26002111 DOI: 10.1016/S0140-6736(14)62223-6]

22 **Luyckx M**, Leblanc E, Filleron T, Morice P, Darai E, Classe JM, Ferron G, Stoeckle E, Pomel C, Vinet B, Chereau E, Bergzoll C, Querleu D. Maximal cytoreduction in patients with FIGO stage IIIC to stage IV ovarian, fallopian, and peritoneal cancer in day-to-day practice: a Retrospective French Multicentric Study. *Int J Gynecol*

Cancer 2012; **22**: 1337-1343 [PMID: 22964527 DOI: 10.1097/IGC.0b013e31826a3559]
23 **Crawford SC**, Vasey PA, Paul J, Hay A, Davis JA, Kaye SB. Does aggressive surgery only benefit patients with less advanced ovarian cancer? Results from an international comparison within the SCOTROC-1 Trial. *J Clin Oncol* 2005; **23**: 8802-8811 [PMID: 16314640 DOI: 10.1200/JCO.2005.02.1287]
24 **Yang JY**, Yoshihara K, Tanaka K, Hatae M, Masuzaki H, Itamochi H; Cancer Genome Atlas (TCGA) Research Network, Takano M, Ushijima K, Tanyi JL, Coukos G, Lu Y, Mills GB, Verhaak RG. Predicting time to ovarian carcinoma recurrence using protein markers. *J Clin Invest* 2013; **123**: 3740-3750 [PMID: 23945238 DOI: 10.1172/JCI68509]
25 **Salani R**, Bristow RE. Surgical management of epithelial ovarian cancer. *Clin Obstet Gynecol* 2012; **55**: 75-95 [PMID: 22343231 DOI: 10.1097/GRF.0b013e31824b4629]
26 **Brand AH**. Ovarian cancer debulking surgery: a survey of practice in Australia and New Zealand. *Int J Gynecol Cancer* 2011; **21**: 230-235 [PMID: 21270606 DOI: 10.1097/IGC.0b013e318205fb4f]
27 **Redman C**, Duffy S, Bromham N, Francis K; Guideline Development Group. Recognition and initial management of ovarian cancer: summary of NICE guidance. *BMJ* 2011; **342**: d2073 [PMID: 21511784 DOI: 10.1136/bmj.d2073]
28 **Nelson RC**, Chezmar JL, Hoel MJ, Buck DR, Sugarbaker PH. Peritoneal carcinomatosis: preoperative CT with intraperitoneal contrast material. *Radiology* 1992; **182**: 133-138 [PMID: 1727276 DOI: 10.1148/radiology.182.1.1727276]
29 **Bristow RE**, Duska LR, Lambrou NC, Fishman EK, O'Neill MJ, Trimble EL, Montz FJ. A model for predicting surgical outcome in patients with advanced ovarian carcinoma using computed tomography. *Cancer* 2000; **89**: 1532-1540 [PMID: 11013368 DOI: 10.1002/1097-0142(20001001)89:7<1532::AID-CNCR17>3.0.CO;2-A]
30 **Dowdy SC**, Mullany SA, Brandt KR, Huppert BJ, Cliby WA. The utility of computed tomography scans in predicting suboptimal cytoreductive surgery in women with advanced ovarian carcinoma. *Cancer* 2004; **101**: 346-352 [PMID: 15241833 DOI: 10.1002/cncr.20376]
31 **Hynninen J**, Kemppainen J, Lavonius M, Virtanen J, Matomäki J, Oksa S, Carpén O, Grénman S, Seppänen M, Auranen A. A prospective comparison of integrated FDG-PET/contrast-enhanced CT and contrast-enhanced CT for pretreatment imaging of advanced epithelial ovarian cancer. *Gynecol Oncol* 2013; **131**: 389-394 [PMID: 23994535 DOI: 10.1016/j.ygyno.2013.08.023]
32 **Low RN**, Barone RM. Combined diffusion-weighted and gadolinium-enhanced MRI can accurately predict the peritoneal cancer index preoperatively in patients being considered for cytoreductive surgical procedures. *Ann Surg Oncol* 2012; **19**: 1394-1401 [PMID: 22302265 DOI: 10.1245/s10434-012-2236-3]
33 **Espada M**, Garcia-Flores JR, Jimenez M, Alvarez-Moreno E, De Haro M, Gonzalez-Cortijo L, Hernandez-Cortes G, Martinez-Vega V, Sainz De La Cuesta R. Diffusion-weighted magnetic resonance imaging evaluation of intra-abdominal sites of implants to predict likelihood of suboptimal cytoreductive surgery in patients with ovarian carcinoma. *Eur Radiol* 2013; **23**: 2636-2642 [PMID: 23604800 DOI: 10.1007/s00330-013-2837-7]
34 **Michielsen K**, Vergote I, Op de Beeck K, Amant F, Leunen K, Moerman P, Deroose C, Souverijns G, Dymarkowski S, De Keyzer F, Vandecaveye V. Whole-body MRI with diffusion-weighted sequence for staging of patients with suspected ovarian cancer: a clinical feasibility study in comparison to CT and FDG-PET/CT. *Eur Radiol* 2014; **24**: 889-901 [PMID: 24322510 DOI: 10.1007/s00330-013-3083-8]
35 **Thrall MM**, Goff BA, Symons RG, Flum DR, Gray HJ. Thirty-day mortality after primary cytoreductive surgery for advanced ovarian cancer in the elderly. *Obstet Gynecol* 2011; **118**: 537-547 [PMID: 21860281 DOI: 10.1097/AOG.0b013e31822a6d56]
36 **Aletti GD**, Eisenhauer EL, Santillan A, Axtell A, Aletti G, Holschneider C, Chi DS, Bristow RE, Cliby WA. Identification of patient groups at highest risk from traditional approach to ovarian cancer treatment. *Gynecol Oncol* 2011; **120**: 23-28 [PMID: 20933255 DOI: 10.1016/j.ygyno.2010.09.010]
37 **Fagotti A**, Ferrandina G, Fanfani F, Ercoli A, Lorusso D, Rossi M, Scambia G. A laparoscopy-based score to predict surgical outcome in patients with advanced ovarian carcinoma: a pilot study. *Ann Surg Oncol* 2006; **13**: 1156-1161 [PMID: 16791447 DOI: 10.1245/ASO.2006.08.021]
38 **Fagotti A**, Vizzielli G, De Iaco P, Surico D, Buda A, Mandato VD, Petruzzelli F, Ghezzi F, Garzarelli S, Mereu L, Viganò R, Tateo S, Fanfani F, Scambia G. A multicentric trial (Olympia-MITO 13) on the accuracy of laparoscopy to assess peritoneal spread in ovarian cancer. *Am J Obstet Gynecol* 2013; **209**: 462.e1-462.e11 [PMID: 23891632 DOI: 10.1016/j.ajog.2013.07.016]
39 **Fagotti A**, Vizzielli G, Fanfani F, Costantini B, Ferrandina G, Gallotta V, Gueli Alletti S, Tortorella L, Scambia G. Introduction of staging laparoscopy in the management of advanced epithelial ovarian, tubal and peritoneal cancer: impact on prognosis in a single institution experience. *Gynecol Oncol* 2013; **131**: 341-346 [PMID: 23938372 DOI: 10.1016/j.ygyno.2013.08.005]
40 **Rutten MJ**, Gaarenstroom KN, Van Gorp T, van Meurs HS, Arts HJ, Bossuyt PM, Ter Brugge HG, Hermans RH, Opmeer BC, Pijnenborg JM, Schreuder HW, Schutter EM, Spijkerboer AM, Wensveen CW, Zusterzeel P, Mol BW, Kenter GG, Buist MR. Laparoscopy to predict the result of primary cytoreductive surgery in advanced ovarian cancer patients (LapOvCa-trial): a multicentre randomized controlled study. *BMC Cancer* 2012; **12**: 31 [PMID: 22264278 DOI: 10.1186/1471-2407-12-31]
41 **Engelen MJ**, Kos HE, Willemse PH, Aalders JG, de Vries EG, Schaapveld M, Otter R, van der Zee AG. Surgery by consultant gynecologic oncologists improves survival in patients with ovarian carcinoma. *Cancer* 2006; **106**: 589-598 [PMID: 16369985 DOI: 10.1002/cncr.21616]
42 **Paulsen T**, Kjaerheim K, Kaern J, Tretli S, Tropé C. Improved short-term survival for advanced ovarian, tubal, and peritoneal cancer patients operated at teaching hospitals. *Int J Gynecol Cancer* 2006; **16** Suppl 1: 11-17 [PMID: 16515561 DOI: 10.1111/j.1525-1438.2006.00319.x]
43 **Junor EJ**, Hole DJ, McNulty L, Mason M, Young J. Specialist gynaecologists and survival outcome in ovarian cancer: a Scottish national study of 1866 patients. *Br J Obstet Gynaecol* 1999; **106**: 1130-1136 [PMID: 10549956 DOI: 10.1111/j.1471-0528.1999.tb08137.x]
44 **Earle CC**, Schrag D, Neville BA, Yabroff KR, Topor M, Fahey A, Trimble EL, Bodurka DC, Bristow RE, Carney M, Warren JL. Effect of surgeon specialty on processes of care and outcomes for ovarian cancer patients. *J Natl Cancer Inst* 2006; **98**: 172-180 [PMID: 16449677 DOI: 10.1093/jnci/djj019]
45 **Tingulstad S**, Skjeldestad FE, Hagen B. The effect of centralization of primary surgery on survival in ovarian cancer patients. *Obstet Gynecol* 2003; **102**: 499-505 [PMID: 12962932 DOI: 10.1016/S0029-7844(03)00579-9]

P- Reviewer: Hutz RJ, Peng Y, Wan TTH **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.203

World J Methodol 2015 December 26; 5(4): 203-211
ISSN 2222-0682 (online)

MINIREVIEWS

Helicobacter pylori and allergy: Update of research

Ilva Daugule, Jelizaveta Zavoronkova, Daiga Santare

Ilva Daugule, Jelizaveta Zavoronkova, Daiga Santare, Faculty of Medicine, University of Latvia, LV1586 Riga, Latvia

Author contributions: Daugule I conceived and designed the review, made data analysis and interpretation and wrote the paper; Zavoronkova J made data acquisition and classification; Santare D drafted the article, and made critical revisions.

Conflict-of-interest statement: There is no conflict of interest.

Correspondence to: Ilva Daugule, MD PhD, Faculty of Medicine, University of Latvia, Raina bulvaris 19, LV1586 Riga, Latvia. ilva_daugule@hotmail.com
Telephone: +371-26-320374
Fax: +371-67-034369

Received: May 11, 2015
Peer-review started: May 12, 2015
First decision: June 18, 2015
Revised: September 18, 2015
Accepted: October 16, 2015
Article in press: October 19, 2015
Published online: December 26, 2015

Abstract

Recently a lot of literature has been published about the possible preventive action of *Helicobacter pylori* (*H. pylori*) against allergy. The present review summarizes research data about the association between *H. pylori* and allergic diseases, as well as discusses possible hypotheses about the preventive action of *H. pylori* against atopy. There is evidence from observational studies to support a weak inverse association between prevalence of *H. pylori* infection and allergy. However, confounders like some unidentified socioeconomic factors, antibiotic use and others could bias the association. Although data from cohort studies point to a possible association of *H. pylori* with some of the allergic diseases, no definite proof for causal relationship has been clearly demonstrated yet. A biological mechanism proposed to explain the preventive action of *H. pylori* to allergy is reduced exposure to a major stimulus for the generation of Treg cells in individuals without *H. pylori* infection. In addition, *H. pylori* could be an indicator for changes in gut microbiome, reflecting the complex interaction between microbes and immune system.

Key words: *Helicobacter pylori*; Allergy; Atopy

Core tip: Review summarizes research data about the association between *Helicobacter pylori* (*H. pylori*) and allergic diseases. Results from observational studies support a weak inverse association between prevalence of *H. pylori* and allergy. However, different confounders like unidentified socioeconomic factors, antibiotic use and others could bias the observed association. Further, no definite proof for causal relationship has been clearly demonstrated yet, although data from cohort studies point to a possible association of *H. pylori* with some of the allergic diseases. Finally, microbiological studies show that *H. pylori* could be an indicator for changes in gut microbiome during recent decades, reflecting the complex interaction between microbes and immune system.

Daugule I, Zavoronkova J, Santare D. *Helicobacter pylori* and allergy: Update of research. *World J Methodol* 2015; 5(4): 203-211 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/203.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.203

INTRODUCTION

Although *Helicobacter pylori* (*H. pylori*) infection is supposed to be associated with gastric and duodenal

ulcer, gastric adenocarcinoma, MALT lymphoma and even recognized as grade-1 carcinogen[1-4], only minority of infected patients will develop a serious disease. Moreover, some researchers even suggest possible preventive effect of *H. pylori* against several diseases like gastro-esophageal reflux disease and Barret's adenocarcinoma, obesity, autoimmune diseases, allergy and others[5,6].

The latest Maastricht consensus states that the evidence available shows no definite causative protective effect of *H. pylori* against asthma and atopy nor that its eradication causes or worsens them and further research is needed[7]. Thus, new data appear constantly about the possible role of bacterium in the development of allergic diseases. The present review summarizes research data about the association between *H. pylori* and allergic diseases.

EVIDENCE FOR OPPOSITE PREVALENCE TREND BETWEEN *H. PYLORI* AND ALLERGIC DISEASES

The idea about a possible protective role of *H. pylori* against allergy has arisen observing opposite prevalence trends between *H. pylori* and allergic diseases, showing that the prevalence of *H. pylori* in industrialized countries is decreasing while the prevalence of asthma and other allergic diseases is increasing[6].

Ironically, the first case-control studies showed a positive association between *H. pylori* infection and allergy. For example, in 1998 a study from Italy identified higher prevalence of *H. pylori* IgG antibodies among allergic patients compared to patients with inflammatory bowel disease[8]. Further, Figura *et al*[9] identified higher prevalence of anti-CagA antibodies in *H. pylori* infected persons with food allergy compared to controls (62.5% *vs* 28.0%, respectively; $P = 0.03$). In addition, the mean IgE level to the most common alimentary antigens was increased in CagA-positive individuals compared to CagA-negative patients. This made the authors suggest that mucosal and inflammatory lesions found in individuals infected with CagA-positive *H. pylori* strains could increase the epithelial permeability and promote the passage of allergens, which, in atopic persons, could directly stimulate an IgE response.

Higher prevalence of *H. pylori* among patients with allergic diseases observed in some studies raised question about the role of inflammation (observed in the presence of *H. pylori* infection) in the development of allergy. A positive association between *H. pylori* and allergic diseases is still being discussed in respect to urticaria. Moreover, International guidelines in Urticaria state that in some cases of chronic spontaneous urticaria eradication of infections, such as *H. pylori*, bowel parasites and bacterial infections of the nasopharynx, have shown to provide a benefit in the management of the disease[10].

One of the first well designed and controlled studies showing opposite prevalence trends between *H. pylori* and atopy comes from Finland, demonstrating 3.5-fold increase of total and allergen-specific IgE antibody level in random population from 1973 to 1994 (OR = 5.12, 95%CI: 2.32-11.3)[11]. However, increase of IgE was observed mainly in the subgroup with no *H. pylori* antibodies, thus raising the hypothesis that *H. pylori* could influence the development of atopic diseases[11].

A huge study from United States based on database containing information about asthma and *H. pylori* status in 7663 subjects showed an inverse association between cag-positive *H. pylori* strain and asthma (OR = 0.79, 95%CI: 0.63-0.99), with a stronger association in younger individuals[12]. An inverse association was found also with other allergic disorders (allergic rhinitis and sensitization to different allergens). Further, the authors tested the association in children up till 20 years of age ($n = 7412$) and again found inverse association with wheezing, allergic rhinitis and eczema[13].

Up to now many cross-sectional and case-control studies have been performed, thus, showing controversial results. Results are summarized in Tables 1 and 2.

Basing on the published studies, several meta-analyses have tested the association. Although in 2012 Wang *et al*[14] demonstrated no association between asthma and *H. pylori* infection, analyzing five studies with 770 cases and 785 controls (OR = 1.1, 95%CI: 0.82-1.24), another meta-analysis by the same authors showed pooled OR of all included studies (nine cross-sectional, seven case-control and three cohort studies) for asthma and *H. pylori* to be 0.81 (95%CI: 0.72-0.91); while pooled OR for asthma and *H. pylori* infection in cross-sectional studies was 0.84 (95%CI: 0.74-0.96), in case-control studies - 0.94 (95%CI:79-1.12)[15].

Similarly, Zhou *et al*[16] analyzing 14 studies with 28283 persons demonstrated a weak inverse association between *H. pylori* and asthma 0.84 (95%CI: 0.73-0.96). Taye *et al*[17] have performed meta-analysis of 16 studies ($n = 21348$) about the association of atopy with *H. pylori.* The authors found an inverse association with atopy (OR = 0.82, 95%CI: 0.73-0.91) as well as with increased level of specific IgE (OR = 0.75, 95%CI: 0.62-0.92).

Detailed overview about case-control and cross-sectional studies, as well as meta-analysis of studies, has been recently published by Lionetti *et al*[18]. The authors concluded that pooled results of case-control studies showed a significant inverse association of *H. pylori* infection with atopy/allergic disease (or with atopy, but not with allergic disease), while pooled results of cross-sectional studies showed only a significant association between allergic disease and *H. pylori* infection.

However, the analysis and comparison of studies is complicated and should be evaluated with caution due to differences among study designs. The authors of the meta-analysis argue that different diagnostic criteria for allergic disease and atopy are used - in some studies asthma was diagnosed by physician tests or by symptoms, while in others the authors evaluated self-reported disease or used only laboratory tests[18].

Table 1 Association between *Helicobacter pylori* and allergy in cross-sectional studies

Ref.	Country	Studied population	*n*	Age (yr)	*H. pylori* detection	Allergy diagnosis	Main finding: OR (95%CI) in relation to *H. pylori*
Lee *et al*[35]	South Korea	Routine check-up	3376	Adults	IgG	Physician diagnosed allergy; use of anti-allergic medication; IgE	No association with allergic disease: 1.05 (0.86-1.28); Inverse association with IgE hypersensitivity: 1.32 (0.98-1.31)
Zevit *et al*[46]	Israel	National referral laboratory	6959	5-18	UBT	Physician diagnosed asthma; use of anti-allergic medication	Inverse association with asthma: 0.82 (0.69-0.08)
Imamura *et al*[47]	Japan	Healthy volunteers	211	Adults	IgG	Specific IgE, polinosis symptoms	Inverse association with polinosis: 0.15 (0.05-0.48)
Fullerton *et al*[21]	United Kingdom	General population	2437	Adults	IgG	Symptoms of wheeze, hay fever; lung function tests; bronchial reactivity; SPT; IgE	No association with asthma: 1.09 (0.77-1.54), atopy: 0.92 (0.74-1.15); hay fever: 1.00 (0.79-1.26), wheeze: 0.94 (0.74-1.19)
Pfefferle *et al*[48]	Germany	Employees of two companies	500	Adults	SAT	Self-reported physician diagnosed allergy, use of anti-allergic medication specific skin sensitization	Inverse association with allergy diagnosis: 0.26 (0.08-0.84)
Chen *et al*[13]	United States	Data from health and nutrition examination survey	7412	3-19	IgG; CagA	Self-reported asthma	Inverse association with asthma: 0.69 (0.45-1.06) Subgroup < 5 yr: 0.58 (0.38-0.88); 3-13 yr: 0.14 (0.24-0.69)
Shiotani *et al*[49]	Japan	University students	1953	Adults	IgG	Self-reported atopic dermatitis, asthma, allergic rhinitis, urticaria	Inverse association with allergic diseases: 0.49 (0.27-0.89)
Baccioglu *et al*[50]	Turkey	Patients with upper gastrointestinal endoscopy	90	Adults	Gastric tissue microscopy	SPT, total IgE, questionnaire	No association with allergic disease: 1.0 (0.1-18.9) Inverse association with asthma/CagA$^+$ cases: 0.79 (0.63-0.99);
Chen *et al*[12]	United States	Data from health and nutrition examination survey	7663	Adults	CagA	Self-reported asthma, allergen-specific skin sensitization	Inverse association with allergic rhinitis/CagA$^+$: 0.77 (0.62-0.96)
Herbarth *et al*[51]	Germany	School starters	3347	5-7	UBT	Eczema	Inverse association with eczema: OR 0.31
Kolho *et al*[52]	Finland	Patients with upper gastrointestinal endoscopy	97	5-15	Histology data	Specific IgE	No association with IgE, asthma, hay fever;
Jarvis *et al*[20]	United Kingdom	Health service registry	1121	Adults	IgG	Specific IgE, symptoms	Inverse association with sensitization to grass: 0.65 (0.43-0.99)
Uter *et al*[53]	Germany	University students	1368	18-20	IgG	Physician diagnosed asthma	No association with asthma: 0.99 (0.57-1.64)
McCune *et al*[54]	United Kingdom	Community-based population	3244	Adults	UBT	Use of asthma medication	Inverse association with asthma: 0.78 (0.59-1.05)

H. pylori: *Helicobacter pylori*; UBT: 13C-urea breath test; SAT: Stool antigen test; SPT: Skin prick test.

Further, difference between detection of active infection by urea breath test or stool antigen test and detection of *H. pylori* antibodies should be noted. In addition, the age of study population should also be taken into account since the time between *H. pylori* colonization and allergen sensitization is difficult to evaluate, therefore *H. pylori* negative adult patient could have been colonized since childhood and *vice versa*[18].

Therefore we could conclude, that, although evidence from observational studies show an inverse association between allergic disease and *H. pylori,* the association is weak and not consistent.

IS *H. PYLORI* TRULY INDEPENDENTLY INVERSELY ASSOCIATED WITH ALLERGIC DISEASE?

The idea about the inverse association of *H. pylori* with allergic diseases has been strongly criticized arguing, that *H. pylori* could be merely a marker of socio-economic status, known to be also associated with allergic disease[19].

Although Blaser *et al*[6] report, that the inverse association between *H. pylori* and asthma was observed independent of socioeconomic status, age, gender,

Table 2 Association between *Helicobacter pylori* and allergy in case-control studies

Ref.	Country	Cases (*n*)	Controls (*n*)	Age (yr)	*H. pylori* detection	Allergy diagnosis	Main finding: OR (95%CI) in relation to *H. pylori*$^+$
Pedullà *et al*[55]	Italy	Food allergy + atopic dermatitis (88)	Atopic dermatitis (202)	2-11.8	IgG, SAT	Physician diagnosed food allergy, IgE	Inverse association with food allergy: 0.32 (0.11-0.95)
Elitsur *et al*[56]	United States	Eosinophil esophagitis (62)	Esophagitis (268); idiopathic gastritis (480)	Children	Histology data	Upper endoscopy, histology data	Inverse association with eosinophil esophagitis: 0.096 (013-0.72)
Karimi *et al*[57]	Iran	Asthma (98)	Healthy children (98)	6-12	UBT	Physician diagnosed asthma	No association with asthma: *H. pylori* positivity 18% (cases) *vs* 23% (controls)
Reibman *et al*[58]	United States	Asthma (318)	Non-asthma controls (208)	Adults	IgG, CagA	Physician diagnosed asthma; IgE; spirometry	Inverse association with asthma for CagA$^+$ cases: 0.57 (0.36-0.89)
Konturek *et al*[59]	Germany	Food allergy (42)	Healthy controls (20)	Adults	UBT, IgG	Physician diagnosed food allergy; SPT, IgE, N-tele-methylhistamine urinary excretion	Inverse association with food allergy: *H. pylori* positivity 33% (cases) *vs* 40%(controls)
Annagür *et al*[60]	Turkey	Asthma (79)	Healthy children (36)	5-15	IgM and IgG	Pulmonary function tests, SPT, total IgE	No association with asthma: 1.69 (0.62-4.67)
Jaber *et al*[61]	Sauda Arabia	Asthma (220)	Asymptomatic children (543)	1-10	IgG	Physician diagnosed asthma	Inverse association with asthma: 0.84 (0.56-1.25)
Jun *et al*[62]	Japan	Asthma (46)	Peptic ulcer patients (48) + healthy controls (48)	Adults	IgG, CagA	Physician diagnosed asthma	No association with asthma: 1.20 (0.53-2.72)
Pessi *et al*[63]	Finland	Asthma (245)	Matched controls (405)	Adults	IgG	Physician diagnosed asthma	Inverse association with asthma: 0.86 (0.63-1.19)
Bartuzi *et al*[64]	Poland	Food allergy with GI symptoms (110)	Chronic gastritis (40)	Adults	Biopsy, histology	Physician diagnosed food allergy, IgE	In atopic patients *H. pylori* increases intensity of gastric inflammation
Tsang *et al*[65]	China	Asthma (90)	Healthy controls (97)	Adults	IgG	Physician diagnosed asthma	No association with asthma: 1.55 (0.87-2.78)
Corrado *et al*[66]	Italy	Atopic dermatitis (30) + atopic dermatitis with GI symptoms (30)	Asthma (30)	4-12	IgG; CagA	Physician diagnosed allergy	Positive association with atopic dermatitis compared to asthma: 56% and 37% (cases) *vs* 10% (controls)
Matricardi *et al*[67]	Italy	Atopic cases (240)	Non-atopic controls (240)	17-24	IgG	Physician diagnosed allergic rhinitis and asthma; IgE	Inverse association with atopy: 0.76 (0.47-1.24)
Figura *et al*[9]	Italy	Food allergy (38)	Matched controls (53)	4-12	IgG, CagA	Physician diagnosed food allergy; IgE	Positive association with food allergy in CagA$^+$ cases: 4.29
Corrado *et al*[8]	Norway	Food allergy (30) + asthma (30)	Inflammatory bowel disease (30)	5-14	IgG, CagA	Physician diagnosed food allergy and asthma	Positive association: 37% (cases) *vs* 10% controls

H. pylori: *Helicobacter pylori*; UBT: ^{13}C-urea breath test; SAT: Stool antigen test; SPT: Skin prick test; GI: Gastrointestinal.

ethnic background, smoking status, and hepatitis A infection, several studies indirectly suggest that other factors could influence the opposite prevalence trends and could play a role in the development of allergy.

For example, Jarvis *et al*[20] showed no association between presence of *H. pylori* antibodies and night cough, hay fever, wheezing within last 12 mo as well as sensitization to five allergens, after adjusting the patient sample for age, gender, area, number of siblings, social class. In addition, the authors observed a marked negative association of both hepatitis A and *H. pylori* with family size only in seropositive individuals (but not in those who were seronegative). This made authors suggest that those without either infection are likely to have grown up in hygienic environments, possibly less overcrowded and with a better diet.

Similarly, a well-designed cross sectional study from United Kingdom (also controlled for social class) could identify only lower lung function in individuals with *H. pylori* seropositivity. However, after adjustment for either height or social class the size of the association was reduced. No association was observed with wheezing, chronic bronchitis, self-reported asthma, atopy or bronchial hyper-reactivity[21].

No association with a group of infections was observed among Roma children living in poor hygienic

conditions compared to non-Roma children in Greece[22]. Although Roma children were found significantly more often seropositive for *Toxoplasma gondii*, hepatitis A, *H. pylori*, herpes simplex virus-1 (HSV-1), cytomegalovirus and Hepatitis B, no statistically significant differences were found between Roma and non-Roma children in respect to atopy or specific IgE level. Despite the higher numbers of exposure to infectious agents among Roma children, no protective effect for allergic disease development was evident. Even more, a positive association of the cumulative index of exposure to infections with atopy was found in the non-Roma children (OR = 1.38, 95%CI: 1.08-1.75) and in the total population (OR = 1.42, 95%CI: 1.11-1.83).

An interesting study on schoolchildren with similar genetic background but different socioeconomic environment (Finland and Russian Karelia) showed higher prevalence of allergen-specific IgE in Finnish children, while in Russian children higher prevalence of antibodies to *coxsackivirus B4*, *H. pylori, Toxoplasma gondi* and hepatitis A was detected. However, an inverse association between infections and prevalence of atopy was observed only in Russian Karelian children and the biggest effect was observed for enterovirus. However, the authors also hypothised that some other factors could be associated with infections in Russian but not in Finnish populations are responsible for the effect[23].

Finally, in Malaysia low *H. pylori* prevalence goes together with low prevalence of wheezing among 6-7 and 13-14 years old children (5.4% and 5.7%, respectively)[24], therefore scientists have concluded that *H. pylori* is only a marker for poor hygiene[25]. Although no study has been performed yet comparing the prevalence of *H. pylori* among patients with and without asthma in Malaysia, Raj *et al*[26] consider that available data speak against the unique role for *H. pylori* infection as a protective factor against asthma.

To summarize, there is evidence that *H. pylori* could not be independently inversely associated with allergic disease, but just reflect changes in environment and/or diet. The inverse association between prevalence of *H. pylori* infection and allergic diseases observed in studies could also be biased by some other uncontrolled factors.

POSSIBLE CAUSAL RELATIONSHIP BETWEEN *H. PYLORI* AND ALLERGIC DISEASE

Although a weak inverse association between *H. pylori* and allergy can be recognized, scientists argue that opposite prevalence, possibly evident from observational studies, does not mean a causal relationship[19]. However, demonstration of a possible causal relationship between *H. pylori* and allergy is extremely complicated.

A biological mechanism proposed to explain the preventive association of *H. pylori* to allergy is reduced exposure to a major stimulus for the generation of Treg cells in individuals without *H. pylori* infection[27]. One of the latest ideas involves neutrophil-activating protein of *H. pylori* that could inhibit Th2-mediated bronchial inflammation in patients with allergic asthma[5]. Possible immunomodulatory properties of *H. pylori* are well described by Arnold *et al*[28].

Fulfilment of Bradford Hill criteria

Blaser *et al*[6] used Bradford Hill criteria to support the evidence about the inverse association between *H. pylori* and asthma. Hill's criteria consist of several conditions fulfillment of which can provide evidence of a causal relationship between an incidence (*H. pylori* prevalence) and a possible consequence (asthma)[29].

To prove the causal link Blaser *et al*[6] mentioned the small but consistent trend demonstrated in several studies as well as the fact, that inverse causation is not likely and the decline is preceding the increase in asthma. However, although there is a weak trend showing inverse association between allergy and *H. pylori,* not all studies approve it. Further, Blaser *et al*[6] considered that the inverse association observed with early life asthma (not with long-standing asthma seen in adults) supported the role of *H. pylori,* since the effect of *H. pylori* might be less important in adult-onset asthma due to much more heterogeneous nature of adult asthma. However, confounding factors that could influence the association are not fully ruled out.

Further, one of the Bradford criteria states that there is no other likely explanation of disease - the more specific an association between a factor and an effect is, the bigger the probability of a causal relationship. However, at present allergologists consider asthma as a multifactorial disease associated with several other risk factors (like urban outdoor and indoor pollution, allergens, *etc.*) rather than *H. pylori* infection[30]. Therefore the fulfillment of Bradford Hill's criteria, demonstrated by Blaser *et al*[6], should be interpreted with caution and should be considered only as one of the arguments for protection of *H. pylori* against asthma.

Data from cohort studies

Since it is impossible to perform interventional studies to test the link between *H. pylori* and allergy, some knowledge about a possible causal association could be driven from cohort studies. However, it should be noted that such studies are not conclusive and they give only a better insight about a possible causality.

Holster *et al*[31] detected the presence of *H. pylori* antibodies in 7-9 years old children who were followed from birth and assessed by yearly questionnaires about allergic symptoms and possible risk factors. The authors observed no association between *H. pylori* and atopic dermatitis, allergic rhinitis and asthma. A borderline association was found only between *H. pylori* and wheezing. However, the authors admit, that they were not able to detect if *H. pylori* infection preceded the diagnosis of allergic disease, since presence of *H. pylori* infection was diagnosed only at the age of 7-9 years.

Further, a cohort study in Ethiopia followed children

since birth, detecting presence of allergic symptoms with questionnaires and performing allergic skin tests and *H. pylori* stool antigen tests at the age of one, there and five years. The sample was controlled for potential confounders. After three year follow-up the authors found only a borderline association with eczema[32]. Further, following the same cohort for five years an inverse association was observed only with eczema[33]. No association was observed with asthma or other allergic disease. Interestingly, that in the same cohort the association between paracetamolum therapy and allergic symptoms was analyzed separately and an inverse association was observed between use of paracetamolum and wheezing and eczema[34].

Development of allergy after H. pylori eradication

Several studies demonstrated development of an allergic disease or increase of IgE after *H. pylori* eradication. Korean study demonstrated increased levels of IgE related, non IgE related allergy as well as subclinical raise of IgE levels in patients after *H. pylori* eradication compared to *H. pylori* positive patients without eradication and *H. pylori* negative controls[35]. However, this could also be related to the change in gastric acidity due to treatment with proton pump inhibitors, used together with eradication therapy. In addition, some patients continue use of acid lowering drugs even after eradication therapy.

Data from animal studies

Finally, a possible causal relationship can be demonstrated in animal models. One of the first studies showing causal relationship was the study by Arnold *et al*[36], showing that animals infected with *H. pylori* infection had lower airway hyper-responsiveness, tissue inflammation, and goblet cell metaplasia. Further studies supported the finding that *H. pylori* infection could protect mice from development of allergic asthma[37]. However, effect observed in animal models quite often is not observed also in humans.

H. PYLORI AS A PART OF COMPLEX INTERACTION BETWEEN MICROBES AND HUMAN IMMUNE SYSTEM

H. pylori and other infectious agents

Blaser *et al*[6] speculate, that *H. pylori* could be merely a marker for other phenomena, for example, early life antibiotic use could eliminate *H. pylori* as well as other microbes that actually could be the protective agents. Therefore, the question arises if *H. pylori per se* plays the crucial role in the development of allergy or it is just a marker of frequent infections or other factors, since several other microbes have been shown to be inversely associated with allergic disease[38].

This could be indirectly supported by study, showing that seropositivity to *H. pylori* and Hepatitis A was unrelated to atopic status, while multivariate analysis showed that both the effect of having two or more younger siblings (OR = 0.1, 95%CI: 0.03-0.8) and of acquiring measles up to the age of three (OR = 0.2, CI: 0.03-0.8) were significantly related to a lower risk of asthma[39]. The finding indicates that frequent infections observed more often in families with siblings are more important than *H. pylori i*nfection *per se*. Further, Janson *et al*[40] demonstrated that combination of different infectious agents [hepatitis A, *H. pylori*, *Toxoplasmosis gondii*, HSV, Chlamydia pneumoniae, Ebstein Barr virus (EBV) and cytomegalovirus] was an independent risk factor for atopy (OR = 1.43, 95%CI: 1.06-1.93), allergic asthma (OR = 1.82, 95%CI: 1.12-2.98), and allergic rhinitis (OR = 1.69, 95%CI: 1.21-2.37).

Importance of several pathogens (*Ascaris lumbricoides, T. gondii,* HSV and EBV) for prevention of atopy has been shown in a study by Alcantara-Neves *et al*[41]: Children with three or fewer infection markers had a higher prevalence of specific IgE and skin prick test reactivity compared with those with four or more infection markers. On the contrary, isolated infections were not associated with the prevalence of atopic or non-atopic wheeze.

Therefore, evidence from studies suggests that *H. pylori* could be just a part of complex interaction between immune system and pathogens, as proposed by Janson *et al*[40].

H. pylori as a part of gut microbiome

This goes together with the idea that the increase in allergic diseases could be caused by changes in the composition of gut microflora due to global changes of environmental, socioeconomic and life style factors[27]. Data exist, showing lower diversity of microflora in allergic patients compared to healthy controls: A study in Sweden reports a lower diversity of the total microbiota at one month in infants with IgE-associated eczema[42].

Although previously *H. pylori* was considered as the major inhabitant of stomach, at present up-to-date sequence based molecular methods have allowed identifying gastric microbiota more precisely. von Rosenvinge *et al*[43] have shown that such phyla as *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria* and *Fusobacteria* dominate in gastric fluid samples. In a review Engstrand *et al*[44] have also summarized that gastric micro-biota contains a large variety of genera including *Staphylococcus, Streptococcus*, *Pervotella, Lactobacillus* and some others, therefore *H. pylori* could be considered only a part of a complex microbial flora in the stomach.

Further, Rosenvinge has identified that treatment with proton pump inhibitors promotes bacterial overgrowth, while antibacterial treatment is associated with reduced bacterial diversity[43]. Decreased microbiota diversity in patients after *H. pylori* eradication therapy has been identified also by Jakobsson *et al*[45]. Therefore, one can conclude, that after *H. pylori* eradication the diversity of gastric microflora decreases that could possibly be

associated with development of allergy.

Therefore we could hypothise that loss of *H. pylori* results in a small (possibly significant) reduction of stimulation of immune system, as proposed by other authors[44]. Importance of other microorganisms should also be considered in the complex interaction between the human immune system and microbes. However, how *H. pylori* specifically and the entire human microbial ecosystem affect human health is still questionable.

CONCLUSION

Evidence from observational studies supports a weak inverse association between prevalence of *H. pylori* infection and allergy. However, it could be biased by confounders like socioeconomic factors, antibiotic use and others. No definite proof for causal relationship has been clearly demonstrated yet, although data from cohort studies point to a possible association of *H. pylori* with some allergic diseases. In addition, *H. pylori* could be an indicator for changes in gut microbiome during recent decades, reflecting the complex interaction between microbes and immune system.

Summarizing the data, it seems that *H. pylori* infection alone cannot prevent development of allergy in all infected individuals, similarly like bacterium is not causing a serious gastrointestinal disease in all infected patients. In both conditions genetic and environmental factors (diet, other microbes, microflora, *etc.*) are of importance next to the recognized role of the bacterium.

Nevertheless, the intensive research in *H. pylori* field has brought a new insight into the interaction between microbes and immune system and the microbial - host relationship, supporting the idea that microbes could play a role in the development of allergy.

REFERENCES

1 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241 [PMID: 7715068]

2 **Parsonnet J**, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med* 1994; **330**: 1267-1271 [PMID: 8145781 DOI: 10.1056/NEJM199405053301803]

3 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131 [PMID: 1891020 DOI: 10.1056/NEJM199110173251603]

4 **Peterson WL**. Helicobacter pylori and peptic ulcer disease. *N Engl J Med* 1991; **324**: 1043-1048 [PMID: 2005942 DOI: 10.1056/NEJM199104113241507]

5 **Pacifico L**, Osborn JF, Tromba V, Romaggioli S, Bascetta S, Chiesa C. Helicobacter pylori infection and extragastric disorders in children: a critical update. *World J Gastroenterol* 2014; **20**: 1379-1401 [PMID: 24587617 DOI: 10.3748/wjg.v20.i6.1379]

6 **Blaser MJ**, Chen Y, Reibman J. Does Helicobacter pylori protect against asthma and allergy? *Gut* 2008; **57**: 561-567 [PMID: 18194986 DOI: 10.1136/gut.2007.133462]

7 **Malfertheiner P**, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012; **61**: 646-664 [PMID: 22491499 DOI: 10.1136/gutjnl-2012-302084]

8 **Corrado G**, Luzzi I, Lucarelli S, Frediani T, Pacchiarotti C, Cavaliere M, Rea P, Cardi E. Positive association between Helicobacter pylori infection and food allergy in children. *Scand J Gastroenterol* 1998; **33**: 1135-1139 [PMID: 9867089]

9 **Figura N**, Perrone A, Gennari C, Orlandini G, Giannace R, Lenzi C, Vagliasindi M, Bianciardi L, Rottoli P. CagA-positive Helicobacter pylori infection may increase the risk of food allergy development. *J Physiol Pharmacol* 1999; **50**: 827-831 [PMID: 10695562]

10 **Zuberbier T**. A Summary of the New International EAACI/GA2-LEN/EDF/WAO Guidelines in Urticaria. *World Allergy Organ J* 2012; **5** Suppl 1: S1-S5 [PMID: 23282889 DOI: 10.1097/WOX.0-b013e3181f13432]

11 **Kosunen TU**, Höök-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to Helicobacter pylori infections. *Clin Exp Allergy* 2002; **32**: 373-378 [PMID: 11940066]

12 **Chen Y**, Blaser MJ. Inverse associations of Helicobacter pylori with asthma and allergy. *Arch Intern Med* 2007; **167**: 821-827 [PMID: 17452546 DOI: 10.1001/archinte.167.8.821]

13 **Chen Y**, Blaser MJ. Helicobacter pylori colonization is inversely associated with childhood asthma. *J Infect Dis* 2008; **198**: 553-560 [PMID: 18598192 DOI: 10.1086/590158]

14 **Wang Y**, Bi Y, Zhang L, Wang C. Is Helicobacter pylori infection associated with asthma risk? A meta-analysis based on 770 cases and 785 controls. *Int J Med Sci* 2012; **9**: 603-610 [PMID: 23028243 DOI: 10.7150/ijms.4970]

15 **Wang Q**, Yu C, Sun Y. The association between asthma and Helicobacter pylori: a meta-analysis. *Helicobacter* 2013; **18**: 41-53 [PMID: 23067334 DOI: 10.1111/hel.12012]

16 **Zhou X**, Wu J, Zhang G. Association between Helicobacter pylori and asthma: a meta-analysis. *Eur J Gastroenterol Hepatol* 2013; **25**: 460-468 [PMID: 23242126 DOI: 10.1097/MEG.0b013e32835c280a]

17 **Taye B**, Enquselassie F, Tsegaye A, Medhin G, Davey G, Venn A. Is Helicobacter Pylori infection inversely associated with atopy? A systematic review and meta-analysis. *Clin Exp Allergy* 2015; **45**: 882-890 [PMID: 25207960 DOI: 10.1111/cea.12404]

18 **Lionetti E**, Leonardi S, Lanzafame A, Garozzo MT, Filippelli M, Tomarchio S, Ferrara V, Salpietro C, Pulvirenti A, Francavilla R, Catassi C. Helicobacter pylori infection and atopic diseases: is there a relationship? A systematic review and meta-analysis. *World J Gastroenterol* 2014; **20**: 17635-17647 [PMID: 25516679 DOI: 10.3748/wjg.v20.i46.17635]

19 **Graham DY**. Helicobacter pylori update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 2015; **148**: 719-31.e3 [PMID: 25655557 DOI: 10.1053/j.gastro.2015.01.040]

20 **Jarvis D**, Luczynska C, Chinn S, Burney P. The association of hepatitis A and Helicobacter pylori with sensitization to common allergens, asthma and hay fever in a population of young British adults. *Allergy* 2004; **59**: 1063-1067 [PMID: 15355464 DOI: 10.1111/j.1398-9995.2004.00539.x]

21 **Fullerton D**, Britton JR, Lewis SA, Pavord ID, McKeever TM, Fogarty AW. Helicobacter pylori and lung function, asthma, atopy and allergic disease--a population-based cross-sectional study in adults. *Int J Epidemiol* 2009; **38**: 419-426 [PMID: 19109248 DOI: 10.1093/ije/dyn348]

22 **Michos A**, Terzidis A, Kanariou M, Kalampoki V, Koilia C, Giannaki M, Liatsis M, Pangalis A, Petridou E. Association of allergic sensitization with infectious diseases burden in Roma and non-Roma children. *Pediatr Allergy Immunol* 2011; **22**: 243-248 [PMID: 20573034 DOI: 10.1111/j.1399-3038.2010.01086.x]

23 **Seiskari T**, Viskari H, Kaila M, Haapala AM, Koskela P, Hyöty H. Time trends in allergic sensitisation and Helicobacter pylori prevalence in Finnish pregnant women. *Int Arch Allergy Immunol* 2009; **150**: 83-88 [PMID: 19339806 DOI: 10.1159/000210384]

24 **Quah BS**, Wan-Pauzi I, Ariffin N, Mazidah AR. Prevalence of asthma, eczema and allergic rhinitis: two surveys, 6 years apart, in Kota Bharu, Malaysia. *Respirology* 2005; **10**: 244-249 [PMID:

15823193 DOI: 10.1111/j.1440-1843.2005.00645.x]
25 **Lee YY**, Mahendra Raj S, Graham DY. Helicobacter pylori infection--a boon or a bane: lessons from studies in a low-prevalence population. *Helicobacter* 2013; **18**: 338-346 [PMID: 23607896 DOI: 10.1111/hel.12058]
26 **Raj SM**, Choo KE, Noorizan AM, Lee YY, Graham DY. Evidence against Helicobacter pylori being related to childhood asthma. *J Infect Dis* 2009; **199**: 914-915; author reply 915-916 [PMID: 19434934]
27 **Harris PR**, Smythies LE, Smith PD, Perez-Perez GI. Role of childhood infection in the sequelae of H. pylori disease. *Gut Microbes* 2013; **4**: 426-438 [PMID: 24275060 DOI: 10.4161/gmic.26943]
28 **Arnold IC**, Hitzler I, Müller A. The immunomodulatory properties of Helicobacter pylori confer protection against allergic and chronic inflammatory disorders. *Front Cell Infect Microbiol* 2012; **2**: 10 [PMID: 22919602 DOI: 10.3389/fcimb.2012.00010]
29 **Hill AB.** The Environment and Disease: Association or Causation? *Proc R Soc Med* 1965; **58**: 295-300 [PMID: 14283879]
30 **Subbarao P**, Mandhane PJ, Sears MR. Asthma: epidemiology, etiology and risk factors. *CMAJ* 2009; **181**: E181-E190 [PMID: 19752106 DOI: 10.1503/cmaj.080612]
31 **Holster IL**, Vila AM, Caudri D, den Hoed CM, Perez-Perez GI, Blaser MJ, de Jongste JC, Kuipers EJ. The impact of Helicobacter pylori on atopic disorders in childhood. *Helicobacter* 2012; **17**: 232-237 [PMID: 22515362 DOI: 10.1111/j.1523-5378.2012.00934.x]
32 **Amberbir A**, Medhin G, Erku W, Alem A, Simms R, Robinson K, Fogarty A, Britton J, Venn A, Davey G. Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. *Clin Exp Allergy* 2011; **41**: 1422-1430 [PMID: 21831135 DOI: 10.1111/j.1365-2222.2011.03831.x]
33 **Amberbir A**, Medhin G, Abegaz WE, Hanlon C, Robinson K, Fogarty A, Britton J, Venn A, Davey G. Exposure to Helicobacter pylori infection in early childhood and the risk of allergic disease and atopic sensitization: a longitudinal birth cohort study. *Clin Exp Allergy* 2014; **44**: 563-571 [PMID: 24528371 DOI: 10.1111/cea.12289]
34 **Amberbir A**, Medhin G, Hanlon C, Britton J, Davey G, Venn A. Effects of early life paracetamol use on the incidence of allergic disease and sensitization: 5 year follow-up of an Ethiopian birth cohort. *PLoS One* 2014; **9**: e93869 [PMID: 24718577 DOI: 10.1371/journal.pone.0093869]
35 **Lee SP**, Lee SY, Kim JH, Sung IK, Park HS, Shim CS, Moon HW. Correlation between Helicobacter pylori infection, IgE hypersensitivity, and allergic disease in Korean adults. *Helicobacter* 2015; **20**: 49-55 [PMID: 25257099 DOI: 10.1111/hel.12173]
36 **Arnold IC**, Dehzad N, Reuter S, Martin H, Becher B, Taube C, Müller A. Helicobacter pylori infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J Clin Invest* 2011; **121**: 3088-3093 [PMID: 21737881 DOI: 10.1172/JCI45041]
37 **Engler DB**, Reuter S, van Wijck Y, Urban S, Kyburz A, Maxeiner J, Martin H, Yogev N, Waisman A, Gerhard M, Cover TL, Taube C, Müller A. Effective treatment of allergic airway inflammation with Helicobacter pylori immunomodulators requires BATF3-dependent dendritic cells and IL-10. *Proc Natl Acad Sci USA* 2014; **111**: 11810-11815 [PMID: 25074917 DOI: 10.1073/pnas.1410579111]
38 **Liu AH**. Hygiene theory and allergy and asthma prevention. *Paediatr Perinat Epidemiol* 2007; **21** Suppl 3: 2-7 [PMID: 17935569 DOI: 10.1111/j.1365-3016.2007.00878.x]
39 **Bodner C**, Anderson WJ, Reid TS, Godden DJ. Childhood exposure to infection and risk of adult onset wheeze and atopy. *Thorax* 2000; **55**: 383-387 [PMID: 10770819 DOI: 10.1136/thorax.55.5.383]
40 **Janson C**, Asbjornsdottir H, Birgisdottir A, Sigurjonsdottir RB, Gunnbjörnsdottir M, Gislason D, Olafsson I, Cook E, Jögi R, Gislason T, Thjodleifsson B. The effect of infectious burden on the prevalence of atopy and respiratory allergies in Iceland, Estonia, and Sweden. *J Allergy Clin Immunol* 2007; **120**: 673-679 [PMID: 17586034 DOI: 10.1016/j.jaci.2007.05.003]
41 **Alcantara-Neves NM**, Veiga RV, Dattoli VC, Fiaccone RL, Esquivel R, Cruz ÁA, Cooper PJ, Rodrigues LC, Barreto ML. The effect of single and multiple infections on atopy and wheezing in children. *J Allergy Clin Immunol* 2012; **129**: 359-367, 367.e1-3 [PMID: 22035877 DOI: 10.1016/j.jaci.2011.09.015]
42 **Abrahamsson TR**, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema. *J Allergy Clin Immunol* 2012; **129**: 434-440, 440.e1-e2 [PMID: 22153774 DOI: 10.1016/j.jaci.2011.10.025]
43 **von Rosenvinge EC**, Song Y, White JR, Maddox C, Blanchard T, Fricke WF. Immune status, antibiotic medication and pH are associated with changes in the stomach fluid microbiota. *ISME J* 2013; **7**: 1354-1366 [PMID: 23466701 DOI: 10.1038/ismej.2013.33]
44 **Engstrand L**, Lindberg M. Helicobacter pylori and the gastric microbiota. *Best Pract Res Clin Gastroenterol* 2013; **27**: 39-45 [PMID: 23768551 DOI: 10.1016/j.bpg.2013.03.016]
45 **Jakobsson HE**, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS One* 2010; **5**: e9836 [PMID: 20352091 DOI: 10.1371/journal.pone.0009836]
46 **Zevit N**, Balicer RD, Cohen HA, Karsh D, Niv Y, Shamir R. Inverse association between Helicobacter pylori and pediatric asthma in a high-prevalence population. *Helicobacter* 2012; **17**: 30-35 [PMID: 22221613 DOI: 10.1111/j.1523-5378.2011.00895.x]
47 **Imamura S**, Sugimoto M, Kanemasa K, Sumida Y, Okanoue T, Yoshikawa T, Yamaoka Y. Inverse association between Helicobacter pylori infection and allergic rhinitis in young Japanese. *J Gastroenterol Hepatol* 2010; **25**: 1244-1249 [PMID: 20594251 DOI: 10.1111/j.1440-1746.2010.06307.x]
48 **Pfefferle PI**, Krämer A. Helicobacter pylori-infection status and childhood living conditions are associated with signs of allergic diseases in an occupational population. *Eur J Epidemiol* 2008; **23**: 635-640 [PMID: 18704702 DOI: 10.1007/s10654-008-9276-9]
49 **Shiotani A**, Miyanishi T, Kamada T, Haruma K. Helicobacter pylori infection and allergic diseases: epidemiological study in Japanese university students. *J Gastroenterol Hepatol* 2008; **23**: e29-e33 [PMID: 17725593 DOI: 10.1111/j.1440-1746.2007.05107.x]
50 **Baccioglu A**, Kalpaklioglu F, Guliter S, Yakaryilmaz F. Helicobacter pylori in allergic inflammation--fact or fiction? *Allergol Immunopathol* (Madr) 2008; **36**: 85-89 [PMID: 18479660 DOI: 10.1157/13120393]
51 **Herbarth O**, Bauer M, Fritz GJ, Herbarth P, Rolle-Kampczyk U, Krumbiegel P, Richter M, Richter T. Helicobacter pylori colonisation and eczema. *J Epidemiol Community Health* 2007; **61**: 638-640 [PMID: 17568058 DOI: 10.1136/jech.2006.046706]
52 **Kolho KL**, Haapaniemi A, Haahtela T, Rautelin H. Helicobacter pylori and specific immunoglobulin E antibodies to food allergens in children. *J Pediatr Gastroenterol Nutr* 2005; **40**: 180-183 [PMID: 15699693 DOI: 10.1097/00005176-200502000-00018]
53 **Uter W**, Stock C, Pfahlberg A, Guillén-Grima F, Aguinaga-Ontoso I, Brun-Sandiumenge C, Krämer A. Association between infections and signs and symptoms of 'atopic' hypersensitivity--results of a cross-sectional survey among first-year university students in Germany and Spain. *Allergy* 2003; **58**: 580-584 [PMID: 12823114 DOI: 10.1034/j.1398-9995.2003.00102.x]
54 **McCune A**, Lane A, Murray L, Harvey I, Nair P, Donovan J, Harvey R. Reduced risk of atopic disorders in adults with Helicobacter pylori infection. *Eur J Gastroenterol Hepatol* 2003; **15**: 637-640 [PMID: 12840675 DOI: 10.1097/01.meg.0000059127.68845.57]
55 **Pedullà M**, Fierro V, Del Tufo E, Alfano R, Triassi M, Perrone L. Helicobacter pylori immunization and atopic dermatitis in South Italian children. *United European Gastroenterol J* 2014; **2**: 263-267 [PMID: 25083283 DOI: 10.1177/2050640614544314]

56 **Elitsur Y**, Alrazzak BA, Preston D, Demetieva Y. Does Helicobacter pylori protect against eosinophilic esophagitis in children? *Helicobacter* 2014; **19**: 367-371 [PMID: 24750254 DOI: 10.1111/hel.12129]

57 **Karimi A**, Fakhimi-Derakhshan K, Imanzadeh F, Rezaei M, Cavoshzadeh Z, Maham S. Helicobacter pylori infection and pediatric asthma. *Iran J Microbiol* 2013; **5**: 132-135 [PMID: 23825730]

58 **Reibman J**, Marmor M, Filner J, Fernandez-Beros ME, Rogers L, Perez-Perez GI, Blaser MJ. Asthma is inversely associated with Helicobacter pylori status in an urban population. *PLoS One* 2008; **3**: e4060 [PMID: 19112508 DOI: 10.1371/journal.pone.0004060]

59 **Konturek PC**, Rienecker H, Hahn EG, Raithel M. Helicobacter pylori as a protective factor against food allergy. *Med Sci Monit* 2008; **14**: CR452-CR458 [PMID: 18758415]

60 **Annagür A**, Kendirli SG, Yilmaz M, Altintas DU, Inal A. Is there any relationship between asthma and asthma attack in children and atypical bacterial infections; Chlamydia pneumoniae, Mycoplasma pneumoniae and Helicobacter pylori. *J Trop Pediatr* 2007; **53**: 313-318 [PMID: 17535826 DOI: 10.1093/tropej/fmm040]

61 **Jaber SM**. Helicobacter pylori seropositivity in children with chronic disease in Jeddah, Saudi Arabia. *Saudi J Gastroenterol* 2006; **12**: 21-26 [PMID: 19858580]

62 **Jun ZJ**, Lei Y, Shimizu Y, Dobashi K, Mori M. Helicobacter pylori seroprevalence in patients with mild asthma. *Tohoku J Exp Med* 2005; **207**: 287-291 [PMID: 16272799 DOI: 10.1620/tjem.207.287]

63 **Pessi T**, Virta M, Adjers K, Karjalainen J, Rautelin H, Kosunen TU, Hurme M. Genetic and environmental factors in the immunopathogenesis of atopy: interaction of Helicobacter pylori infection and IL4 genetics. *Int Arch Allergy Immunol* 2005; **137**: 282-288 [PMID: 15970635 DOI: 10.1159/000086421]

64 **Bartuzi Z**, Korenkiewicz J, Romański B. Correlation between Helicobacter pylori infection and food allergy in chronic gastritis. *Med Sci Monit* 2000; **6**: 530-538 [PMID: 11208366]

65 **Tsang KW**, Lam WK, Chan KN, Hu W, Wu A, Kwok E, Zheng L, Wong BC, Lam SK. Helicobacter pylori sero-prevalence in asthma. *Respir Med* 2000; **94**: 756-759 [PMID: 10955750 DOI: 10.1053/rmed.2000.0817]

66 **Corrado G**, Luzzi I, Pacchiarotti C, Lucarelli S, Frediani T, Cavaliere M, Rea P, Cardi E. Helicobacter pylori seropositivity in children with atopic dermatitis as sole manifestation of food allergy. *Pediatr Allergy Immunol* 2000; **11**: 101-105 [PMID: 10893012 DOI: 10.1034/j.1399-3038.2000.00028.x]

67 **Matricardi PM**, Rosmini F, Riondino S, Fortini M, Ferrigno L, Rapicetta M, Bonini S. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000; **320**: 412-417 [PMID: 10669445 DOI: 10.1136/bmj.320.7232.412]

P- Reviewer: Allen M, Bernhardt GA, Lee YY **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.212

World J Methodol 2015 December 26; 5(4): 212-215
ISSN 2222-0682 (online)

Monitoring anticoagulant therapy with new oral agents

Allan Ramos-Esquivel

Allan Ramos-Esquivel, Department of Pharmacology, School of Medicine, University of Costa Rica, San José 11501-2060, Costa Rica

Author contributions: The author solely contributed to this paper.

Conflict-of-interest statement: There are not any conflicts of interest to disclose.

Correspondence to: Allan Ramos-Esquivel, MD, MSc, Department of Pharmacology, School of Medicine, University of Costa Rica, Sede Rodrigo Facio, San Pedro Calle 27, San José 11501-2060, Costa Rica. allanramoscr@gmail.com
Telephone: +506-8844-8187
Fax: +506-2237-3930

Received: June 28, 2015
Peer-review started: July 5, 2015
First decision: July 28, 2015
Revised: September 22, 2015
Accepted: October 12, 2015
Article in press: October 13, 2015
Published online: December 26, 2015

Abstract

Thromboembolic disease is a major leading cause of mortality and morbidity in industrialized countries. Currently, the management of these patients is challenging due to the availability of new drugs with proven efficacy and security compared to traditional oral vitamin K antagonists. These compounds are characterized by a predictable pharmacokinetic profile for which blood monitoring is not routinely needed. Nevertheless, some data have suggested inter-patient variability in the anticoagulant effect of these drugs, raising concerns about their effectiveness and safety. Although mass-spectrometry is the gold standard to determine drug plasma concentrations, this method is not widely available in every-day practice and some coagulation assays are commonly used to determine the anticoagulant effect of these drugs. The present review aims to summarize the current knowledge regarding the clinical question of how and when to monitor patients with new anticoagulant oral agents.

Key words: Anticoagulant agents; Apixaban; Dabigatran; Drug monitoring; Rivaroxaban

Core tip: New oral anticoagulants are replacing oral vitamin K antagonists for some practical advantages, like unnecessary monitoring and a better pharmacokinetic profile. Nevertheless, in some circumstances, their anticoagulant activity must be monitored in order to prevent adverse outcomes. In this minireview a list of the available laboratory test are reviewed to better understand the pros and cons of each analysis.

Ramos-Esquivel A. Monitoring anticoagulant therapy with new oral agents. *World J Methodol* 2015; 5(4): 212-215 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/212.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.212

INTRODUCTION

The management of thromboembolic disease has always been challenging since current treatments involve an inherent risk of bleeding that must be counterbalanced by the anticoagulant effect of each drug.

The use of vitamin K antagonists, such as warfarin, implies the monitoring of their anticoagulant effect through frequent blood tests and the education of patients about

Table 1 Pharmacokinetic features of new oral anticoagulants[11,12]

	Apixaban	Rivaroxaban	Dabigatran
Posology	Twice daily	Once daily	Twice daily
Oral bioavailability	45%	> 80%	6%
Half life	12 h	7-11 h	12-17 h
Excretion	25% renal	66% renal (active and inactive)	80% renal

potential drug and food interactions, making their use puzzling and difficult for many clinicians. In contrast, new agents have shown some advantages over vitamin K antagonists, since no dose adjustment and monitoring is routinely needed, as a consequence of a "more favorable pharmacokinetic profile". In addition, these new drugs, usually mentioned as new oral anticoagulants (NOACs), have demonstrated clinical efficacy and a better security profile than warfarin in various clinical trials, making attractive their use in clinical practice[1-3].

However, recent data have emerged regarding a potential role for monitoring the anticoagulant effect of these drugs particularly in patients with specific circumstances and comorbidities in order to reduce side effects and improve efficacy[4-7].

Some recommendations to measure the anticoagulant effect of NOACs include the following scenarios[8,9]: (1) Bleeding or recurrence of thrombosis; (2) Before surgery or any invasive procedure when the patient has taken the drug in the previous 24 h or longer if creatinine clearance is less than 50 mL/min; (3) Identification of supra or subtherapeutic levels in patients taking other drugs with potential interactions, or in patients with extreme body weight; (4) Patients with renal failure or prompt to it; (5) Reversal of anticoagulation; (6) Suspicion of drug overdose; (7) Patients with genetic mutations (*e.g.*, rs2244613 minor allele carriers for dabigatran etexilate); and (8) Assessment of compliance.

Although it is desirable to explore the anticoagulant effect of these drugs in the aforementioned circumstances, some cons have also emerged and detractors of routine monitoring include the following reasons in their arguments[10]: (1) Lack of measures in clinical trials; (2) Wide therapeutic window of some of these agents; (3) There is no a standardized clinical method to detect the anticoagulant effect or it is not yet available; and (4) The interpretation and dose adjustments have not been established.

PHARMACOLOGY OF NOACS

NOACs are categorized according to their site of action; apixaban and rivaroxaban act by inhibiting factor Xa, thereby decreasing the conversion of prothrombin to thrombin. On the other hand, dabigatran acts by directly inhibiting thrombin. Table 1 summarizes some pharmacokinetics features of clinical utility for these agents. Of particular interest is the renal clearance of these drugs that modifies or prohibits their use in case of severe kidney failure.

Although it is thought that there are fewer drug interactions for NOACs than for warfarin, clinical data suggest moderate to severe drug-drug interactions when dabigatran is used in combination with verapamil, amiodarone, and dronedarone[13,14].

Similarly, some other drugs commonly known as CYP inhibitors such as ketoconazole, itraconazole, macrolides, human immunodeficiency virus protease inhibitors can increase the serum NOACs concentration. On the other hand, some CYP inductors, such as phenytoin, phenobarbital, rifampicin and carbamazepine can decrease the anticoagulant effect of NOACs and thus are not generally recommended in these patients.

DETERMINING ANTICOAGULATION LEVELS WITH THE NOACS

The gold standard to measure plasma drug concentrations is mass-spectrometry. Nevertheless, the availability and laboratory expertise for doing this specialized technique is not fulfilled in the majority of clinical centers. For this reason, some other test must be carried out in order to determine the anticoagulant effect of NOACs. Table 2 resumes the advantages and drawbacks of available coagulation tests that have been used to determine the anticoagulat effect of NOACs in clinical settings.

Dabigatran

Activated partial thromboplastin time and thrombin time: These are very sensitive assays that do not accurately reflect plasma dabigatran concentrations. Although they are widely available, they are affected by a lot of variables such as inappropriate collection, improper handling and storage. Besides neither are strong predictors of bleeding, and patients may present any kind of hemorrhage even when the activated partial thromboplastin time and/or thrombin time are within normal range[15].

Diluted thrombin time: Since assays to evaluate thrombin activity are very sensitive to determine the anticoagulant effect of dabigatran, the use of diluted plasma in conjunction with the Hemoclot thrombin inhibitor permits to easily measure dabigatran anticoagulant activity[16].

Ecarin clotting time and ecarin chromogenic assay: These assays are not widely available and calibration is required to perform these tests. They use a metalloprotease called ecarin and are very specific for anti thrombin inhibitors due to the fact that prothrombin is a substrate for these analyses[17].

Rivaroxaban

Prothrombin time: The Subcommittee of Control of Anticoagulation of the Scientific and Standardization Committee recommends that this assay can determine the relative intensity of anticoagulation in patients taking

Table 2 Available coagulation tests to determine the anticoagulant effect of oral anticoagulants

Drug	Coagulation test	Pros	Cons
Dabigatran	aPTT	Highly available	Do not reflect the intensity of coagulation Low specificity
	TT	Highly available	It only determines the effect of dabigatran but lacks specificity
	dTT	Very accurate and precise to estimates plasma concentrations of dabigatran	Requires specific calibrators and controls in specialized laboratories with trained personal Low specificity
	ECT		Requires specific calibrators and controls in specialized laboratories with trained personal Limited standardization and validation required Low specificity Interlot variability reported
	ECA	Very accurate and precise to estimates plasma concentrations of dabigatran	Requires specific calibrators and controls in specialized laboratories with trained personal Low specificity
	DRVV-T		Requires specific calibrators and controls in specialized laboratories with trained personal Low specificity
Rivaroxaban	PT	Highly available	Do not reflect the intensity of coagulation Low specificity
Rivaroxaban and Apixaban	Chromogenic anti-Xa assays	Very accurate and precise to estimates plasma concentrations of dabigatran	Requires specific calibrators and controls in specialized laboratories with trained personal
	DRVV-T		Requires specific calibrators and controls in specialized laboratories with trained personal Low specificity

Any of these tests have been associated with clinical endpoints and data regarding their use in special populations are scarce. aPTT: Activated partial thromboplastin time; DRVV-T: Dilute Russell's viper venom time; dTT: Dilute thrombin time; ECA: Ecarin chromogenic assay; ECT: Ecarin clotting time; PT: Prothrombin time; TT: Thrombin time.

rivaroxaban but it is not useful to extrapolate plasma concentrations. Besides, it has different sensitivities according to the type of the employed reagent with high variability among laboratories[8,18].

Dilute Russell's viper venom time: This is a useful test to determine the anticoagulant effect of Xa and thrombin inhibitors since Russell's viper venom contains a potent activator of factor X and II. Nevertheless, validation and calibrations are technical issues that must be explored in future trials to determine a valid cut off and their sensitivity is low[19].

Chromogenic anti-Xa assay: Plasma concentrations of rivaroxaban and anti-Xa levels correlate fairly well and it is the preferred method to estimate plasma concentrations. This method is less affected by sample handling or clotting factors in patients. However, a major limitation is the standardization and the availability of laboratories with specific calibrators and controls[20].

Apixaban

Dilute Russell's viper venom time: As pointed before, this test is very useful and sensitive to determine the anticoagulant effect of apixaban, but with a low specificity.

Chromogenic anti-Xa assay: It is the most sensitive assay to determine the inhibition of factor Xa by apixaban and it is recommended to estimate plasma concentrations of this drug. Nevertheless, this test is not widely available and it is time consuming[21].

SPECIAL CONSIDERATIONS

It is important to point out that any of these analyses have been tested in special populations such as elderly patients and children, as well as pregnant women and individuals with multiple morbidities[22,23]. Besides, some methodological issues regarding sample collection must be accomplished to avoid misinterpretations and biases. For example, plasma concentrations of the NOACs can vary among 10 to 20 times between peak and trough concentrations. Therefore, the assessment of the anticoagulant activity of each drug should be obtained immediately prior to the next scheduled dose[22]. Furthermore there are few reports regarding a correlation between any of these tests and the efficacy and security of any NOAC and the clinical significance of these analyses must be interpreted cautiously.

CONCLUSION

Despite of the potentials scenarios in which the role of monitoring the anticoagulant effect of NOAC can be clinically valid, it must be point out that there is no trial which has compared results of these drugs with or without coagulation monitoring and there are no guidelines to determine the steps to follow in order to improve the quality of the anticoagulation therapy.

Nevertheless, with the broad use of NOACs in clinical practice we must keep in mind the inter-patient variability of these drugs that can result in loss of efficacy and security.

REFERENCES

1 **Patel MR**, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011; **365**: 883-891 [PMID: 21830957 DOI: 10.1056/NEJMoa1009638]

2 **Granger CB**, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD, Ezekowitz JA, Flaker G, Garcia D, Geraldes M, Gersh BJ, Golitsyn S, Goto S, Hermosillo AG, Hohnloser SH, Horowitz J, Mohan P, Jansky P, Lewis BS, Lopez-Sendon JL, Pais P, Parkhomenko A, Verheugt FW, Zhu J, Wallentin L. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011; **365**: 981-992 [PMID: 21870978 DOI: 10.1056/NEJMoa1107039]

3 **Connolly SJ**, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009; **361**: 1139-1151 [PMID: 19717844 DOI: 10.1056/NEJMoa0905561]

4 **Freyburger G**, Macouillard G, Labrouche S, Sztark F. Coagulation parameters in patients receiving dabigatran etexilate or rivaroxaban: two observational studies in patients undergoing total hip or total knee replacement. *Thromb Res* 2011; **127**: 457-465 [PMID: 21277622 DOI: 10.1016/j.thromres.2011.01.001]

5 **Paré G**, Eriksson N, Lehr T, Connolly S, Eikelboom J, Ezekowitz MD, Axelsson T, Haertter S, Oldgren J, Reilly P, Siegbahn A, Syvanen AC, Wadelius C, Wadelius M, Zimdahl-Gelling H, Yusuf S, Wallentin L. Genetic determinants of dabigatran plasma levels and their relation to bleeding. *Circulation* 2013; **127**: 1404-1412 [PMID: 23467860 DOI: 10.1161/CIRCULATIONAHA.112.001233]

6 **Reilly PA**, Lehr T, Haertter S, Connolly SJ, Yusuf S, Eikelboom JW, Ezekowitz MD, Nehmiz G, Wang S, Wallentin L; RE-LY Investigators. The effect of dabigatran plasma concentrations and patient characteristics on the frequency of ischemic stroke and major bleeding in atrial fibrillation patients: the RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy). *J Am Coll Cardiol* 2014; **63**: 321-328 [PMID: 24076487 DOI: 10.1016/j.jacc.2013.07.104]

7 **Southworth MR**, Reichman ME, Unger EF. Dabigatran and postmarketing reports of bleeding. *N Engl J Med* 2013; **368**: 1272-1274 [PMID: 23484796 DOI: 10.1056/NEJMp1302834]

8 **Baglin T**, Hillarp A, Tripodi A, Elalamy I, Buller H, Ageno W. Measuring Oral Direct Inhibitors (ODIs) of thrombin and factor Xa: A recommendation from the Subcommittee on Control of Anticoagulation of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. *J Thromb Haemost* 2013 Jan 24; Epub ahead of print [PMID: 23347120 DOI: 10.1111/jth.12149]

9 **Douxfils J**, Mani H, Minet V, Devalet B, Chatelain B, Dogné JM, Mullier F. Non-VKA Oral Anticoagulants: Accurate Measurement of Plasma Drug Concentrations. *Biomed Res Int* 2015; **2015**: 345138 [PMID: 26090400 DOI: 10.1155/2015/345138]

10 **Samama MM**, Guinet C, Le Flem L. Do new oral anticoagulants require laboratory monitoring? The clinician point of view. *Thromb Res* 2012; **130** Suppl 1: S88-S89 [PMID: 23026675 DOI: 10.1016/j.thromres.2012.08.286]

11 **Eriksson BI**, Quinlan DJ, Weitz JI. Comparative pharmacodynamics and pharmacokinetics of oral direct thrombin and factor xa inhibitors in development. *Clin Pharmacokinet* 2009; **48**: 1-22 [PMID: 19071881 DOI: 10.2165/0003088-200948010-00001]

12 **Poulsen BK**, Grove EL, Husted SE. New oral anticoagulants: a review of the literature with particular emphasis on patients with impaired renal function. *Drugs* 2012; **72**: 1739-1753 [PMID: 22931521 DOI: 10.2165/11635730-000000000-00000]

13 **Liesenfeld KH**, Lehr T, Dansirikul C, Reilly PA, Connolly SJ, Ezekowitz MD, Yusuf S, Wallentin L, Haertter S, Staab A. Population pharmacokinetic analysis of the oral thrombin inhibitor dabigatran etexilate in patients with non-valvular atrial fibrillation from the RE-LY trial. *J Thromb Haemost* 2011; **9**: 2168-2175 [PMID: 21972820 DOI: 10.1111/j.1538-7836.2011.04498.x]

14 **Mueck W**, Kubitza D, Becka M. Co-administration of rivaroxaban with drugs that share its elimination pathways: pharmacokinetic effects in healthy subjects. *Br J Clin Pharmacol* 2013; **76**: 455-466 [PMID: 23305158 DOI: 10.1111/bcp.12075]

15 **Stangier J**, Rathgen K, Stähle H, Gansser D, Roth W. The pharmacokinetics, pharmacodynamics and tolerability of dabigatran etexilate, a new oral direct thrombin inhibitor, in healthy male subjects. *Br J Clin Pharmacol* 2007; **64**: 292-303 [PMID: 17506785 DOI: 10.1111/j.1365-2125.2007.02899.x]

16 **Hapgood G**, Butler J, Malan E, Chunilal S, Tran H. The effect of dabigatran on the activated partial thromboplastin time and thrombin time as determined by the Hemoclot thrombin inhibitor assay in patient plasma samples. *Thromb Haemost* 2013; **110**: 308-315 [PMID: 23783268 DOI: 10.1160/TH13-04-0301]

17 **Antovic JP**, Skeppholm M, Eintrei J, Boija EE, Söderblom L, Norberg EM, Onelöv L, Rönquist-Nii Y, Pohanka A, Beck O, Hjemdahl P, Malmström RE. Evaluation of coagulation assays versus LC-MS/MS for determinations of dabigatran concentrations in plasma. *Eur J Clin Pharmacol* 2013; **69**: 1875-1881 [PMID: 23784008 DOI: 10.1007/s00228-013-1550-4]

18 **van Veen JJ**, Smith J, Kitchen S, Makris M. Normal prothrombin time in the presence of therapeutic levels of rivaroxaban. *Br J Haematol* 2013; **160**: 859-861 [PMID: 23278675 DOI: 10.1111/bjh.12173]

19 **Exner T**, Ellwood L, Rubie J, Barancewicz A. Testing for new oral anticoagulants with LA-resistant Russells viper venom reagents. An in vitro study. *Thromb Haemost* 2013; **109**: 762-765 [PMID: 23364321 DOI: 10.1160/TH12-11-0842]

20 **Douxfils J**, Tamigniau A, Chatelain B, Chatelain C, Wallemacq P, Dogné JM, Mullier F. Comparison of calibrated chromogenic anti-Xa assay and PT tests with LC-MS/MS for the therapeutic monitoring of patients treated with rivaroxaban. *Thromb Haemost* 2013; **110**: 723-731 [PMID: 23846172 DOI: 10.1160/TH13-04-0274]

21 **Frost C**, Nepal S, Wang J, Schuster A, Byon W, Boyd RA, Yu Z, Shenker A, Barrett YC, Mosqueda-Garcia R, Lacreta F. Safety, pharmacokinetics and pharmacodynamics of multiple oral doses of apixaban, a factor Xa inhibitor, in healthy subjects. *Br J Clin Pharmacol* 2013; **76**: 776-786 [PMID: 23451769 DOI: 10.1111/bcp.12106]

22 **Garcia D**, Barrett YC, Ramacciotti E, Weitz JI. Laboratory assessment of the anticoagulant effects of the next generation of oral anticoagulants. *J Thromb Haemost* 2013; **11**: 245-252 [PMID: 23216682 DOI: 10.1111/jth.12096]

23 **Favaloro EJ**, Lippi G. The new oral anticoagulants and the future of haemostasis laboratory testing. *Biochem Med* (Zagreb) 2012; **22**: 329-341 [PMID: 23092064 DOI: 10.11613/BM.2012.035]

P- Reviewer: Fukuda S, Redondo PC **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.216

World J Methodol 2015 December 26; 5(4): 216-222
ISSN 2222-0682 (online)

Biomarkers of oxidative stress in erythrocytes as a function of human age

Pawan Kumar Maurya, Prabhanshu Kumar, Pranjal Chandra

Pawan Kumar Maurya, Interdisciplinary Laboratory of Clinical Neuroscience (LINC), Department of Psychiatry, Federal University of São Paulo, São Paulo 04039-032, Brazil

Pawan Kumar Maurya, Prabhanshu Kumar, Amity Institute of Biotechnology, Amity University Uttar Pradesh, Noida 201303, India

Pranjal Chandra, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

Author contributions: Maurya PK had conceived the idea; Maurya PK and Chandra P set up the frame of the review article and wrote up manuscript; Kumar P edited the entire manuscript.

Supported by Fellowship (to Pawan Kumar Maurya) (Science without Borders-Level A) from coordination of Improvement of Higher Education Personnel (CAPES), National Counsel of Technological and Scientific Development (CNPq), Brazil.

Conflict-of-interest statement: The authors report no conflicts of interest.

Correspondence to: Dr. Pranjal Chandra, MTech, PhD, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Kamrup, Guwahati 781039, Assam, India. pchandra13@iitg.ernet.in
Telephone: +91-361-2583207
Fax: +91-361-2582249

Received: April 8, 2015
Peer-review started: April 9, 2015
First decision: June 24, 2015
Revised: September 1, 2015
Accepted: September 16, 2015
Article in press: September 18, 2015
Published online: December 26, 2015

Abstract

Despite more than 300 theories to explain the aging process, oxidative stress theory offers the best mechanism to explain aging and age related disorders. Several studies has shown the importance of oxidative stress during aging. PubMed, Science Direct and Springer online data bases are taken into consideration to write this mini-review. Human erythrocytes are most abundant and specialized cells in the body. Erythrocytes were extensively studied due to their metabolism and gas transport functions. Recent studies on erythrocytes have provided us detailed information of cell membrane and its structural organization that may help in studying the aging and age associated changes. The susceptibility of an organism is associated with the antioxidant potential of the body. Erythrocytes have potent antioxidant protection consisting of enzymatic and non-enzymatic pathways that counteract with reactive oxygen species, thus maintaining the redox regulation in the body. The non-enzymatic and enzymatic antioxidants and other biomarkers associated with erythrocyte membrane transport functions are the main content of this review. Biomarkers of oxidative stress in erythrocytes and its membrane were taken into the consideration during human aging that will be the main subject of this mini-review.

Key words: Biomarkers; Humans; Aging; Oxidative stress; Erythrocytes; Erythrocyte membrane

Core tip: The aim of present review is to summarize important oxidative stress biomarkers in erythrocytes during human aging. Erythrocyte membrane is rich in

lipids and proteins which are easy targets of reactive oxygen species. Erythrocytes are also equipped with antioxidant defense system. Studies on biomarkers of oxidative stress are important in the establishment of reference values in different populations and in studies involving their role in different disease conditions.

Maurya PK, Kumar P, Chandra P. Biomarkers of oxidative stress in erythrocytes as a function of human age. *World J Methodol* 2015; 5(4): 216-222 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/216.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.216

INTRODUCTION

Aging is a characterized by alterations that takes place in a single cell or in the whole organ system. The exact process of aging is still not well understood but many evidences support that it is associated with excess production of free radicals in the form of reactive oxygen species (ROS) and reactive nitrogen species (RNS) throughout life[1]. During aerobic respiration, ROS/RNS are produced from electron transport chain present inside mitochondria (Table 1). Excess ROS/RNS damages proteins, lipids and nucleic acids, when enzymatic and non-enzymatic antioxidants of the body are unable to scavenge free radicals[2]. Even under normal metabolic conditions, certain amount of oxidative damage to cell and its membrane takes place, but its rate increases with the increase of OS during aging, as the antioxidant variation machinery gets diminished[3,4]. Besides many recent studies at molecular level like telomere shortening contributes to the accumulation of DNA damage during cellular aging[5], erythrocytes cell as a whole and its membrane has its own importance in aging and age associated diseases.

Human erythrocytes or red blood cells (RBCs) are in the circulatory system for 120 d[6], which transport oxygen from the lungs to all other tissues of the body and carbon dioxide (CO_2) from the body tissues back to the lungs. These erythrocytes are produced in the bone marrow by differentiation process and hematopoietic stem cells differentiate to form nucleate erythrocytes. After degradation of endoplasmic reticulum and formation of nuclei, reticulocytes appear in the circulation. An erythrocyte is a disc shaped, 8 μm biconcave structures bounded by a plasma membrane. The protein and lipid bilayer to erythrocytes changes throughout the whole life. This can be particularly seen at the stage of its plasma membrane[7], since it is made up of protein-lipid bilayer. Erythrocyte contains a conjugate protein in the form of hemoglobin. The main function of hemoglobin is the binding and releasing oxygen and carbon dioxide, for this reason the membrane of RBC is extremely important. The plasma membrane is a two dimensional meshwork of protein called as spectrin membrane skeleton. It helps in maintenance of structure of erythrocytes. Because of the above mentioned facts of erythrocyte and the plasma membrane, this cell type has been studied extensively.

Table 1 Reactive oxygen species

Oxygen centered radicals	Oxygen centered non-radicals
$O_2\cdot$	H_2O_2
$\cdot OH$	
$HOO\cdot$	$^1O_2$
$ROO\cdot$	

RBCs as oxygen carriers are continuously exposed to high oxygen tension. Oxidative stress, which decreases the antioxidant capacity, irreversibly damages erythrocytes, resulting in their eventual damage by hemolysis and their removal by circulation. Because mature RBCs are cells without nucleus and other cell organelles, they have no capacity to repair the damaged components. Celedón *et al*[8], showed that biochemical alteration which takes place during acute hypobaric hypoxia, make erythrocyte susceptible to oxidative stress. During erythrocyte life span there are many changes in size and deformability, lipid and protein content in the membrane, ion exchange and action of enzyme. This review will also address the effect of OS in erythrocytes during normal aging based on the structural alteration in the erythrocytes and its membrane. The widely studied clinical biomarkers of oxidative stress and its mechanism in human erythrocytes has been represented in Figure 1.

BIOMARKERS RELATED OF OXIDATIVE STRESS

Lipid peroxidation-malonaldehyde

Malonaldehyde, is a byproduct of lipid per-oxidation. It reacts with amino acids, membrane proteins, phosphatides, DNA and RNA of the cell, which leads to structural and functional modification. Excess level of malonaldehyde (MDA) levels has been reported when it undergoes cell damage or in several diseased conditions. Red blood cell membrane is made up of 60% of phosphatides. Cholesterol (non-esterifies) represents about 30% of the lipidic erythrocyte composition, and the last 10% are carbohydrate containing lipids[9]. Due to presence of polyunsaturated fatty acids (PUFA), cell membrane becomes more susceptible to free radicals and its oxidation takes place that breaks double bonds of PUFA and generate malonaldehyde. Previously, we have reported elevated level of erythrocyte MDA in normal elderly population[10]. Increased MDA level was supported by other findings, such as, decreased membrane sulfhydryl (-SH) groups and total antioxidant potential in older individuals compared to younger individuals[11]. The increases in the MDA level are found in diabetes, hypertension, inflammation, coronary heart and liver disease[12]. The free radical chain reaction is the mechanism involved in the process of lipid peroxidation. It increased with the increased production of ROS/RNS.

The erythrocyte membrane is rich in phosphatides

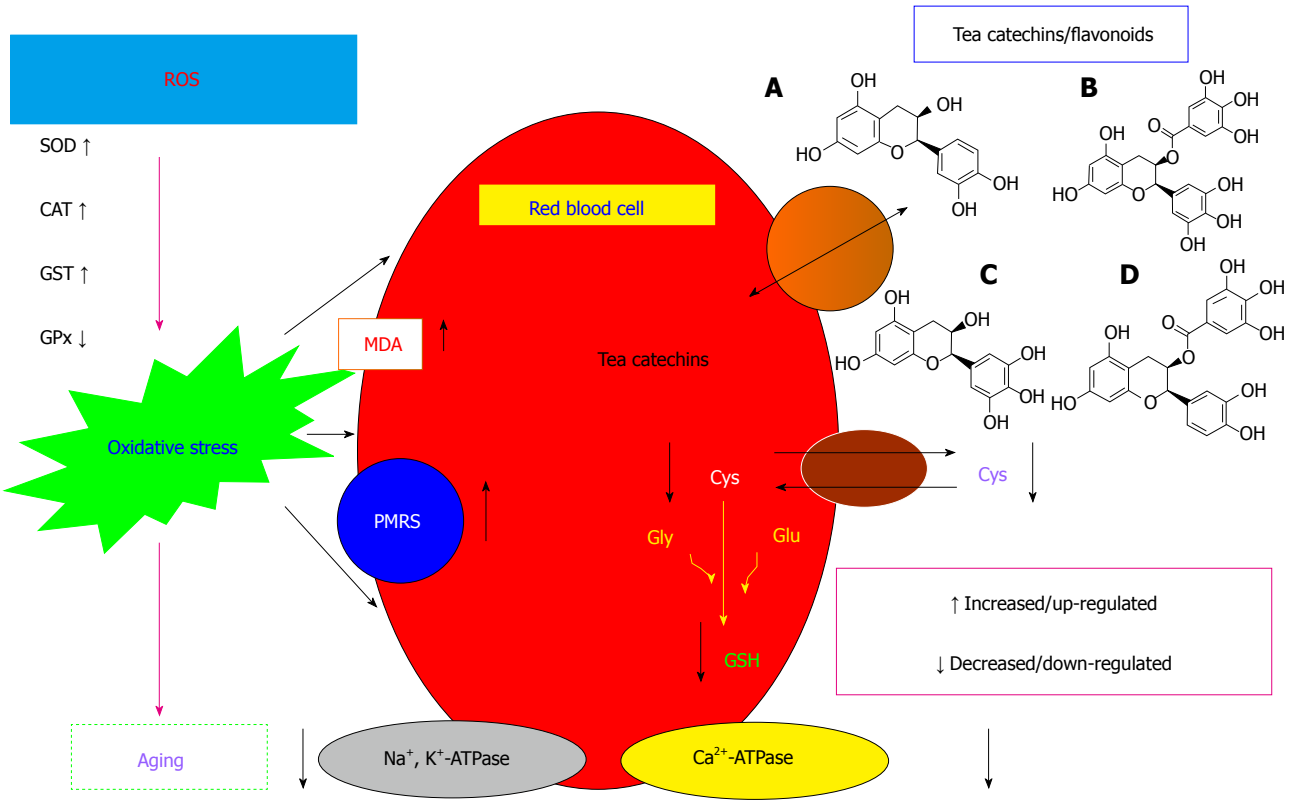

Figure 1 Aerobic cell produce reactive oxygen species as a byproduct of cellular respiration. Erythrocytes are equipped with antioxidant defense system for overcome excess production of ROS. The activity of SOD, CAT, GST and PMRS is upregulated while GPx activity is down regulated during human aging. Increased oxidative stress during aging results in elevated MDA levels and decreased GSH levels. Cysteine influx and efflux decreases during aging, which is an important amino acid for GSH biosynthesis. Red blood cell membrane bound enzymes (Na^+, K^+-ATPase; Ca^{2+}-ATPase) activity decreases as a function of human age. Dietary flavonoids like tea catechins modulate various biomarkers of oxidative stress and are protective in nature. Chemical structures of epicatechin (A), epigallocatechin gallate (B), epigallocatechin (C), and epicatechin gallate (D). Cys: Cysteine; Glu: Glutamic acid; Gly: Glycine; GSH: Reduced gluathione; GSSG: Oxidised glutathione; MDA: Malonaldehyde; SOD: Superoxide dismutase; CAT: Catalase; GST: Glutathione-S-transferase; GPx: Glutathione peroxidase; PMRS: Plasma membrane redox system; ROS: Reactive oxygen species.

containing proteins present outside the membrane. The proteins present in the outer membrane are easy target for free radicals which results in the formation of MDA. Increased MDA level results in alterations of the cell membrane polarity, charge sharing across lipid phase surface and oligomer formation. Further elevation in lipid peroxidation, results in decline resistance towards denaturation process, decreased number of membrane-SH groups and altered mobility of lipids. Several studies reported the importance of peroxidation of lipids in caloric restriction and longevity in different populations[13]. It has been observed that animals and avians have long life because they contains less number of unsaturated fatty acids in its plasma membranes, so they are not easy targets for ROS/RNS and have lower degree of MDA formation and less modification in proteins in long life of these species. Results clearly indicate that the animals/species having low degree of unsaturated fatty acids/unsaturation have longer life span.

Reduced glutathione

Reduced glutathione (GSH) is a primary antioxidant of erythrocytes. It is a tripeptide containing there amino acids and is an intracellular non-protein sulphydryl (-SH) compound. A significant decrease in erythrocyte GSH level has been reported in human erythrocytes[10]. Erythrocyte GSH level negatively correlated between decline GSH and age has been observed, this decrease is also correlates with total antioxidant potential of plasma[14]. Erythrocytes contain most important hydrophilic antioxidant in form of GSH[15]. Reduced glutathione contains sulphur containing amino acid cysteine which is the rate liming amino acid in GSH biosynthesis. Due to presence of cysteine amino acid, GSH helps in maintaining reduced status of SH group of cell membrane, including many other biological functions. OS results in the oxidized form of SH groups, which will results in many cellular and functional dysfunctions. A study carried out in European subjects also demonstrate a significant decline in erythrocyte GSH level during aging[16], while other reports on the same study did not show significant alterations in intracellular glutathione[17]. Oxidized form of glutathione (GSSG) is not favourable for body. A recent study on age associated change in the glutathione level in brain of rat shows decline in glutathione in almost all regions of the brain. The conversion of reduced glutathione to oxidized glutathione was further increased and the ratio of these two (GSH/GSSG) was

also reported to decrease, which measures the oxidation/reduction status of the cell[18]. The GSH/GSSG ratio has many biological functions including oxidation/reduction signaling and in the protection of antioxidants. The ratio also helps in providing a link to influence of environment with elderly population[19].

Membrane-SH group

The human RBCs are rich in membrane SH groups. The -SH group play a major role in maintenance of oxidation-reduction status of the cell[20]. The OS caused by ROS/RNS in erythrocytes effect cell membrane and its mechanical characteristics. Any oxidative damage to the plasma membrane-SH group of erythrocyte will induce the alterations in micro-elasticity under pathological and physiological state of OS[21]. Alterations in the oxidation/reduction balance during normal aging can modify several enzyme activates and proteins within the cell. Most of the protein molecules contain sulphur containing amino acids, methionine and cysteine which are subject to reox changes. We reported as related decrease in erythrocyte membranes-SH group during human aging[10]. Enzymes and proteins having -SH group are easy target for ROS/RNS. Age related changes in protein oxidation have been documented in erythrocytes and plasma[22]. Nitric oxide is highly reactive having half life of few seconds yet it can diffuse cell membrane freely which make NO ideal for a transient signal molecule. It is known to be involved in aging process[23].

ENZYMATIC ANTIOXIDANTS

RBCs are exposed permanently with potentially damaging level of ROS/RNS, but their metabolic processes are capable of reversing this oxidative damage under normal conditions. However, it is well known that variety of physiological and pathological factors may increase ROS/RNS which induces the oxidative stress. In addition, hemoglobin is known to stimulate lipid peroxidation[24]. Erythrocytes are equipped by antioxidant defense system in form of enzymatic and non-enzymatic antioxidants[25]. This protective system in form of enzymatic antioxidants includes superoxide dismutase (SOD)[26], which detoxify the effect of superoxide radical ($O_2^{\cdot-}$) catalase (CAT)[27], which is involved in the conversion of H_2O_2 to H_2O, and other enzymatic antioxidants like glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-transferases (GSTs)[28,29]. Two enzymes are shared in H_2O_2 detoxification: CAT and GPx but their relative significance in H_2O_2 scavenging is still not clear[30]. The reduced glutathione (GSH) is a non-enzymatic antioxidant.

In human subjects there is a considerable disagreement in age-related changes of erythrocyte SOD and CAT activity[31,32]. Lower activities of CAT and SOD were shown in premature infants during first 72 h of their life in comparison with full-term infants and even during aging[33,34]. Less than 10% of normal erythrocyte CAT activity was observed in homozygous carrier of inherited CAT deficiency-acatalasemia[35], and less than 50% in heterozygous subject's hypocatalasemia[36]. An elevated SOD and CAT activities during aging in human erythrocytes has been reported[33]. Increased OS during normal aging was compensated by elevation in the activities of these enzymes. Elevated CAT and SOD activities may be a manifestation of more production of ROS/RNS during aging in humans. Several contradictory results have been shown in published reports for SOD and CAT role in normal aging process, the reason for which is hard to explain[37,38]. As far as pathological processes are concerned; decreased CAT activities were found in erythrocytes from human patients of different ages with several types of brain disorders including dementia, stroke and Parkinson disease[39,40].

Oxidative stress with alterations in profile of antioxidant enzymes in erythrocytes is also related to many others specific pathologies[41,42]. Cell requires certain enzymes to detoxify the toxicants. GSTs are group of enzymes that play a very important role in the detoxification of dangerous compounds to less toxic compounds[43]. Age associated changes in GST has been reported[44,45]. GSTs also play a significant role in drug resistant development in tumor cells, Alzheimer's and Parkinson's disease, atherosclerosis[46,47]. GST are involved in many biological functions in mammals which includes detoxification of toxicants, catalysis of several biological processes, several functions associated with metabolism, resistance towards drugs and inhibiting age associated disorders[48,49].

Age associated changes in the activity of GPx has been shown in human erythrocytes and correlated with total antioxidant capacity[50]. Several studies have been reported having conflicting data as to how GPx activity is changes with age[51]. Increased GPx activity have been shown in smaller population studies, while decreased activity has been reported in most large studies as a function of age[52]. GPx activity decreases in presence of more H_2O_2, which ultimately leads to direct cell/tissue damage and activation of nuclear factor-κB - related inflammatory pathways[53,54].

ALTERATIONS IN ERYTHROCYTE MEMBRANE TRANSPORT FUNCTIONS

In most of the eukaryotic cell membrane, the lipids are distributed in asymmetric manner across the bilayer plan. This kind of structural organization is referred as trans asymmetry. This trans-asymmetric distribution play very important role in structural and functional aspect of cell membrane. Homeostasis is very important for the cell. Transport of ion across the cell membrane are regulated by various kind of cell membrane enzymes such as Na^+, K^+-ATPase and Ca^{2+}-ATPase[55]. A recent study reports a significant decreased activity of these two enzymes during human aging[56]. Transport of dietary flavonoids across cell membrane is well documented[57]. Recently, we reported the beneficial effect of tea catechins in erythrocytes during human aging[58,59]. L-cysteine is a

sulphur containing amino acid that has free functional -SH group which is important in oxidation/reduction reactions. Free -SH group in erythrocyte membrane will help in the regulation and maintenance of intracellular redox status of erythrocytes and other cell types. Human erythrocyte does not have any machinery to synthesize protein inside the cell. Synthesis of GSH take place inside erythrocyte and it require L-cysteine, a semi-essential amino acid. GSH is a tripeptide containing glutamic acid, cysteine and glycine joined together with the help of peptide bonds. All above said amino acids are required for the biosynthesis of GSH, but the rate depends on only the availability of L-cysteine. We report L-cysteine influx and efflux across human erythrocytes during aging[60,61]. L-cysteine is the amino acid which provides free functional -SH group to GSH which play a very important role in antioxidant defense system. GSH is a soluble antioxidant which protect cell from ROS/RNS caused oxidative damage. Recently, there are substantial evidences in literature which supports that normal aging is accompanied with higher level of OS. Incorporation of flavonoids and cysteine in diet has been shown to alter several OS biomarkers which are known to be associated with aging and age related disorders[62]. We report a significant decreased efflux of L-cysteine in erythrocytes during aging[63]. Since, GSH biosynthesis in erythrocytes is dependent on L-cysteine bioavailability; the decreased influx and efflux may explain the low GSH level reported in erythrocytes as a function of age[10]. L-cysteine transported into erythrocytes only when there is properly reduced membrane lipid and protein thiol. Aging is associated with reduced antioxidant capacity which results in induction of conformational and structural changes in the cell membrane and in the amino acid transporters that finally results in L-cysteine influx and efflux.

CONCLUSION

Red blood cells are the main cell types present in blood. It is reviewed in this mini-review that erythrocytes have a very particular membrane structure and composition which alters during aging and that support their features and functions to study human aging. It has been shown that RBCs have ability to encounter various oxidative stressors to prevent oxidative stress. They are good model to study various plant products to evaluate their anti-aging properties. These findings of biomarkers of OS during normal aging will help in the establishment of reference values for biomarkers of OS in elderly peoples and several other parameters. These reference values will help in studies that involve the role of biomarkers in various other diseased conditions.

ACKNOWLEDGMENTS

Pranjal Chandra thanks Indian Institute of Technology, Guwahati for providing necessary facility for completion of this research work.

REFERENCES

1 **Fransen M**, Nordgren M, Wang B, Apanasets O. Role of peroxisomes in ROS/RNS-metabolism: implications for human disease. *Biochim Biophys Acta* 2012; **1822**: 1363-1373 [PMID: 22178243 DOI: 10.1016/j.bbadis.2011.12.001]
2 **Dröge W**. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; **82**: 47-95 [PMID: 11773609]
3 **Kumawat M**, Sharma TK, Singh I, Singh N, Singh SK, Ghalaut VS, Shankar V, Vardey SK. Decrease in antioxidant status of plasma and erythrocytes from geriatric population. *Dis Markers* 2012; **33**: 303-308 [PMID: 23089922 DOI: 10.3233/DMA-2012-00938]
4 **Michalak A**, Krzeszowiak J, Markiewicz-Górka I. [The correlations between aging of the human body, oxidative stress and reduced efficiency of repair systems]. *Postepy Hig Med Dosw* (Online) 2014; **68**: 1483-1491 [PMID: 25531712]
5 **Ju Z**, Lenhard Rudolph K. Telomere dysfunction and stem cell ageing. *Biochimie* 2008; **90**: 24-32 [PMID: 18029082]
6 **Cancelas JA**, Dumont LJ, Maes LA, Rugg N, Herschel L, Whitley PH, Szczepiokowski ZM, Siegel AH, Hess JR, Zia M. Additive solution-7 reduces the red blood cell cold storage lesion. *Transfusion* 2015; **55**: 491-498 [PMID: 25233911 DOI: 10.1111/trf.12867]
7 **de Oliveira S**, Saldanha C. An overview about erythrocyte membrane. *Clin Hemorheol Microcirc* 2010; **44**: 63-74 [PMID: 20134094 DOI: 10.3233/CH-2010-1253]
8 **Celedón G**, González G, Sotomayor CP, Behn C. Membrane lipid diffusion and band 3 protein changes in human erythrocytes due to acute hypobaric hypoxia. *Am J Physiol* 1998; **275**: C1429-C1431 [PMID: 9843702]
9 **Yawata Y**. Cell Membrane: The Red Blood Cell as a Model, Wiley, 2003 [DOI: 10.1002/3527601538]
10 **Rizvi SI**, Maurya PK. Markers of oxidative stress in erythrocytes during aging in humans. *Ann N Y Acad Sci* 2007; **1100**: 373-382 [PMID: 17460201]
11 **Andriollo-Sanchez M**, Hininger-Favier I, Meunier N, Venneria E, O'Connor JM, Maiani G, Coudray C, Roussel AM. Age-related oxidative stress and antioxidant parameters in middle-aged and older European subjects: the ZENITH study. *Eur J Clin Nutr* 2005; **59** Suppl 2: S58-S62 [PMID: 16254584]
12 **Leitinger N**. The role of phospholipid oxidation products in inflammatory and autoimmune diseases: evidence from animal models and in humans. *Subcell Biochem* 2008; **49**: 325-350 [PMID: 18751917 DOI: 10.1007/978-1-4020-8830-8_12]
13 **Hulbert AJ**. Metabolism and longevity: is there a role for membrane fatty acids? *Integr Comp Biol* 2010; **50**: 808-817 [PMID: 21558243 DOI: 10.1093/icb/icq007]
14 **Rizvi SI**, Jha R, Maurya PK. Erythrocyte plasma membrane redox system in human aging. *Rejuvenation Res* 2006; **9**: 470-474 [PMID: 17105387]
15 **Smeyne M**, Smeyne RJ. Glutathione metabolism and Parkinson's disease. *Free Radic Biol Med* 2013; **62**: 13-25 [PMID: 23665395 DOI: 10.1016/j.freeradbiomed.2013.05.001]
16 **Zhu Y**, Carvey PM, Ling Z. Age-related changes in glutathione and glutathione-related enzymes in rat brain. *Brain Res* 2006; **1090**: 35-44 [PMID: 16647047]
17 **Franzoni F**, Ghiadoni L, Galetta F, Plantinga Y, Lubrano V, Huang Y, Salvetti G, Regoli F, Taddei S, Santoro G, Salvetti A. Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men. *Am J Hypertens* 2005; **18**: 510-516 [PMID: 15831361]
18 **Zhang C**, Rodriguez C, Spaulding J, Aw TY, Feng J. Age-dependent and tissue-related glutathione redox status in a mouse model of Alzheimer's disease. *J Alzheimers Dis* 2012; **28**: 655-666 [PMID: 22045490 DOI: 10.3233/JAD-2011-111244]
19 **Ghosh D**, Levault KR, Brewer GJ. Relative importance of redox

buffers GSH and NAD(P)H in age-related neurodegeneration and Alzheimer disease-like mouse neurons. *Aging Cell* 2014; **13**: 631-640 [PMID: 24655393 DOI: 10.1111/acel.12216]
20 **Reglinski J**, Hoey S, Smith WE, Sturrock RD. Cellular response to oxidative stress at sulfhydryl group receptor sites on the erythrocyte membrane. *J Biol Chem* 1988; **263**: 12360-12366 [PMID: 3410845]
21 **Wang X**, Wu Z, Song G, Wang H, Long M, Cai S. Effects of oxidative damage of membrane protein thiol groups on erythrocyte membrane viscoelasticities. *Clin Hemorheol Microcirc* 1999; **21**: 137-146 [PMID: 10599597]
22 **Pandey KB**, Mehdi MM, Maurya PK, Rizvi SI. Plasma protein oxidation and its correlation with antioxidant potential during human aging. *Dis Markers* 2010; **29**: 31-36 [PMID: 20826915 DOI: 10.3233/DMA-2010-0723]
23 **Maurya PK**, Rizvi SI. Alterations in plasma nitric oxide during aging in humans. *Indian J Biochem Biophys* 2009; **46**: 130-132 [PMID: 19374267]
24 **Arashiki N**, Kimata N, Manno S, Mohandas N, Takakuwa Y. Membrane peroxidation and methemoglobin formation are both necessary for band 3 clustering: mechanistic insights into human erythrocyte senescence. *Biochemistry* 2013; **52**: 5760-5769 [PMID: 23889086 DOI: 10.1021/bi400405p]
25 **Kurata M**, Suzuki M, Agar NS. Antioxidant systems and erythrocyte life-span in mammals. *Comp Biochem Physiol B* 1993; **106**: 477-487 [PMID: 8281748]
26 **Rowiński R**, Kozakiewicz M, Kędziora-Kornatowska K, Hübner-Woźniak E, Kędziora J. Markers of oxidative stress and erythrocyte antioxidant enzyme activity in older men and women with differing physical activity. *Exp Gerontol* 2013; **48**: 1141-1146 [PMID: 23911531 DOI: 10.1016/j.exger.2013.07.010]
27 Mladenov M, Gokik M, Hadzi-Petrushev N, Gjorgoski I, Jankulovski N. The relationship between antioxidant enzymes and lipid peroxidation in senescent rat erythrocytes. *Physiol Res* 2015 [PMID: 26047376]
28 **Johnson RM**, Goyette G, Ravindranath Y, Ho YS. Red cells from glutathione peroxidase-1-deficient mice have nearly normal defenses against exogenous peroxides. *Blood* 2000; **96**: 1985-1988 [PMID: 10961904]
29 **Nagababu E**, Chrest FJ, Rifkind JM. Hydrogen-peroxide-induced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. *Biochim Biophys Acta* 2003; **1620**: 211-217 [PMID: 12595091]
30 **Rocha S**, Gomes D, Lima M, Bronze-da-Rocha E, Santos-Silva A. Peroxiredoxin 2, glutathione peroxidase, and catalase in the cytosol and membrane of erythrocytes under H2O2-induced oxidative stress. *Free Radic Res* 2015; **49**: 990-1003 [PMID: 25786472]
31 **Mariani E**, Mangialasche F, Feliziani FT, Cecchetti R, Malavolta M, Bastiani P, Baglioni M, Dedoussis G, Fulop T, Herbein G, Jajte J, Monti D, Rink L, Mocchegiani E, Mecocci P. Effects of zinc supplementation on antioxidant enzyme activities in healthy old subjects. *Exp Gerontol* 2008; **43**: 445-451 [PMID: 18078731]
32 **Wojciech Ł**, Ewa Z, Elzbieta S. Influence of green tea on erythrocytes antioxidant status of different age rats intoxicated with ethanol. *Phytother Res* 2010; **24**: 424-428 [PMID: 19777505 DOI: 10.1002/ptr.2986]
33 **Rizvi SI**, Maurya PK. Alterations in antioxidant enzymes during aging in humans. *Mol Biotechnol* 2007; **37**: 58-61 [PMID: 17914165]
34 **Ochoa JJ**, Ramirez-Tortosa MC, Quiles JL, Palomino N, Robles R, Mataix J, Huertas JR. Oxidative stress in erythrocytes from premature and full-term infants during their first 72 h of life. *Free Radic Res* 2003; **37**: 317-322 [PMID: 12688427]
35 **Góth L**. A new type of inherited catalase deficiencies: its characterization and comparison to the Japanese and Swiss type of acatalasemia. *Blood Cells Mol Dis* 2001; **27**: 512-517 [PMID: 11500062]
36 **Vitai M**, Góth L. Reference ranges of normal blood catalase activity and levels in familial hypocatalasemia in Hungary. *Clin Chim Acta* 1997; **261**: 35-42 [PMID: 9187503]
37 **Fabian E**, Bogner M, Elmadfa I. Age-related modification of antioxidant enzyme activities in relation to cardiovascular risk factors. *Eur J Clin Invest* 2012; **42**: 42-48 [PMID: 21722101 DOI: 10.1111/j.1365-2362.2011.02554.x]
38 **Andersen HR**, Nielsen JB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem* 1997; **43**: 562-568 [PMID: 9105255]
39 **Kasapoglu M**, Ozben T. Alterations of antioxidant enzymes and oxidative stress markers in aging. *Exp Gerontol* 2001; **36**: 209-220 [PMID: 11226737]
40 **Kosenko EA**, Aliev G, Tikhonova LA, Li Y, Poghosyan AC, Kaminsky YG. Antioxidant status and energy state of erythrocytes in Alzheimer dementia: probing for markers. *CNS Neurol Disord Drug Targets* 2012; **11**: 926-932 [PMID: 22998137]
41 **Matés JM**, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. *Clin Biochem* 1999; **32**: 595-603 [PMID: 10638941]
42 **Sailaja YR**, Baskar R, Saralakumari D. The antioxidant status during maturation of reticulocytes to erythrocytes in type 2 diabetics. *Free Radic Biol Med* 2003; **35**: 133-139 [PMID: 12853069]
43 **Dourado DF**, Fernandes PA, Ramos MJ. Mammalian cytosolic glutathione transferases. *Curr Protein Pept Sci* 2008; **9**: 325-337 [PMID: 18691123]
44 **Maurya PK**, Rizvi SI. Age-dependent changes in glutathione-s-transferase: correlation with total plasma antioxidant potential and red cell intracellular glutathione. *Indian J Clin Biochem* 2010; **25**: 398-400 [PMID: 21966113 DOI: 10.1007/s12291-010-0047-5]
45 **Mazzetti AP**, Fiorile MC, Primavera A, Lo Bello M. Glutathione transferases and neurodegenerative diseases. *Neurochem Int* 2015; **82**: 10-18 [PMID: 25661512 DOI: 10.1016/j.neuint.2015.01.008]
46 **Goncharova ND**, Shmaliy AV, Marenin VY, Smelkova SA. Hypothalamic-pituitary-adrenal system and enzymes of the glutathione-dependent antioxidant system during stress and aging. *Bull Exp Biol Med* 2007; **144**: 730-733 [PMID: 18683509]
47 **Tchaikovskaya T**, Fraifeld V, Urphanishvili T, Andorfer JH, Davies P, Listowsky I. Glutathione S-transferase hGSTM3 and ageing-associated neurodegeneration: relationship to Alzheimer's disease. *Mech Ageing Dev* 2005; **126**: 309-315 [PMID: 15621212]
48 **Perez-Pastene C**, Graumann R, Díaz-Grez F, Miranda M, Venegas P, Godoy OT, Layson L, Villagra R, Matamala JM, Herrera L, Segura-Aguilar J. Association of GST M1 null polymorphism with Parkinson's disease in a Chilean population with a strong Amerindian genetic component. *Neurosci Lett* 2007; **418**: 181-185 [PMID: 17403576]
49 **Zimniak P**. Detoxification reactions: relevance to aging. *Ageing Res Rev* 2008; **7**: 281-300 [PMID: 18547875]
50 **Maurya PK**, Kumar P, Siddiqui N, Tripathi P, Rizvi SI. Age-associated changes in erythrocyte glutathione peroxidase activity: correlation with total antioxidant potential. *Indian J Biochem Biophys* 2010; **47**: 319-321 [PMID: 21280570]
51 **Harisa GI**. Naringin mitigates erythrocytes aging induced by paclitaxel: an in vitro study. *J Biochem Mol Toxicol* 2014; **28**: 129-136 [PMID: 24375949 DOI: 10.1002/jbt.21544]
52 **Trevisan M**, Browne R, Ram M, Muti P, Freudenheim J, Carosella AM, Armstrong D. Correlates of markers of oxidative status in the general population. *Am J Epidemiol* 2001; **154**: 348-356 [PMID: 11495858]
53 **Yu BP**, Chung HY. Adaptive mechanisms to oxidative stress during aging. *Mech Ageing Dev* 2006; **127**: 436-443 [PMID: 16497363]
54 **Meyer M**, Pahl HL, Baeuerle PA. Regulation of the transcription factors NF-kappa B and AP-1 by redox changes. *Chem Biol Interact* 1994; **91**: 91-100 [PMID: 8194138]
55 **Kumar N**, Kant R, Maurya PK, Rizvi SI. Concentration dependent effect of (-)-Epicatechin on Na(+) /K(+) -ATPase and Ca(2+) -ATPase inhibition induced by free radicals in hypertensive patients: comparison with L-ascorbic acid. *Phytother Res* 2012; **26**: 1644-1647 [PMID: 22371366 DOI: 10.1002/ptr.4624]
56 **Maurya PK**, Prakash S. Decreased activity of Ca(++)-ATPase and Na(+)/K(+)-ATPase during aging in humans. *Appl Biochem Biotechnol* 2013; **170**: 131-137 [PMID: 23483411 DOI: 10.1007/

s12010-013-0172-8]
57 **Maurya PK**, Prakash S. Intracellular uptake of (-)epicatechin by human erythrocytes as a function of human age. *Phytother Res* 2011; **25**: 944-946 [PMID: 21626601 DOI: 10.1002/ptr.3343]
58 **Kumar P**, Maurya PK. Epigallocatechin-3-Gallate Protects Erythrocyte Ca(2+)-ATPase and Na(+)/K(+)-ATPase Against Oxidative Induced Damage During Aging in Humans. *Adv Pharm Bull* 2014; **4**: 443-447 [PMID: 25364660 DOI: 10.5681/apb.2014.065]
59 **Maurya PK**, Rizvi SI. Protective role of tea catechins on erythrocytes subjected to oxidative stress during human aging. *Nat Prod Res* 2009; **23**: 1072-1079 [PMID: 18846469 DOI: 10.1080/14786410802267643]
60 **Yildiz D**, Uslu C, Cakir Y, Oztas H. L-Cysteine influx and efflux: a possible role for red blood cells in regulation of redox status of the plasma. *Free Radic Res* 2006; **40**: 507-512 [PMID: 16551577]
61 **Rizvi SI**, Maurya PK. L-cysteine influx in erythrocytes as a function of human age. *Rejuvenation Res* 2008; **11**: 661-665 [PMID: 18593284 DOI: 10.1089/rej.2007.0652]
62 **Dröge W**. Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? *Philos Trans R Soc Lond B Biol Sci* 2005; **360**: 2355-2372 [PMID: 16321806]
63 **Kumar P**, Maurya PK. L-cysteine efflux in erythrocytes as a function of human age: correlation with reduced glutathione and total anti-oxidant potential. *Rejuvenation Res* 2013; **16**: 179-184 [PMID: 23442131 DOI: 10.1089/rej.2012.1394]

P- Reviewer: Haidara M, Mechri A **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.223

World J Methodol 2015 December 26; 5(4): 223-229
ISSN 2222-0682 (online)

MINIREVIEWS

Forkhead box protein A2 and T helper type 2-mediated pulmonary inflammation

Ling Sun, Xiao-Ju Tang, Feng-Ming Luo

Ling Sun, Xiao-Ju Tang, Laboratory of Cardiovascular Diseases Research Center of Regeneration Medicine, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Feng-Ming Luo, Department of Pulmonary Diseases, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Feng-Ming Luo, Department of Family Medicine, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Author contributions: Sun L and Tang XJ generated the figures and wrote the manuscript; Luo FM designed the aim of the editorial and wrote the manuscript.

Supported by The National Natural Science Foundation of China, Nos. 30871118, 30971325, 81270129 and 81470268 (FL); and grants from Department of Science and Technology of Sichuan Province, Nos. 09ZQ026-020 and 2009SZ0190 (FL).

Conflict-of-interest statement: None.

Correspondence to: Feng-Ming Luo, MD, PhD, Department of Family Medicine, West China Hospital, Sichuan University, 37 GuoXue Xiang, Chengdu 610041, Sichuan Province, China. luofengming@hotmail.com
Telephone: +86-28-85422759
Fax: +86-28-85422788

Received: January 29, 2015
Peer-review started: January 30, 2015
First decision: March 6, 2015
Revised: March 24, 2015
Accepted: September 29, 2015

Article in press: September 30, 2015
Published online: December 26, 2015

Abstract

The transcription factor forkhead box protein A2 (FOXA2, also known as hepatocyte nuclear factor 3β or transcription factor 3β), has been found to play pivotal roles in multiple phases of mammalian life, from the early development to the organofaction, and subsequently in homeostasis and metabolism in the adult. In the embryonic development period, FOXA2 is require d for the formation of the primitive node and notochord, and its absence results in embryonic lethality. Moreover, FOXA2 plays an important role not only in lung development, but also in T helper type 2 (Th2)-mediated pulmonary inflammation and goblet cell hyperplasia. In this article, the role of FOXA2 in lung development and Th2-mediated pulmonary inflammation, as well as in goblet cell hyperplasia, is reviewed. FOXA2 deletion in airway epithelium results into Th2-mediated pulmonary inflammation and goblet cell hyperplasia in developing lung. Leukotriene pathway and signal transducers and activators of transcription 6 pathway may mediate this inflammation through recruitment and activation of denditric cell during lung developments. FOXA2 is a potential treatment target for lung diseases with Th2 inflammation and goblet cell hyperplasia, such as asthma and chronic obstructive pulmonary disease.

Key words: Forkhead box protein A2; T helper type 2 inflammation; Pulmonary; Development; Goblet cell hyperplasia

Core tip: The transcription factor forkhead box protein A2 (FOXA2) plays pivotal roles in embryonic development and

organogenesis. Conditional deletion of FOXA2 in airway epithelial cells during the early stage of lung development will result in abnormal morphology of the lung and T helper type 2-mediated pulmonary inflammation. In addition, FOXA2 regulates the goblet cell differentiation during lung development and in pulmonary diseases such as asthma and chronic obstructive pulmonary disease. FOXA2 may be a new target for the treatment of lung disease.

Sun L, Tang XJ, Luo FM. Forkhead box protein A2 and T helper type 2-mediated pulmonary inflammation. *World J Methodol* 2015; 5(4): 223-229 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/223.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.223

INTRODUCTION

The transcription factor forkhead box protein A2 (FOXA2), also known as hepatocyte nuclear factor 3β (HNF3β), is first identified by its ability to regulate liver-specific gene expression[1]. FOXA2 is a part of the large Forkhead box (*FOX*) gene family that all members have the DNA binding "winged helix domain"[2]. The gene of *FOXA2* is located in chromosome 20p11.21. FOXA2 is able to bind to specific DNA sequence[3], activate or inhibit the transcriptional activity of target genes, and also participate in cellular signal transduction[4] and metabolism regulation[5]. Meanwhile, it plays a key role in the development[6] and mature of tissues and organs[7].

With the development of mouse embryos, the first active *FOXA* gene is FOXA2 whose RNA and protein are detected on day 6.5 of gestation in the primitive streak and node[8], suggesting that FOXA2 plays an essential role in the formation of the primitive streak and endoderm[9]. The research has indicated that FOXA2 is required for the maintenance of dopaminergic properties in ventral midbrain neurons at late embryonic stage[10]. The expression of FOXA2 is also found in the liver, pancreas, lung, intestine, thyroid gland and prostate[11], implying that FOXA2 not only regulates the organogenesis and development of liver[7,12,13] and lung[14,15], but also participates in the process of glucose[16] and lipid metabolism[17]. Many studies have also shown that FOXA2 has a close relationship with the occurrence and metastasis of tumor[18-20]. For the past few years, FOXA2 is found to participate in regulating the lung development, Th2-mediated pulmonary inflammation and goblet cell hyperplasia[21,22].

STRUCTURE OF FOXA2

FOXA2 gene is located in chromosome 20p11.21 and its length is 2242 bp. As a member of the FOXA family, FOXA2 has a forkhead domain (FHD) complexed to a target DNA. The first 3D structure of FHD resolved by X-ray crystallography was that of FOXA3/HNF3γ in 1993[3]. Subsequently, the FHD structures of other members were resolved, which are similar to that of FOXA3[23], so is FOXA2. The FHD contains three N-terminal α-helices (H1-3), three β-strands and two loops (W1-2) towards its C-terminal region[24]. The recent data about the FOXA function have identified the FOXA proteins as "pioneer factors" whose binding to promoters and enhancers enable chromatin access for other factors[25-27]. It is unique in that FOXA2 is the only one in the family which contains an AKT2/PKB phosphorylation site at the N terminus of the FHD[27]. FOXA2 has two nuclear localization sequences (NLS) which are located at both ends of the FHD[28], one of the two NLS in H1 while the other in W2[24] (Figure 1).

ROLE OF FOXA2 IN DEVELOPMENT

Research has shown that the FOX superfamily express in many kinds of organism from invertebrate to vertebrate and its subfamily FOXA participants in the whole process of the embryonic development[29]. FOXA2, a member of FOXA family, is the first gene of this family to be expressed in the progress of embryogenesis[30]. In the study of mouse embryogenesis, the expression of the *FOXA2* gene appears first at the anterior of the primitive streak. After the primitive node has formed, FOXA2 expression is localized in the primitive node, notochord and neural plate[31]. Mice lacking FOXA2 die by E10 to E11 and show marked defects in structures related to embryogenesis, without forming a distinct primitive node, aberrant somites and neural tube resulting from the absence of the notochord, and failure to form the gut tube, although endoderm cells are present[6]. The defects of the notochord and neural tube, can be ascribed to a deficiency of Sonic hedgehog, as FOXA2 cooperates with the homeobox gene Goosecoid in the activation of this gene[32]. Furthermore, FOXA2 can activate the canonical WNT-β-catenin pathway and subsequently induce the primitive extraembryonic endoderm by directly up-regulating the Wnt6[33].

With the embryonic development, the expression of *FOXA2* is also detected in definitive endoderm and endoderm-derived apparatus such as liver, pancreas, and prostatic gland, where it persists through development to adulthood[30,31,33-36]. As to the lung, *FOXA2* expresses in the endoderm which later differentiates into the lung buds, where it expresses continuously in the pulmonary epithelium to adulthood[37]. *FOXA2* is found in specific subsets of respiratory epithelial cells. In the respiratory epithelium, *FOXA2* can activate the transcription of thyroid transcription factor-1 (TTF-1), clara cell secretary protein and surfactant proteins (A-D), which mark the differentiation of epithelial cells[38,39]. Moreover, the surfactant proteins A-D play critical roles in surfactant function and homeostasis[40]. In the mice lacking *FOXA2* in conducting airways, pulmonary abnormalities are not observed by light microscopy at E18.5. However, the decreased alveolar septation and peripheral saccules appear at PN3. At PN15 and later, pulmonary

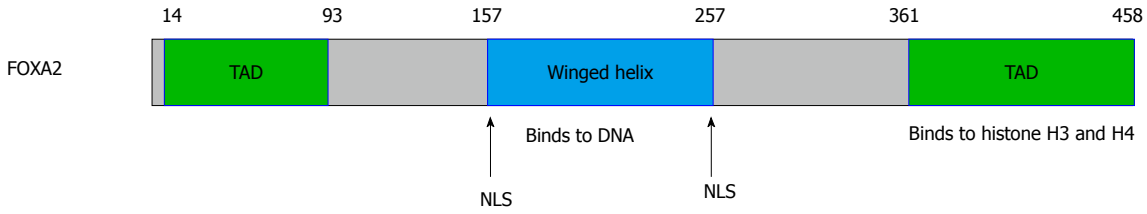

Figure 1 Structure of forkhead box protein A2 protein. FOXA2 has two transactivation domains and two nuclear localization sequences. There is a winged helix in the central of FOXA2. FOXA: Forkhead box protein A; TAD: Transcription activation domain; NLS: Nuclear localization sequences.

abnormalities including emphysema in distal airways and goblet cell hyperplasia in bronchi and bronchioles are observed in the FOXA2$^{\Delta/\Delta}$ mice[15,41]. *FOXA2* is indeed a positive regulator for E-cadherin gene[42], a cell adhesion molecule required for normal lung branching morphogenesis and cell differentiation[43,44]. Mildred *et al*[45] verified that the temporal-spatial expression patterns of *FOXA2* in the developing and regenerating of lung fit in with their proposed function in epithelial cell differentiation and regeneration, and surfactant protein gene expression. In summary, *FOXA2* plays a critical role in the development of lung.

ROLE OF FOXA2 IN TH2-MEDIATED PULMONARY INFLAMMATION AND GOBLET CELL HYPERPLASIA

Evidences showed that respiratory epithelial cells lining conducting airways regulate the inflammatory responses caused by allergens, pathogens and injurious agents[46,47]. Disruption of FOXA2 in respiratory epithelial cells results in airspace enlargement, neutrophilic pulmonary infiltrates, mucus hypersecretion, goblet cell hyperplasia and metaplasia (GCHM)[15], associated with the activation of pro-GCHM Stat6 and epidermal growth factor receptor signaling pathways[15,21,48]. Further study demonstrates that lack of FOXA2 in airway epithelial cells results in Th2-mediated pulmonary inflammation, including infiltration of eosinophiles, up-regulation of Th2 cytokines and chemokines, goblet cell hyperplasia and mucus hypersecretion, accompanied by the activation of leukotriene pathway at PN15[21]. All these findings are common in the lung of asthma patients. Therefore, FOXA2 expression in the lung may be disturbed in asthma. In fact, decreased expression of FOXA2 in the lung is found in asthma patients compared with control subjects[49]. The decreased expression of FOXA2 was negatively correlated with increased expression of mucin-5ac (MUC5ac) and chloride channel accessory 1[49]. Furthermore, the expression of FOXA2 in airway epithelial cell is inhibited by allergen challenge, and by over-expression of Th2 cytokines such as interleukin (IL)-4 and IL-13 in mouse airway epithelium[15], and also by IL-13 stimulation in human bronchial epithelial cells[48]. All these results indicate that FOXA2 plays a critical role in Th2-mediated pulmonary inflammation in developing lung. Although it inhibits goblet cell hyperplasia and metaplasia, conditional over-expression of FOXA2 in the respiratory epithelium in adult mice prior to ovalbumin (OVA) sensitization cannot alter Th2 cytokine production or inflammation in the lung[21]. Inflammatory cell counts, as well as IL-4, IL-5, IL-13, IL-10, and interferon-γ concentrations, are similar in bronchoalveolar lavage fluid (BALF) from FOXA2 over-expressing and control mice after OVA exposure[21]. Tang *et al*[22] also demonstrate that in the very early stage (from PN0 to PN10) of lung development after birth, Th2-mediated inflammation is missing in the lung of mice with FOXA2 deletion in airway epithelium. The Th2 related cytokines and chemokines are up-regulated from PN7 and Th2 inflammation in the lung is obvious on PN15. All the results indicate that Th2-mediated pulmonary inflammation induced by deletion of FOXA2 in airway epithelial cells is development-depended.

MECHANISM OF FOXA2 REGULATING TH2-MEDIATED PULMONARY INFLAMMATION AND GOBLET CELL HYPERPLASIA

The mechanism of Th2-mediated pulmonary inflammation induced by deletion of FOXA2 in airway epithelial cells remains unknown at present. Dendritic cell (DC) plays a very important role in Th2-mediated pulmonary inflammation. Chen *et al*[21] investigates the role of myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) in this inflammation. They found that the frequency of both DCs and mDC/pDC ratio are significant increased in the lung of *FOXA2*$^{\Delta/\Delta}$ mice[21]. They also found that frequencies of mDCs expressing B7-DC, B7-H1, and CD86 are significantly elevated[21]. The results indicate that increased recruitment and activation of pulmonary mDCs may mediate the Th2 inflammation in the lung in *FOXA2*$^{\Delta/\Delta}$ mice during development. However, the mechanism of the recruitment and activation of pulmonary mDCs after FOXA2 deletion in airway epithelial cells is not clear. Previous studies have provided direct evidence that cysteinyl leukotrienes (cys-LTs) plays an important role in regulating Th2 cell-dependent pulmonary inflammation[50,51]. Further study discloses that FOXA2 regulates 15-lipoxygenase (Alox15) and Alox5 gene transcription associating with leukotrienes (LTs) biosynthesis and lung inflammation[52,53]. Montelukast,

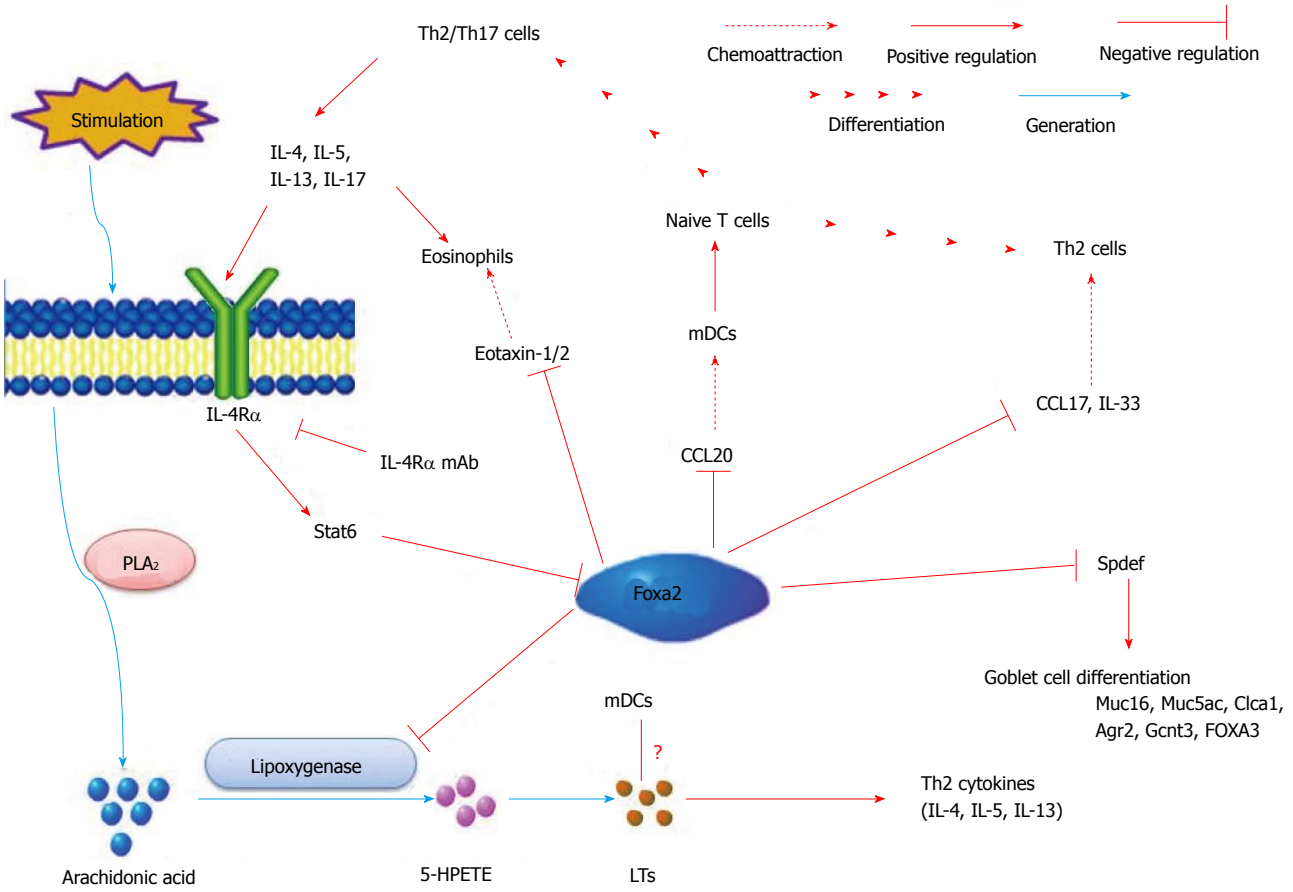

Figure 2 Network of forkhead box protein A2 regulating T helper type 2 inflammation and goblet cell hyperplasia. IL: Interleukin; mDC: Myeloid dendritic cell; CCL: Chemokine (C-C motif) ligand; Stat6: Signal transducers and activators of transcription 6; PLA2: Phosolipase A2; HPETE: Hydroperoxyeicosatetraenoic acid; LTs: Leukotrienes; MUC: Mucin; FOXA: Forkhead box protein A; Gcnt3: Glucosaminyl (N-Acetyl) transferase 3.

a selective inhibitor of the CysLT$_1$ receptor[54,55], suppresses the Th2-mediated inflammation arising from the ablation of FOXA2 in the developing mice lung. In developing FOXA2$^{\Delta/\Delta}$ mice, the increased expression of Th2 cytokines followed the activation of LT pathway. In brief, these findings uncover that FOXA2 is required for the repression of Th2-mediated pulmonary inflammation during lung development *via* its regulation to CysLT pathway[22]. Therefore, deletion of FOXA2 in the early stage of lung development leads to the spontaneous activation of LTs pathway. The activated LTs pathway may increase the recruitment and activation of pulmonary mDCs and then mediate the Th2 inflammation in the lung of *FOXA2*$^{\Delta/\Delta}$ mice during development. However, this hypothesis needs more direct evidences (Figure 2).

IL-13/IL-4-STAT6 pathway plays a critical role in Th2-mediated pulmonary inflammation[56,57]. Chen *et al*[21] tests whether Th2-mediated pulmonary inflammation and goblet cell differentiation caused by conditional deletion of FOXA2 in the airway epithelium is depend upon IL-4R–mediated signaling. The results indicate that administration of IL-4Rα mAb, an antibody which blocks IL-4Rα (a key molecular in IL-13/IL-4-STAT6 signaling pathway), significantly inhibits eosinophilic inflammation and goblet cell metaplasia and mucus hyper-production in *FOXA2*$^{\Delta/\Delta}$ mice. These results indicates that IL-4Rα-STAT6 pathway mediated the Th2 pulmonary inflammation and goblet cell hyperplasia in *FOXA2*$^{\Delta/\Delta}$ mice during lung development[21]. Wan *et al*[15] found that intratracheal administration IL-4 resulted in the decrease expression of FOXA2 and this effect is STAT6-depended. However, over-expression of FOXA2 in airway epithelium of adult mice inhibis goblet cell metaplasia and mucus hyper-production caused by OVA, but not Th2-mediated pulmonary inflammation[21]. These results indicate that the interaction between FOXA2 and IL-13/IL-4-STAT6 signaling pathway may be reciprocal in Th2-mediated inflammation and goblet cell hyperplasia in the lung.

REGULATION OF *FOXA2* EXPRESSION

FOXA2 in airway epithelial cells plays important role in lung development and Th2-mediated pulmonary inflammation, as well as in goblet cell hyperplasia. Therefore, regulation of *FOXA2* expression in airway epithelial cells may have potential role in the pathogenesis and treatment of lung diseases, such as asthma and chronic

obstructive pulmonary disease (COPD). Unfortunately, No medicine up-regulating *FOXA2* expression has been investigated in animal model or patients with COPD and asthma. Recent study indicated that Tetrapeptide Ala-Asp-Glu-Leu, a peptide which is effective on models of acute bacterial lung inflammation, fibrosis, and toxic lung damage, could increase the expression of *FOXA2* and decrease expression of MUC5ac in cultured bronchial epithelium[58]. Whether this peptide has the same effect *in vivo* has not been tested yet.

Thioredoxin-interacting protein (TXNIP) increases the expression of human islet amyloid polypeptide (IAPP) in beta-cell. TXNIP-induced FOXA2 transcription factor expression is conferring this effect by promoting FOXA2 enrichment at the proximal FOXA2 site in the IAPP promoter[59]. TXNIP can down-regulate miR-124a expression, which can directly target FOXA2. Indeed, miR-124a overexpression led to decreased FOXA2 expression and also can be effectively inhibited by TXNIP[59]. Thus, this study identifies a novel TXNIP/miR-124a/FOXA2/IAPP signaling cascade linking the critical beta-cell signaling pathway. However, whether this pathway also plays a role in airway epithelial cells and thus regulates the goblet cell hyperplasia and mucus production remains unknown.

Recent study demonstrates that over expression of NK2 homeobox 1 (NKX2-1, also known as TTF-1), inhibits allergen-induced goblet cell hyperplasia and airway inflammation[60]. Further study indicates that loss of FOXA2 in airway epithelial cell is prevented by over expression of NKX2-1 at the same time[60]. All these results suggest that NKX2-1 may regulate the FOXA2 expression in airway epithelial cell.

CONCLUSION

In conclusion, as a member of the FOX superfamily, FOXA2 participates in the formation and development of organs. Meanwhile, FOXA2 plays a very important role in lung development, Th2-mediated pulmonary inflammation and goblet cell hyperplasia. Lose of FOXA2 in the early stage of lung development will result in abnormal morphology of the lung and Th2-mediated pulmonary inflammation. FOXA2 regulates the goblet cell differentiation during lung development and in pulmonary diseases such as asthma and COPD. LTs pathway and STAT6 pathway which are regulated by FOXA2 mediate the Th2 pulmonary inflammation and goblet cell hyperplasia. Moreover, other transcription factors, such as NXK-2-1, may cooperate with FOXA2 in lung development, Th2-mediated pulmonary inflammation, and also in lung diseases with goblet cell hyperplasia.

REFERENCES

1 **Lai E**, Prezioso VR, Smith E, Litvin O, Costa RH, Darnell JE. HNF-3A, a hepatocyte-enriched transcription factor of novel structure is regulated transcriptionally. *Genes Dev* 1990; **4**: 1427-1436 [PMID: 2227418 DOI: 10.1101/gad.4.8.1427]

2 **Lai E**, Clark KL, Burley SK, Darnell JE. Hepatocyte nuclear factor 3/fork head or "winged helix" proteins: a family of transcription factors of diverse biologic function. *Proc Natl Acad Sci USA* 1993; **90**: 10421-10423 [PMID: 8248124 DOI: 10.1073/pnas.90.22.10421]

3 **Clark KL**, Halay ED, Lai E, Burley SK. Co-crystal structure of the HNF-3/fork head DNA-recognition motif resembles histone H5. *Nature* 1993; **364**: 412-420 [PMID: 8332212 DOI: 10.1038/364412a0]

4 **Maruyama R**, Grevengoed E, Stempniewicz P, Andrew DJ. Genome-wide analysis reveals a major role in cell fate maintenance and an unexpected role in endoreduplication for the Drosophila FoxA gene Fork head. *PLoS One* 2011; **6**: e20901 [PMID: 21698206 DOI: 10.1371/journal.pone.0020901]

5 **Wolfrum C**, Asilmaz E, Luca E, Friedman JM, Stoffel M. Foxa2 regulates lipid metabolism and ketogenesis in the liver during fasting and in diabetes. *Nature* 2004; **432**: 1027-1032 [PMID: 15616563 DOI: 10.1038/nature03047]

6 **Weinstein DC**, Ruiz i Altaba A, Chen WS, Hoodless P, Prezioso VR, Jessell TM, Darnell JE. The winged-helix transcription factor HNF-3 beta is required for notochord development in the mouse embryo. *Cell* 1994; **78**: 575-588 [PMID: 8069910 DOI: 10.1016/0092-8674(94)90523-1]

7 **Lee CS**, Friedman JR, Fulmer JT, Kaestner KH. The initiation of liver development is dependent on Foxa transcription factors. *Nature* 2005; **435**: 944-947 [PMID: 15959514 DOI: 10.1038/nature03649]

8 **Ruiz i Altaba A**, Prezioso VR, Darnell JE, Jessell TM. Sequential expression of HNF-3 beta and HNF-3 alpha by embryonic organizing centers: the dorsal lip/node, notochord and floor plate. *Mech Dev* 1993; **44**: 91-108 [PMID: 8155584 DOI: 10.1016/0925-4773(93)90060-B]

9 **Ang SL**, Rossant J. HNF-3 beta is essential for node and notochord formation in mouse development. *Cell* 1994; **78**: 561-574 [PMID: 8069909 DOI: 10.1016/0092-8674(94)90522-3]

10 **Stott SR**, Metzakopian E, Lin W, Kaestner KH, Hen R, Ang SL. Foxa1 and foxa2 are required for the maintenance of dopaminergic properties in ventral midbrain neurons at late embryonic stages. *J Neurosci* 2013; **33**: 8022-8034 [PMID: 23637192 DOI: 10.1523/JNEUROSCI.4774-12.2013]

11 **Clevidence DE**, Overdier DG, Tao W, Qian X, Pani L, Lai E, Costa RH. Identification of nine tissue-specific transcription factors of the hepatocyte nuclear factor 3/forkhead DNA-binding-domain family. *Proc Natl Acad Sci USA* 1993; **90**: 3948-3952 [PMID: 7683413 DOI: 10.1073/pnas.90.9.3948]

12 **Xu C**, Lu X, Chen EZ, He Z, Uyunbilig B, Li G, Ma Y, Hui L, Xie B, Gao Y, Ding X, Hu Y, Hu P, Han JD, Wang X. Genome-wide roles of Foxa2 in directing liver specification. *J Mol Cell Biol* 2012; **4**: 420-422 [PMID: 22750789 DOI: 10.1093/jmcb/mjs037]

13 **Zaret KS**, Watts J, Xu J, Wandzioch E, Smale ST, Sekiya T. Pioneer factors, genetic competence, and inductive signaling: programming liver and pancreas progenitors from the endoderm. *Cold Spring Harb Symp Quant Biol* 2008; **73**: 119-126 [PMID: 19028990 DOI: 10.1101/sqb.2008.73.040]

14 **Porter JL**, Bukey BR, Geyer AJ, Willnauer CP, Reynolds PR. Immunohistochemical detection and regulation of α5 nicotinic acetylcholine receptor (nAChR) subunits by FoxA2 during mouse lung organogenesis. *Respir Res* 2011; **12**: 82 [PMID: 21682884 DOI: 10.1186/1465-9921-12-82]

15 **Wan H**, Kaestner KH, Ang SL, Ikegami M, Finkelman FD, Stahlman MT, Fulkerson PC, Rothenberg ME, Whitsett JA. Foxa2 regulates alveolarization and goblet cell hyperplasia. *Development* 2004; **131**: 953-964 [PMID: 14757645 DOI: 10.1242/dev.00966]

16 **Pandey AK**, Bhardwaj V, Datta M. Tumour necrosis factor-alpha attenuates insulin action on phosphoenolpyruvate carboxykinase gene expression and gluconeogenesis by altering the cellular localization of Foxa2 in HepG2 cells. *FEBS J* 2009; **276**: 3757-3769 [PMID: 19769745 DOI: 10.1111/j.1742-4658.2009.07091.x]

17 **von Meyenn F**, Porstmann T, Gasser E, Selevsek N, Schmidt A, Aebersold R, Stoffel M. Glucagon-induced acetylation of Foxa2 regulates hepatic lipid metabolism. *Cell Metab* 2013; **17**: 436-447

[PMID: 23416070 DOI: 10.1016/j.cmet.2013.01.014]
18 **Tang Y**, Shu G, Yuan X, Jing N, Song J. FOXA2 functions as a suppressor of tumor metastasis by inhibition of epithelial-to-mesenchymal transition in human lung cancers. *Cell Res* 2011; **21**: 316-326 [PMID: 20820189 DOI: 10.1038/cr.2010.126]
19 **Birnbaum DJ**, Adélaïde J, Mamessier E, Finetti P, Lagarde A, Monges G, Viret F, Gonçalvès A, Turrini O, Delpero JR, Iovanna J, Giovannini M, Birnbaum D, Chaffanet M. Genome profiling of pancreatic adenocarcinoma. *Genes Chromosomes Cancer* 2011; **50**: 456-465 [PMID: 21412932 DOI: 10.1002/gcc.20870]
20 **Yu X**, Wang Y, DeGraff DJ, Wills ML, Matusik RJ. Wnt/β-catenin activation promotes prostate tumor progression in a mouse model. *Oncogene* 2011; **30**: 1868-1879 [PMID: 21151173 DOI: 10.1038/onc.2010.560]
21 **Chen G**, Wan H, Luo F, Zhang L, Xu Y, Lewkowich I, Wills-Karp M, Whitsett JA. Foxa2 programs Th2 cell-mediated innate immunity in the developing lung. *J Immunol* 2010; **184**: 6133-6141 [PMID: 20483781 DOI: 10.4049/jimmunol.1000223]
22 **Tang X**, Liu XJ, Tian C, Su Q, Lei Y, Wu Q, He Y, Whitsett JA, Luo F. Foxa2 regulates leukotrienes to inhibit Th2-mediated pulmonary inflammation. *Am J Respir Cell Mol Biol* 2013; **49**: 960-970 [PMID: 23822876 DOI: 10.1165/rcmb.2013-0122OC]
23 **Carlsson P**, Mahlapuu M. Forkhead transcription factors: key players in development and metabolism. *Dev Biol* 2002; **250**: 1-23 [PMID: 12297093 DOI: 10.1006/dbio.2002.0780]
24 **Benayoun BA**, Caburet S, Veitia RA. Forkhead transcription factors: key players in health and disease. *Trends Genet* 2011; **27**: 224-232 [PMID: 21507500 DOI: 10.1016/j.tig.2011.03.003]
25 **Zaret KS**, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. *Genes Dev* 2011; **25**: 2227-2241 [PMID: 22056668 DOI: 10.1101/gad.176826.111]
26 **Sekiya T**, Muthurajan UM, Luger K, Tulin AV, Zaret KS. Nucleosome-binding affinity as a primary determinant of the nuclear mobility of the pioneer transcription factor FoxA. *Genes Dev* 2009; **23**: 804-809 [PMID: 19339686 DOI: 10.1101/gad.1775509]
27 **Friedman JR**, Kaestner KH. The Foxa family of transcription factors in development and metabolism. *Cell Mol Life Sci* 2006; **63**: 2317-2328 [PMID: 16909212 DOI: 10.1007/s00018-006-6095-6]
28 **Romanelli MG**, Tato' L, Lorenzi P, Morandi C. Nuclear localization domains in human thyroid transcription factor 2. *Biochim Biophys Acta* 2003; **1643**: 55-64 [PMID: 14654228 DOI: 10.1016/j.bbamcr.2003.09.002]
29 **Wang DY**, Kumar S, Hedges SB. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proc Biol Sci* 1999; **266**: 163-171 [PMID: 10097391 DOI: 10.1098/rspb.1999.0617]
30 **Monaghan AP**, Kaestner KH, Grau E, Schütz G. Postimplantation expression patterns indicate a role for the mouse forkhead/HNF-3 alpha, beta and gamma genes in determination of the definitive endoderm, chordamesoderm and neuroectoderm. *Development* 1993; **119**: 567-578 [PMID: 8187630]
31 **Sasaki H**, Hogan BL. Differential expression of multiple fork head related genes during gastrulation and axial pattern formation in the mouse embryo. *Development* 1993; **118**: 47-59 [PMID: 8375339]
32 **Filosa S**, Rivera-Pérez JA, Gómez AP, Gansmuller A, Sasaki H, Behringer RR, Ang SL. Goosecoid and HNF-3beta genetically interact to regulate neural tube patterning during mouse embryogenesis. *Development* 1997; **124**: 2843-2854 [PMID: 9226455]
33 **Hwang JT**, Kelly GM. GATA6 and FOXA2 regulate Wnt6 expression during extraembryonic endoderm formation. *Stem Cells Dev* 2012; **21**: 3220-3232 [PMID: 22607194 DOI: 10.1089/scd.2011.0492]
34 **Mirosevich J**, Gao N, Matusik RJ. Expression of Foxa transcription factors in the developing and adult murine prostate. *Prostate* 2005; **62**: 339-352 [PMID: 15389796 DOI: 10.1002/pros.20131]
35 **Sasaki H**, Hogan BL. Enhancer analysis of the mouse HNF-3 beta gene: regulatory elements for node/notochord and floor plate are independent and consist of multiple sub-elements. *Genes Cells* 1996; **1**: 59-72 [PMID: 9078367 DOI: 10.1046/j.1365-2443.1996.04004.x]
36 **Yasui K**, Sasaki H, Arakaki R, Uemura M. Distribution pattern of HNF-3beta proteins in developing embryos of two mammalian species, the house shrew and the mouse. *Dev Growth Differ* 1997; **39**: 667-676 [PMID: 9493826 DOI: 10.1046/j.1440-169X.1997.t01-5-00002.x]
37 **Wan H**, Dingle S, Xu Y, Besnard V, Kaestner KH, Ang SL, Wert S, Stahlman MT, Whitsett JA. Compensatory roles of Foxa1 and Foxa2 during lung morphogenesis. *J Biol Chem* 2005; **280**: 13809-13816 [PMID: 15668254 DOI: 10.1074/jbc.M414122200]
38 **Wan H**, Xu Y, Ikegami M, Stahlman MT, Kaestner KH, Ang SL, Whitsett JA. Foxa2 is required for transition to air breathing at birth. *Proc Natl Acad Sci USA* 2004; **101**: 14449-14454 [PMID: 15452354 DOI: 10.1073/pnas.0404424101]
39 **Ikeda K**, Shaw-White JR, Wert SE, Whitsett JA. Hepatocyte nuclear factor 3 activates transcription of thyroid transcription factor 1 in respiratory epithelial cells. *Mol Cell Biol* 1996; **16**: 3626-3636 [PMID: 8668179]
40 **Whitsett JA**, Weaver TE. Hydrophobic surfactant proteins in lung function and disease. *N Engl J Med* 2002; **347**: 2141-2148 [PMID: 12501227 DOI: 10.1056/NEJMra022387]
41 **Besnard V**, Wert SE, Hull WM, Whitsett JA. Immunohistochemical localization of Foxa1 and Foxa2 in mouse embryos and adult tissues. *Gene Expr Patterns* 2004; **5**: 193-208 [PMID: 15567715 DOI: 10.1016/j.modgep.2004.08.006]
42 **Liu YN**, Lee WW, Wang CY, Chao TH, Chen Y, Chen JH. Regulatory mechanisms controlling human E-cadherin gene expression. *Oncogene* 2005; **24**: 8277-8290 [PMID: 16116478 DOI: 10.1038/sj.onc.1208991]
43 **Ye H**, Kelly TF, Samadani U, Lim L, Rubio S, Overdier DG, Roebuck KA, Costa RH. Hepatocyte nuclear factor 3/fork head homolog 11 is expressed in proliferating epithelial and mesenchymal cells of embryonic and adult tissues. *Mol Cell Biol* 1997; **17**: 1626-1641 [PMID: 9032290]
44 **Carraro G**, El-Hashash A, Guidolin D, Tiozzo C, Turcatel G, Young BM, De Langhe SP, Bellusci S, Shi W, Parnigotto PP, Warburton D. miR-17 family of microRNAs controls FGF10-mediated embryonic lung epithelial branching morphogenesis through MAPK14 and STAT3 regulation of E-Cadherin distribution. *Dev Biol* 2009; **333**: 238-250 [PMID: 19559694 DOI: 10.1016/j.ydbio.2009.06.020]
45 **Stahlman MT**, Gray ME, Whitsett JA. Temporal-spatial distribution of hepatocyte nuclear factor-3beta in developing human lung and other foregut derivatives. *J Histochem Cytochem* 1998; **46**: 955-962 [PMID: 9671445 DOI: 10.1177/002215549804600809]
46 **Diamond G**, Legarda D, Ryan LK. The innate immune response of the respiratory epithelium. *Immunol Rev* 2000; **173**: 27-38 [PMID: 10719665 DOI: 10.1034/j.1600-065X.2000.917304.x]
47 **Kato A**, Schleimer RP. Beyond inflammation: airway epithelial cells are at the interface of innate and adaptive immunity. *Curr Opin Immunol* 2007; **19**: 711-720 [PMID: 17928212 DOI: 10.1016/j.coi.2007.08.004]
48 **Zhen G**, Park SW, Nguyenvu LT, Rodriguez MW, Barbeau R, Paquet AC, Erle DJ. IL-13 and epidermal growth factor receptor have critical but distinct roles in epithelial cell mucin production. *Am J Respir Cell Mol Biol* 2007; **36**: 244-253 [PMID: 16980555 DOI: 10.1165/rcmb.2006-0180OC]
49 **Park SW**, Verhaeghe C, Nguyenvu LT, Barbeau R, Eisley CJ, Nakagami Y, Huang X, Woodruff PG, Fahy JV, Erle DJ. Distinct roles of FOXA2 and FOXA3 in allergic airway disease and asthma. *Am J Respir Crit Care Med* 2009; **180**: 603-610 [PMID: 19628779 DOI: 10.1164/rccm.200811-1768OC]
50 **Kim DC**, Hsu FI, Barrett NA, Friend DS, Grenningloh R, Ho IC, Al-Garawi A, Lora JM, Lam BK, Austen KF, Kanaoka Y. Cysteinyl leukotrienes regulate Th2 cell-dependent pulmonary inflammation. *J Immunol* 2006; **176**: 4440-4448 [PMID: 16547282 DOI: 10.4049/jimmunol.176.7.4440]
51 **Ogawa Y**, Calhoun WJ. The role of leukotrienes in airway inflammation. *J Allergy Clin Immunol* 2006; **118**: 789-798; quiz 799-800 [PMID: 17030228 DOI: 10.1016/j.jaci.2006.08.009]
52 **Andersson CK**, Claesson HE, Rydell-Törmänen K, Swedmark

S, Hällgren A, Erjefält JS. Mice lacking 12/15-lipoxygenase have attenuated airway allergic inflammation and remodeling. *Am J Respir Cell Mol Biol* 2008; **39**: 648-656 [PMID: 18511709 DOI: 10.1165/rcmb.2007-0443OC]

53 **Rådmark O**, Samuelsson B. 5-Lipoxygenase: mechanisms of regulation. *J Lipid Res* 2009; **50** Suppl: S40-S45 [PMID: 18987389 DOI: 10.1194/jlr.R800062-JLR200]

54 **Profita M**, Sala A, Bonanno A, Siena L, Ferraro M, Di Giorgi R, Montalbano AM, Albano GD, Gagliardo R, Gjomarkaj M. Cysteinyl leukotriene-1 receptor activation in a human bronchial epithelial cell line leads to signal transducer and activator of transcription 1-mediated eosinophil adhesion. *J Pharmacol Exp Ther* 2008; **325**: 1024-1030 [PMID: 18305014 DOI: 10.1124/jpet.107.131649]

55 **Meliton AY**, Munoz NM, Leff AR. Blockade of avidity and focal clustering of beta 2-integrin by cysteinyl leukotriene antagonism attenuates eosinophil adhesion. *J Allergy Clin Immunol* 2007; **120**: 1316-1323 [PMID: 17904626 DOI: 10.1016/j.jaci.2007.07.038]

56 **Hacha J**, Tomlinson K, Maertens L, Paulissen G, Rocks N, Foidart JM, Noel A, Palframan R, Gueders M, Cataldo DD. Nebulized anti-IL-13 monoclonal antibody Fab' fragment reduces allergen-induced asthma. *Am J Respir Cell Mol Biol* 2012; **47**: 709-717 [PMID: 22904197 DOI: 10.1165/rcmb.2012-0031OC]

57 **Oh CK**, Geba GP, Molfino N. Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma. *Eur Respir Rev* 2010; **19**: 46-54 [PMID: 20956165 DOI: 10.1183/09059180.00007609]

58 **Khavinson VKh**, Tendler SM, Vanyushin BF, Kasyanenko NA, Kvetnoy IM, Linkova NS, Ashapkin VV, Polyakova VO, Basharina VS, Bernadotte A. Peptide regulation of gene expression and protein synthesis in bronchial epithelium. *Lung* 2014; **192**: 781-791 [PMID: 25015171 DOI: 10.1007/s00408-014-9620-7]

59 **Jing G**, Westwell-Roper C, Chen J, Xu G, Verchere CB, Shalev A. Thioredoxin-interacting protein promotes islet amyloid polypeptide expression through miR-124a and FoxA2. *J Biol Chem* 2014; **289**: 11807-11815 [PMID: 24627476 DOI: 10.1074/jbc.M113.525022]

60 **Maeda Y**, Chen G, Xu Y, Haitchi HM, Du L, Keiser AR, Howarth PH, Davies DE, Holgate ST, Whitsett JA. Airway epithelial transcription factor NK2 homeobox 1 inhibits mucous cell metaplasia and Th2 inflammation. *Am J Respir Crit Care Med* 2011; **184**: 421-429 [PMID: 21562130 DOI: 10.1164/rccm.201101-0106OC]

P- Reviewer: Ciccone MM, Iyngkaran P, Peng Y
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.230

World J Methodol 2015 December 26; 5(4): 230-237
ISSN 2222-0682 (online)

ORIGINAL ARTICLE

Basic Study

Recombinant outer membrane protein F-B subunit of LT protein as a prophylactic measure against *Pseudomonas aeruginosa* burn infection in mice

Hassan Heydari Farsani, Iraj Rasooli, Seyed Latif Mousavi Gargari, Shahram Nazarian, Shakiba Darwish Alipour Astaneh

Hassan Heydari Farsani, Iraj Rasooli, Seyed Latif Mousavi Gargari, Department of Biology, Shahed University, Tehran-Qom Express Way, Opposite Imam Khomeini's Shrine, Tehran 3319118651, Iran

Shakiba Darwish Alipour Astaneh, Faculty of Biotechnology, University of Semnan, Semnan 3513119111, Iran

Iraj Rasooli, Molecular Microbiology Research Center, Shahed University, Tehran 1417953836, Iran

Shahram Nazarian, Department of Biology, Faculty Of Science, Imam Hussein University, Tehran 1489684511, Iran

Author contributions: Rasooli I contributed to the conception and design of the study, or acquisition of data, or analysis and interpretation of data; Farsani HH carried out the experimental part of the project; Astaneh SDA supervised the experiments; Nazarian S drafted the article; Gargari SLM revised it critically for important intellectual content; final approval of the version to be submitted was made by Rasooli I.

Institutional review board statement: This work was carried out as a part of M.Sc thesis of Mr. Hassan Heydari Farsani at Shahed University. The thesis proposal was approved by postgraduate committee in Biology department of the aforementioned University.

Institutional animal care and use committee statement: All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the Shahed University (IACUC protocol number: 917584001).

Animal care and use statement: The animals were housed in clean standard animal care facility of Shahed University. The research was carried out in compliance with the Animal Welfare Act and regulations related to experiments involving animals. The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions *viz*; 25 ℃, equal daily light/dark hours, 50% humidity and ad libitum access to food and water, for two wk prior to experimentation. The thermal injury was brought about under anaesthesia. All animals were euthanized by 150 mg/kg pentobarbital sodium for tissue collection.

Conflict-of-interest statement: The authors declare no conflict of interests.

Data sharing statement: Technical details and dataset available from the corresponding author at rasooli@shahed.ac.ir. No additional data are available.

Correspondence to: Iraj Rasooli, Professor, Department of Biology, Shahed University, Tehran-Qom Express Way, Opposite Imam Khomeini's Shrine, Tehran 3319118651, Iran. rasooli@shahed.ac.ir
Telephone: +98-21-51212200
Fax: +98-21-51212201

Received: May 9, 2015
Peer-review started: May 11, 2015
First decision: July 6, 2015
Revised: November 10, 2015
Accepted: December 1, 2015
Article in press: December 2, 2015
Published online: December 26, 2015

Abstract

AIM: To study immunogenicity of outer membrane

protein F (OprF) fused with B subunit of LT (LTB), against *Pseudomonas aeruginosa* (*P. aeruginosa*).

METHODS: The OprF, a major surface exposed outer membrane protein that is antigenically conserved in various strains of *P. aeruginosa*, is a promising immunogen against *P. aeruginosa*. In the present study recombinant OprF and OprF-LTB fusion gene was cloned, expressed and purified. BALB/c mice and rabbits were immunized using recombinant OprF and OprF-LTB and challenged at the burn site with *P. aeruginosa* lethal dose of 10^4 CFU. The protective efficacy of rabbit anti OprF IgG against *P. aeruginosa* burn infection was investigated by passive immunization.

RESULTS: It has been well established that the LTB is a powerful immunomodulator with strong adjuvant activity. LTB as a bacterial adjuvant enhanced immunogenicity of OprF and anti OprF IgG titer in serum was increased. Experimental findings showed significantly higher average survival rate in burned mice immunized with OprF-LTB than immunized with OprF or the control group. Rabbits anti OprF IgG brought about 75% survival of mice following challenge with *P. aeruginosa*. Post challenge hepatic and splenic tissues of mice group immunized with OprF-LTB had significantly lower bacterial load than those immunized with OprF or the control groups.

CONCLUSION: These results demonstrate that LTB-fused OprF might be a potential candidate protein for a prophylactic measure against *P. aeruginosa* in burn infection.

Key words: *Pseudomonas aeruginosa*; Outer membrane protein F; B subunit of LT; Immunization; Burn

Core tip: *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen that infects hospitalized, burned and immunosuppressed patients. Vaccination of high-risk groups may reduce the incidence and spread of infection. In this study outer membrane protein F (OprF) and fusion genes containing OprF and B subunit of LT were cloned and expressed. The proteins were administered to the experimental mice challenged at the burn site with lethal dose of *P. aeruginosa*. Significant protection was noted in immunized animals.

Farsani HH, Rasooli I, Gargari SLM, Nazarian S, Astaneh SDA. Recombinant outer membrane protein F-B subunit of LT protein as a prophylactic measure against *Pseudomonas aeruginosa* burn infection in mice. *World J Methodol* 2015; 5(4): 230-237 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/230.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.230

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is a major cause of morbidity and mortality in hospital acquired infections. The pathogen invades the host tissue of mainly immunocompromised patients. *This pathogen is one of the* most common organisms isolated in nosocomial pneumonia, urinary tract infection, surgical site infection, burn wounds, the cornea and the lower respiratory tract and those with the cystic fibrosis (CF)[1]. *P. aeruginosa* infecting strains are initially nonmucoid. The organism converts to mucoid and alginate producing followed by development of biofilm that enhance its antibiotic resistance[2]. The eradication of Pseudomonas frequently proves difficult due to antibiotic resistance and the ability to form a biofilm in case of chronic infection[3]. Antibiotic resistance and biofilm formation on mucosal surfaces further complicates the therapy. Hospital-derived *P. aeruginosa* strains can become colonized in burn patients that survive the initial burn trauma, are not easily eradicated with antibiotic therapy[4,5]. The initial clinical trials on *P. aeruginosa* vaccines established vaccine safety, however the limited effectiveness in preventing subsequent infection clearly evidenced the need for reevaluating correlates of vaccine efficacy[6]. Although a significant humoral response was elicited by lipopolysaccharide (LPS) vaccination, it was not able to prevent subsequent infection brought about by *P. aeruginosa*[7,8]. However, both LPS vaccines did not meet the approval for routine clinical use because of their toxicity associated with their lipid A fraction[9]. This was due in large part to the inability of the vaccine to provide protection against a broad range of *P. aeruginosa* serotypes[10]. Outer membrane proteins (OMPs), LPS and flagellin have been evaluated as vaccine candidates[11,12]. Conserved region from amino acids of flagellin and two OPMs, *i.e.*, outer membrane protein F (OprF) and OprI in clinical isolates from CF patients have been studied for their protective immunogenicity[6,13]. This sequence was used to create a vaccine preparation that was successfully tested for the ability to protect humans against *P. aeruginosa* infection[14]. Three fold increase in antigen specific IgG was reported following immunization of CF patients with an OprF-OprI fusion protein[12]. Very high IgG titers were induced in adult mice against OprF, OprI and flagellin following immunization with OprF epitope 8 (amino acid residues 311-341)-OprI-flagellins[15]. As an adjuvant the recombinant flagellin potentially affected the vaccine efficacy[16]. Despite the attractiveness of mucosal vaccination, mucosally administered antigens are frequently not immunogenic. The *P. aeruginosa* OprF protein, a major outer membrane protein that is surface exposed and antigenically conserved in various strains of *P. aeruginosa*, is a promising antigen for a vaccine[17]. It is assumed that the most active epitopes of OprF are located in the C-terminal region, because this part of the protein is located on the surface of the bacterial cell[18]. The pure recombinant and synthetic antigens used in modern day vaccines are generally less immunogenic than older style live/attenuated and killed whole organism vaccines. One can improve the quality of vaccine production by incorporating immunomodulators or adjuvants with modified delivery vehicle[19]. It has

Table 1 Primers and linkers used to amplify and fuse outer membrane protein F and B subunit of LT

Name	Sequence (5′-3′)	Restriction site
OprF-F	TTAA *AAGCTT* ATGAAACTGAAGAACACCTTAG	Hind Ⅲ
OprF-R	TATA *CTCGAG* TTACTTGGCTTCRGCTTCT	Xho Ⅰ
Linker-EAAAK-F	ATAT *AAGCTT* GAAGCTGCGGCAAAA ATGAAACTGAAGAAC	Hind Ⅲ
Linker-EAAAK-R	ATAT *CTCGAG* TTACTTGGCTTCGGCTTCTACTTCGGCTTC	Xho Ⅰ
LTB-F	ATAA*GAATTC*ATGGCTCCGCAAAG	EcoR Ⅰ
LTB-R	ATTA*AAGCTT*TTAGTTTTCCATCGAGATG	Hind Ⅲ

Restriction site sequences are shown in italic.

been well established that the B subunit of LT (LTB) and cholera toxin are powerful immunomodulators with strong adjuvant activity[20,21]. The recombinant LTB is safely and commonly used as an adjuvant to stimulate antibody responses to co-administered protein antigens, and its GM1-binding function is essential for both immunogenicity and adjuvanticity[22]. The aim of this study was to produce a recombinant chimeric protein composed of the OprF fused to LTB in order to evaluate the capacity of this fusion protein to induce a specific immunity in mice burn model against *P. aeruginosa*.

MATERIALS AND METHODS

Kits, enzymes and reagents

Plasmid extraction and gel purification kits were from Bioneer (Daejeon, South Korea). The designed primers were synthesized by Bioneer. Nickel-nitrilotriacetic acid (Ni-NTA) agarose was procured from Qiagen (Valencia, United States). Restriction endonucleases were purchased from Cinnagen (Tehran, Iran). T4 DNA ligase (Fermentas, Vilnius, Lithuania), anti-polyhistidine antibodies and anti-mouse IgG conjugated with HRP (RAY Biotech), nitrocellulose membrane (PROTRAN), microtiter plates (Nunc, United States) were used. All other chemical reagents were from Merck (Darmstadt, Germany) or Sigma (Munich, Germany).

Bacterial strains and growth conditions

E. coli BL21 (DE3) (Invitrogen) and *P. aeruginosa* (PAO1) were grown in Luria Bertani (LB) medium incubated on a shaker at 37 ℃ and 150 rpm.

Construction of OprF and LTB-OprF fusion gene

The *oprF* gene (GenBank accession No.: JX040481.1) was amplified from its genomic DNA by PCR using the OprF-F and OprF-R primers (Table 1). Forward primer was designed to contain a Hind Ⅲ site and reverse primers carried an Xho Ⅰ site. The *OprF* gene was amplified by PCR. Cyclic conditions were initiated at 95 ℃ for 5 min followed by 35 cycles of 94 ℃ for 30 s, 58 ℃ for 1 min, 72 ℃ for 90 s, and a final extension at 72 ℃ for 5 min. The amplified fragments were analyzed on 1% agarose gel. The pET28a (+) vector and PCR products were double digested with Hind Ⅲ and Xho Ⅰ and were then purified using the Bioneer Gel extraction kit. The ligation of OprF into pET28a (+) was performed using T4 DNA ligase. A helix-forming peptide linkers EAAAK was introduced between OPRF and LTB proteins. For the gene fusion with OprF and LTB, DNA was amplified using the *P. aeruginosa* chromosome as a template and oligonucleotide pairs Link-EAAAK-F and OprF-R (Table 1) as primers for the LTB-EAAAK-OprF fusion. Forward primer was designed to contain a Hind Ⅲ site and reverse primers carried an Xho Ⅰ site. In order to construct LTB-OprF fusion Gene, the *OprF* gene with a linker was inserted in Hind Ⅲ and Xho Ⅰ sites of pET28a (+) vector containing LTB gene in EcoR Ⅰ and Hind Ⅲ sites[23]. The recombinant DNA plasmids, OprF-pET28a and LTB-OprF-pET28a were transformed into *E. coli* strain BL21(DE3). The expression host was grown for 12 h at 37 ℃ in LB agar containing 70 μg/mL kanamycin.

OprF and LTB-OprF expression and purification

E. coli BL21 cells harboring the OprF-pET28a and LTB-OprF-pET28a constructs were grown at 37 ℃ under constant shaking at 200 rpm overnight in 10 mL of LB medium containing 70 mg/mL Kanamycin. The culture was then used to inoculate 200 mL of LB medium. 1 mmol/L isopropyl b-D-thiogalactoside (IPTG) was added at the optical density of 0.6 at 600 nm to induce expression. The cells were further incubated for 6 h at 37 ℃ followed by centrifugation at 10000 × *g* for 10 min at 4 ℃. The cell pellet resuspended in lysis buffer (100 mmol/L NaH_2PO_4, 10 mmol/L Tris-Cl, 8 M urea) was sonicated at 200 W at 1 min intervals for five times. The cell suspension was centrifuged at 8000 × *g* for 30 min at 4 ℃ to separate the supernatant from cellular debris. Affinity chromatography was employed to purify the protein from the supernatant using Ni^{2+}-NTA agarose under denaturation condition (Qiagen, CA). A stepwise dialysis was carried out to remove the denaturant (8 mol urea). The fractions were analyzed by SDS-PAGE. Bradford method was used for determination of protein concentration[24].

Western blotting

The purified recombinant OprF, OprF-LTB and bovine serum albumin were electrophoresed on a 12% SDS-PAGE gel to analyze the cross-reactivity. The proteins were blotted onto nitrocellulose membranes. The membrane strips were blocked with 5% nonfat dried milk and washed whit phosphate buffered saline (PBS)

(137 mmol/L NaCl, 2.7 mmol/L KCl and 4.3 mmol/L Na_2HPO_4). The membrane was incubated with rabbit anti-6X His tag antibody (Abcam). This was followed by incubation in 1:10000 dilution of horseradish peroxidase-conjugated (HRP) goat anti-rabbit IgG antibody (Sigma). Detection was carried out using HRP staining solution.

Animals husbandry

The animal care protocol was approved by Shahed University. Four-six weeks old (16-22 g) BALB/c mice procured from the Razi Institute, Tehran, Iran were housed in clean standard animal care facility of Shahed University. The research was carried out in compliance with the Animal Welfare Act and regulations related to experiments involving animals.

Immunization of mice

Mice were divided into three groups of five mice each. Ten micrograms of the recombinant protein was injected subcutaneously (sc) per mouse on days 0, 15, 30 and 45. Equal amount of Freund's complete adjuvant was used at the first dose and incomplete adjuvant in the subsequent doses. Negative control was a group of mice injected with 20 μL of PBS each time simultaneously with the test group.

Determination of Anti OprF and Anti OprF-ltb antibody titers by ELISA

Five micrograms of the recombinant proteins were coated to the surface of each well of 96-well microtiter plates and incubated overnight at 4 ℃. Sera serial dilutions from 1:250 to 1:100000 were added to each well. HRP conjugated anti-mouse IgG (100 μL) diluted to 1:3000 was added to each well as secondary antibody. Orthophenylenediamine (OPD) was added and the reaction was stopped with H_2SO_4 (2 mol/L) in order to detect the immunoreaction. The plates were analyzed at OD_{492} by ELISA reader.

Thermal injuries/ burned mouse model

The mice burned model was carried out according to Stieritz and Holder[25] with slight modifications. Six-eight-week-old female BALB/c mice (22-25 g; Razi Vaccine and Serum Research Institute, Iran) were anaesthetised intraperitoneally using a mixture of ketamine hydrochloride (100 mg/mL; Alfasan, Woerden-Holand) and xylazine (20 mg/mL; Alfasan, Woerden-Holand) in distilled water. A uniform thermal injury of 120 ℃ was brought about by exposing the depilated area for 5 s to a round brass probe of 28 mm diameter heated to thermal equilibration with boiling tap water[26]. Sterile saline (2 mL *i.p.*) was administered to support fluid balance during recovery. Mice were supervised until full recovery. Control mice were shaved but no thermal injury was performed[26].

Determination of bacterial lethal dose (LD_{50})

The mice were inoculated intraperitoneally (*i.p.*) with *P. aeruginosa*. Two hundred microliters of the bacterial suspensions at 3×10^4 to 3×10^9 CFU/mL concentrations were administered subeschar at the burn site to six groups of five BALB/c mice per group. Mortality rate was recorded for three consecutive post-challenge days. LD_{50} was defined as the volume (CFU/mL) of bacterial load that brought about death in half of the population size.

Animal challenge with P. aeruginosa (PAO1)

The microbial population was precipitated at $3000 \times g$ and was then suspended in 0.2 mL PBS. The immunized and control mice were challenged subeschar at the burn site with 10^4 CFU of *P. aeruginosa (PAO1)*.

Bacteriological examination of spleen and liver

Ten immunized and non-immunized mice were sacrificed after 72 h and spleen and liver were removed under aseptic conditions. The surfaces of the samples were washed thoroughly with sterile PBS to remove non adherent bacteria. Tissues were homogenized, and incubated in 1 mL selenite cysteine broth and were subsequently plated on SS agar plates.

In vivo neutralization assays

Antisera to the OprF and OprF-LTB proteins were raised in a New Zealand White male rabbits (Razi institute, Iran) by injecting 100 μg of OprF and OprF-LTB per animal. Lethal dose (10^4 CFU) of *P. aeruginosa* was mixed with 200 μL of immune rabbit serum (diluted to 1:800 in PBS) and was allowed to stand for 30 min at 37 ℃. Groups of five mice were injected intra-peritoneally with the lethal dose of *P. aeruginosa* to study the neutralization[27]. In order to verify that natural antibodies in rabbit serum do not offer any resistance to *P. aeruginosa in vivo*, groups of mice received mixture of lethal dose of *P. aeruginosa* and normal rabbit serum as control[28]. The animals were monitored for mortality for seven days.

Statistical analysis

The data derived from the experiments carried out in triplicate were expressed as mean ± SD. Student's *t* test was used to calculate the *P* values in order to determine the significance of differences in the experimental groups. *P* values of < 0.05 were considered as significant.

RESULTS

Construction and characterization of OprF and LTB-OprF fusion gene

The OprF and Linker-OprF genes of *P. aeruginosa* (PAO1) were successfully amplified by PCR and the fragments of appropriate size and digestion patterns of amplified genes were observed on 1% agarose gel. The amplified OprF gene was cloned into pET28a (+) and amplified Linker-OprF gene was cloned in frame, at the 3' end of the LTB gene carried by plasmid pET28a (+) and

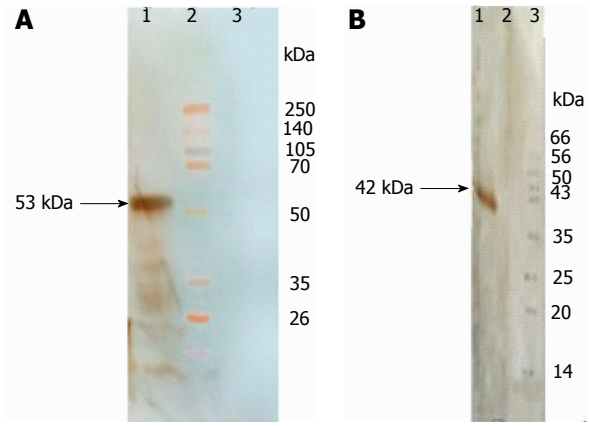

Figure 1 Western blot analysis of recombinant outer membrane protein F (A) and outer membrane protein F-B subunit of LT (B).

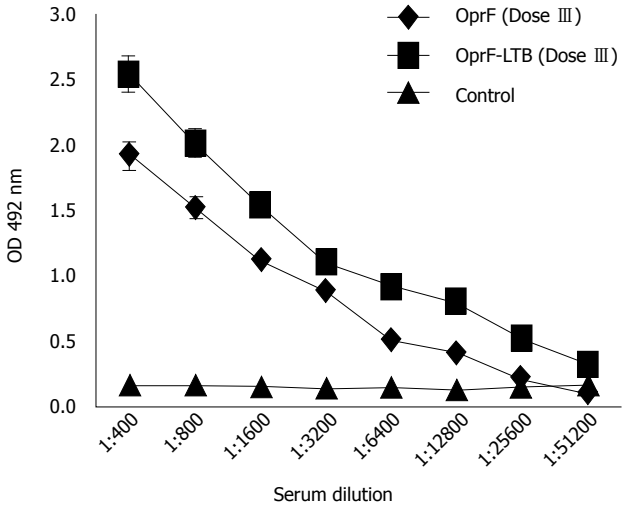

Figure 2 Comparison of IgG titer in mice sera after third dose of immunization with outer membrane protein F and outer membrane protein F- B subunit of LT. OprF: Outer membrane protein F; LTB: B subunit of LT.

confirmed by DNA sequencing. The OprF and OprF-LTB were expressed in *E. coli* BL21 (DE3). The recombinant protein was verified in insoluble pellets by SDS-PAGE. The expression of recombinant proteins was confirmed by reaction with the anti-His-tag antibodies by Western Blotting (Figure 1). Purification of the proteins were carried out under denaturation condition and SDS-PAGE analysis revealed the presence of the approximately 42 kDa (OprF), 53 kDa (OprF-LTB) proteins in the eluted fraction.

Immunogenic property of the recombinant proteins

Animals remained healthy and showed no signs of abnormal behavior after vaccination. Following immunization, mice elicited significant IgG antibodies in sera samples compared to control mice ($P < 0.05$). The antibody titer increased after the second booster, whereas animals received adjuvant and PBS, as a control had no porin-specific antibodies in sera. The combined protein administration had no significant difference with those of the single protein injections (Figure 2).

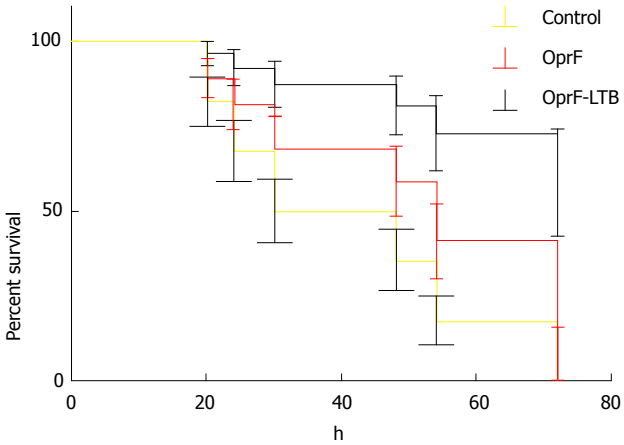

Figure 3 Survival of mice groups challenged with *Pseudomonas aeruginosa*. OprF: Outer membrane protein F; LTB: B subunit of LT.

Animal challenge with P. aeruginosa

LD_{50} was determined as 1×10^3 CFU/mL per mouse *via* injection at the burn site. Percent immunized mice survived is summarized in Figure 3. Control mice died within 24 h of challenge. Mice immunized with OprF-LTB could survive longer than mice immunized with OprF. A significant ($P < 0.01$) survival was observed in mice group immunized with OprF-LTB. The analysis of variance showed significant differences ($P < 0.01$) among the treatments.

Effect of immunization on bacterial uptake in liver and spleen

Mice immunized with OprF-LTB showed high levels of bacteria in outset and reduced gradually to $(1.4 \pm 0.33) \times 10^2$ and $(5.2 \pm 1.11) \times 10^2$ CFU per gram of spleen and liver respectively after nine days. Bacterial loads were detected per gram of spleen and liver of the mice group immunized with OprF $(4.13 \pm 1.06) \times 10^2$ and $(6.1 \pm 0.41) \times 10^3$ CFU respectively. Unimmunized mice exhibited bacterial load of $(6.00 \pm 1.00) \times 10^6$ and $(2.00 \pm 1.00) \times 10^6$ CFU per gram of spleen and liver respectively over the two-week sampling period.

In vivo neutralization assay

The antibody level raised against OprF and OprF-LTB increased in the vaccinated rabbits (Figure 4). Neutralization test was performed to determine if sera from immunized rabbits could protect naive mice against bacterial challenge. As shown in Figure 5, all experimental mice groups were significantly ($P < 0.05$) protected. All mice receiving normal rabbit serum succumbed.

DISCUSSION

P. aeruginosa still remains as a problem in the therapeutic management of nosocomial infections. Today, active

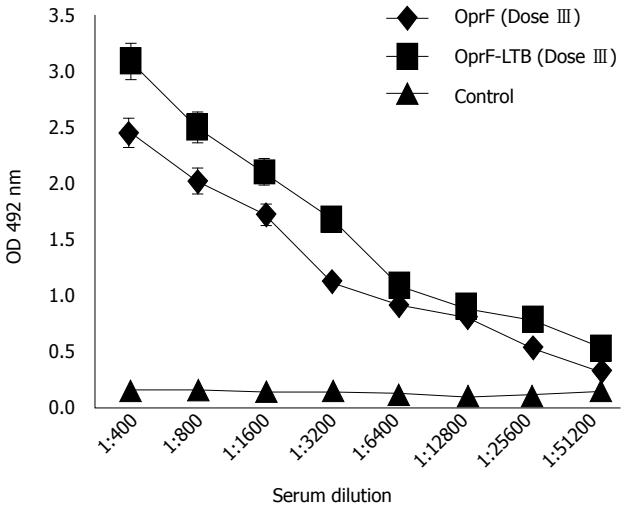

Figure 4 Comparison of IgG titer in rabbit sera after third dose of immunization with outer membrane protein F and outer membrane protein F-B subunit of LT. OprF: Outer membrane protein F; LTB: B subunit of LT.

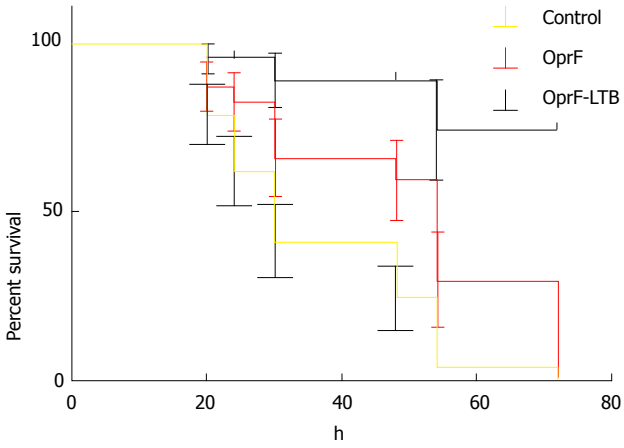

Figure 5 Passive immunization against *Pseudomonas aeruginosa*. OprF: Outer membrane protein F; LTB: B subunit of LT.

and passive immunoprophylaxis against *P. aeruginosa* infections have been widely considered because antibiotic therapy has provided extensive inherent and acquired resistance. As there is a time limitation for burned patients to respond to infectious agents, passive immunization seems to be the best therapeutic pathway for the prevention and treatment of these patients. In this study OprF and fusion genes containing OprF and LTB were cloned and expressed. The results indicate that the LTB adjuvant may enhance the efficacy of the vaccine candidate. A principal effect of LTB interaction with mammalian cells is the stable cross-linking of GM1 at the cell surface, resulting in the uptake of co administered proteins[29], and enhancement of both humoral and cellular immune responses[30-32]. In order to confirm as to whether or not the OprF-LTB and Oprf were immunogenic in mice, serum IgG antibodies were investigated. The serum IgG titers of mice group immunized with OprF-LTB was significantly increased compared to the group immunized with OprF or control group at the 6th week ($P < 0.05$). These results indicate that immunogenicity of LTB in mice. Major structural proteins such as VP2 and VP4 of porcine parvovirus were expressed in *Lactobacillus casei* fused with LTB as a mucosal adjuvant[33,34]. IgG and sIgA levels from mice orally immunized with the fusion proteins were significantly higher than those from mice receiving VP2 or VP4 only without LTB. Our results showed an enhancement the protective efficacy against *P. aeruginosa*. Experimental findings showed significantly higher survival average rate of 75% in burned mice immunized with OprF-LTB than with OprF as well as the control group. Furthermore, the challenge strain isolated from the hepatic and splenic tissues of mice group immunized with OprF-LTB post challenge was significantly lower than those from the group immunized with OprF. However both immunized groups showed significant reduction of bacterial load in spleen and liver compared to the control group. A divided by eight hundred sera dilution from rabbits immunized with OprF and OprF-LTB protected 95% and 75% mice population when mixed with 10 × LD_{50} bacterial load. Antibodies alone can provide relative protective immunity against infection that may partly be related to efficiency of opsonization in deracination of infection[35]. OprF-LTB seems to be used as a vaccine candidate where Pseudomonas infections are potential threat in burn patients.

The results demonstrate that LTB-fused OprF might be a potential candidate as a protective immunogen against *P. aeruginosa*.

ACKNOWLEDGMENTS

The authors wish to thank Shahed University for supporting this work.

COMMENTS

Background

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen that infects hospitalized, burned and immunosuppressed patients. Vaccination of high-risk groups may reduce the incidence and spread of infection. The outer membrane protein F (OprF), a major surface exposed outer membrane protein that is antigenically conserved in various strains of *P. aeruginosa*, is a promising immunogen against *P. aeruginosa*. It has been well established that the B subunit of LT (LTB) is a powerful immunomodulator with strong adjuvant activity. In order to confirm as to whether or not the OprF-LTB and Oprf were immunogenic in mice, serum IgG antibodies were investigated.

Research frontiers

A principal effect of LTB interaction with mammalian cells is the stable cross-linking of GM1 at the cell surface, resulting in the uptake of co administered proteins, and enhancement of both humoral and cellular immune responses.

Innovations and breakthroughs

The initial clinical trials on *P. aeruginosa* vaccines established vaccine safety, however the limited effectiveness in preventing subsequent infection clearly evidenced the need for reevaluating correlates of vaccine efficacy. Although a significant humoral response was elicited by lipopolysaccharide (LPS) vaccination, it was not able to prevent subsequent infection brought about by *P. aeruginosa*. However, both LPS vaccines did not meet the approval for routine clinical use

because of their toxicity associated with their lipid A fraction. Outer membrane proteins, LPS and flagellin have been evaluated as vaccine candidates.

Applications

The results demonstrate that LTB-fused OprF might be a potential candidate as a protective immunogen against *P. aeruginosa*. The *oprF* gene may be cloned into plasmid vector, and the plasmid vaccines could be delivered to mice to find its immunogenicity as a DNA vaccine.

Peer-review

This is an interesting study regarding the use of recombinant OprF-LTB protein to prevent *P. aeruginosa* burn infection in mice. The subject is clinically relevant, and the findings of this study are significant.

REFERENCES

1 **Driscoll JA**, Brody SL, Kollef MH. The epidemiology, pathogenesis and treatment of Pseudomonas aeruginosa infections. *Drugs* 2007; **67**: 351-368 [PMID: 17335295 DOI: 10.2165/00003495-200767030-00003]

2 **Li Z**, Kosorok MR, Farrell PM, Laxova A, West SE, Green CG, Collins J, Rock MJ, Splaingard ML. Longitudinal development of mucoid Pseudomonas aeruginosa infection and lung disease progression in children with cystic fibrosis. *JAMA* 2005; **293**: 581-588 [PMID: 15687313 DOI: 10.1001/jama.293.5.581]

3 **Elmanama AA**, Laham NA, Tayh GA. Antimicrobial susceptibility of bacterial isolates from burn units in Gaza. *Burns* 2013; **39**: 1612-1618 [PMID: 23664775 DOI: 10.1016/j.burns.2013.04.011]

4 **Agnihotri N**, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms--a five-year study. *Burns* 2004; **30**: 241-243 [PMID: 15082351 DOI: 10.1016/j.burns.2003.11.010]

5 **Erol S**, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. *Burns* 2004; **30**: 357-361 [PMID: 15145194 DOI: 10.1016/j.burns.2003.12.013]

6 **Weimer ET**, Lu H, Kock ND, Wozniak DJ, Mizel SB. A fusion protein vaccine containing OprF epitope 8, OprI, and type A and B flagellins promotes enhanced clearance of nonmucoid Pseudomonas aeruginosa. *Infect Immun* 2009; **77**: 2356-2366 [PMID: 19349426 DOI: 10.1128/IAI.00054-09]

7 **Zuercher AW**, Horn MP, Que JU, Ruedeberg A, Schoeni MH, Schaad UB, Marcus P, Lang AB. Antibody responses induced by long-term vaccination with an octovalent conjugate Pseudomonas aeruginosa vaccine in children with cystic fibrosis. *FEMS Immunol Med Microbiol* 2006; **47**: 302-308 [PMID: 16831219 DOI: 10.1111/j.1574-695X.2006.00103.x]

8 **Lang AB**, Rüdeberg A, Schöni MH, Que JU, Fürer E, Schaad UB. Vaccination of cystic fibrosis patients against Pseudomonas aeruginosa reduces the proportion of patients infected and delays time to infection. *Pediatr Infect Dis J* 2004; **23**: 504-510 [PMID: 15194830 DOI: 10.1097/01.inf.0000129688.50588.ac]

9 **Baumann U**, Mansouri E, von Specht BU. Recombinant OprF-OprI as a vaccine against Pseudomonas aeruginosa infections. *Vaccine* 2004; **22**: 840-847 [PMID: 15040936 DOI: 10.1016/j.vaccine.2003.11.029]

10 **DiGiandomenico A**, Rao J, Harcher K, Zaidi TS, Gardner J, Neely AN, Pier GB, Goldberg JB. Intranasal immunization with heterologously expressed polysaccharide protects against multiple Pseudomonas aeruginosa infections. *Proc Natl Acad Sci USA* 2007; **104**: 4624-4629 [PMID: 17360574 DOI: 10.1073/pnas.0608657104]

11 **Döring G**, Meisner C, Stern M. A double-blind randomized placebo-controlled phase III study of a Pseudomonas aeruginosa flagella vaccine in cystic fibrosis patients. *Proc Natl Acad Sci USA* 2007; **104**: 11020-11025 [PMID: 17585011 DOI: 10.1073/pnas.0702403104]

12 **Sorichter S**, Baumann U, Baumgart A, Walterspacher S, von Specht BU. Immune responses in the airways by nasal vaccination with systemic boosting against Pseudomonas aeruginosa in chronic lung disease. *Vaccine* 2009; **27**: 2755-2759 [PMID: 19366571 DOI: 10.1016/j.vaccine.2009.03.010]

13 **Arnold H**, Bumann D, Felies M, Gewecke B, Sörensen M, Gessner JE, Freihorst J, von Specht BU, Baumann U. Enhanced immunogenicity in the murine airway mucosa with an attenuated Salmonella live vaccine expressing OprF-OprI from Pseudomonas aeruginosa. *Infect Immun* 2004; **72**: 6546-6553 [PMID: 15501786 DOI: 10.1128/IAI.72.11.6546-6553.2004]

14 **Mansouri E**, Blome-Eberwein S, Gabelsberger J, Germann G, von Specht BU. Clinical study to assess the immunogenicity and safety of a recombinant Pseudomonas aeruginosa OprF-OprI vaccine in burn patients. *FEMS Immunol Med Microbiol* 2003; **37**: 161-166 [PMID: 12832120 DOI: 10.1016/S0928-8244(03)00072-5]

15 **Weimer ET**, Ervin SE, Wozniak DJ, Mizel SB. Immunization of young African green monkeys with OprF epitope 8-OprI-type A- and B-flagellin fusion proteins promotes the production of protective antibodies against nonmucoid Pseudomonas aeruginosa. *Vaccine* 2009; **27**: 6762-6769 [PMID: 19744586 DOI: 10.1016/j.vaccine.2009.08.080]

16 **Mizel SB**, Graff AH, Sriranganathan N, Ervin S, Lees CJ, Lively MO, Hantgan RR, Thomas MJ, Wood J, Bell B. Flagellin-F1-V fusion protein is an effective plague vaccine in mice and two species of nonhuman primates. *Clin Vaccine Immunol* 2009; **16**: 21-28 [PMID: 18987167 DOI: 10.1128/CVI.00333-08]

17 **Stanislavsky ES**, Lam JS. Pseudomonas aeruginosa antigens as potential vaccines. *FEMS Microbiol Rev* 1997; **21**: 243-277 [PMID: 9451816 DOI: 10.1111/j.1574-6976.1997.tb00353.x]

18 **Hedstrom RC**, Pavlovskis OR, Galloway DR. Antibody response of infected mice to outer membrane proteins of Pseudomonas aeruginosa. *Infect Immun* 1984; **43**: 49-53 [PMID: 6418663]

19 **Mohan T**, Verma P, Rao DN. Novel adjuvants & amp; delivery vehicles for vaccines development: a road ahead. *Indian J Med Res* 2013; **138**: 779-795 [PMID: 24434331]

20 **Hur J**, Lee JH. Enhancement of immune responses by an attenuated Salmonella enterica serovar Typhimurium strain secreting an Escherichia coli heat-labile enterotoxin B subunit protein as an adjuvant for a live Salmonella vaccine candidate. *Clin Vaccine Immunol* 2011; **18**: 203-209 [PMID: 21159921 DOI: 10.1128/CVI.00407-10]

21 **Norton EB**, Lawson LB, Freytag LC, Clements JD. Characterization of a mutant Escherichia coli heat-labile toxin, LT(R192G/L211A), as a safe and effective oral adjuvant. *Clin Vaccine Immunol* 2011; **18**: 546-551 [PMID: 21288994 DOI: 10.1128/CVI.00538-10]

22 **Williams NA**, Hirst TR, Nashar TO. Immune modulation by the cholera-like enterotoxins: from adjuvant to therapeutic. *Immunol Today* 1999; **20**: 95-101 [PMID: 10098329 DOI: 10.1016/S0167-5699(98)01397-8]

23 **Nazarian S**, Gargari SL, Rasooli I, Hasannia S, Pirooznia N. A PLGA-encapsulated chimeric protein protects against adherence and toxicity of enterotoxigenic Escherichia coli. *Microbiol Res* 2014; **169**: 205-212 [PMID: 23906742 DOI: 10.1016/j.micres.2013.06.005]

24 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254 [PMID: 942051 DOI: 10.1016/0003-2697(76)90527-3]

25 **Neely AN,** Holder IA, Warden GD. Then and now: studies using a burned mouse model reflect trends in burn research over the past 25 years. *Burns* 1999; **25**: 603-9 [DOI: 10.1016/S0305-4179(99)00068-6]

26 **Nichols DP**, Caceres S, Caverly L, Fratelli C, Kim SH, Malcolm K, Poch KR, Saavedra M, Solomon G, Taylor-Cousar J, Moskowitz S, Nick JA. Effects of azithromycin in Pseudomonas aeruginosa burn wound infection. *J Surg Res* 2013; **183**: 767-776 [PMID: 23478086 DOI: 10.1016/j.jss.2013.02.003]

27 **Stiles BG**, Sexton FW. Immunoreactivity, epitope mapping and protection studies with anti-conotoxin GI sera and various conotoxins. *Toxicon* 1992; **30**: 367-377 [PMID: 1378233 DOI:

10.1016/0041-0101(92)90533-B]
28 **Bentancor LV**, O'Malley JM, Bozkurt-Guzel C, Pier GB, Maira-Litrán T. Poly-N-acetyl-β-(1-6)-glucosamine is a target for protective immunity against Acinetobacter baumannii infections. *Infect Immun* 2012; **80**: 651-656 [PMID: 22104104 DOI: 10.1128/IAI.05653-11]
29 **Freytag LC**, Clements JD. Mucosal adjuvants. *Vaccine* 2005; **23**: 1804-1813 [PMID: 15734046 DOI: 10.1016/j.vaccine.2004.11.010]
30 **Fingerut E**, Gutter B, Goldway M, Eliahoo D, Pitcovski J. B subunit of E. coli enterotoxin as adjuvant and carrier in oral and skin vaccination. *Vet Immunol Immunopathol* 2006; **112**: 253-263 [PMID: 16701905 DOI: 10.1016/j.vetimm.2006.03.005]
31 **Moyle PM**, McGeary RP, Blanchfield JT, Toth I. Mucosal immunisation: adjuvants and delivery systems. *Curr Drug Deliv* 2004; **1**: 385-396 [PMID: 16305400 DOI: 10.2174/1567201043334588]
32 **Williams NA**. Immune modulation by the cholera-like enterotoxin B-subunits: from adjuvant to immunotherapeutic. *Int J Med Microbiol* 2000; **290**: 447-453 [PMID: 11111925 DOI: 10.1016/S1438-4221(00)80062-4]
33 **Liu D**, Wang X, Ge J, Liu S, Li Y. Comparison of the immune responses induced by oral immunization of mice with Lactobacillus casei-expressing porcine parvovirus VP2 and VP2 fused to Escherichia coli heat-labile enterotoxin B subunit protein. *Comp Immunol Microbiol Infect Dis* 2011; **34**: 73-81 [PMID: 20226529 DOI: 10.1016/j.cimid.2010.02.004]
34 **Qiao X**, Li G, Wang X, Li X, Liu M, Li Y. Recombinant porcine rotavirus VP4 and VP4-LTB expressed in Lactobacillus casei induced mucosal and systemic antibody responses in mice. *BMC Microbiol* 2009; **9**: 249 [PMID: 19958557 DOI: 10.1186/1471-2180-9-249]
35 **Taborda CP**, Rivera J, Zaragoza O, Casadevall A. More is not necessarily better: prozone-like effects in passive immunization with IgG. *J Immunol* 2003; **170**: 3621-3630 [PMID: 12646626 DOI: 10.4049/jimmunol.170.7.3621]

P- Reviewer: Chen GS, Rogers JV **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

World Journal of Methodology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.5662/wjm.v5.i4.238

World J Methodol 2015 December 26; 5(4): 238-254
ISSN 2222-0682 (online)

Laparoscopic surgery: A qualified systematic review

Alexander Buia, Florian Stockhausen, Ernst Hanisch

Alexander Buia, Florian Stockhausen, Department of General and Visceral Surgery, St. Elisabethen-Krankenhaus, Academic Teaching Hospital Goethe-University Frankfurt, D-60487 Frankfurt, Germany

Ernst Hanisch, Department of Visceral and Thoracic Surgery, Asklepios Klinik Langen, Academic Teaching Hospital Goethe-University Frankfurt, D-63225 Langen, Germany

Author contributions: Buia A, Stockhausen F and Hanisch E contributed equally to this paper; Hanisch E performed the systematic search of the literature; Buia A and Stockhausen F reviewed the full-text findings; Buia A wrote the paper; Stockhausen F and Hanisch E reviewed the final version of the text.

Conflict-of-interest statement: All the authors declare no conflict of interest.

Data sharing statement: No additional data are available.

Correspondence to: Dr. Ernst Hanisch, Professor, Department of Visceral and Thoracic Surgery, Asklepios Klinik Langen, Academic Teaching Hospital Goethe-Universität Frankfurt, Röntgenstr 20, D-63225 Langen, Germany. e.hanisch@asklepios.com
Telephone: +49-6103-9121309
Fax: +49-6103-9121814

Received: May 5, 2015
Peer-review started: May 8, 2015
First decision: June 18, 2015
Revised: August 28, 2015
Accepted: November 17, 2015
Article in press: November 25, 2015
Published online: December 26, 2015

Abstract

AIM: To review current applications of the laparoscopic surgery while highlighting the standard procedures across different fields.

METHODS: A comprehensive search was undertaken using the PubMed Advanced Search Builder. A total of 321 articles were found in this search. The following criteria had to be met for the publication to be selected: Review article, randomized controlled trials, or meta-analyses discussing the subject of laparoscopic surgery. In addition, publications were hand-searched in the Cochrane database and the high-impact journals. A total of 82 of the findings were included according to matching the inclusion criteria. Overall, 403 full-text articles were reviewed. Of these, 218 were excluded due to not matching the inclusion criteria.

RESULTS: A total of 185 relevant articles were identified matching the search criteria for an overview of the current literature on the laparoscopic surgery. Articles covered the period from the first laparoscopic application through its tremendous advancement over the last several years. Overall, the biggest advantage of the procedure has been minimizing trauma to the abdominal wall compared with open surgery. In the case of cholecystectomy, fundoplication, and adrenalectomy, the procedure has become the gold standard without being proven as a superior technique over the open surgery in randomized controlled trials. Faster recovery, reduced hospital stay, and a quicker return to normal activities are the most evident advantages of the laparoscopic surgery. Positive outcomes, efficiency, a lower rate of wound infections, and reduction in the perioperative morbidity of minimally invasive procedures have been shown in most indications.

CONCLUSION: Improvements in surgical training and developments in instruments, imaging, and surgical techniques have greatly increased safety and feasibility of the laparoscopic surgical procedures.

Key words: Laparoscopic surgery; Endoscopic surgery; Pancreatic surgery; Rectal resection; Gastrectomy; Gastric cancer; Colon resection; Appendectomy; Esophagectomy; Cholecystectomy

Core tip: This review investigates different applications of the laparoscopic approach on the basis of the current literature and summarizes studies concerning laparoscopic surgery in the different medical fields.

Buia A, Stockhausen F, Hanisch E. Laparoscopic surgery: A qualified systematic review. *World J Methodol* 2015; 5(4): 238-254 Available from: URL: http://www.wjgnet.com/2222-0682/full/v5/i4/238.htm DOI: http://dx.doi.org/10.5662/wjm.v5.i4.238

INTRODUCTION

Laparoscopic surgery has existed since the development of diagnostic laparoscopy in the 1960s. The pioneers of laparoscopic surgery Semm K[1] and Muehe E[1] changed it from a diagnostic to a surgical procedure at the beginning of the 1980s, and it has since become a frequently applied technique for a wide field of indications. The procedure has become the gold standard for many organ systems, with some of the most common being reproductive (particularly gynecological) and digestive (as for cholecystectomy). Significant improvements in surgical training, as well as developments of instruments, imaging, and surgical techniques, have made laparoscopic surgery safe and feasible across different medical fields.

This review summarizes studies on the laparoscopic surgery across different fields and highlights the state of the art, standard procedures in laparoscopic surgery.

MATERIALS AND METHODS

A comprehensive search was undertaken using the PubMed Advanced Search Builder. The exact search strings are listed in Tables 1 and 2. In addition, publications were hand-searched in the Cochrane database and in the high-impact journals such as *Ann Surg*, *Lancet*, *BMJ*, *Brit J Surg*, *World J Gastroenterol*, *Surg Endosc*, *World J Surg*, *Am J Gastroenterol*, *Hernia*, *Am J Surg*, *Langenbecks Arch Surg*, *Arch Surg, Chirurg*, *J Am Coll Surg*, and *Colorectal Dis*. Publications had to meet the following criteria to be selected: review articles, randomized controlled trials, or meta-analyses discussing the subject of laparoscopic surgery (Figure 1). A total of 403 articles were identified and downloaded for full-text review. Of these, 218 articles were excluded for not matching the inclusion criteria and 185 articles were determined to match the search criteria and included in the analysis.

The systematic review was conducted in compliance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines[2]. The study was registered on the PROSPERO international prospective register of systematic reviews (Registration Number: CRD42015019334).

RESULTS

Appendectomy

The systematic reviews and meta-analysis that compared the clinical outcome of laparoscopic *vs* open appendectomy clearly showed an advantage of the laparoscopic procedure[3]. Laparoscopic appendectomy has been proven to be a safe and feasible procedure in the treatment of acute appendicitis and even complicated appendicitis[4,5]. Supporters of the laparoscopic appendectomy maintain that in addition to an improved diagnostic accuracy, the procedure lowers the number of wound infections, results in less pain, faster recovery, and an earlier return to work[6]. However, there seems to be an increase in the operating time for the laparoscopic *vs* open surgery[7]. Earlier reviews reported an increase in incidences of intra-abdominal abscess in the laparoscopic group[8]; later reviews, however, did not find any significant difference between the laparoscopic and open surgery groups[6]. The authors explained the discrepancy in the data as a result of an adapted learning curve, better instruments and imaging quality, and of course more experienced surgeons[6].

Regarding the complication rates of laparoscopic *vs* open appendectomy, there were no significant differences in the overall postoperative complications, pulmonary complications, or postoperative ileus[3]. Considering the postoperative ileus situation after a laparoscopic appendectomy, a significant reduction in the short-term bowel obstruction in pediatric patients and patients with perforated appendicitis were described.

Regarding the long-term bowel obstruction or bowel obstruction requiring surgery, there was a significantly better outcome in the laparoscopic appendectomy group[9]. Patients who underwent the laparoscopic procedure for their appendectomy had shorter hospital stays and were able to "return to normal activity" and "return to work" faster than those who underwent open surgery[3]. The initial higher charges and operating costs in the laparoscopic group were compensated through reduced costs required outside the hospital stay[10].

In the obese patients, the outcome parameters, wound infection rate and overall postoperative complication rate were significantly decreased compared to the open appendectomy group. The operation time did increase, most likely due to technical challenges associated with patients who have a body mass index > 30 kg/m^2. Despite this, the advantages of the laparoscopic approach outweigh the disadvantages for the treatment of appendectomy in obese patients[11].

In pregnant patients, there is a higher risk of fetal loss (although with little evidence) and a slightly increased rate of preterm labor, which was not significant and was without clinical importance[12].

Table 1 Search strategy using publication within the past 5 years

	And	And	And	And	Results	Selected
Laparoscopic surgery	Pancreatic surgery	Whipple	Meta-analysis	Analysis	1	1
Laparoscopic surgery	Rectal resection	Meta-analysis			37	13
Laparoscopic surgery	Gastrectomy	Gastric cancer	Meta-analysis		65	20
Laparoscopic surgery	Liver resection	Liver surgery	Meta-analysis		30	11
Laparoscopic surgery	Colon resection	Meta-analysis			16	6
Laparoscopic appendectomy	Open appendectomy	Meta-analysis			26	13
Laparoscopic surgery	Esophagectomy	Meta-analysis			9	3

Table 2 Search strategy using unrestricted publication dates

	And	And	And	Results	Selected
Laparoscopic surgery	Open cholecystectomy	Meta-analysis		61	15
Laparoscopic surgery	Endoscopic surgery	Groin hernia	Meta-analysis	15	6
Laparoscopic surgery	Endoscopic surgery	Incisional hernia	Meta-analysis	19	6
Laparoscopic surgery	Fundoplication	Meta-analysis		42	9

Newer techniques of reduced ports surgery and single-incision surgery as a less invasive alternative are also performed as a procedure for laparoscopic appendectomy.

The reviews comparing single-incision *vs* conventional laparoscopic appendectomy show a significantly longer operation time with an outcome similar to a conventional laparoscopic procedure with no difference in wound infection rate, conversion to open surgery, reoperation, intra-abdominal collection of fluid, or overall complications[13,14]. Single-incision appendectomy is considered a safe and feasible procedure with comparable clinical outcomes when undertaken by experienced surgeons[15,16]. Results related to better cosmetics and an earlier return to work should be considered with caution due to the small number of studies reporting these two items, along with the short follow-ups used for evaluation of cosmetic results[17]. Critics of the approach have stated that there is no benefit in a single-incision access for appendectomy since clinical outcomes were related to increased operation time, higher frequency of technical failure, and potentially higher costs[18].

Cholecystectomy

Patients who underwent cholecystectomy *via* laparoscopic procedure had no significant difference in morbidity and mortality compared to those who underwent open surgery. In fact, they had faster recovery and shorter hospital stay[19]. Although mini-laparotomy had a similar overall outcome, the authors regard the minimalized open technique as a viable and safe option for healthcare providers without financial resources for laparoscopic equipment and without appropriate, trained surgeons[20]. Later studies showed no difference between laparoscopic and minimally open and conventional open surgery in terms of morbidity and mortality[21].

Laparoscopic cholecystectomy is proven as a safe and feasible technique; concerning cardiac and respiratory complications, it shows superiority over open surgery in elderly patients[22].

Patients with child A or B stadium of liver cirrhosis who underwent laparoscopic cholecystectomy had a similar or even better outcome than those who underwent open surgery. As a result, these patients experienced a lower incidence of postoperative complications[23], such as blood loss and bacterial contamination of ascites - an important cause of in-hospital morbidity and mortality[23]. The postoperative incidence of hepatic insufficiency was not different. These findings have to be regarded with caution, however, because some of the studies had small patient groups with heterogeneous patient populations and criteria[24].

The routine on-table use of intraoperative cholangiography shows no advantage in preventing injuries of the common bile duct or retained stones[25]. There is a significantly longer surgery time with the recommendation not to use routine on-table cholangiography when there are no clinical, radiological or biochemical signs of common bile duct stones[26]. In the management of suspected common bile duct stones, there is a slight advantage concerning complications after sphincterotomy in patients, where the endoscopic sphincterotomy is performed intraoperatively rather than preoperatively[27].

With laparoscopic cholecystectomy, the goal is to minimize trauma to the abdominal wall as much as possible by using the upcoming techniques of reduced port[28-30] or single-port surgery[31] or hybrid NOTES techniques. These techniques have yet to prove their feasibility, safety, and possible superiority over the established 4-port technique of laparoscopic cholecystectomy. In the case of single-incision techniques, the findings varied depending on the number of cases included in the studies. Some of the earlier studies that had been performed when single-incision surgery was newly introduced often lacked evidence and were thus biased.

Some of the studies showed better postoperative pain scores after single-incision procedure *vs* laparoscopic

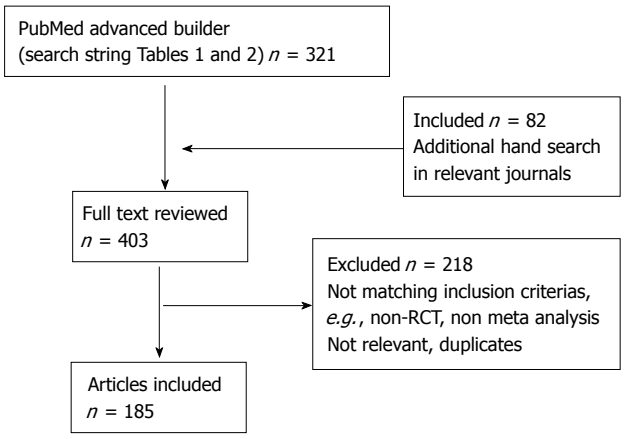

Figure 1 Selection of articles for the review.

procedure[32,33], while others showed no difference[34,35]. Because there was no significant difference in mortality, morbidity, short-term surgical outcome, length of hospital stay, or return to normal activity, many authors consider single-incision cholecystectomy safe and feasible, with a better outcome for patients' cosmetic satisfaction. Others do not recommend single-incision techniques because there is no apparent advantage over conventional laparoscopic cholecystectomy, which has longer operation times and requires additional ports[36]. Hybrid NOTES did not differ in overall complications, surgery-related complications, or postoperative pain, but did have a significantly better outcome in "return to normal activity"[37].

Laparoscopic cholecystectomy is accepted as the gold standard, although studies have shown that mini-laparotomy is similar in outcome, with decreased surgery time and lower cost[19,21]. Single-incision techniques and hybrid NOTES must prove their advantage or similarity in the same way that laparoscopic cholecystectomy has had to do.

Esophageal surgery

Minimally invasive surgery for esophageal cancer is a technically challenging procedure, and the great variability in surgical techniques makes it difficult to interpret the findings and outcomes reported in the current literature. Many studies have a small number of patients, and no randomized controlled trials are available to verify the data[38].

Patients with esophageal cancer who underwent laparoscopy experienced longer surgery time, less blood loss, shorter hospital stay, and reduced overall morbidity[39,40].

One of the difficulties in comparing results across different studies was a lack of definitions regarding postoperative complications or operative techniques. Many studies did not perform or describe a radical lymphadenectomy, and lymph node harvest was lower than the recommended minimum number[41]. Other studies have found a similar lymph node harvest[42] or even more lymph nodes in laparoscopic treatment compared with open surgery[43]. The reported minor complications associated with laparoscopy were similar to open surgery, but the major complications were significantly lower in the laparoscopic group. The lower number of harvested lymph nodes showed a similar percentage of positive lymph nodes, and the lymph node harvest did not impact the survival rate[44], even in the case of the long-term results[45]. A reported adjustment in the surgical techniques (the "Ivor Lewis approach") led to better short-term and long-term outcomes for patients undergoing minimally invasive surgery with an acceptable lymph node harvest and a similar oncological outcome to open surgery[46,47].

Burdall *et al*[48] found significant independent parameters that had an impact on outcome in overall survival. These included the use of neoadjuvant chemotherapy, tumor size (T3 or T4), positivity of lymph nodes, R1-resection, and the surgical approach. Overall, patients who underwent laparoscopic treatment had a significantly better overall survival.

Reflux surgery

The laparoscopic approach has become the gold standard for surgical management of gastroesophageal reflux disease (GERD). Laparoscopic surgery in the treatment of GERD led to reduced hospital stay, faster recovery, faster return to normal activity, and a significant reduction of perioperative morbidity compared with open surgery[49,50]. Laparoscopy has been shown to more effectively treat symptoms, such as reduction of heartburn and regurgitation, and patients reported higher quality of life than achieved with other medical treatments such as proton-pump inhibitor drug alone[51].

The different surgical techniques in the surgical management of GERD are still under discussion. The functional outcome appears similar whether the short gastric vessels are divided or not; therefore, this step in the reflux surgery procedure seems unnecessary and may reduce the operation time if avoided[52-54]. Posterior surgical techniques - the Nissen 360° and Toupet 270° procedures - gave patients a satisfactory control of reflux symptoms. Dysphagia symptoms were more prevalent after the Nissen procedure, while Toupet operations had a higher rate of gas-related symptoms, such as inability to belch and bloating[55]. The Toupet's procedure seems to have superior level 1a evidence than the Nissen procedure. However, these findings should be taken with caution since the study had a Jadad score of 2 with a corresponding possibility of bias[55]. Another evidence-based appraisal for these posterior methods in reflux surgery showed adequate symptom relief after both procedures with a slightly reduced rate of adverse results in the Toupet group[56].

The anterior approach in the treatment of GERD often leads to a longer esophagus acid exposure time, persistent heartburn symptoms and a higher rate of reoperation in the short-term outcomes compared with the posterior approach. However, these symptoms are counterbalanced by the less severe dysphagia

symptoms. For the long-term outcomes, persistence of heartburn and higher use of antacids prevail, with dysphagia scores and gas-related symptoms being similar to those in the posterior group. Hence, there is level 1a support for the usage of posterior techniques in reflux surgery[57].

Robotic surgery used in surgical treatment of GERD is a feasible alternative to laparoscopic surgery. The approach has similar results in terms of symptom relief and surgical outcome. However, lack of obvious advantages with respect to operating time and length of hospital stay and cost do not support the expansion of robotic surgery to other applications in clinics[58].

Gastric surgery

Laparoscopic gastric surgery is a demanding procedure. Results of the surgery depend on the stage of the gastric tumor (early *vs* advanced), whether a partial or total gastrectomy was performed, and presence of gastrointestinal stromal tumors (GIST)[59]. Laparoscopic resections have an overall better short-term outcome with less blood loss, shorter hospital stay, faster recovery of bowel movement with shorter time to first flatus, and fewer serious perioperative complications. However, there was an overall longer time for surgery[60]. Wound complications and surgical site infections were less significant in the laparoscopic group. For severe postoperative complications, such as anastomotic leakage, stenosis, bleeding and postoperative ileus, there was no difference from open surgery[61].

Clearance of harvested lymph nodes is an important factor influencing long-term survival in gastric cancer. Laparoscopic distal gastric resection for early gastric cancer shows fewer or a similar amount of harvested lymph nodes compared with open surgery[60,62,63]. Viñuela *et al*[64] had significantly more lymph nodes harvested in the open surgery group, and the proportion of patients with < 15 lymph nodes was similar to that in the laparoscopic group. The authors were of the opinion that adequate pathological lymph node staging was not compromised by the laparoscopic technique.

Further improvements in the laparoscopic technique show no difference between D2-lymphadenectomy performed for advanced gastric cancer compared to open surgery[65,66]. The surgical approach is not the only parameter that determines the types of or frequency of complications, as well as an acceptable long-term outcome. Other factors such as patient's age, comorbidities (*e.g.,* chronic liver disease, chronic renal failure) and additional organ resection have a significant effect on complications and outcome[67].

In advanced gastric cancer, the total laparoscopic gastrectomy consistently showed the advantages of laparoscopic surgery, having a better short-term outcome than open surgery. Incidence of the short-term complications appears to be correlated with intraoperative blood loss; hence, many studies directed their attention to this parameter. The results in all studies were consistently in favor of the laparoscopic surgery[68].

Concerning the long-term follow-up, the results of laparoscopic surgery were suggested to be similar to open procedures[69]. Current clinical evidence showed that laparoscopic gastric surgery is not inferior to open techniques, even in the treatment of advanced gastric cancer, if the surgeon is experienced[70]. The laparoscopic gastric resection had acceptable results concerning oncological safety[71]. These findings were confirmed in gastric stromal tumors. Laparoscopic surgery was superior to open techniques regarding short-term postoperative outcome without compromising oncological safety and long-term oncological outcome[72]. Furthermore, these long-term results depended mainly on the tumor itself rather than the performed surgical technique[73].

The results of robotic gastric surgery are comparable to laparoscopic surgery. The robotic gastric surgery could be favorably effective considering the increased number of harvested lymph nodes and D2-lymphadenectomy in patients with high body mass index[74]. Data on the long-term outcome and survival are rare, so randomized clinical trials are required[75].

Colorectal surgery

Laparoscopic colorectal surgery is well established as a safe procedure and must be differentiated between the surgical treatment of the benign and malignant disease. The review of current literature shows an ongoing learning curve and an improvement in the management of post-operative care, such as developing fast-track concepts and pathways in "enhanced recovery after surgery" (ERAS). This combination of the surgical procedures (minimally invasive access) reducing major morbidities and standardized postoperative treatment concepts led to a satisfying outcome for patients undergoing colorectal surgery[76]. Colorectal cancer patients who underwent laparoscopic surgery within a multimodal rehabilitation protocol experienced the shortest hospital stay and the lowest morbidity[77].

The general benefits for patients undergoing laparoscopic surgery are the minimal trauma of access, reduced pain, accelerated postoperative return of bowel function, faster return to activity, and better cosmesis. Evidence for potential benefits such as reduced adhesions and a lower rate of incisional hernias was difficult to find[78].

For the ulcerative colitis surgery, the results showed a reduced morbidity in colectomy with a lower mortality in the laparoscopic group compared to open surgery[79].

Surgical treatment of Crohn's disease showed a trend towards lower rate of wound infections, with a lower rate of reoperation in non-disease related complications in the laparoscopic group[80]. In regard to the perioperative morbidity and convalescence, there is evidence that laparoscopic surgery is favored over open surgery for the treatment of Crohn's disease. The data on the lower incidence of small bowel obstruction

and Crohn's recurrence following a minimally invasive surgery was of poor quality[81].

A laparoscopic surgical approach to diverticular disease was associated with longer operation time and reduced postoperative pain. In the elective cases, the reported quality of life score and reduced major morbidity rates were superior to open surgery[82-84]. The decreased incidence of wound infection, the lower need for blood transfusion, and the lower ileus rates led to acceptance of laparoscopic surgery in treatment of elective cases of recurrent diverticulitis[85]. Although laparoscopy minimized the short-term complication rate, a meta-analysis of randomized clinical trials found 42% morbidity in the elective cases, a worrisome finding[86].

The use of hand-assisted devices led to a decrease of conversion rates without difference in the duration of surgery or complication rates. However, all these studies had methodical limitations, along with the additional cost associated with these devices[87]. These findings were less certain in the case of emergency surgery. In generalized peritonitis due to perforated diverticulitis, the approach to drain without resection reduced the incidence of Hartmann's procedure without increasing morbidity and mortality. This finding may lead to a shift in the management of acute perforated diverticulitis in the future[88].

Natural orifice specimen extraction after colon or sigmoid resection is feasible and seems to have an advantage in minimizing trauma to the abdominal wall by avoiding a small incision for specimen extraction. Current literature, however, shows that this technique is not standardized, and no pooled analysis or meta-analysis is practicable, so evidence in the literature is weak[89].

Colon cancer

For colon cancer, the laparoscopic approach is regarded safe and feasible without compromising the oncological outcome. The short-term outcome benefits were lower blood loss, earlier recovery of bowel movement, earlier resumption of oral intake, and reduced hospital stay, as well as lower overall morbidity. The lymph node harvest and results of oncological outcome were similar to open surgery. Thus, laparoscopic surgery was considered superior to open surgery in the short-term outcome of colon cancer[90].

Expert opinion in the literature shows a growing acceptance of laparoscopic surgery for treatment of colorectal cancer, which is further supported by the large randomized clinical trials[91]. Evaluation of the long-term outcomes for non-metastatic colon cancer shows no difference in the occurrence of port site metastasis/wound recurrence, cancer-related mortality, tumor recurrence, or overall mortality[92]. Considering the anatomical circumstances and the oncological outcome, complete mesocolic excision is also recommended in the minimally invasive surgery[93].

For the right hemicolectomy, in regard to the benign and malignant disease, the intraoperative and postoperative results were favorable for the laparoscopic group. There was no difference between the laparoscopic treatment and open surgery in regard to the short-term oncological outcome, including lymph node harvest and length of specimen, and the long-term oncological outcome[94].

In transverse colon tumors, the evidence is not as clear as in other types of colorectal cancer. The benefits of the laparoscopic approach have been demonstrated; however, the data need to be interpreted with caution due to the heterogeneity in the included studies[95].

For long-term survival following laparoscopic colectomy, there was no difference in the overall survival between the laparoscopic group and open surgery. Large randomized controlled trials (excluding transverse colon cancer) demonstrated that laparoscopic surgery was not inferior to open surgery in the long-term oncological outcomes[96]. There was no significant difference in overall mortality, total recurrence rate, or 5-year disease-free and overall survival[97].

Conversion to open surgery seems to have a worse oncological long-term outcome than completion with laparoscopic surgery or starting with an open procedure from the outset. Factors for conversion include higher body mass index, male sex, T3-T4 tumor size, or positive lymph nodal disease[98].

Robotic surgery has short-term outcomes similar to laparoscopic surgery. For blood loss, conversion rate, and time to recover bowel function, there were significant findings in favor of robotic surgery compared with laparoscopy[99].

Rectal surgery

The current data is insufficient to favor a certain technique over another for treatment of rectal prolapse. Laparoscopic rectopexy had fewer postoperative complications and a shorter hospital stay compared with open surgery. The different rectopexy fixation methods did not differ in their outcomes[100]. Compared with a perineal approach (Delorme procedure), the laparoscopic group had higher overall morbidity[101].

Rectal cancer

Rectal cancer laparoscopic surgery has the benefit of minimally invasive access. In the laparoscopic group, blood loss, time to first bowel movement, intake of oral fluids and wound infection were significantly lower after the laparoscopic approach. There were no differences between the laparoscopic approach and the open surgery in terms of complications such as ureter injury, urinary retention, ileus, anastomotic leakage, or an incisional hernia. Oncological outcomes in the short-term and long-term results, such as length of specimen, circumferential resection margin, regional recurrence, port/wound metastasis and distant metastasis, also showed no significant difference[102]. Three-year, 5-year and 10-year

disease-free and overall survival was similar between the two treatments[103].

For early rectal cancer, preoperative staging determines the surgical approach to the rectum. In tumors with good prognostic features and a limited risk of relapse, which can safely be removed while preserving the rectum, the endoscopic mucosal resection or a transanal microsurgery can be performed. If local excision is pathologically difficult or risky, a complete total mesorectal excision (TME) must be done. The laparoscopic approach showed a better short-term outcome with a similar oncological outcome[104].

Contrary to the findings for colon cancer, the clinical outcome was not worse when a laparoscopic procedure was converted to open surgery in rectal cancer. The conversion had no impact on unplanned hospital readmission or redo surgery, nor on 3-year disease-free survival and local recurrence compared with a laparoscopic procedure[105].

Minimally invasive TME can also be performed safely and efficiently after neoadjuvant radiochemotherapy, with a rate similar to open surgery for postoperative complications and a comparable rate of positive circumferential resection margin[106].

Considering postoperative bladder and sexual function in patients undergoing rectal surgery for cancer, there is no difference between the laparoscopic and open surgery group for men in the postoperative ejaculatory and erectile function or bladder function. No difference in the sexual or bladder function between open and laparoscopic surgery was found in women either. Therefore, none of the techniques are superior to the other. Since there is limited data available for these results, they must be viewed with caution[107].

The oncological outcome in laparoscopic surgery was controversial when laparoscopic TME was first introduced. However, the latest results that focus on equivalent oncological outcome prove this topic. The involvement of a circumferential resection margin is equivalent to open TME. Harvest of lymph nodes in the open surgery group was described as slightly higher without having an impact on oncological outcome. In subgroup analysis, there was no difference in R0-resection, distal margin clearance, integrity of the mesorectal fascia, or in local recurrence after 5 years[108,109].

Laparoscopic rectal surgery is a challenging procedure for experienced surgeons. For trainees being supervised by an experienced surgeon, there are similar rates of anastomotic leakage with no difference in the conversion, R0-resection, local recurrence and 30-d mortality compared with experienced surgeons. Furthermore, there were no differences in the cancer-specific survival between the experienced group and the supervised trainees. Therefore, the procedure can be performed by supervised trainees with an adequate learning curve[110].

An upcoming technique is a transanal approach for performing the TME in combination with a laparoscopic approach. In the current literature, the reported TME quality with intact mesorectal fascia is satisfactorily complete, and the number of harvested lymph nodes was $\geqslant$ 12, so the oncological safety parameters seem to be adequate[111].

Robotic procedures in the rectal surgery show the known merits of robotic surgery with a favorable perioperative outcome considering the conversion rate. There were no significant differences with laparoscopic surgery in terms of surgery length, blood loss, recovery of bowel movement, hospital stay, short-term complications and details on the pathological performance such as harvested lymph nodes, resection margin, and circumferential resection margin. These results must be viewed with caution, however, because most of the studies had small sample size or a low level of evidence[112-114]. More high-quality studies are necessary to show the benefit of robotic over laparoscopic surgery and to justify its costs[115]. The long-term benefits of robotic surgery in colon and rectal cancers are still unknown[116].

Liver surgery

Laparoscopic resection of the liver is performed mainly when treating metastasis of colorectal carcinoma or primary hepatocellular carcinoma. Other indications such as cyst resection are rare. The minimally invasive approach is widely applied to patients with limited disease, such as those with solitary findings of 3-4 metastases in the liver. Findings in the liver segments Ⅱ-Ⅳ are better suited for a laparoscopic approach than those in segments Ⅶ, Ⅷ and Ⅳa, which are difficult to reach. Detailed preoperative imaging and an intraoperative ultrasound are helpful[117].

Laparoscopic liver resection has advantages for short-term outcomes such as lower blood loss with a lower rate of transfusion, shorter postoperative hospital stay, and lower rates of positive resection margin and perioperative complications. Patient selection may have influenced some of the observed outcomes[118].

Left lateral resection is described as safe and feasible, and due to lower complication rates and the known advantages of minimally invasive access, some authors claim that it should be used as a standard technique for left resection. At this time, however, there is a lack of randomized controlled trials to support such a statement[119].

Laparoscopic resection with positive short-term results and improved surgical outcome[120] do not compromise oncological outcome or 5-year disease-free and overall survival compared to patients with similar results from open surgery[121,122].

There were fewer postoperative complications in the laparoscopic group concerning short-term outcomes of hepatocellular carcinoma resection, positive resection margin rate, and tumor recurrence, with no significant difference between laparoscopic and open surgery. In short-term results, no tumor recurrence in the site of resection margin and no peritoneal dissemination or

trocar site metastasis were found in the laparoscopic group[123].

The incidence of postoperative ascites and liver failure in the laparoscopic surgery group was lower, benefiting patients with severe liver disease and especially for those with hepatocellular carcinoma[124]. For laparoscopic surgery in the treatment of liver cirrhosis, the advantages for the short-term and long-term outcomes persist[125]. Although laparoscopic liver resection is described as a safe and feasible alternative to open surgery, with favorable outcomes, there is a need for future randomized controlled trials[126] as the current studies do not provide enough support[127].

The laparoscopic approach for radiofrequency ablation is used for hepatocellular carcinoma. Intraoperative laparoscopic ultrasound led to an oncological upstaging in up to 32% in a small case series[128]. In the laparoscopic resection of liver metastases for colorectal carcinoma, these findings do not show any true benefit for the patient, so here, laparoscopy as a diagnostic method appears more as a useful instrument when a peritoneal disease is suspected[129].

Blood loss with a significant reduction of transfusion and the overall complication rate were lower in the laparoscopic group. No difference in the long-term oncological results in 1-, 3- and 5-year survival was found compared with open surgery[130,131]. Some analyses even showed a lower incidence of R1 resection in the laparoscopic group[132].

Resection of liver metastasis can be performed synchronously with colectomy, with a favorable outcome of the short-time results, but there was no difference in the long-term results in regard to the survival or recurrence. The authors consider synchronous resection of liver metastasis to be an option for selected cases, but the included studies lacked standardized inclusion criteria, so the results must be interpreted carefully[133].

In fenestration of congenital hepatic cysts, a favorable short-term outcome of the laparoscopic approach is described without a difference in postoperative complications or cyst recurrence rates, so the minimally invasive surgery is favored in these cases[134].

In living donor liver transplantation, the laparoscopic approach is seen as comparable to the open approach for liver procurement in terms of donor safety. Lower blood loss was shown in the laparoscopic group[135].

Surgery of the adrenal glands

Minimally invasive surgery of the adrenal glands has become the gold standard in resection of benign and malignant disease. Potential laparoscopic techniques are the lateral transperitoneal approach and the posterior retroperitoneal approach. Regarding outcome of these approaches, compared to each other, the posterior retroperitoneal seems to be a comparable[136] or superior[137] approach based on the operation time, blood loss, pain score, hospital stay, and time to return to normal activity[138]. The conversion rate and surgical complication rate are similar[139]. Both techniques have replaced open access to the adrenal gland in tumors $\leqslant$ 8 cm. For the retroperitoneal approach, the findings support a faster convalescence compared with the lateral transperitoneal approach. Since the difference is not significant the authors could not make a recommendation for either approach[140].

Minimally invasive adrenalectomy is the mainstay of operative options for adrenal tumors. Despite lack of evidence for the technique in the randomized controlled trials, it has gained acceptance in the surgical world. Adrenalectomy is performed in hormone-active tumors, lesions > 4 cm, and benign disease with untypical imaging[141].

Studies have shown that the laparoscopic approach can be an acceptable option for the primary neoplasm or adrenal metastasis with no evidence of local invasion[142]. If there is any doubt before the operation that the tumor cannot be removed with an intact capsule, open surgery must be considered[143].

Surgical therapy of primary aldosteronism (Conn's disease), compared with medical treatment, leads to a significantly better decrease in blood pressure, with no difference in the cardiovascular complications in qualitative analyses with heterogeneous protocols. Despite the absence of randomized controlled trials, there is support for unilateral resection of unilateral disease[144].

For subclinical Cushing's disease, unilateral laparoscopic adrenalectomy can resolve the hypercortisolism with low morbidity and can provide significant benefit to blood pressure, glucometabolic control and obesity *vs* a conservative medical treatment. However, these findings lack evidence are from studies that include data that is too low quality for a systematic review, so the authors could not give a definitive recommendation[145].

In the treatment of refractory Cushing's disease, bilateral adrenalectomy plays a crucial role in cases where transsphenoidal surgery of ACTH-producing tumors does not lead to normalization of hypercortisolism. In these cases, laparoscopic bilateral resection is safe and effective[146].

The single-incision technique showed similar benefits to the conventional laparoscopic approach. The studies found no difference in blood loss, length of hospital stay, or time to oral intake, with similar outcomes in cosmetic satisfaction, rate of complication, rate of conversion to open surgery, and need for transfusion[147]; longer duration of surgery, lower pain perception, and slightly faster recovery were also reported[148].

Robotic surgery is used in the retroperitoneal posterior and transabdominal lateral approach. A significantly lower blood loss was found in the robotic group compared with the group who underwent laparoscopic surgery, with no difference in the short-term surgical outcomes or complications[149]. With longer surgery and selection of patients, the widespread robotic techniques will be difficult[150] even with lower conversion rate for large tumors compared with laparoscopic surgery[151]. Training and costs associated with the technique are the

major drawbacks of robotic surgery in the treatment of adrenal glands[152].

Pancreatic surgery

Laparoscopic surgery of the pancreas is a challenging procedure for experienced surgeons. Most of the studies had a small sample size, and patients were highly selected. Laparoscopic left-side resection of pathologies of the distal pancreas or laparoscopic enucleation for benign lesions has been reported as safe and feasible, with morbidity comparable to open surgery[153]. Left-side resection is the most common laparoscopic procedure in pancreas surgery because it is technically easier to perform. The duration of surgery, surgical morbidity, rate of R1-resection and rate of major complications like pancreatic fistula are similar to the open surgery group; hospital stay and blood loss were reduced[154]. Long-term outcomes of the left resection are equivalent[155] even for malignant findings[156].

For laparoscopic necrosectomy, therapeutic drainage is the best option for conservative management. The literature findings were difficult to compare because of differences in the techniques used, as well as in timings of the interventions[154].

Laparoscopic pancreaticoduodenectomy is performed as a laparoscopic-assisted or total laparoscopic procedure[157]. The outcomes were comparable to open surgery in overall morbidity and surgical-related complications; oncological criteria were fulfilled in terms of number of harvested lymph nodes and resection margin. Due to a lack of randomized controlled trials and the small sample sizes of highly selected patients[158], there was a selection bias so the results should be viewed with caution[154].

Robotic surgery was reported only in the case reports or cohort series, making it difficult to compare. Trials were heterogeneous so data could not be analyzed. In the selected patients, results were comparable to laparoscopic surgery[159] with a trend to improve spleen preservation[160].

Surgery of the spleen

Laparoscopic surgery has become standard for splenectomy in elective cases. The laparoscopic approach showed a reduced morbidity, especially in the pulmonary complications after surgery and wound infection compared with open surgery[161].

In large spleens, a hand-assisted device showed an advantage to open surgery in terms of reduced pain and reduced length of hospital stay, with a surgical outcome similar to laparoscopic surgery. Furthermore, compared with laparoscopic splenectomy, the hand-assisted surgery of the spleen had a lower conversion rate to open surgery[162].

Information on the alternative methods of access like NOTES or single-incision laparoscopic splenectomy is rare. These methods are feasible, however, there is a high risk of bias in the early case reports regarding the alternative approaches in highly selected patients[163]. Only single cases on robotic surgery for splenectomy have been reported[164].

Laparoscopic hernia surgery

Groin hernia: Operative and perioperative management of hernia repair represent a wide field of surgical research. Of the tissue repair techniques, the Shouldice procedure is the only technique with recurrence rates that are lower than other open non-mesh techniques. Mesh repair is evidently superior to tissue reconstruction[165]. The Lichtenstein's procedure is the gold standard in open hernia mesh repair, and has minimal recurrence and morbidity.

There are two minimally invasive surgeries available for a groin hernia: A transabdominal preperitoneal repair (TAPP) and a totally extraperitoneal hernia repair (TEP). While the mesh is placed in the same position, the approach through the abdominal cavity (TAPP) or in front of the peritoneum (TEP) is different. Both techniques are described as effective treatments of a groin hernia but with a slight increase in perioperative morbidity in comparison to open mesh techniques[165].

Comparing the two techniques, TEP is associated with a slightly faster postoperative recovery; the TAPP technique has a significantly higher incidence of operative morbidity. For incidence of recurrence, long-term neuralgia, duration of surgery, and length of hospital stay, both laparoscopic approaches seem to be similar[166].

Comparison of the minimally invasive approach with the open mesh repair (Lichtenstein) in unilateral hernias, the TAPP technique had a similar recurrence rate and was associated with greater perioperative morbidity. The TEP technique had a slightly increased recurrence rate and a comparable incidence of perioperative complications. The minimally invasive techniques had a significantly reduced risk of chronic groin pain and groin stiffness[167]. In recurrent hernia repair, neither of the laparoscopic procedures were considered superior to open surgery in terms of recurrence and chronic pain[168].

Use of lightweight mesh in hernia repair reduced the risk of chronic groin pain[169] with no effect on recurrence[170] and showed a reduced risk of stiffness and groin body sensation[169,171].

The type of mesh fixation method in laparoscopic hernia repair led to different outcomes. Fibrin glue showed a reduced risk of chronic groin pain and a higher patient satisfaction[172] without impact on recurrence rate[173]. Use of staplers/tacks had a higher incidence of postoperative and groin pain[174].

In the TEP procedure, mesh fixation was not necessary. In cases where mesh fixation was not used, there was no difference in recurrence, incidence of seroma, or postoperative or chronic pain[175].

In the guidelines for endoscopic hernia repair, the EAES Consensus (European Association of Endoscopic Surgeons), and the International Endohernia Society recommend technical points for a tailored approach to

specific hernias on the basis of evidence[176-178].

Incisional hernia: Laparoscopic surgery for repair of an incisional hernia shows no difference in recurrence or length of hospital stay compared with open surgery. There were significantly fewer wound infections and complications requiring mesh removal[179]. Risk of bowel injury increased, while postoperative pain score showed no significant difference. Long-term results were not available from the current data[180].

A comparison of the data was difficult. There were many studies, reviews and meta-analyses regarding ventral hernia and incisional hernia, although the origin of these hernias was different. Furthermore, the optimal mesh was not found and neither was the optimal mesh fixation[181], which is why long-term results with a high level of evidence are not available[182].

The results on incisional hernia were highly biased due to methodical limitations. It was hard to distinguish whether patient-related factors or technique-related factors, such as type of mesh and fixation, influenced the outcome[183]. Compared with open surgery, there was a longer duration of operation with no difference in the short-term adverse events[184]. The incidence of wound infection was significantly decreased[185]. There was no difference in the overall recurrence. There was a lack of randomized controlled trials with a standardized technique to demonstrate one technique's superiority over the other[186].

DISCUSSION

Laparoscopic surgery has continuously developed over the past years, advancing from an invasive diagnostic tool to an efficient instrument for surgical treatment of benign and malignant disease. Ongoing training, experience, and development in imaging and laparoscopic instruments have facilitated extension of the applications of laparoscopic surgery.

The overall advantage of minimizing trauma to the abdominal wall has been reproducible in many of the laparoscopic procedures compared with open surgery. Faster convalescence, reduced hospital stay, and faster return to normal activity are the most evident advantages of laparoscopic surgery. The outcome, efficiency, decreased incidence of wound infections, and reduced perioperative morbidity of minimally invasive procedures have been shown across different applications, *e.g.,* cholecystectomy, fundoplication, and adrenalectomy. Despite the lack of randomized controlled trials to support laparoscopic applications, these procedures are accepted as the gold standard for surgical intervention. The laparoscopic surgeries are longer than the open ones. However, the duration has decreased over the years through experience and the learning curve. There has been no evidence that open surgery is superior to laparoscopic surgery in terms of oncological short- and long-term outcomes.

Interpretation of the data in meta-analyses or reviews was at times difficult. Different laparoscopic techniques were mixed up under one indication, *e.g.,* in incisional hernia repair. The use of different mesh and types of fixation led to heterogeneity in the compared studies. The possibility of bias as a methodical limitation was high.

Demonstrating the superiority of laparoscopy over other techniques, such as single-incision surgery, NOTES, and robotic surgery was difficult. Cost and effort were the major drawbacks in distribution of these techniques, particularly for the robotic surgery.

Randomized controlled trials in some fields of laparoscopic surgery could not be performed. There is a lack of sufficient evidence, because, for example, sample size calculation to achieve a certain power is too high to conduct such a study in daily surgical routine. There is a need for randomized controlled studies and standardization because many results were interpreted from case reports or cohort series, and were difficult to compare. This is especially needed in technically demanding procedures like laparoscopic gastric, pancreatic and esophagus surgeries.

COMMENTS

Background

Laparoscopic surgery is a technique with a widespread field of applications. In some medical fields, the laparoscopic approach is a standard, *e.g.,* cholecystectomy. Due to the continuous surgical training and the ongoing development of instruments, imaging systems and surgical techniques, highly complex laparoscopic procedures are possible, such as laparoscopic gastrectomy. The primary aim of this review is to show a variety of different laparoscopic surgeries and to assess their benefits and outcomes in benign and malignant diseases.

Research frontiers

This review is a clinical evaluation of laparoscopic surgery and its role in the surgical treatment of benign and malignant disease of different organ systems. Since the first laparoscopic appendectomy and the first laparoscopic cholecystectomy, there has been an overwhelming development of the technique. Therefore, this review shows a complete summary of the most frequent procedures used for digestive diseases and performed with a minimally invasive approach.

Innovations and breakthroughs

No other surgical technique has gone through such advances as laparoscopic surgery over the years, including approaches such as robotic or single-port surgery. This review summarizes the current literature (review articles, meta-analyses, and randomized trials) to give an overview of the laparoscopic procedures.

Applications

Laparoscopic surgery has become a state of the art technique in many fields of surgical treatment, providing better clinical outcomes than open surgery without compromising the oncological results.

Peer-review

The authors have performed a good study. The manuscript is interesting.

REFERENCES

1 **Reynolds W**. The first laparoscopic cholecystectomy. *JSLS* 2001; **5**: 89-94 [PMID: 11304004]

2 **Liberati A**, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700 [PMID: 19622552 DOI: 10.1136/bmj.b2700]

3 **Ohtani H**, Tamamori Y, Arimoto Y, Nishiguchi Y, Maeda K, Hirakawa K. Meta-analysis of the results of randomized controlled trials that compared laparoscopic and open surgery for acute appendicitis. *J Gastrointest Surg* 2012; **16**: 1929-1939 [PMID: 22890606 DOI: 10.1007/s11605-012-1972-9]

4 **Thomson JE**, Kruger D, Jann-Kruger C, Kiss A, Omoshoro-Jones JA, Luvhengo T, Brand M. Laparoscopic versus open surgery for complicated appendicitis: a randomized controlled trial to prove safety. *Surg Endosc* 2015; **29**: 2027-2032 [PMID: 25318368 DOI: 10.1007/s00464-014-3906-y]

5 **Markides G**, Subar D, Riyad K. Laparoscopic versus open appendectomy in adults with complicated appendicitis: systematic review and meta-analysis. *World J Surg* 2010; **34**: 2026-2040 [PMID: 20549210 DOI: 10.1007/s00268-010-0669-z]

6 **Li X**, Zhang J, Sang L, Zhang W, Chu Z, Li X, Liu Y. Laparoscopic versus conventional appendectomy--a meta-analysis of randomized controlled trials. *BMC Gastroenterol* 2010; **10**: 129 [PMID: 21047410 DOI: 10.1186/1471-230X-10-129]

7 **Wei B**, Qi CL, Chen TF, Zheng ZH, Huang JL, Hu BG, Wei HB. Laparoscopic versus open appendectomy for acute appendicitis: a metaanalysis. *Surg Endosc* 2011; **25**: 1199-1208 [PMID: 20848140 DOI: 10.1007/s00464-010-1344-z]

8 **Golub R**, Siddiqui F, Pohl D. Laparoscopic versus open appendectomy: a metaanalysis. *J Am Coll Surg* 1998; **186**: 545-553 [PMID: 9583695 DOI: 10.1016/S1072-7515(98)00080-5]

9 **Markar SR**, Penna M, Harris A. Laparoscopic approach to appendectomy reduces the incidence of short- and long-term post-operative bowel obstruction: systematic review and pooled analysis. *J Gastrointest Surg* 2014; **18**: 1683-1692 [PMID: 24950775 DOI: 10.1007/s11605-014-2572-7]

10 **Sauerland S**, Lefering R, Neugebauer EA. Laparoscopic versus open surgery for suspected appendicitis. *Cochrane Database Syst Rev* 2004; **(4)**: CD001546 [PMID: 15495014 DOI: 10.1002/14651858.CD001546.pub2]

11 **Woodham BL**, Cox MR, Eslick GD. Evidence to support the use of laparoscopic over open appendicectomy for obese individuals: a meta-analysis. *Surg Endosc* 2012; **26**: 2566-2570 [PMID: 22437955 DOI: 10.1007/s00464-012-2233-4]

12 **Wilasrusmee C**, Sukrat B, McEvoy M, Attia J, Thakkinstian A. Systematic review and meta-analysis of safety of laparoscopic versus open appendicectomy for suspected appendicitis in pregnancy. *Br J Surg* 2012; **99**: 1470-1478 [PMID: 23001791 DOI: 10.1002/bjs.8889]

13 **Antoniou SA**, Koch OO, Antoniou GA, Lasithiotakis K, Chalkiadakis GE, Pointner R, Granderath FA. Meta-analysis of randomized trials on single-incision laparoscopic versus conventional laparoscopic appendectomy. *Am J Surg* 2014; **207**: 613-622 [PMID: 24370108 DOI: 10.1016/j.amjsurg.2013.07.045]

14 **Li P**, Chen ZH, Li QG, Qiao T, Tian YY, Wang DR. Safety and efficacy of single-incision laparoscopic surgery for appendectomies: a meta-analysis. *World J Gastroenterol* 2013; **19**: 4072-4082 [PMID: 23840155 DOI: 10.3748/wjg.v19.i25.4072]

15 **Markar SR**, Karthikesalingam A, Di Franco F, Harris AM. Systematic review and meta-analysis of single-incision versus conventional multiport appendicectomy. *Br J Surg* 2013; **100**: 1709-1718 [PMID: 24227355 DOI: 10.1002/bjs.9296]

16 **Xu AM**, Huang L, Li TJ. Single-incision versus three-port laparoscopic appendectomy for acute appendicitis: systematic review and meta-analysis of randomized controlled trials. *Surg Endosc* 2015; **29**: 822-843 [PMID: 25106718 DOI: 10.1007/s00464-014-3735-z]

17 **Clerveus M**, Morandeira-Rivas A, Moreno-Sanz C, Herrero-Bogajo ML, Picazo-Yeste JS, Tadeo-Ruiz G. Systematic review and meta-analysis of randomized controlled trials comparing single incision versus conventional laparoscopic appendectomy. *World J Surg* 2014; **38**: 1937-1946 [PMID: 24682257 DOI: 10.1007/s00268-014-2535-x]

18 **Qiu J**, Yuan H, Chen S, He Z, Wu H. Single-port laparoscopic appendectomy versus conventional laparoscopic appendectomy: evidence from randomized controlled trials and nonrandomized comparative studies. *Surg Laparosc Endosc Percutan Tech* 2014; **24**: 12-21 [PMID: 24487152 DOI: 10.1097/SLE.0b013e3182937da4]

19 **Keus F**, de Jong JA, Gooszen HG, van Laarhoven CJ. Laparoscopic versus open cholecystectomy for patients with symptomatic cholecystolithiasis. *Cochrane Database Syst Rev* 2006; **(4)**: CD006231 [PMID: 17054285 DOI: 10.1002/14651858.CD006231]

20 **Purkayastha S**, Tilney HS, Georgiou P, Athanasiou T, Tekkis PP, Darzi AW. Laparoscopic cholecystectomy versus mini-laparotomy cholecystectomy: a meta-analysis of randomised control trials. *Surg Endosc* 2007; **21**: 1294-1300 [PMID: 17516122 DOI: 10.1007/s00464-007-9210-3]

21 **Keus F**, Gooszen HG, van Laarhoven CJ. Open, small-incision, or laparoscopic cholecystectomy for patients with symptomatic cholecystolithiasis. An overview of Cochrane Hepato-Biliary Group reviews. *Cochrane Database Syst Rev* 2010; **(1)**: CD008318 [PMID: 20091665 DOI: 10.1002/14651858.CD008318]

22 **Antoniou SA**, Antoniou GA, Koch OO, Pointner R, Granderath FA. Meta-analysis of laparoscopic vs open cholecystectomy in elderly patients. *World J Gastroenterol* 2014; **20**: 17626-17634 [PMID: 25516678 DOI: 10.3748/wjg.v20.i46.17626]

23 **de Goede B**, Klitsie PJ, Hagen SM, van Kempen BJ, Spronk S, Metselaar HJ, Lange JF, Kazemier G. Meta-analysis of laparoscopic versus open cholecystectomy for patients with liver cirrhosis and symptomatic cholecystolithiasis. *Br J Surg* 2013; **100**: 209-216 [PMID: 23034741 DOI: 10.1002/bjs.8911]

24 **Laurence JM**, Tran PD, Richardson AJ, Pleass HC, Lam VW. Laparoscopic or open cholecystectomy in cirrhosis: a systematic review of outcomes and meta-analysis of randomized trials. *HPB* (Oxford) 2012; **14**: 153-161 [PMID: 22321033 DOI: 10.1111/j.1477-2574.2011.00425.x]

25 **Ford JA**, Soop M, Du J, Loveday BP, Rodgers M. Systematic review of intraoperative cholangiography in cholecystectomy. *Br J Surg* 2012; **99**: 160-167 [PMID: 22183717 DOI: 10.1002/bjs.7809]

26 **Sajid MS**, Leaver C, Haider Z, Worthington T, Karanjia N, Singh KK. Routine on-table cholangiography during cholecystectomy: a systematic review. *Ann R Coll Surg Engl* 2012; **94**: 375-380 [PMID: 22943325 DOI: 10.1308/003588412X13373405385331]

27 **Gurusamy K**, Sahay SJ, Burroughs AK, Davidson BR. Systematic review and meta-analysis of intraoperative versus preoperative endoscopic sphincterotomy in patients with gallbladder and suspected common bile duct stones. *Br J Surg* 2011; **98**: 908-916 [PMID: 21472700 DOI: 10.1002/bjs.7460]

28 **Sun S**, Yang K, Gao M, He X, Tian J, Ma B. Three-port versus four-port laparoscopic cholecystectomy: meta-analysis of randomized clinical trials. *World J Surg* 2009; **33**: 1904-1908 [PMID: 19597878 DOI: 10.1007/s00268-009-0108-1]

29 **Sajid MS**, Khan MA, Ray K, Cheek E, Baig MK. Needlescopic versus laparoscopic cholecystectomy: a meta-analysis. *ANZ J Surg* 2009; **79**: 437-442 [PMID: 19566866 DOI: 10.1111/j.1445-2197.2009.04945.x]

30 **Li L**, Tian J, Tian H, Sun R, Wang Q, Yang K. The efficacy and safety of different kinds of laparoscopic cholecystectomy: a network meta analysis of 43 randomized controlled trials. *PLoS One* 2014; **9**: e90313 [PMID: 24587319 DOI: 10.1371/journal.pone.0090313]

31 **Pisanu A**, Reccia I, Porceddu G, Uccheddu A. Meta-analysis of prospective randomized studies comparing single-incision laparoscopic cholecystectomy (SILC) and conventional multiport laparoscopic cholecystectomy (CMLC). *J Gastrointest Surg* 2012; **16**: 1790-1801 [PMID: 22767084 DOI: 10.1007/s11605-012-1956-9]

32 **Geng L**, Sun C, Bai J. Single incision versus conventional laparoscopic cholecystectomy outcomes: a meta-analysis of randomized controlled trials. *PLoS One* 2013; **8**: e76530 [PMID:

24098522 DOI: 10.1371/journal.pone.0076530]
33 **Arezzo A**, Scozzari G, Famiglietti F, Passera R, Morino M. Is single-incision laparoscopic cholecystectomy safe? Results of a systematic review and meta-analysis. *Surg Endosc* 2013; **27**: 2293-2304 [PMID: 23355161 DOI: 10.1007/s00464-012-2763-9]
34 **Qiu J**, Yuan H, Chen S, He Z, Han P, Wu H. Single-port versus conventional multiport laparoscopic cholecystectomy: a meta-analysis of randomized controlled trials and nonrandomized studies. *J Laparoendosc Adv Surg Tech A* 2013; **23**: 815-831 [PMID: 24079960 DOI: 10.1089/lap.2013.0040]
35 **Zehetner J**, Pelipad D, Darehzereshki A, Mason RJ, Lipham JC, Katkhouda N. Single-access laparoscopic cholecystectomy versus classic laparoscopic cholecystectomy: a systematic review and meta-analysis of randomized controlled trials. *Surg Laparosc Endosc Percutan Tech* 2013; **23**: 235-243 [PMID: 23751985 DOI: 10.1097/SLE.0b013e31828b8b4e]
36 **Sajid MS**, Ladwa N, Kalra L, Hutson KK, Singh KK, Sayegh M. Single-incision laparoscopic cholecystectomy versus conventional laparoscopic cholecystectomy: meta-analysis and systematic review of randomized controlled trials. *World J Surg* 2012; **36**: 2644-2653 [PMID: 22855214 DOI: 10.1007/s00268-012-1719-5]
37 **Sodergren MH**, Markar S, Pucher PH, Badran IA, Jiao LR, Darzi A. Safety of transvaginal hybrid NOTES cholecystectomy: a systematic review and meta-analysis. *Surg Endosc* 2015; **29**: 2077-2090 [PMID: 25424364 DOI: 10.1007/s00464-014-3915-x]
38 **Herbella FA**, Patti MG. Minimally invasive esophagectomy. *World J Gastroenterol* 2010; **16**: 3811-3815 [PMID: 20698044 DOI: 10.1007/978-3-540-68866-2_14]
39 **Butler N**, Collins S, Memon B, Memon MA. Minimally invasive oesophagectomy: current status and future direction. *Surg Endosc* 2011; **25**: 2071-2083 [PMID: 21298548 DOI: 10.1007/s00464-010-1511-2]
40 **Watanabe M**, Baba Y, Nagai Y, Baba H. Minimally invasive esophagectomy for esophageal cancer: an updated review. *Surg Today* 2013; **43**: 237-244 [PMID: 22926551 DOI: 10.1007/s00595-012-0300-z]
41 **Hanna GB**, Arya S, Markar SR. Variation in the standard of minimally invasive esophagectomy for cancer--systematic review. *Semin Thorac Cardiovasc Surg* 2012; **24**: 176-187 [PMID: 23200072 DOI: 10.1053/j.semtcvs.2012.10.004]
42 **Safranek PM**, Cubitt J, Booth MI, Dehn TC. Review of open and minimal access approaches to oesophagectomy for cancer. *Br J Surg* 2010; **97**: 1845-1853 [PMID: 20922782 DOI: 10.1002/bjs.7231]
43 **Dantoc MM**, Cox MR, Eslick GD. Does minimally invasive esophagectomy (MIE) provide for comparable oncologic outcomes to open techniques? A systematic review. *J Gastrointest Surg* 2012; **16**: 486-494 [PMID: 22183862 DOI: 10.1007/s11605-011-1792-3]
44 **Cash JC**, Zehetner J, Hedayati B, Bildzukewicz NA, Katkhouda N, Mason RJ, Lipham JC. Outcomes following laparoscopic transhiatal esophagectomy for esophageal cancer. *Surg Endosc* 2014; **28**: 492-499 [PMID: 24100862 DOI: 10.1007/s00464-013-3230-y]
45 **Dantoc M**, Cox MR, Eslick GD. Evidence to support the use of minimally invasive esophagectomy for esophageal cancer: a meta-analysis. *Arch Surg* 2012; **147**: 768-776 [PMID: 22911078 DOI: 10.1001/archsurg.2012.1326]
46 **Zhang J**, Wang R, Liu S, Luketich JD, Chen S, Chen H, Schuchert MJ. Refinement of minimally invasive esophagectomy techniques after 15 years of experience. *J Gastrointest Surg* 2012; **16**: 1768-1774 [PMID: 22777054 DOI: 10.1007/s11605-012-1950-2]
47 **Luketich JD**, Pennathur A, Awais O, Levy RM, Keeley S, Shende M, Christie NA, Weksler B, Landreneau RJ, Abbas G, Schuchert MJ, Nason KS. Outcomes after minimally invasive esophagectomy: review of over 1000 patients. *Ann Surg* 2012; **256**: 95-103 [PMID: 22668811 DOI: 10.1097/SLA.0b013e3182590603]
48 **Burdall OC**, Boddy AP, Fullick J, Blazeby J, Krysztopik R, Streets C, Hollowood A, Barham CP, Titcomb D. A comparative study of survival after minimally invasive and open oesophagectomy. *Surg Endosc* 2015; **29**: 431-437 [PMID: 25125095 DOI: 10.1007/s00464-014-3694-4]
49 **Peters MJ**, Mukhtar A, Yunus RM, Khan S, Pappalardo J, Memon B, Memon MA. Meta-analysis of randomized clinical trials comparing open and laparoscopic anti-reflux surgery. *Am J Gastroenterol* 2009; **104**: 1548-1561; quiz 1547, 1562 [PMID: 19491872 DOI: 10.1038/ajg.2009.176]
50 **Memon MA**, Subramanya MS, Hossain MB, Yunus RM, Khan S, Memon B. Laparoscopic anterior versus posterior fundoplication for gastro-esophageal reflux disease: a meta-analysis and systematic review. *World J Surg* 2015; **39**: 981-996 [PMID: 25446479 DOI: 10.1007/s00268-014-2889-0]
51 **Rickenbacher N**, Kötter T, Kochen MM, Scherer M, Blozik E. Fundoplication versus medical management of gastroesophageal reflux disease: systematic review and meta-analysis. *Surg Endosc* 2014; **28**: 143-155 [PMID: 24018760 DOI: 10.1007/s00464-013-3140-z]
52 **Khatri K**, Sajid MS, Brodrick R, Baig MK, Sayegh M, Singh KK. Laparoscopic Nissen fundoplication with or without short gastric vessel division: a meta-analysis. *Surg Endosc* 2012; **26**: 970-978 [PMID: 22042586 DOI: 10.1007/s00464-011-1979-4]
53 **Markar SR**, Karthikesalingam AP, Wagner OJ, Jackson D, Hewes JC, Vyas S, Hashemi M. Systematic review and meta-analysis of laparoscopic Nissen fundoplication with or without division of the short gastric vessels. *Br J Surg* 2011; **98**: 1056-1062 [PMID: 21560121 DOI: 10.1002/bjs.7519]
54 **Engström C**, Jamieson GG, Devitt PG, Watson DI. Meta-analysis of two randomized controlled trials to identify long-term symptoms after division of the short gastric vessels during Nissen fundoplication. *Br J Surg* 2011; **98**: 1063-1067 [PMID: 21618497 DOI: 10.1002/bjs.7563]
55 **Broeders JA**, Mauritz FA, Ahmed Ali U, Draaisma WA, Ruurda JP, Gooszen HG, Smout AJ, Broeders IA, Hazebroek EJ. Systematic review and meta-analysis of laparoscopic Nissen (posterior total) versus Toupet (posterior partial) fundoplication for gastro-oesophageal reflux disease. *Br J Surg* 2010; **97**: 1318-1330 [PMID: 20641062 DOI: 10.1002/bjs.7174]
56 **Shan CX**, Zhang W, Zheng XM, Jiang DZ, Liu S, Qiu M. Evidence-based appraisal in laparoscopic Nissen and Toupet fundoplications for gastroesophageal reflux disease. *World J Gastroenterol* 2010; **16**: 3063-3071 [PMID: 20572311 DOI: 10.3748/wjg.v16.i24.3063]
57 **Broeders JA**, Roks DJ, Ahmed Ali U, Draaisma WA, Smout AJ, Hazebroek EJ. Laparoscopic anterior versus posterior fundoplication for gastroesophageal reflux disease: systematic review and meta-analysis of randomized clinical trials. *Ann Surg* 2011; **254**: 39-47 [PMID: 21543968 DOI: 10.1097/SLA.0b013e31821d4ba0]
58 **Mi J**, Kang Y, Chen X, Wang B, Wang Z. Whether robot-assisted laparoscopic fundoplication is better for gastroesophageal reflux disease in adults: a systematic review and meta-analysis. *Surg Endosc* 2010; **24**: 1803-1814 [PMID: 20112116 DOI: 10.1007/s00464-009-0873-9]
59 **Parisi A**, Nguyen NT, Reim D, Zhang S, Jiang ZW, Brower ST, Azagra JS, Facy O, Alimoglu O, Jackson PG, Tsujimoto H, Kurokawa Y, Zang L, Coburn NG, Yu PW, Zhang B, Qi F, Coratti A, Annecchiarico M, Novotny A, Goergen M, Lequeu JB, Eren T, Leblebici M, Al-Refaie W, Takiguchi S, Ma J, Zhao YL, Liu T, Desiderio J. Current status of minimally invasive surgery for gastric cancer: A literature review to highlight studies limits. *Int J Surg* 2015; **17**: 34-40 [PMID: 25758348 DOI: 10.1016/j.ijsu.2015.02.021]
60 **Zhang CD**, Chen SC, Feng ZF, Zhao ZM, Wang JN, Dai DQ. Laparoscopic versus open gastrectomy for early gastric cancer in Asia: a meta-analysis. *Surg Laparosc Endosc Percutan Tech* 2013; **23**: 365-377 [PMID: 23917592 DOI: 10.1097/SLE.0b013e31828e3e6e]
61 **Inokuchi M**, Sugita H, Otsuki S, Sato Y, Nakagawa M, Kojima K. Laparoscopic distal gastrectomy reduced surgical site infection as compared with open distal gastrectomy for gastric cancer in a meta-analysis of both randomized controlled and case-controlled studies. *Int J Surg* 2015; **15**: 61-67 [PMID: 25644544 DOI: 10.1016/j.ijsu.2015.01.030]
62 **Sun J**, Li J, Wang J, Pan T, Zhou J, Fu X, Zhang S. Meta-analysis of randomized controlled trials on laparoscopic gastrectomy vs.

open gastrectomy for distal gastric cancer. *Hepatogastroenterology* 2012; **59**: 1699-1705 [PMID: 22626787 DOI: 10.5754/hge12259]
63 **Zeng YK**, Yang ZL, Peng JS, Lin HS, Cai L. Laparoscopy-assisted versus open distal gastrectomy for early gastric cancer: evidence from randomized and nonrandomized clinical trials. *Ann Surg* 2012; **256**: 39-52 [PMID: 22664559 DOI: 10.1097/SLA.0b013e3182583e2e]
64 **Viñuela EF**, Gonen M, Brennan MF, Coit DG, Strong VE. Laparoscopic versus open distal gastrectomy for gastric cancer: a meta-analysis of randomized controlled trials and high-quality nonrandomized studies. *Ann Surg* 2012; **255**: 446-456 [PMID: 22330034 DOI: 10.1097/SLA.0b013e31824682f4]
65 **Lu C**, Zhou S, Peng Z, Chen L. Quality of D2 lymphadenectomy for advanced gastric cancer: is laparoscopic-assisted distal gastrectomy as effective as open distal gastrectomy? *Surg Endosc* 2015; **29**: 1537-1544 [PMID: 25294526 DOI: 10.1007/s00464-014-3838-6]
66 **Ding J**, Liao GQ, Liu HL, Liu S, Tang J. Meta-analysis of laparoscopy-assisted distal gastrectomy with D2 lymph node dissection for gastric cancer. *J Surg Oncol* 2012; **105**: 297-303 [PMID: 21952834 DOI: 10.1002/jso.22098]
67 **Oh SY**, Kwon S, Lee KG, Suh YS, Choe HN, Kong SH, Lee HJ, Kim WH, Yang HK. Outcomes of minimally invasive surgery for early gastric cancer are comparable with those for open surgery: analysis of 1,013 minimally invasive surgeries at a single institution. *Surg Endosc* 2014; **28**: 789-795 [PMID: 24263458 DOI: 10.1007/s00464-013-3256-1]
68 **Kunisaki C**, Makino H, Takagawa R, Kimura J, Ota M, Ichikawa Y, Kosaka T, Akiyama H, Endo I. A systematic review of laparoscopic total gastrectomy for gastric cancer. *Gastric Cancer* 2015; **18**: 218-226 [PMID: 25666184 DOI: 10.1007/s10120-015-0474-3]
69 **Lee HJ**, Yang HK. Laparoscopic gastrectomy for gastric cancer. *Dig Surg* 2013; **30**: 132-141 [PMID: 23867590 DOI: 10.1159/000350884]
70 **Choi YY**, Bae JM, An JY, Hyung WJ, Noh SH. Laparoscopic gastrectomy for advanced gastric cancer: are the long-term results comparable with conventional open gastrectomy? A systematic review and meta-analysis. *J Surg Oncol* 2013; **108**: 550-556 [PMID: 24115104 DOI: 10.1002/jso.23438]
71 **Chen XZ**, Wen L, Rui YY, Liu CX, Zhao QC, Zhou ZG, Hu JK. Long-term survival outcomes of laparoscopic versus open gastrectomy for gastric cancer: a systematic review and meta-analysis. *Medicine* (Baltimore) 2015; **94**: e454 [PMID: 25634185 DOI: 10.1097/MD.0000000000000454]
72 **Koh YX**, Chok AY, Zheng HL, Tan CS, Chow PK, Wong WK, Goh BK. A systematic review and meta-analysis comparing laparoscopic versus open gastric resections for gastrointestinal stromal tumors of the stomach. *Ann Surg Oncol* 2013; **20**: 3549-3560 [PMID: 23793362 DOI: 10.1245/s10434-013-3051-1]
73 **Zheng L**, Ding W, Zhou D, Lu L, Yao L. Laparoscopic versus open resection for gastric gastrointestinal stromal tumors: a meta-analysis. *Am Surg* 2014; **80**: 48-56 [PMID: 24401515 DOI: 10.1186/1477-7819-12-206]
74 **Lee J,** Kim Y-M, Woo Y, Obama K, Noh SH, Hyung WJ. Robotic distal subtotal gastrectomy with D2 lymphadenectomy for gastric cancer patients with high body mass index: comparison with conventional laparoscopic distal subtotal gastrectomy with D2 lymphadenectomy. *Surg Endosc* 2015 Jan 29; Epub ahead of print [PMID: 25631106 DOI: 10.1007/s00464-015-4069-1]
75 **Xiong B**, Ma L, Zhang C. Robotic versus laparoscopic gastrectomy for gastric cancer: a meta-analysis of short outcomes. *Surg Oncol* 2012; **21**: 274-280 [PMID: 22789391 DOI: 10.1016/j.suronc.2012.05.004]
76 **Spanjersberg WR,** van Sambeeck JDP, Bremers A, Rosman C, van Laarhoven CJHM. Systematic review and meta-analysis for laparoscopic versus open colon surgery with or without an ERAS programme. *Surg Endosc* 2015 Mar 24; Epub ahead of print [PMID: 25801106 DOI: 10.1007/s00464-015-4148-3]
77 **Esteban F**, Cerdan FJ, Garcia-Alonso M, Sanz-Lopez R, Arroyo A, Ramirez JM, Moreno C, Morales R, Navarro A, Fuentes M. A multicentre comparison of a fast track or conventional postoperative protocol following laparoscopic or open elective surgery for colorectal cancer surgery. *Colorectal Dis* 2014; **16**: 134-140 [PMID: 24164975 DOI: 10.1111/codi.12472]
78 **Jones OM**, Lindsey I, Cunningham C. Laparoscopic colorectal surgery. *BMJ* 2011; **343**: d8029 [PMID: 22207042 DOI: 10.1136/bmj.d8029]
79 **Tan JJ**, Tjandra JJ. Laparoscopic surgery for ulcerative colitis - a meta-analysis. *Colorectal Dis* 2006; **8**: 626-636 [PMID: 16970571 DOI: 10.1111/j.1463-1318.2006.00971.x]
80 **Dasari BV**, McKay D, Gardiner K. Laparoscopic versus Open surgery for small bowel Crohn's disease. *Cochrane Database Syst Rev* 2011; **(1)**: CD006956 [PMID: 21249684 DOI: 10.1002/14651858.CD006956.pub2]
81 **Antoniou SA**, Antoniou GA, Koch OO, Pointner R, Granderath FA. Is laparoscopic ileocecal resection a safe option for Crohn's disease? Best evidence topic. *Int J Surg* 2014; **12**: 22-25 [PMID: 24246171 DOI: 10.1016/j.ijsu.2013.11.003]
82 **Gervaz P**, Inan I, Perneger T, Schiffer E, Morel P. A prospective, randomized, single-blind comparison of laparoscopic versus open sigmoid colectomy for diverticulitis. *Ann Surg* 2010; **252**: 3-8 [PMID: 20505508 DOI: 10.1097/SLA.0b013e3181dbb5a5]
83 **Klarenbeek BR**, Veenhof AA, Bergamaschi R, van der Peet DL, van den Broek WT, de Lange ES, Bemelman WA, Heres P, Lacy AM, Engel AF, Cuesta MA. Laparoscopic sigmoid resection for diverticulitis decreases major morbidity rates: a randomized control trial: short-term results of the Sigma Trial. *Ann Surg* 2009; **249**: 39-44 [PMID: 19106674 DOI: 10.1097/SLA.0b013e31818e416a]
84 **Jones OM**, Stevenson AR, Clark D, Stitz RW, Lumley JW. Laparoscopic resection for diverticular disease: follow-up of 500 consecutive patients. *Ann Surg* 2008; **248**: 1092-1097 [PMID: 19092355 DOI: 10.1097/SLA.0b013e3181884923]
85 **Siddiqui MR**, Sajid MS, Qureshi S, Cheek E, Baig MK. Elective laparoscopic sigmoid resection for diverticular disease has fewer complications than conventional surgery: a meta-analysis. *Am J Surg* 2010; **200**: 144-161 [PMID: 20637347 DOI: 10.1016/j.amjsurg.2009.08.021]
86 **Cirocchi R,** Farinella E, Trastulli S, Sciannameo F, Audisio RA. Elective sigmoid colectomy for diverticular disease. Laparoscopic vs open surgery: A systematic review. *Color Dis* 2012; **14**: 671-683 [PMID: 21689339 DOI: 10.1111/j.1463-1318.2011.02666.x]
87 **Moloo H**, Haggar F, Coyle D, Hutton B, Duhaime S, Mamazza J, Poulin EC, Boushey RP, Grimshaw J. Hand assisted laparoscopic surgery versus conventional laparoscopy for colorectal surgery. *Cochrane Database Syst Rev* 2010; **(10)**: CD006585 [PMID: 20927747 DOI: 10.1002/14651858.CD006585.pub2]
88 **Myers E**, Hurley M, O'Sullivan GC, Kavanagh D, Wilson I, Winter DC. Laparoscopic peritoneal lavage for generalized peritonitis due to perforated diverticulitis. *Br J Surg* 2008; **95**: 97-101 [PMID: 18076019 DOI: 10.1002/bjs.6024]
89 **Wolthuis AM**, Penninckx F, D'Hoore A. Laparoscopic sigmoid resection with transrectal specimen extraction has a good short-term outcome. *Surg Endosc* 2011; **25**: 2034-2038 [PMID: 21136110 DOI: 10.1111/j.1463-1318.2011.02869.x]
90 **Lorenzon L**, La Torre M, Ziparo V, Montebelli F, Mercantini P, Balducci G, Ferri M. Evidence based medicine and surgical approaches for colon cancer: evidences, benefits and limitations of the laparoscopic vs open resection. *World J Gastroenterol* 2014; **20**: 3680-3692 [PMID: 24707154 DOI: 10.3748/wjg.v20.i13.3680]
91 **Martel G**, Crawford A, Barkun JS, Boushey RP, Ramsay CR, Fergusson DA. Expert opinion on laparoscopic surgery for colorectal cancer parallels evidence from a cumulative meta-analysis of randomized controlled trials. *PLoS One* 2012; **7**: e35292 [PMID: 22532846 DOI: 10.1371/journal.pone.0035292]
92 **Kuhry E**, Schwenk W, Gaupset R, Romild U, Bonjer J. Long-term outcome of laparoscopic surgery for colorectal cancer: a cochrane systematic review of randomised controlled trials. *Cancer Treat Rev* 2008; **34**: 498-504 [PMID: 18468803 DOI: 10.1016/j.ctrv.2008.03.011]
93 **Søndenaa K**, Quirke P, Hohenberger W, Sugihara K, Kobayashi

H, Kessler H, Brown G, Tudyka V, D'Hoore A, Kennedy RH, West NP, Kim SH, Heald R, Storli KE, Nesbakken A, Moran B. The rationale behind complete mesocolic excision (CME) and a central vascular ligation for colon cancer in open and laparoscopic surgery: proceedings of a consensus conference. *Int J Colorectal Dis* 2014; **29**: 419-428 [PMID: 24477788 DOI: 10.1007/s00384-013-1818-2]

94 **Rondelli F**, Trastulli S, Avenia N, Schillaci G, Cirocchi R, Gullà N, Mariani E, Bistoni G, Noya G. Is laparoscopic right colectomy more effective than open resection? A meta-analysis of randomized and nonrandomized studies. *Colorectal Dis* 2012; **14**: e447-e469 [PMID: 22540533 DOI: 10.1111/j.1463-1318.2012.03054.x]

95 **Chand M**, Siddiqui MR, Rasheed S, Brown G, Tekkis P, Parvaiz A, Qureshi T. A systematic review and meta-analysis evaluating the role of laparoscopic surgical resection of transverse colon tumours. *Surg Endosc* 2014; **28**: 3263-3272 [PMID: 24962859 DOI: 10.1007/s00464-014-3634-3]

96 **Theophilus M**, Platell C, Spilsbury K. Long-term survival following laparoscopic and open colectomy for colon cancer: a meta-analysis of randomized controlled trials. *Colorectal Dis* 2014; **16**: O75-O81 [PMID: 24206016 DOI: 10.1111/codi.12483]

97 **Di B**, Li Y, Wei K, Xiao X, Shi J, Zhang Y, Yang X, Gao P, Zhang K, Yuan Y, Zhang D, Wei X, Liu S, Wang J, Wang X, Zhang Y, Cai H. Laparoscopic versus open surgery for colon cancer: a meta-analysis of 5-year follow-up outcomes. *Surg Oncol* 2013; **22**: e39-e43 [PMID: 23643698 DOI: 10.1016/j.suronc.2013.03.002]

98 **Clancy C**, O'Leary DP, Burke JP, Redmond HP, Coffey JC, Kerin MJ, Myers E. A meta-analysis to determine the oncological implications of conversion in laparoscopic colorectal cancer surgery. *Colorectal Dis* 2015; **17**: 482-490 [PMID: 25524157 DOI: 10.1111/codi.12875]

99 **Liao G**, Zhao Z, Lin S, Li R, Yuan Y, Du S, Chen J, Deng H. Robotic-assisted versus laparoscopic colorectal surgery: a meta-analysis of four randomized controlled trials. *World J Surg Oncol* 2014; **12**: 122 [PMID: 24767102 DOI: 10.1186/1477-7819-12-122]

100 **Tou S**, Brown SR, Malik AI, Nelson RL. Surgery for complete rectal prolapse in adults. *Cochrane Database Syst Rev* 2008; **(4)**: CD001758 [PMID: 18843623 DOI: 10.1002/14651858.CD001758.pub2]

101 **Young MT**, Jafari MD, Phelan MJ, Stamos MJ, Mills S, Pigazzi A, Carmichael JC. Surgical treatments for rectal prolapse: how does a perineal approach compare in the laparoscopic era? *Surg Endosc* 2015; **29**: 607-613 [PMID: 25052123 DOI: 10.1007/s00464-014-3707-3]

102 **Zhao J,** Chen N, Zheng J, He S, Sun X. Laparoscopic versus open surgery for rectal cancer: Results of a systematic review and meta-analysis on clinical efficacy. *Mol Clin Oncol* 2014; **2**: 1097-1102 [PMID: 25279204 DOI: 10.3892/mco.2014.345]

103 **Zhang FW**, Zhou ZY, Wang HL, Zhang JX, Di BS, Huang WH, Yang KH. Laparoscopic versus open surgery for rectal cancer: a systematic review and meta-analysis of randomized controlled trials. *Asian Pac J Cancer Prev* 2014; **15**: 9985-9996 [PMID: 25520140 DOI: 10.7314/APJCP.2014.15.22.9985]

104 **Morino M**, Risio M, Bach S, Beets-Tan R, Bujko K, Panis Y, Quirke P, Rembacken B, Rullier E, Saito Y, Young-Fadok T, Allaix ME. Early rectal cancer: the European Association for Endoscopic Surgery (EAES) clinical consensus conference. *Surg Endosc* 2015; **29**: 755-773 [PMID: 25609317 DOI: 10.1007/s00464-015-4067-3]

105 **Keller DS**, Khorgami Z, Swendseid B, Champagne BJ, Reynolds HL, Stein SL, Delaney CP. Laparoscopic and converted approaches to rectal cancer resection have superior long-term outcomes: a comparative study by operative approach. *Surg Endosc* 2014; **28**: 1940-1948 [PMID: 24515259 DOI: 10.1007/s00464-014-3419-8]

106 **Chen H**, Zhao L, An S, Wu J, Zou Z, Liu H, Li G. Laparoscopic versus open surgery following neoadjuvant chemoradiotherapy for rectal cancer: a systematic review and meta-analysis. *J Gastrointest Surg* 2014; **18**: 617-626 [PMID: 24424713 DOI: 10.1007/s11605-014-2452-1]

107 **Lim RS**, Yang TX, Chua TC. Postoperative bladder and sexual function in patients undergoing surgery for rectal cancer: a systematic review and meta-analysis of laparoscopic versus open resection of rectal cancer. *Tech Coloproctol* 2014; **18**: 993-1002 [PMID: 25056719 DOI: 10.1007/s10151-014-1189-x]

108 **Arezzo A**, Passera R, Salvai A, Arolfo S, Allaix ME, Schwarzer G, Morino M. Laparoscopy for rectal cancer is oncologically adequate: a systematic review and meta-analysis of the literature. *Surg Endosc* 2015; **29**: 334-348 [PMID: 25007974 DOI: 10.1007/s00464-014-3686-4]

109 **Breukink S**, Pierie J, Wiggers T. Laparoscopic versus open total mesorectal excision for rectal cancer. *Cochrane Database Syst Rev* 2006; **(4)**: CD005200 [PMID: 17054246 DOI: 10.1002/14651858.CD005200.pub2]

110 **Kelly M**, Bhangu A, Singh P, Fitzgerald JE, Tekkis PP. Systematic review and meta-analysis of trainee- versus expert surgeon-performed colorectal resection. *Br J Surg* 2014; **101**: 750-759 [PMID: 24760684 DOI: 10.1002/bjs.9472]

111 **Araujo SE**, Crawshaw B, Mendes CR, Delaney CP. Transanal total mesorectal excision: a systematic review of the experimental and clinical evidence. *Tech Coloproctol* 2015; **19**: 69-82 [PMID: 25380741 DOI: 10.1007/s10151-014-1233-x]

112 **Lin S**, Jiang HG, Chen ZH, Zhou SY, Liu XS, Yu JR. Meta-analysis of robotic and laparoscopic surgery for treatment of rectal cancer. *World J Gastroenterol* 2011; **17**: 5214-5220 [PMID: 22215947 DOI: 10.3748/wjg.v17.i47.5214]

113 **Trastulli S**, Farinella E, Cirocchi R, Cavaliere D, Avenia N, Sciannameo F, Gullà N, Noya G, Boselli C. Robotic resection compared with laparoscopic rectal resection for cancer: systematic review and meta-analysis of short-term outcome. *Colorectal Dis* 2012; **14**: e134-e156 [PMID: 22151033 DOI: 10.1111/j.1463-1318.2011.02907.x]

114 **Antoniou SA**, Antoniou GA, Koch OO, Pointner R, Granderath FA. Robot-assisted laparoscopic surgery of the colon and rectum. *Surg Endosc* 2012; **26**: 1-11 [PMID: 21858568 DOI: 10.1007/s00464-011-1867-y]

115 **Mak TW**, Lee JF, Futaba K, Hon SS, Ngo DK, Ng SS. Robotic surgery for rectal cancer: A systematic review of current practice. *World J Gastrointest Oncol* 2014; **6**: 184-193 [PMID: 24936229 DOI: 10.4251/wjgo.v6.i6.184]

116 **Baek SK**, Carmichael JC, Pigazzi A. Robotic surgery: colon and rectum. *Cancer J* 2013; **19**: 140-146 [PMID: 23528722 DOI: 10.1097/PPO.0b013e31828ba0fd]

117 **Winslow E**, Hawkins WG. Laparoscopic resection of the liver for cancer. *Surg Oncol Clin N Am* 2013; **22**: 75-89, vi [PMID: 23158086 DOI: 10.1016/j.soc.2012.08.005]

118 **Fancellu A**, Rosman AS, Sanna V, Nigri GR, Zorcolo L, Pisano M, Melis M. Meta-analysis of trials comparing minimally-invasive and open liver resections for hepatocellular carcinoma. *J Surg Res* 2011; **171**: e33-e45 [PMID: 21920552 DOI: 10.1016/j.jss.2011.07.008]

119 **Rao A**, Rao G, Ahmed I. Laparoscopic left lateral liver resection should be a standard operation. *Surg Endosc* 2011; **25**: 1603-1610 [PMID: 21136115 DOI: 10.1007/s00464-010-1459-2]

120 **Rao A**, Rao G, Ahmed I. Laparoscopic or open liver resection? Let systematic review decide it. *Am J Surg* 2012; **204**: 222-231 [PMID: 22245507 DOI: 10.1016/j.amjsurg.2011.08.013]

121 **Zhou Y**, Xiao Y, Wu L, Li B, Li H. Laparoscopic liver resection as a safe and efficacious alternative to open resection for colorectal liver metastasis: a meta-analysis. *BMC Surg* 2013; **13**: 44 [PMID: 24083369 DOI: 10.1186/1471-2482-13-44]

122 **Yin Z**, Fan X, Ye H, Yin D, Wang J. Short- and long-term outcomes after laparoscopic and open hepatectomy for hepatocellular carcinoma: a global systematic review and meta-analysis. *Ann Surg Oncol* 2013; **20**: 1203-1215 [PMID: 23099728 DOI: 10.1245/s10434-012-2705-8]

123 **Li N**, Wu YR, Wu B, Lu MQ. Surgical and oncologic outcomes following laparoscopic versus open liver resection for hepatocellular carcinoma: A meta-analysis. *Hepatol Res* 2012; **42**: 51-59 [PMID: 21988222 DOI: 10.1111/j.1872-034X.2011.00890.x]

124 **Morise Z**, Ciria R, Cherqui D, Chen KH, Belli G, Wakabayashi G. Can we expand the indications for laparoscopic liver resection? A systematic review and meta-analysis of laparoscopic liver resection for patients with hepatocellular carcinoma and chronic liver disease.

J Hepatobiliary Pancreat Sci 2015; **22**: 342-352 [PMID: 25663288 DOI: 10.1002/jhbp.215]

125 **Twaij A**, Pucher PH, Sodergren MH, Gall T, Darzi A, Jiao LR. Laparoscopic vs open approach to resection of hepatocellular carcinoma in patients with known cirrhosis: systematic review and meta-analysis. *World J Gastroenterol* 2014; **20**: 8274-8281 [PMID: 25009403 DOI: 10.3748/wjg.v20.i25.8274]

126 **Xiong JJ**, Altaf K, Javed MA, Huang W, Mukherjee R, Mai G, Sutton R, Liu XB, Hu WM. Meta-analysis of laparoscopic vs open liver resection for hepatocellular carcinoma. *World J Gastroenterol* 2012; **18**: 6657-6668 [PMID: 23236242 DOI: 10.3748/wjg.v18.i45.6657]

127 **Mizuguchi T**, Kawamoto M, Meguro M, Shibata T, Nakamura Y, Kimura Y, Furuhata T, Sonoda T, Hirata K. Laparoscopic hepatectomy: a systematic review, meta-analysis, and power analysis. *Surg Today* 2011; **41**: 39-47 [PMID: 21191689 DOI: 10.1007/s00595-010-4337-6]

128 **Herbold T**, Wahba R, Bangard C, Demir M, Drebber U, Stippel DL. The laparoscopic approach for radiofrequency ablation of hepatocellular carcinoma--indication, technique and results. *Langenbecks Arch Surg* 2013; **398**: 47-53 [PMID: 23093087 DOI: 10.1007/s00423-012-1018-5]

129 **Hariharan D**, Constantinides V, Kocher HM, Tekkis PP. The role of laparoscopy and laparoscopic ultrasound in the preoperative staging of patients with resectable colorectal liver metastases: a meta-analysis. *Am J Surg* 2012; **204**: 84-92 [PMID: 22244586 DOI: 10.1016/j.amjsurg.2011.07.018]

130 **Schiffman SC**, Kim KH, Tsung A, Marsh JW, Geller DA. Laparoscopic versus open liver resection for metastatic colorectal cancer: a metaanalysis of 610 patients. *Surgery* 2015; **157**: 211-222 [PMID: 25282529 DOI: 10.1016/j.surg.2014.08.036]

131 **Parks KR**, Kuo YH, Davis JM, O' Brien B, Hagopian EJ. Laparoscopic versus open liver resection: a meta-analysis of long-term outcome. *HPB* (Oxford) 2014; **16**: 109-118 [PMID: 23672270 DOI: 10.1111/hpb.12117]

132 **Luo LX**, Yu ZY, Bai YN. Laparoscopic hepatectomy for liver metastases from colorectal cancer: a meta-analysis. *J Laparoendosc Adv Surg Tech A* 2014; **24**: 213-222 [PMID: 24571350 DOI: 10.1089/lap.2013.0399]

133 **Wei M**, He Y, Wang J, Chen N, Zhou Z, Wang Z. Laparoscopic versus open hepatectomy with or without synchronous colectomy for colorectal liver metastasis: a meta-analysis. *PLoS One* 2014; **9**: e87461 [PMID: 24489916 DOI: 10.1371/journal.pone.0087461]

134 **Qiu JG**, Wu H, Jiang H, Huang JW, Pankaj P, Xu YL, Wang JZ, Zeng Y. Laparoscopic fenestration vs open fenestration in patients with congenital hepatic cysts: a meta-analysis. *World J Gastroenterol* 2011; **17**: 3359-3365 [PMID: 21876626 DOI: 10.3748/wjg.v17.i28.3359]

135 **Bekheit M,** Khafagy P-A, Bucur P, Katri K, Elgendi A, Abdel-salam WN, Vibert E, El-kayal E. Donor safety in live donor laparoscopic liver procurement: systematic review and meta-analysis. *Surg Endosc* 2015 Jan 1; Epub ahead of print [PMID: 25552233 DOI: 10.1007/s00464-014-4045-1]

136 **Nigri G**, Rosman AS, Petrucciani N, Fancellu A, Pisano M, Zorcolo L, Ramacciato G, Melis M. Meta-analysis of trials comparing laparoscopic transperitoneal and retroperitoneal adrenalectomy. *Surgery* 2013; **153**: 111-119 [PMID: 22939744 DOI: 10.1016/j.surg.2012.05.042]

137 **Constantinides VA**, Christakis I, Touska P, Palazzo FF. Systematic review and meta-analysis of retroperitoneoscopic versus laparoscopic adrenalectomy. *Br J Surg* 2012; **99**: 1639-1648 [PMID: 23023976 DOI: 10.1002/bjs.8921]

138 **Chen W**, Li F, Chen D, Zhu Y, He C, Du Y, Tan W. Retroperitoneal versus transperitoneal laparoscopic adrenalectomy in adrenal tumor: a meta-analysis. *Surg Laparosc Endosc Percutan Tech* 2013; **23**: 121-127 [PMID: 23579504 DOI: 10.1097/SLE.0b013e3182827b57]

139 **Chai YJ**, Kwon H, Yu HW, Kim SJ, Choi JY, Lee KE, Youn YK. Systematic Review of Surgical Approaches for Adrenal Tumors: Lateral Transperitoneal versus Posterior Retroperitoneal and Laparoscopic versus Robotic Adrenalectomy. *Int J Endocrinol* 2014; **2014**: 918346 [PMID: 25587275 DOI: 10.1155/2014/918346]

140 **Walz MK**. [Minimally invasive adrenal gland surgery]. *Chirurg* 1998; **69**: 613-620 [PMID: 9676363 DOI: 10.1007/s00104-011-2194-5]

141 **Bittner JG**, Brunt LM. Evaluation and management of adrenal incidentaloma. *J Surg Oncol* 2012; **106**: 557-564 [PMID: 22623268 DOI: 10.1002/jso.23161]

142 **Creamer J**, Matthews BD. Laparoscopic adrenalectomy for cancer. *Surg Oncol Clin N Am* 2013; **22**: 111-124, vi-vii [PMID: 23158088 DOI: 10.1016/j.soc.2012.08.006]

143 **Bickenbach KA**, Strong VE. Laparoscopic transabdominal lateral adrenalectomy. *J Surg Oncol* 2012; **106**: 611-618 [PMID: 22933307 DOI: 10.1002/jso.23250]

144 **Muth A**, Ragnarsson O, Johannsson G, Wängberg B. Systematic review of surgery and outcomes in patients with primary aldosteronism. *Br J Surg* 2015; **102**: 307-317 [PMID: 25605481 DOI: 10.1002/bjs.9744]

145 **Iacobone M**, Citton M, Scarpa M, Viel G, Boscaro M, Nitti D. Systematic review of surgical treatment of subclinical Cushing's syndrome. *Br J Surg* 2015; **102**: 318-330 [PMID: 25640696 DOI: 10.1002/bjs.9742]

146 **Wong A**, Eloy JA, Liu JK. The role of bilateral adrenalectomy in the treatment of refractory Cushing's disease. *Neurosurg Focus* 2015; **38**: E9 [PMID: 25639327 DOI: 10.3171/2014.10.FOCUS14684]

147 **Wang L**, Wu Z, Li M, Cai C, Liu B, Yang Q, Sun Y. Laparoendoscopic single-site adrenalectomy versus conventional laparoscopic surgery: a systematic review and meta-analysis of observational studies. *J Endourol* 2013; **27**: 743-750 [PMID: 23611672 DOI: 10.1089/end.2012.0599]

148 **Hu Q**, Gou Y, Sun C, Xu K, Xia G, Ding Q. A systematic review and meta-analysis of current evidence comparing laparoendoscopic single-site adrenalectomy and conventional laparoscopic adrenalectomy. *J Endourol* 2013; **27**: 676-683 [PMID: 23391020 DOI: 10.1089/end.2012.0687]

149 **Brandao LF**, Autorino R, Laydner H, Haber GP, Ouzaid I, De Sio M, Perdonà S, Stein RJ, Porpiglia F, Kaouk JH. Robotic versus laparoscopic adrenalectomy: a systematic review and meta-analysis. *Eur Urol* 2014; **65**: 1154-1161 [PMID: 24079955 DOI: 10.1016/j.eururo.2013.09.021]

150 **Tang K**, Li H, Xia D, Yu G, Guo X, Guan W, Xu H, Ye Z. Robot-assisted versus laparoscopic adrenalectomy: a systematic review and meta-analysis. *J Laparoendosc Adv Surg Tech A* 2015; **25**: 187-195 [PMID: 25763475 DOI: 10.1089/lap.2014.0431]

151 **Agcaoglu O**, Aliyev S, Karabulut K, Mitchell J, Siperstein A, Berber E. Robotic versus laparoscopic resection of large adrenal tumors. *Ann Surg Oncol* 2012; **19**: 2288-2294 [PMID: 22396002 DOI: 10.1245/s10434-012-2296-4]

152 **Moinzadeh A**, Gill IS. Robotic adrenalectomy. *Urol Clin North Am* 2004; **31**: 753-756 [PMID: 15474602 DOI: 10.1002/jso.23132]

153 **Giger U**, Michel JM, Wiesli P, Schmid C, Krähenbühl L. Laparoscopic surgery for benign lesions of the pancreas. *J Laparoendosc Adv Surg Tech A* 2006; **16**: 452-457 [PMID: 17004867 DOI: 10.1089/lap.2006.16.452]

154 **Subar D**, Gobardhan PD, Gayet B. Laparoscopic pancreatic surgery: An overview of the literature and experiences of a single center. *Best Pract Res Clin Gastroenterol* 2014; **28**: 123-132 [PMID: 24485260 DOI: 10.1016/j.bpg.2013.11.011]

155 **Kooby DA**, Gillespie T, Bentrem D, Nakeeb A, Schmidt MC, Merchant NB, Parikh AA, Martin RC, Scoggins CR, Ahmad S, Kim HJ, Park J, Johnston F, Strouch MJ, Menze A, Rymer J, McClaine R, Strasberg SM, Talamonti MS, Staley CA, McMasters KM, Lowy AM, Byrd-Sellers J, Wood WC, Hawkins WG. Left-sided pancreatectomy: a multicenter comparison of laparoscopic and open approaches. *Ann Surg* 2008; **248**: 438-446 [PMID: 18791364 DOI: 10.1097/SLA.0b013e318185a990]

156 **Kooby DA**, Hawkins WG, Schmidt CM, Weber SM, Bentrem DJ, Gillespie TW, Sellers JB, Merchant NB, Scoggins CR, Martin RC, Kim HJ, Ahmad S, Cho CS, Parikh AA, Chu CK, Hamilton NA, Doyle CJ, Pinchot S, Hayman A, McClaine R, Nakeeb A, Staley

CA, McMasters KM, Lillemoe KD. A multicenter analysis of distal pancreatectomy for adenocarcinoma: is laparoscopic resection appropriate? *J Am Coll Surg* 2010; **210**: 779-785, 786-787 [PMID: 20421049 DOI: 10.1016/j.jamcollsurg.2009.12.033]

157 **Gagner M**, Palermo M. Laparoscopic Whipple procedure: review of the literature. *J Hepatobiliary Pancreat Surg* 2009; **16**: 726-730 [PMID: 19636494 DOI: 10.1007/s00534-009-0142-2]

158 **Gumbs AA**, Rodriguez Rivera AM, Milone L, Hoffman JP. Laparoscopic pancreatoduodenectomy: a review of 285 published cases. *Ann Surg Oncol* 2011; **18**: 1335-1341 [PMID: 21207166 DOI: 10.1245/s10434-010-1503-4]

159 **Lai EC**, Tang CN. Current status of robot-assisted laparoscopic pancreaticoduodenectomy and distal pancreatectomy: a comprehensive review. *Asian J Endosc Surg* 2013; **6**: 158-164 [PMID: 23710970 DOI: 10.1111/ases.12040]

160 **Cirocchi R**, Partelli S, Trastulli S, Coratti A, Parisi A, Falconi M. A systematic review on robotic pancreaticoduodenectomy. *Surg Oncol* 2013; **22**: 238-246 [PMID: 24060451 DOI: 10.1016/j.suronc.2013.08.003]

161 **Feldman LS**. Laparoscopic splenectomy: standardized approach. *World J Surg* 2011; **35**: 1487-1495 [PMID: 21424869 DOI: 10.1007/s00268-011-1059-x]

162 **Qian D**, He Z, Hua J, Gong J, Lin S, Song Z. Hand-assisted versus conventional laparoscopic splenectomy: a systematic review and meta-analysis. *ANZ J Surg* 2014; **84**: 915-920 [PMID: 24712437 DOI: 10.1111/ans.12597]

163 **Makino T**, Zhou J, Pankaj P, Peng B. Comparative treatment and literature review for laparoscopic splenectomy alone versus preoperative splenic artery embolization splenectomy. *Surg Endosc* 2012; **26**: 2758-2766 [PMID: 22580870 DOI: 10.1007/s00464-012-2270-z]

164 **Wu Z**, Reza M, Mayol JA, Blasco JA, Guerra M, Andradas E, Plana MN. Efficacy of the Da Vinci surgical system in abdominal surgery compared with that of laparoscopy: a systematic review and meta-analysis. *Ann Surg* 2010; **252**: 254-262 [PMID: 20622659 DOI: 10.1097/SLA.0b013e3181e6239e]

165 **Antoniou SA**, Pointner R, Granderath FA. Current treatment concepts for groin hernia. *Langenbecks Arch Surg* 2014; **399**: 553-558 [PMID: 24824799 DOI: 10.1007/s00423-014-1212-8]

166 **Antoniou SA**, Antoniou GA, Bartsch DK, Fendrich V, Koch OO, Pointner R, Granderath FA. Transabdominal preperitoneal versus totally extraperitoneal repair of inguinal hernia: a meta-analysis of randomized studies. *Am J Surg* 2013; **206**: 245-252.e1 [PMID: 23768695 DOI: 10.1016/j.amjsurg.2012.10.041]

167 **O'Reilly EA**, Burke JP, O'Connell PR. A meta-analysis of surgical morbidity and recurrence after laparoscopic and open repair of primary unilateral inguinal hernia. *Ann Surg* 2012; **255**: 846-853 [PMID: 22470068 DOI: 10.1097/SLA.0b013e318264c801]

168 **Karthikesalingam A**, Markar SR, Holt PJ, Praseedom RK. Meta-analysis of randomized controlled trials comparing laparoscopic with open mesh repair of recurrent inguinal hernia. *Br J Surg* 2010; **97**: 4-11 [PMID: 20013926 DOI: 10.1002/bjs.6902]

169 **Sajid MS**, Kalra L, Parampalli U, Sains PS, Baig MK. A systematic review and meta-analysis evaluating the effectiveness of lightweight mesh against heavyweight mesh in influencing the incidence of chronic groin pain following laparoscopic inguinal hernia repair. *Am J Surg* 2013; **205**: 726-736 [PMID: 23561639 DOI: 10.1016/j.amjsurg.2012.07.046]

170 **Currie A**, Andrew H, Tonsi A, Hurley PR, Taribagil S. Lightweight versus heavyweight mesh in laparoscopic inguinal hernia repair: a meta-analysis. *Surg Endosc* 2012; **26**: 2126-2133 [PMID: 22311304 DOI: 10.1007/s00464-012-2179-6]

171 **Sajid MS**, Leaver C, Baig MK, Sains P. Systematic review and meta-analysis of the use of lightweight versus heavyweight mesh in open inguinal hernia repair. *Br J Surg* 2012; **99**: 29-37 [PMID: 22038579 DOI: 10.1002/bjs.7718]

172 **Berney CR**, Yeo AE. Mesh fixation with fibrin sealant during endoscopic totally extraperitoneal inguinal hernia approach: a review of 640 repairs. *Hernia* 2013; **17**: 709-717 [PMID: 23344667 DOI: 10.1007/s10029-012-1034-y]

173 **Sajid MS**, Ladwa N, Kalra L, McFall M, Baig MK, Sains P. A meta-analysis examining the use of tacker mesh fixation versus glue mesh fixation in laparoscopic inguinal hernia repair. *Am J Surg* 2013; **206**: 103-111 [PMID: 23388426 DOI: 10.1016/j.amjsurg.2012.09.003]

174 **Kaul A**, Hutfless S, Le H, Hamed SA, Tymitz K, Nguyen H, Marohn MR. Staple versus fibrin glue fixation in laparoscopic total extraperitoneal repair of inguinal hernia: a systematic review and meta-analysis. *Surg Endosc* 2012; **26**: 1269-1278 [PMID: 22350225 DOI: 10.1007/s00464-011-2025-2]

175 **Teng YJ**, Pan SM, Liu YL, Yang KH, Zhang YC, Tian JH, Han JX. A meta-analysis of randomized controlled trials of fixation versus nonfixation of mesh in laparoscopic total extraperitoneal inguinal hernia repair. *Surg Endosc* 2011; **25**: 2849-2858 [PMID: 21487873 DOI: 10.1007/s00464-011-1668-3]

176 **Poelman MM**, van den Heuvel B, Deelder JD, Abis GS, Beudeker N, Bittner RR, Campanelli G, van Dam D, Dwars BJ, Eker HH, Fingerhut A, Khatkov I, Koeckerling F, Kukleta JF, Miserez M, Montgomery A, Munoz Brands RM, Morales Conde S, Muysoms FE, Soltes M, Tromp W, Yavuz Y, Bonjer HJ. EAES Consensus Development Conference on endoscopic repair of groin hernias. *Surg Endosc* 2013; **27**: 3505-3519 [PMID: 23708718 DOI: 10.1007/s00464-013-3001-9]

177 **Bittner R**, Arregui ME, Bisgaard T, Dudai M, Ferzli GS, Fitzgibbons RJ, Fortelny RH, Klinge U, Kockerling F, Kuhry E, Kukleta J, Lomanto D, Misra MC, Montgomery A, Morales-Conde S, Reinpold W, Rosenberg J, Sauerland S, Schug-Pass C, Singh K, Timoney M, Weyhe D, Chowbey P. Guidelines for laparoscopic (TAPP) and endoscopic (TEP) treatment of inguinal hernia [International Endohernia Society (IEHS)]. *Surg Endosc* 2011; **25**: 2773-2843 [PMID: 21751060 DOI: 10.1007/s00464-011-1799-6]

178 **Bittner R**, Montgomery MA, Arregui E, Bansal V, Bingener J, Bisgaard T, Buhck H, Dudai M, Ferzli GS, Fitzgibbons RJ, Fortelny RH, Grimes KL, Klinge U, Köckerling F, Kumar S, Kukleta J, Lomanto D, Misra MC, Morales-Conde S, Reinpold W, Rosenberg J, Singh K, Timoney M, Weyhe D, Chowbey P. Update of guidelines on laparoscopic (TAPP) and endoscopic (TEP) treatment of inguinal hernia (International Endohernia Society). *Surg Endosc* 2015; **29**: 289-321 [PMID: 25398194 DOI: 10.1007/s00464-014-3917-8]

179 **Forbes SS**, Eskicioglu C, McLeod RS, Okrainec A. Meta-analysis of randomized controlled trials comparing open and laparoscopic ventral and incisional hernia repair with mesh. *Br J Surg* 2009; **96**: 851-858 [PMID: 19591158 DOI: 10.1002/bjs.6668]

180 **Sauerland S**, Walgenbach M, Habermalz B, Seiler CM, Miserez M. Laparoscopic versus open surgical techniques for ventral or incisional hernia repair. *Cochrane Database Syst Rev* 2011; **(3)**: CD007781 [PMID: 21412910 DOI: 10.1002/14651858.CD007781.pub2]

181 **Sajid MS**, Bokhari SA, Mallick AS, Cheek E, Baig MK. Laparoscopic versus open repair of incisional/ventral hernia: a meta-analysis. *Am J Surg* 2009; **197**: 64-72 [PMID: 18614144 DOI: 10.1016/j.amjsurg.2007.12.051]

182 **Kapischke M**, Schulz T, Schipper T, Tensfeldt J, Caliebe A. Open versus laparoscopic incisional hernia repair: something different from a meta-analysis. *Surg Endosc* 2008; **22**: 2251-2260 [PMID: 18320281 DOI: 10.1007/s00464-008-9773-7]

183 **Müller-Riemenschneider F**, Roll S, Friedrich M, Zieren J, Reinhold T, von der Schulenburg JM, Greiner W, Willich SN. Medical effectiveness and safety of conventional compared to laparoscopic incisional hernia repair: a systematic review. *Surg Endosc* 2007; **21**: 2127-2136 [PMID: 17763905 DOI: 10.1007/s00464-007-9513-4]

184 **Sains PS**, Tilney HS, Purkayastha S, Darzi AW, Athanasiou T, Tekkis PP, Heriot AG. Outcomes following laparoscopic versus open repair of incisional hernia. *World J Surg* 2006; **30**: 2056-2064 [PMID: 17058029 DOI: 10.1007/s00268-006-0026-4]

185 **Zhang Y**, Zhou H, Chai Y, Cao C, Jin K, Hu Z. Laparoscopic versus open incisional and ventral hernia repair: a systematic review

and meta-analysis. *World J Surg* 2014; **38**: 2233-2240 [PMID: 24777660 DOI: 10.1007/s00268-014-2578-z]

186 **Awaiz A**, Rahman F, Hossain MB, Yunus RM, Khan S, Memon B, Memon MA. Meta-analysis and systematic review of laparoscopic versus open mesh repair for elective incisional hernia. *Hernia* 2015; **19**: 449-463 [PMID: 25650284 DOI: 10.1007/s10029-015-1351-z]

P- Reviewer: Chen GS, Ciccone MM, Neri V **S- Editor**: Song XX
L- Editor: A **E- Editor**: Lu YJ

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