ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2018 October 7; 24(37): 4217-4296

Published by Baishideng Publishing Group Inc

World Journal of Gastroenterology

Contents

Weekly Volume 24 Number 37 October 7, 2018

ABOUT COVER

Editorial board member of *World Journal of Gastroenterology*, Cesare Tosetti, MD, Doctor, Professor, Department of Primary Care, Health Care Agency of Bologna, Porretta Terme 40046, Bologna, Italy

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology (*WJG*) is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central and Directory of Open Access Journals. The 2018 edition of Journal Citation Reports® cites the 2017 impact factor for *WJG* as 3.300 (5-year impact factor: 3.387), ranking *WJG* as 35th among 80 journals in gastroenterology and hepatology (quartile in category Q2).

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITORIAL OFFICE
Ze-Mao Gong, Director
World Journal of Gastroenterology
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

PUBLICATION DATE
October 7, 2018

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/bpg/gerinfo/204

ONLINE SUBMISSION
http://www.f6publishing.com

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4217-4223

DOI: 10.3748/wjg.v24.i37.4217

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Focus on the gut-brain axis: Multiple sclerosis, the intestinal barrier and the microbiome

Carlos R Camara-Lemarroy, Luanne M Metz, V Wee Yong

Carlos R Camara-Lemarroy, Luanne M Metz, V Wee Yong, Department of Clinical Neurosciences, Cumming School of Medicine, University of Calgary, Calgary T2N 2T9, Canada

Carlos R Camara-Lemarroy, Luanne M Metz, V Wee Yong, Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary T2N 2T9, Canada

ORCID number: Carlos R Camara-Lemarroy (0000-0003-0676-6675); Luanne M Metz (0000-0002-9868-6842); V Wee Yong (0000-0002-2600-3563).

Author contributions: Camara-Lemarroy CR conceived the study and drafted the manuscript; Metz LM and Yong VW revised and approved the final version of the article.

Supported by the Lejoie-Lake Fellowship (to Camara-Lemarroy CR) awarded by the Hotchkiss Brain Institute.

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

Manuscript source: Invited manuscript

Correspondence to: Carlos R Camara-Lemarroy, MD, Academic Fellow, Postdoctoral Fellow, Department of Clinical Neurosciences, Cumming School of Medicine, University of Calgary, 1403 29 Street NW, Calgary T2N 2T9, Canada. carlos.camara-lemarroy@albertahealthservices.ca
Telephone: +1-403-9444253
Fax: +1-403-2707162

Received: July 5, 2018
Peer-review started: July 5, 2018
First decision: July 25, 2018
Revised: July 30, 2018
Accepted: August 1, 2018
Article in press: August 1, 2018
Published online: October 7, 2018

Abstract

The brain-gut axis serves as the bidirectional connection between the gut microbiome, the intestinal barrier and the immune system that might be relevant for the pathophysiology of inflammatory demyelinating diseases. People with multiple sclerosis have been shown to have an altered microbiome, increased intestinal permeability and changes in bile acid metabolism. Experimental evidence suggests that these changes can lead to profound alterations of peripheral and central nervous system immune regulation. Besides being of pathophysiological interest, the brain-gut axis could also open new avenues of therapeutic targets. Modification of the microbiome, the use of probiotics, fecal microbiota transplantation, supplementation with bile acids and intestinal barrier enhancers are all promising candidates. Hopefully, pre-clinical studies and clinical trials will soon yield significant results.

Key words: Multiple sclerosis; Microbiome; Intestinal barrier; Bile acids; Gut-brain axis

Core tip: Many studies suggest that the brain-gut connection can contribute to our knowledge of the pathophysiology of neurological conditions. Recent evidence suggests that people with multiple sclerosis have changes in their gut microbiome, their intestinal barrier and even in the metabolism of bile acids. All of these represent relevant therapeutic targets that could feasibly be addressed by pre-clinical and clinical studies. This knowledge acquired in the bench might soon be translated to the bedside.

Camara-Lemarroy CR, Metz LM, Yong VW. Focus on the gut-brain axis: Multiple sclerosis, the intestinal barrier and the microbiome. *World J Gastroenterol* 2018; 24(37): 4217-4223 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4217.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4217

INTRODUCTION

Clinical and preclinical studies have shown bidirectional interactions within the brain-gut axis and the gut microbiome, the intestinal barrier and the immune system, both in health and disease. These complex interactions might be relevant for the pathophysiology of inflammatory demyelinating diseases, and in particular, multiple sclerosis, where much interest has been placed in the recent literature.

THE GUT MICROBIOME

Much interest has been placed recently on the possible role of the gut microbiome in multiple sclerosis (MS) pathophysiology. Many review articles on this subject have recently been published[1-3], perhaps more than original research articles that actually characterize the gut microbiome in patients with MS. This research is in keeping with the essential role that the gut microbiome has in regulating the development of the immune system[4]. This area of research has also been the subject of recent symposia in international MS conferences[5,6].

Much of the experimental evidence is derived from studies using the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Modifying the gut microbiota with either antibiotic cocktails or probiotics leads to EAE attenuation as well as a multitude of regulatory immune responses[7-9]. Animals bred in germ-free conditions are resistant to EAE induction and show an attenuated immunological response[10,11], an effect lost when mice are repopulated with gut commensals[11]. In recent intriguing experiments, transgenic mice prone to spontaneous brain autoimmunity developed severe disease when transplanted with fecal microbiota from MS patients, as opposed to mice that received fecal microbiota from healthy matching twins[12]. Germ-free mice receiving similar transplants also developed severe EAE, while showing altered peripheral immune responses[13].

From studies attempting to characterize the composition of the microbiome, it is clear there are some differences in people with MS compared to controls. People with relapsing-remitting MS (RRMS) have an abundance of *Anaerostipes*, *Faecalibacterium*, *Pseudomonas*, *Mycoplasma*, *Haemophilus*, *Blautia*, and *Dorea* and a relative decrease of *Bacteroides*, *Prevotella*, *Parabacteroides* and *Adlercreutzia*[14-16]. In pediatric MS, patients have higher levels of members of *Desulfovibrionaceae* and depletion in *Lachnospiraceae* and *Ruminococcaceae*[17,18]. Issues are further complicated by complex analyses at the taxa, phylum and species levels, and a myriad of microbes have been implicated. For example, studies have found a significant depletion in clostridial species[15,19], *Butyricimonas*[20], *Roseburia*[21] and an increase in *Streptococcus*[22], *Methanobrevibacter*, *Akkermansia* and *Coprococcus*[14,20].

However, there are some limitations to these studies. The methods used to analyze the microbiome have been heterogeneous, with most (but not all) studies using a variation of 16S sequencing. There are differences in sample processing, DNA extraction, choice of primers, databases and hyper-variable regions analyzed across studies. Furthermore, close to two thirds of patients with MS have gastrointestinal symptoms such as constipation, dyspepsia and other functional gastrointestinal disorders[23], and many of these have been also associated with an altered gut microbiota[24]. Studies so far have not properly accounted for these symptoms or other relevant variables such as diet. An ongoing International MS Microbiome study aims to define a "core microbiome"[3]. It might shine some light into this complicated field.

Nonetheless, there is mounting experimental evidence that the gut microbiome may play a role in MS pathophysiology and human studies suggest that patients have a different microbiome compared to controls. Of course, the true significance of the results obtained so far is unclear, considering that there has often been a failure to replicate microbiome animal studies in humans. But the next question that comes to mind is whether this can also constitute a relevant therapeutic target. Although this appears to be the case in experimental models, translation to clinical practice may prove challenging.

Modifying the microbiome through medications, possibly antibiotics, could be the simplest method, but several issues arise that question the feasibility of this approach. Targeting specific commensals might prove difficult and requires appropriate identification of these targets. The case of minocycline is an interesting example. Recently shown to delay the occurrence of a second demyelinating event in patients with a clinically isolated syndrome[25], minocycline is an antibiotic known to alter the gut microbiome[26]. Whether this is an additional mechanism of action remains unknown; it is noteworthy that the initial rationale for testing minocycline in early MS is based on its various immune-modifying properties[27]. On the other hand, there is also evidence that MS disease modifying therapies (DMTs) may alter the microbiome directly[26], and indeed, it also appears that a multitude of other medications such as antidepressants, antipsychotics and immune modulators may also do so[28]. Issues such as the generation of resistant strains are also worthy of consideration.

Probiotics are a popular option but there are various issues with their practical implementation. Probiotics do not modify the host microbiome in a robust and persistent manner, although they are purported to be able to influence gut immunity and homeostasis. Despite success

in showing a benefit for probiotics in animal models[29,30], there are only a handful of clinical trials in MS. Results have been preliminary, with some modest beneficial trends in clinical variables and some biochemical markers of changes in peripheral immune function[31-33]. However, they have included very small numbers of patients and the duration of these trials have been too short to shed any light onto clinically meaningful outcomes. There are many barriers to be overcome, such as selecting the appropriate formulation, dose and study design. There is also a lesson to be learned from the multiple clinical trials in inflammatory bowel disease (IBD), where despite a wealth of available studies (although heterogeneous in design and quality), the evidence supporting their clinical use is limited to carefully selected subpopulations[34,35].

Fecal microbiota transplantation (FMT) would constitute the optimal strategy to modify the gut microbiome. It has proven to be remarkably effective in managing *C. difficile* colitis, and isolated case reports describe beneficial effects over MS disease course, through mechanisms that remain unclear[36,37]. A clinical trial of FMT is underway[38], but even before its completion, many questions arise. It is unclear which population should be studied and what characterizes an ideal donor, not to mention the dose, route of administration and dose scheduling (single dose *vs* multiple doses). Patient with *C. diff* colitis who undergo FMT have been previously treated with antibiotics such as vancomycin and metronidazole, and presumably, have had some of their microbiota depleted. Would patients with MS require "ablation" of their microbiome before FMT? DMTs have immune modulating properties and they may also directly alter the microbiome[26], so their possible effects on the "engraftment" cannot be underestimated.

THE INTESTINAL BARRIER

The intestinal barrier is the physical and functional zone of interaction between the gut microbiome and the organism. It is a complex multi-layered structure that includes major portions of the gut immunological system and is essential for homeostasis[26]. However, it has been comparatively ignored regarding its possible role in MS pathophysiology.

In experimental models, mice undergoing EAE show an altered intestinal barrier, with increased permeability and various gross morphological changes, as well as alterations in the expression of tight junction proteins in the intestinal mucosa[39]. The peak of intestinal barrier dysfunction mirrors the peak of EAE clinical severity and preventing intestinal barrier breakdown leads to attenuation of EAE[40]. These alterations have also been associated with several abnormal immunological responses.

Patients with MS also have an altered intestinal barrier. Almost 2 decades ago, investigators found that patients with MS had increased intestinal permeability when compared to controls, using an *in vivo* mannitol/lactulose ratio test[41]. Increased intestinal permeability was also found to be associated with the number of peripheral CD45RO+ B cells[41]. A more recent study confirmed this finding; up to 70% of MS patients had increased intestinal permeability[42]. It has been hypothesized that an altered intestinal barrier might lead to bacterial translocation thus allowing the passage of noxious molecules such as microbial associated molecular patterns. This could then alter peripheral immune responses or allow these molecules to enter the CNS and alter neuroimmunity[26,43].

Although the evidence linking the intestinal barrier with MS is much more limited than evidence linking MS with alterations of the gut microbiome, the question of whether it constitutes a viable therapeutic target is the same. Of course, the issue is complicated by the fact that the microbiome is essential in the regulation of intestinal barrier function, so it could be arbitrary to think of them as separate entities. An altered intestinal barrier is also a crucial aspect of the pathophysiology of IBD and celiac disease, so research from these fields has shed light on possible strategies to maintain intestinal barrier integrity.

One of the first components of the intestinal barrier is a thick mucus layer forming a protective film, enriched by secretory IgA and antimicrobial peptides and proteins. Oral supplementation with lecithin and phosphatidylcholine can adhere to the intestinal mucosa, strengthening the mucus layer and improving barrier function[44-46]. Regulators of tight junctions, such as larazotide, are under development. Larazotide is a peptide able to re-arrange tight junctions and prevent intestinal barrier dysfunction. It has been studied in patients with celiac disease with promising results[47-49]. Designing pre-clinical studies using these methods to enhance barrier function in the setting of autoimmune neurological disease should be straightforward.

Although probiotics may not be the ideal method to modify the microbiome, they have been suggested to play a significant role in modulating barrier function. *E. coli* strain *nissle* has been marketed in Europe for many years as a probiotic with beneficial effects on the intestinal barrier[50]. It has moderate evidence from randomized trials showing it may lead to remission in ulcerative colitis[51] and in the EAE mouse model of MS it reduced disease severity by maintaining intestinal barrier function[40]. VSL#3 is another probiotic mixture with putative barrier-protecting properties[52]. There is evidence of clinical effectiveness in the management of chronic pouchitis in patients with ulcerative colitis[35,53]. VSL#3 administered to a small number of MS patients leads to an anti-inflammatory peripheral immune response[33]. These two probiotic agents would be good candidates for a large, well-designed clinical trial.

Finally, we go full circle and return to FMT. It is believed that after successful modification of the microbiome, this strategy might lead to improved intestinal barrier function[54]. The gut microbiome is essential for the regulation of intestinal barrier homeostasis[55], partly through the production of short chain fatty acids (SCFA) such as butyrate, propionate and acetate. SCFAs can modulate tight junctions in the gut and modulate inflammatory responses in the intestinal mucosa[44,55]. Other interesting alternatives have also recently been described

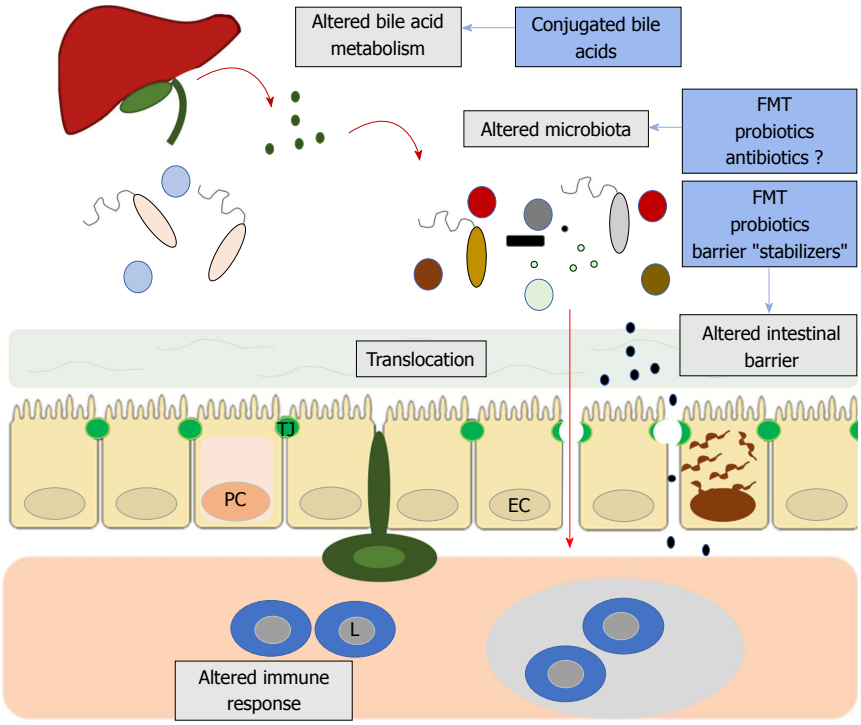

Figure 1 Alterations in intestinal homeostasis described in multiple sclerosis as therapeutic targets. Altered bile acid metabolism, altered microbiota and alterations in intestinal barrier function all lead to local and systemic alterations in immune responses that could negatively impact MS pathophysiology (grey squares). Bile acid supplementation, fecal microbiota transplantation, probiotics, antibiotics and barrier protectors are all possible therapeutic interventions (blue squares). MS: Multiple sclerosis; FMT: Fecal microbiota transplantation; PC: Paneth cells; EC: Epithelial cells; TJ: Tight junctions; L: Lymphocytes.

including the use of stool substitute preparations made from purified intestinal bacterial cultures derived from a single healthy donor[56]. Of course, many questions would need to be settled before clinical trials as discussed above.

BILE ACIDS

Bile acids are the main regulators of fat and fat-soluble vitamins digestion. They also significantly affect gut physiology and homeostasis. Bile acids can modulate the intestinal barrier function through complex mechanisms[57,58], and can shape the gut microbiota community. In turn, the microbiome can change bile acid metabolism[59]. Remarkably, bile acids may also modulate inflammatory signaling in the central nervous system. Ursodeoxycholic acid can inhibit the inflammatory activity of microglia *in vitro*[60], and tauroursodeoxycholic acid can shift microglia phenotypes towards an anti-inflammatory state through activation of the G protein-coupled bile acid receptor 1/Takeda G protein-coupled receptor 5[61]. Bile acids are also agonists of the nuclear hormone receptor farnesoid X receptor. Bile acid farnesoid X agonism led to attenuation of EAE and modulation of neuroinflammatory responses[62]. Mice fed a high-fat diet show dysregulated bile acid synthesis, gut dysbiosis and increased microglial activation[63]. Furthermore, metabolomics studies have found alterations in bile acids in patients with MS compared to healthy controls[64]. Conjugated bile acids such as ursodeoxycholic acid have been used in the management of some gastrointestinal diseases for decades. A clinical trial of bile acid supplementation in MS is underway[65].

CONCLUSION

Exciting research suggests that the brain-gut axis, once an almost esoteric concept, might yield novel therapeutic targets in neuroimmunological diseases such as MS (Figure 1). The often-symbiotic roles of the gut microbiome, intestinal barrier and even bile acids in the regulation of neuroimmune responses is beginning to be elucidated. If future pre-clinical and clinical studies confirm the relevance of intestinal barrier dysfunction, bile acid metabolism and the gut microbiome in the pathophysiology of MS, the next step will be to translate these findings into therapeutics. Only well designed clinical trials will answer whether interventions such as FMT, probiotics or barrier protectors yield clinically meaningful results. The time is right to assess whether the gut-brain axis can be transferred from the bench to the bedside.

REFERENCES

1 **Freedman SN**, Shahi SK, Mangalam AK. The "Gut Feeling": Breaking Down the Role of Gut Microbiome in Multiple Sclerosis. *Neurotherapeutics* 2018; **15**: 109-125 [PMID: 29204955 DOI: 10.1007/s13311-017-0588-x]

2 **Calvo-Barreiro L**, Eixarch H, Montalban X, Espejo C. Combined therapies to treat complex diseases: The role of the gut microbiota in multiple sclerosis. *Autoimmun Rev* 2018; **17**: 165-174 [PMID:

29191793 DOI: 10.1016/j.autrev.2017.11.019]
3 **Pröbstel AK**, Baranzini SE. The Role of the Gut Microbiome in Multiple Sclerosis Risk and Progression: Towards Characterization of the "MS Microbiome". *Neurotherapeutics* 2018; **15**: 126-134 [PMID: 29147991 DOI: 10.1007/s13311-017-0587-y]
4 **Thaiss CA**, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. *Nature* 2016; **535**: 65-74 [PMID: 27383981 DOI: 10.1038/nature18847]
5 Hot Topic 6: Gut microbiota in multiple sclerosis. Symposium held at the 7th Joint ECTRIMS-ACTRIMS meeting, 25-28 October, Paris, France. Available from: URL: https://www.ectrims-congress.eu/2017/scientific-programme/scientific-programme.html
6 Brain-Gut Axis: a focus on finding a cure for Multiple Sclerosis. University of Alberta MS Centre Research Symposium, held May 4, 2018, Edmonton, Alberta, Canada. Available from: URL: https://cloudfront.ualberta.ca/-/media/medicine/ms-centre/2018-ualberta-ms-centre-program-25apr18-pdfa.pdf
7 **Ochoa-Repáraz J**, Mielcarz DW, Haque-Begum S, Kasper LH. Induction of a regulatory B cell population in experimental allergic encephalomyelitis by alteration of the gut commensal microflora. *Gut Microbes* 2010; **1**: 103-108 [PMID: 21326918 DOI: 10.4161/gmic.1.2.11515]
8 **Colpitts SL**, Kasper EJ, Keever A, Liljenberg C, Kirby T, Magori K, Kasper LH, Ochoa-Repáraz J. A bidirectional association between the gut microbiota and CNS disease in a biphasic murine model of multiple sclerosis. *Gut Microbes* 2017; **8**: 561-573 [PMID: 28708466 DOI: 10.1080/19490976.2017.1353843]
9 **Wang Y**, Begum-Haque S, Telesford KM, Ochoa-Repáraz J, Christy M, Kasper EJ, Kasper DL, Robson SC, Kasper LH. A commensal bacterial product elicits and modulates migratory capacity of CD39(+) CD4 T regulatory subsets in the suppression of neuroinflammation. *Gut Microbes* 2014; **5**: 552-561 [PMID: 25006655 DOI: 10.4161/gmic.29797]
10 **Berer K**, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C, Wekerle H, Krishnamoorthy G. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. *Nature* 2011; **479**: 538-541 [PMID: 22031325 DOI: 10.1038/nature10554]
11 **Lee YK**, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 2011; **108** Suppl 1: 4615-4622 [PMID: 20660719 DOI: 10.1073/pnas.1000082107]
12 **Berer K**, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE, Kümpfel T, Hohlfeld R, Krishnamoorthy G, Wekerle H. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. *Proc Natl Acad Sci USA* 2017; **114**: 10719-10724 [PMID: 28893994 DOI: 10.1073/pnas.1711233114]
13 **Cekanaviciute E**, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R, Bencosme Y, Lee YK, Hauser SL, Crabtree-Hartman E, Sand IK, Gacias M, Zhu Y, Casaccia P, Cree BAC, Knight R, Mazmanian SK, Baranzini SE. Gut bacteria from multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse models. *Proc Natl Acad Sci USA* 2017; **114**: 10713-10718 [PMID: 28893978 DOI: 10.1073/pnas.1711235114]
14 **Cantarel BL**, Waubant E, Chehoud C, Kuczynski J, DeSantis TZ, Warrington J, Venkatesan A, Fraser CM, Mowry EM. Gut microbiota in multiple sclerosis: possible influence of immunomodulators. *J Investig Med* 2015; **63**: 729-734 [PMID: 25775034 DOI: 10.1097/JIM.0000000000000192]
15 **Miyake S**, Kim S, Suda W, Oshima K, Nakamura M, Matsuoka T, Chihara N, Tomita A, Sato W, Kim SW, Morita H, Hattori M, Yamamura T. Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. *PLoS One* 2015; **10**: e0137429 [PMID: 26367776 DOI: 10.1371/journal.pone.0137429]
16 **Chen J**, Chia N, Kalari KR, Yao JZ, Novotna M, Paz Soldan MM, Luckey DH, Marietta EV, Jeraldo PR, Chen X, Weinshenker BG, Rodriguez M, Kantarci OH, Nelson H, Murray JA, Mangalam AK. Multiple sclerosis patients have a distinct gut microbiota compared to healthy controls. *Sci Rep* 2016; **6**: 28484 [PMID: 27346372 DOI: 10.1038/srep28484]
17 **Tremlett H**, Fadrosh DW, Faruqi AA, Zhu F, Hart J, Roalstad S, Graves J, Lynch S, Waubant E; US Network of Pediatric MS Centers. Gut microbiota in early pediatric multiple sclerosis: a case-control study. *Eur J Neurol* 2016; **23**: 1308-1321 [PMID: 27176462 DOI: 10.1111/ene.13026]
18 **Tremlett H**, Fadrosh DW, Faruqi AA, Hart J, Roalstad S, Graves J, Lynch S, Waubant E; US Network of Pediatric MS Centers. Gut microbiota composition and relapse risk in pediatric MS: A pilot study. *J Neurol Sci* 2016; **363**: 153-157 [PMID: 27000242 DOI: 10.1016/j.jns.2016.02.042]
19 **Rumah KR**, Linden J, Fischetti VA, Vartanian T. Isolation of Clostridium perfringens type B in an individual at first clinical presentation of multiple sclerosis provides clues for environmental triggers of the disease. *PLoS One* 2013; **8**: e76359 [PMID: 24146858 DOI: 10.1371/journal.pone.0076359]
20 **Jangi S**, Gandhi R, Cox LM, Li N, von Glehn F, Yan R, Patel B, Mazzola MA, Liu S, Glanz BL, Cook S, Tankou S, Stuart F, Melo K, Nejad P, Smith K, Topçuolu BD, Holden J, Kivisäkk P, Chitnis T, De Jager PL, Quintana FJ, Gerber GK, Bry L, Weiner HL. Alterations of the human gut microbiome in multiple sclerosis. *Nat Commun* 2016; **7**: 12015 [PMID: 27352007 DOI: 10.1038/ncomms12015]
21 **Swidsinski A**, Dörffel Y, Loening-Baucke V, Gille C, Göktas Ö, Reißhauer A, Neuhaus J, Weylandt KH, Guschin A, Bock M. Reduced Mass and Diversity of the Colonic Microbiome in Patients with Multiple Sclerosis and Their Improvement with Ketogenic Diet. *Front Microbiol* 2017; **8**: 1141 [PMID: 28702003 DOI: 10.3389/fmicb.2017.01141]
22 **Cosorich I**, Dalla-Costa G, Sorini C, Ferrarese R, Messina MJ, Dolpady J, Radice E, Mariani A, Testoni PA, Canducci F, Comi G, Martinelli V, Falcone M. High frequency of intestinal TH17 cells correlates with microbiota alterations and disease activity in multiple sclerosis. *Sci Adv* 2017; **3**: e1700492 [PMID: 28706993 DOI: 10.1126/sciadv.1700492]
23 **Levinthal DJ**, Rahman A, Nusrat S, O'Leary M, Heyman R, Bielefeldt K. Adding to the burden: gastrointestinal symptoms and syndromes in multiple sclerosis. *Mult Scler Int* 2013; **2013**: 319201 [PMID: 24163768 DOI: 10.1155/2013/319201]
24 **De Palma G**, Collins SM, Bercik P. The microbiota-gut-brain axis in functional gastrointestinal disorders. *Gut Microbes* 2014; **5**: 419-429 [PMID: 24921926 DOI: 10.4161/gmic.29417]
25 **Metz LM**, Li DKB, Traboulsee AL, Duquette P, Eliasziw M, Cerchiaro G, Greenfield J, Riddehough A, Yeung M, Kremenchutzky M, Vorobeychik G, Freedman MS, Bhan V, Blevins G, Marriott JJ, Grand'Maison F, Lee L, Thibault M, Hill MD, Yong VW; Minocycline in MS Study Team. Trial of Minocycline in a Clinically Isolated Syndrome of Multiple Sclerosis. *N Engl J Med* 2017; **376**: 2122-2133 [PMID: 28564557 DOI: 10.1056/NEJMoa1608889]
26 **Camara-Lemarroy CR**, Metz L, Meddings JB, Sharkey KA, Wee Yong V. The intestinal barrier in multiple sclerosis: implications for pathophysiology and therapeutics. *Brain* 2018; **141**: 1900-1916 [PMID: 29860380 DOI: 10.1093/brain/awy131]
27 **Yong VW**, Wells J, Giuliani F, Casha S, Power C, Metz LM. The promise of minocycline in neurology. *Lancet Neurol* 2004; **3**: 744-751 [PMID: 15556807 DOI: 10.1016/S1474-4422(04)00937-8]
28 **Maier L**, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR, Fernandez KC, Dose H, Mori H, Patil KR, Bork P, Typas A. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018; **555**: 623-628 [PMID: 29555994 DOI: 10.1038/nature25979]
29 **Kwon HK**, Kim GC, Kim Y, Hwang W, Jash A, Sahoo A, Kim JE, Nam JH, Im SH. Amelioration of experimental autoimmune encephalomyelitis by probiotic mixture is mediated by a shift in T helper cell immune response. *Clin Immunol* 2013; **146**: 217-227 [PMID: 23416238 DOI: 10.1016/j.clim.2013.01.001]
30 **Lavasani S**, Dzhambazov B, Nouri M, Fåk F, Buske S, Molin G, Thorlacius H, Alenfall J, Jeppsson B, Weström B. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. *PLoS One* 2010; **5**: e9009 [PMID: 20126401 DOI: 10.1371/

journal.pone.0009009]
31 **Kouchaki E**, Tamtaji OR, Salami M, Bahmani F, Daneshvar Kakhaki R, Akbari E, Tajabadi-Ebrahimi M, Jafari P, Asemi Z. Clinical and metabolic response to probiotic supplementation in patients with multiple sclerosis: A randomized, double-blind, placebo-controlled trial. *Clin Nutr* 2017; **36**: 1245-1249 [PMID: 27669638 DOI: 10.1016/j.clnu.2016.08.015]
32 **Tamtaji OR**, Kouchaki E, Salami M, Aghadavod E, Akbari E, Tajabadi-Ebrahimi M, Asemi Z. The Effects of Probiotic Supplementation on Gene Expression Related to Inflammation, Insulin, and Lipids in Patients With Multiple Sclerosis: A Randomized, Double-Blind, Placebo-Controlled Trial. *J Am Coll Nutr* 2017; **36**: 660-665 [PMID: 28922099 DOI: 10.1080/07315724.2017.1347074]
33 **Tankou SK**, Regev K, Healy BC, Cox LM, Tjon E, Kivisakk P, Vanande IP, Cook S, Gandhi R, Glanz B, Stankiewicz J, Weiner HL. Investigation of probiotics in multiple sclerosis. *Mult Scler* 2018; **24**: 58-63 [PMID: 29307299 DOI: 10.1177/1352458517737390]
34 **Abraham BP**, Quigley EMM. Probiotics in Inflammatory Bowel Disease. *Gastroenterol Clin North Am* 2017; **46**: 769-782 [PMID: 29173520 DOI: 10.1016/j.gtc.2017.08.003]
35 **Derwa Y**, Gracie DJ, Hamlin PJ, Ford AC. Systematic review with meta-analysis: the efficacy of probiotics in inflammatory bowel disease. *Aliment Pharmacol Ther* 2017; **46**: 389-400 [PMID: 28653751 DOI: 10.1111/apt.14203]
36 **Borody TJ**, Leis SM, Campbell J, Torres M, Nowak A. Fecal microbiota transplantation (FMT) in multiple sclerosis (MS). *Am J Gastroenterol* 2011; **106**: S352
37 **Makkawi S**, Camara-Lemarroy C, Metz L. Fecal microbiota transplantation associated with 10 years of stability in a patient with SPMS. *Neurol Neuroimmunol Neuroinflamm* 2018; **5**: e459 [PMID: 29619403 DOI: 10.1212/NXI.0000000000000459]
38 **Kremenchutzky M**. Fecal Microbial Transplantation in Relapsing Multiple Sclerosis Patients. cited 2018-05-22; In ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT03183869
39 **Nouri M**, Bredberg A, Weström B, Lavasani S. Intestinal barrier dysfunction develops at the onset of experimental autoimmune encephalomyelitis, and can be induced by adoptive transfer of auto-reactive T cells. *PLoS One* 2014; **9**: e106335 [PMID: 25184418 DOI: 10.1371/journal.pone.0106335]
40 **Secher T**, Kassem S, Benamar M, Bernard I, Boury M, Barreau F, Oswald E, Saoudi A. Oral Administration of the Probiotic Strain *Escherichia coli* Nissle 1917 Reduces Susceptibility to Neuroinflammation and Repairs Experimental Autoimmune Encephalomyelitis-Induced Intestinal Barrier Dysfunction. *Front Immunol* 2017; **8**: 1096 [PMID: 28959254 DOI: 10.3389/fimmu.2017.01096]
41 **Yacyshyn B**, Meddings J, Sadowski D, Bowen-Yacyshyn MB. Multiple sclerosis patients have peripheral blood CD45RO+ B cells and increased intestinal permeability. *Dig Dis Sci* 1996; **41**: 2493-2498 [PMID: 9011463 DOI: 10.1007/BF02100148]
42 **Buscarinu MC**, Cerasoli B, Annibali V, Policano C, Lionetto L, Capi M, Mechelli R, Romano S, Fornasiero A, Mattei G, Piras E, Angelini DF, Battistini L, Simmaco M, Umeton R, Salvetti M, Ristori G. Altered intestinal permeability in patients with relapsing-remitting multiple sclerosis: A pilot study. *Mult Scler* 2017; **23**: 442-446 [PMID: 27270497 DOI: 10.1177/1352458516652498]
43 **Buscarinu MC**, Romano S, Mechelli R, Pizzolato Umeton R, Ferraldeschi M, Fornasiero A, Reniè R, Cerasoli B, Morena E, Romano C, Loizzo ND, Umeton R, Salvetti M, Ristori G. Intestinal Permeability in Relapsing-Remitting Multiple Sclerosis. *Neurotherapeutics* 2018; **15**: 68-74 [PMID: 29119385 DOI: 10.1007/s13311-017-0582-3]
44 **Sun J**, Shen X, Li Y, Guo Z, Zhu W, Zuo L, Zhao J, Gu L, Gong J, Li J. Therapeutic Potential to Modify the Mucus Barrier in Inflammatory Bowel Disease. *Nutrients* 2016; **8**: [PMID: 26784223 DOI: 10.3390/nu8010044]
45 **Merga Y**, Campbell BJ, Rhodes JM. Mucosal barrier, bacteria and inflammatory bowel disease: possibilities for therapy. *Dig Dis* 2014; **32**: 475-483 [PMID: 24969297 DOI: 10.1159/000358156]
46 **Stremmel W**, Gauss A. Lecithin as a therapeutic agent in ulcerative colitis. *Dig Dis* 2013; **31**: 388-390 [PMID: 24246994 DOI: 10.1159/000354707]
47 **Pearce SC**, Al-Jawadi A, Kishida K, Yu S, Hu M, Fritzky LF, Edelblum KL, Gao N, Ferraris RP. Marked differences in tight junction composition and macromolecular permeability among different intestinal cell types. *BMC Biol* 2018; **16**: 19 [PMID: 29391007 DOI: 10.1186/s12915-018-0481-z]
48 **Gopalakrishnan S**, Tripathi A, Tamiz AP, Alkan SS, Pandey NB. Larazotide acetate promotes tight junction assembly in epithelial cells. *Peptides* 2012; **35**: 95-101 [PMID: 22401910 DOI: 10.1016/j.peptides.2012.02.016]
49 **Khaleghi S**, Ju JM, Lamba A, Murray JA. The potential utility of tight junction regulation in celiac disease: focus on larazotide acetate. *Therap Adv Gastroenterol* 2016; **9**: 37-49 [PMID: 26770266 DOI: 10.1177/1756283X15616576]
50 **Sonnenborn U**. Escherichia coli strain Nissle 1917-from bench to bedside and back: history of a special Escherichia coli strain with probiotic properties. *FEMS Microbiol Lett* 2016; **363**: [PMID: 27619890 DOI: 10.1093/femsle/fnw212]
51 **Scaldaferri F**, Gerardi V, Mangiola F, Lopetuso LR, Pizzoferrato M, Petito V, Papa A, Stojanovic J, Poscia A, Cammarota G, Gasbarrini A. Role and mechanisms of action of Escherichia coli Nissle 1917 in the maintenance of remission in ulcerative colitis patients: An update. *World J Gastroenterol* 2016; **22**: 5505-5511 [PMID: 27350728 DOI: 10.3748/wjg.v22.i24.5505]
52 **Dai C**, Zhao DH, Jiang M. VSL#3 probiotics regulate the intestinal epithelial barrier in vivo and in vitro via the p38 and ERK signaling pathways. *Int J Mol Med* 2012; **29**: 202-208 [PMID: 22089663 DOI: 10.3892/ijmm.2011.839]
53 **Singh S**, Stroud AM, Holubar SD, Sandborn WJ, Pardi DS. Treatment and prevention of pouchitis after ileal pouch-anal anastomosis for chronic ulcerative colitis. *Cochrane Database Syst Rev* 2015; CD001176 [PMID: 26593456 DOI: 10.1002/14651858.CD001176.pub3]
54 **Reinisch W**. Fecal Microbiota Transplantation in Inflammatory Bowel Disease. *Dig Dis* 2017; **35**: 123-126 [PMID: 28147375 DOI: 10.1159/000449092]
55 **Wells JM**, Brummer RJ, Derrien M, MacDonald TT, Troost F, Cani PD, Theodorou V, Dekker J, Méheust A, de Vos WM, Mercenier A, Nauta A, Garcia-Rodenas CL. Homeostasis of the gut barrier and potential biomarkers. *Am J Physiol Gastrointest Liver Physiol* 2017; **312**: G171-G193 [PMID: 27908847 DOI: 10.1152/ajpgi.00048.2015]
56 **Petrof EO**, Gloor GB, Vanner SJ, Weese SJ, Carter D, Daigneault MC, Brown EM, Schroeter K, Allen-Vercoe E. Stool substitute transplant therapy for the eradication of Clostridium difficile infection: 'RePOOPulating' the gut. *Microbiome* 2013; **1**: 3 [PMID: 24467987 DOI: 10.1186/2049-2618-1-3]
57 **Pavlidis P**, Powell N, Vincent RP, Ehrlich D, Bjarnason I, Hayee B. Systematic review: bile acids and intestinal inflammation-luminal aggressors or regulators of mucosal defence? *Aliment Pharmacol Ther* 2015; **42**: 802-817 [PMID: 26223936 DOI: 10.1111/apt.13333]
58 **Keating N**, Keely SJ. Bile acids in regulation of intestinal physiology. *Curr Gastroenterol Rep* 2009; **11**: 375-382 [PMID: 19765365 DOI: 10.1007/s11894-009-0057-8]
59 **Li Y**, Tang R, Leung PSC, Gershwin ME, Ma X. Bile acids and intestinal microbiota in autoimmune cholestatic liver diseases. *Autoimmun Rev* 2017; **16**: 885-896 [PMID: 28698093 DOI: 10.1016/j.autrev.2017.07.002]
60 **Joo SS**, Won TJ, Lee DI. Potential role of ursodeoxycholic acid in suppression of nuclear factor kappa B in microglial cell line (BV-2). *Arch Pharm Res* 2004; **27**: 954-960 [PMID: 15473667 DOI: 10.1007/BF02975850]
61 **Yanguas-Casás N**, Barreda-Manso MA, Nieto-Sampedro M, Romero-Ramírez L. TUDCA: An Agonist of the Bile Acid Receptor GPBAR1/TGR5 With Anti-Inflammatory Effects in Microglial Cells. *J Cell Physiol* 2017; **232**: 2231-2245 [PMID: 27987324 DOI: 10.1002/jcp.25742]

62 **Ho PP**, Steinman L. Obeticholic acid, a synthetic bile acid agonist of the farnesoid X receptor, attenuates experimental autoimmune encephalomyelitis. *Proc Natl Acad Sci USA* 2016; **113**: 1600-1605 [PMID: 26811456 DOI: 10.1073/pnas.1524890113]
63 **Jena PK**, Sheng L, Di Lucente J, Jin LW, Maezawa I, Wan YY. Dysregulated bile acid synthesis and dysbiosis are implicated in Western diet-induced systemic inflammation, microglial activation, and reduced neuroplasticity. *FASEB J* 2018; **32**: 2866-2877 [PMID: 29401580 DOI: 10.1096/fj.201700984RR]
64 **Bhargava P**, Mowry E, Calabresi P. Global metabolomics identifies perturbation of multiple metabolic pathways in Multiple Sclerosis (Abstract). *Neurology* 2015; **84**: P5.242
65 **Bhargave P.** A Trial of Bile Acid Supplementation in Patients With Multiple Sclerosis. Cited 2018-05-22; In ClinicalTrials.gov [Internet]. Bethesda (MD): U.S. National Library of Medicine. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT03423121

P- Reviewer: Abalo R, Quigley EM **S- Editor**: Gong ZM
L- Editor: A **E- Editor**: Bian YN

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4224-4229

DOI: 10.3748/wjg.v24.i37.4224

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Hepatocellular carcinoma in Latin America: Diagnosis and treatment challenges

Federico Piñero, Jaime Poniachik, Ezequiel Ridruejo, Marcelo Silva

Federico Piñero, Ezequiel Ridruejo, Marcelo Silva, Liver Unit, Hospital Universitario Austral, Facultad de Ciencias Biomédicas, Universidad Austral, Pilar, Buenos Aires B1629HJ, Argentina

Federico Piñero, Ezequiel Ridruejo, Marcelo Silva, Latin American Liver Research Educational and Awareness Network, Buenos Aires B1629HJ, Argentina

Jaime Poniachik, Department of Gastroenterology and Hepatology, Hospital Clínico Universidad de Chile, Santiago de Chile, Región Metropolitana Santiago 46010, Chile

Jaime Poniachik, Gastroenterology and Hepatology, Clínica Santa María, Santiago de Chile, Región Metropolitana Santiago 834-0518, Chile

Ezequiel Ridruejo, Hepatology Section, Centro de Educación Médica e Investigaciones Clínicas Norberto Quirno, Ciudad de Buenos Aires C1425ASG, Argentina

ORCID number: Federico Piñero (0000-0002-9528-2279); Jaime Poniachik (0000-0001-7958-3357); Ezequiel Ridruejo (0000-0002-3321-0683); Marcelo Silva (0000-0002-2287-7351).

Author contributions: Piñero F and Poniachik J contributed to concept and writing of the article; Ridruejo E and Silva M contributed to critical review and final approval of the article.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to declare.

Manuscript source: Invited manuscript

Correspondence to: Federico Piñero, MD, MSCE, Academic Research, Doctor, Hepatology and Liver Transplant Unit, Hospital Universitario Austral, Av. Presidente Perón 1500, Pilar, Buenos Aires B1629HJ, Argentina. fpinerof@cas.austral.edu.ar
Telephone: +54-230-4482000
Fax: +54-230-4482236

Received: July 5, 2018
Peer-review started: July 5, 2018
First decision: August 1, 2018
Revised: August 2, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

Latin America, a region with a population greater than 600000000 individuals, is well known due to its wide geographic, socio-cultural and economic heterogeneity. Access to health care remains as the main barrier that challenges routine screening, early diagnosis and proper treatment of hepatocellular carcinoma (HCC). Therefore, identification of population at risk, implementation of surveillance programs and access to curative treatments has been poorly obtained in the region. Different retrospective cohort studies from the region have shown flaws in the implementation process of routine surveillance and early HCC diagnosis. Furthermore, adherence to clinical practice guidelines recommendations assessed in two studies from Brazil and Argentina demonstrated that there is also room for improvement in this field, similarly than the one observed in Europe and the United States. In summary, Latin America shares difficulties in HCC decision-making processes similar to those from developed countries. However, a transversal limitation in the region is the poor access to health care with the consequent limitation to standard treatments for overall population. Specifically, universal health care access to the different World Health Organization levels is crucial, including improvement in research, education and continuous

medical training in order to expand knowledge and generation of data promoting a continuous improvement in the care of HCC patients.

Key words: Latin America; Liver cancer; Limitations; Challenge

Core tip: Which are the implications in regard to clinical decision making processes related to hepatocellular carcinoma (HCC) in daily practice in Latin America? Should we consider making these decisions taking into account both, local experiences and their feasibility together with the best available evidence in parallel with patient preferences? These decision-making processes must be individualized according to local barriers to health care systems. Primary prevention programs of liver diseases, surveillance for HCC and intervention programs following the best evidence will be possible only if we are aware of local barriers and develop efficient strategies to overcome them.

Piñero F, Poniachik J, Ridruejo E, Silva M. Hepatocellular carcinoma in Latin America: Diagnosis and treatment challenges. *World J Gastroenterol* 2018; 24(37): 4224-4229 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4224.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4224

INTRODUCTION

Latin American comprises a region of the Americas of Latin origin, in which the most common speaking languages are Spanish and Portuguese. The region accounts for more than twenty million square kilometers of surface area, with more than six hundred million population. Due to its geographic extension, Latin America has a great socio-cultural heterogeneity and an important socio-economic difference among countries. While there are high earners like Chile and Uruguay with a gross domestic product (GDP) per capita over $20000, others like Haiti and Honduras have GDPs per capita lower than $ 5000[1]. At the same time, each country in itself is highly unequal, presenting some of the highest Globalization of Inequality (GINI) scores in the world. Brazil, Chile, Ecuador and Colombia all present GINIs above 0.45 for the year 2016; Argentina and Uruguay having slightly better scores[1]. In comparison, Sweden, Norway, Netherlands and Denmark all have GINI scores less than 0.30[1].

It is in this socio-cultural and economic scenario, where settles a large variety in access to health care systems in the region. These systems are mainly made up of a common payer and provider that is the state. However, in several countries, there are other type of health providers through social security and private insurances and providers. Furthermore, expenditure on access care in many Latin American countries comes from out-of-pocket money among high to middle income people. On the other hand, among low socio-economic classes, expenditure comes purely and exclusively from public services, which in most of the cases provide with low to regular quality of medical care services and shortage of appropriate medical supplies and devices.

WHERE DO WE STAND IN LATIN AMERICA REGARDING HEPATOCELLULAR CARCINOMA?

Hepatocellular carcinoma (HCC) is the second leading cause of cancer related death worldwide and the main cause of cancer in patients with cirrhosis. Incidence of HCC varies according to geographic location, depending on the prevalence of viral hepatitis among the world. The predominant reported causes of HCC in different geographic areas around the world have been related with chronic hepatitis C virus (HCV) or hepatitis B virus (HBV) infection and alcoholic liver disease[2-5]. Heterogeneous data regarding epidemiology of HCC in Latin America has been reported[6-12]. While HCV and alcoholic liver disease are the most frequent etiologies of HCC in the region, HBV is a leading cause in some countries, mainly in Brazil. More recently, we have observed a changing epidemiological trend of HCC towards an increasing non-alcoholic fatty liver disease, becoming an important public health burden in the region[6,7].

As previously proposed by the World Health Organization the structural challenge in the region is the uneven access to health care. To our knowledge there is not even one country with an integrated program to assist on the prevention of chronic liver diseases and early identification of the population at risk for developing HCC. Consequently, the common challenge for scientific societies is to induce regional policy makers to develop interventions and strategies able to identify the population at risk, implement surveillance programs, and improve access to curative and palliative treatments. Once we have assured access to adequate care we should move into next step which is the correct adherence to recommendations from clinical practice guidelines[2-5].

A clinical case scenario as an example of where do we fail in Latin America

The following clinical case demonstrates the regional shortcomings related to HCC diagnosis at late stages and its therapeutic consequences. A 60-year-old male patient with compensated cirrhosis and clinically significant portal hypertension due to chronic HCV infection, who started antiviral treatment with direct-acting antiviral agents, began an erratic path of ultrasound (US) screening for HCC. Surveillance was performed by non-liver expert

Table 1 Surveillance for hepatocellular carcinoma in Latin America

Study	Population	Design	Results
Fassio *et al*[8]	*n* = 240 HCC Brazil, Arg, Colombia, Chile, Uruguay, Venezuela	Prospective cohort (Surveillance retrospectively analyzed)	54% under surveillance; BCLC A 70% *vs* 39% not under surveillance; No survival analysis
Paranaguá-Vezozzo *et al*[9]	*n* = 884 Cirrhosis Child A-B Brazil, Sao Paulo	Retrospective cohort US ± AFP annual	HCC annual incidence 2.9%; 75% under annual surveillance; 80% within Milan, better survival
Piñero *et al*[10]	*n* = 643 Cirrhosis, waiting list for liver transplantation. Argentina	Retrospective cohort Surveillance Failure = incidental HCC in the explant	US accuracy: S 33% and E 99%
Campos Appel-da-Silva *et al*[11]	*n* = 453 Child A-C Cirrhosis Brazil, Porto Alegre	Retrospective cohort US ± AFP every 6 mo	50.7% under surveillance; More BCLC 0-A *vs* no screening; Better survival within Milan criteria
Debes *et al*[12]	*n* = 1336 HCC Brazil, Argentina, Colombia, Peru, Uruguay, Ecuador	Retrospective cohort	47% under surveillance; Better survival *vs* symptomatic diagnosis (adjusted for lead-time bias)

BCLC: Barcelona Clinic Liver Cancer; HCC: Hepatocellular carcinoma; US: Ultrasound.

sonographers due to insurance's related lack of access to academic sites. Initially a 24-mm nodule was visualized and he was recommended to stay on a follow-up visit with no further imaging evaluation. Twelve months later, another US was performed; this time the nodule grew to 38 mm. He performed an abdominal computed tomography (CT) scan with oral contrast only, and the finding of an "uncharacteristic" nodule leaded to a CT-guided biopsy. The pathologic report was "nodules of hepatocellular regeneration separated by broad fibrous septa, cirrhosis". Result: No cancer. His physician suggested him to continue life normally and the patient happily went home.

A year later, a liver specialist suggested him to perform an abdominal CT scan with intravenous contrast. A heterogeneous 80-mm diameter lesion in the right hepatic lobe with "non-characteristic findings" was observed. Not satisfied, the patient looked for a second opinion. A second hepatologist performed a three-phase dynamic abdominal magnetic resonance imaging (MRI). Result: One lesion with arterial enhancement and wash out during portal and late phases: HCC of 83 mm, without vascular invasion. Serum alpha-fetoprotein value was 1200 ng/mL.

In the end, the patient consulted at least 4 medical doctors during a 2-year period, with extended and inadmissible delay in HCC diagnosis that at this point will probably exclude him from potentially curative treatments. Where did we fail?

Early diagnosis of HCC: Challenges and areas of improvement

This case, clearly illustrates some of the reasons for failure in routine surveillance and HCC diagnosis at early stages in Latin America, and as a consequence, failure in the appropriate staging and selection of therapies.

Screening failure entails three important points to be considered. First, absence of early identification of the population at risk, such as chronic HBV or HCV. Second, ineffective application of routine surveillance (semi-annual ultrasound performed by expert operators) and third, errors in interpretation of a positive or negative screening tests, misinterpreting its sensitivity and specificity.

Surveillance for HCC in Latin America demands a continuous improvement. Different retrospective cohort studies have shown flaws in the implementation process of routine surveillance, the consequent failure in the diagnosis in early stages and finally a notorious negative impact upon patient survival[8-12] (Table 1).

Overall, surveillance programs reported to be applied in less than 50% of the patients in Latin America. This number perhaps does not show the "real" regional situation, since most of this data came from academic rather than general hospitals. Consequently, screening failure for HCC in this region might be even greater, demanding strategies to improve its implementation such as application of US done by experts, correct interpretation of imaging tests and finally, adequacy of therapeutic decisions according to the best evidence-based-medicine. Consequently, early HCC diagnosis should be the aim of these strategies.

As exemplified in the clinical case, the misuse of diagnostic tools delays the correct diagnosis. HCC diagnosis implies an appropriate oncologic imaging paradigm, not requiring histological confirmation for diagnosis in most of the cases. However, discordance between images and histology may occur. This situation has been reported up to 10% in Argentina when comparing imaging reports and explanted liver data from liver transplanted patients[13,14]. In a multicenter Latin American cohort study, false positives cases were less than 3%[15]. Two different situations need to be further clarified when discussing imaging accuracy against histological confirmation of HCC. On one hand, when false positives are considered, it should be important to address if complete necrotic nodules were included as false positive cases resulting in a biased report. On the other hand, discrepancy between images

Table 2 Adherence to clinical practice guidelines around the world and in Latin America

Study	Population	Design	Results
Leoni *et al*[20]	*n* = 227 HCV 58% Child A 54%	Retrospective cohort (2005-2010) One center	At HCC diagnosis: BCLC 0-A 55%; Adherence to BCLC 60%; Higher adherence among BCLC A 86%
Gashin *et al*[21]	*n* = 137 HCV 62%	Retrospective cohort (2009-2010) One center	Adherence to BCLC 62%; Better overall survival; Heterogeneous causes of non-adherence
Kim *et al*[22]	*n* = 3515 HBV 77% Child A 82%	Retrospective cohort (2005-2009) One center	At HCC diagnosis: BCLC A 59%; Adherence to BCLC 49%; Better survival for adherence, except BCLC-D (BCLC D who were transplanted were considered "non-adherence")
Wallace *et al*[23]	*n* = 292 OH-HCV 65%	Retrospective cohort (2006-2014) One center	At HCC diagnosis: BCLC 0-A 64%; Adherence to BCLC 48% *vs* HKLC 56% (P.001); No better survival among BCLC adherence *vs* no-adherence but better survival among HKLC (TACE before transplant was considered "no-adherence")
Guarino *et al*[24]	*n* = 1008 HCV Child A 73%	Retrospective cohort (2013-2015) Multicenter study	At HCC diagnosis: BCLC 0-A 59%; Adherence BCLC 71%, lower in BCLC B 36% and C 46%; No better survival (TACE before transplant was considered "no-adherence")
Kikuchi *et al*[25]	*n* = 364 HBV 53% Child A 53%	Retrospective cohort (2010-2012) One center	At HCC diagnosis: BCLC A 36%; Adherence BCLC 52%; Lower adherence in BCLC C-D; No better survival, except in BCLC A (BCLC D who were transplanted were considered "non-adherence")
Piñero *et al*[26]	*n* = 708 HCV 58% Child A 54%	Dual cohort (2009-2016) Multicenter study	At HCC diagnosis: BCLC 0-A 47%; Adherence BCLC 53% initial, 63% subsequently; Adherence to BCLC: better survival HR 0.67 (CI: 0.52-0.87)

BCLC: Barcelona Clinic Liver Cancer; HKLC: Hong Kong Liver Cancer algorithm; HCV: Hepatitis C virus; HBV: Hepatitis B virus; TACE: Transarterial chemoembolization.

and explanted liver should be considered taking into account potential tumor progression, and locoregional response to treatments during the waiting list period.

Nevertheless this led to changes in diagnostic criteria for HCC in patients enrolled for liver transplantation in Argentina aimed to improve imaging diagnostic accuracy. Although the idea was novel, LIRADS criteria implementation leaded even to a greater uncertainty for those cases where HCC diagnosis is probable or possible (LIRADS 3 or 4). Moreover, imaging expert's agreement on LIRADS in the daily practice has been not assessed at all. Thus, LIRADS system seemed to make the clinical decision making process even more complex in daily practice in that country[16,17].

Challenges regarding staging and adherence to recommended treatment options from clinical practice guidelines

HCC staging considering the Barcelona Clinic Liver Cancer (BCLC) algorithm has been recommended in different clinical practice guidelines[3,4], including that from the Latin American Association for the Study of the Liver (ALEH)[3]. However, strict adherence to these therapeutic recommendations is often not feasible in daily practice. This does not contradict the BCLC algorithm, since its explicitly recommends that the therapeutic choice must be individualized considering feasibility, access and preferences of the patients[18]. In addition, there are different guidelines and recommendations, including those from Asia (APASL)[5], Japan and South Korea. Consequently, there is a wide range of treatment algorithms when considering HCC.

The BRIDGE study demonstrated the great heterogeneity in terms of the treatments performed worldwide at each stage and far from that recommended in the ideal situation[19]. Global and individual context makes therapeutic decisions in HCC heterogeneous in real life. Adherence to clinical practice guidelines recommendations varies between 40%-70% in different retrospective cohort studies[20-26]. Two Latin American studies evaluated adherence to BCLC and its impact on survival. In a study from Brazil, adherence to BCLC did not have a favorable impact on survival[25]. However, there was a selection bias when "non-adherence" was categorized in those patients within BCLC-D stage who were candidates for liver transplantation. Precisely, the BCLC clarifies in its footnote that these patients must be transplanted. In a dual cohort study in Argentina, adherence to BCLC was greater than 50%, being associated with better overall survival[26] (Table 2).

In summary, although Latin America shares some difficulties in HCC decision-making processes similar to those reported in some developed countries, we still have big gaps when compared to them. These gaps are seen in medical education, on early and accurate HCC diagnosis, and in universal access to good diagnostic technology and to curative treatments. Until they are corrected, discrepancy on HCC related survival would remain present.

PERSPECTIVE

Consequently, we shall make decisions considering local education, expertise and feasibility together with

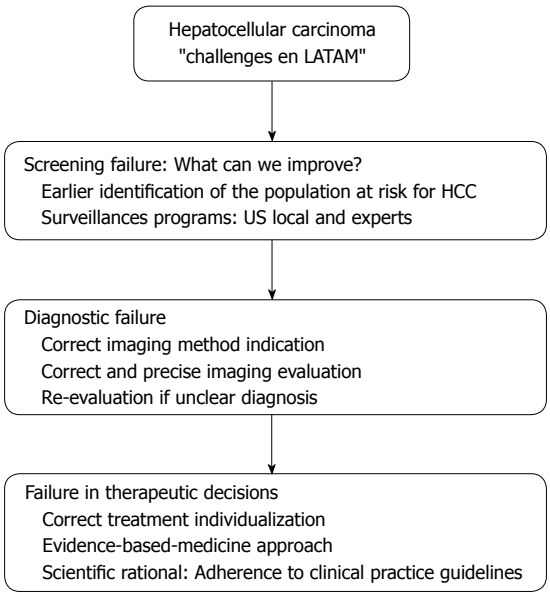

Figure 1 Areas of improvement regarding hepatocellular carcinoma in Latin America. HCC: Hepatocellular carcinoma.

the best available evidence. Ultimately, this decision-making-process must be individualized[27].

Which are the areas for improvement in Latin America? Specifically, universal health care access as per World Health Organization recommendation is crucial. This includes improvement in transmission of information and medical education from academic to primary health care centers, focusing on prevention of development of liver diseases, identification of population at risk for HCC, systematic implementation of routine surveillance programs, improvement in the diagnostic work-up process and finally, promoting overall access to all treatments strategies which have shown improvement in patient's survival (Figure 1). Finally, an important field to promote in the region is the development of research consortia such as the Latin American Liver Research Educational and Awareness Network, through which we can multiply medical education and generation of regional data necessary to develop efficient health interventions for improvement the care of patients with HCC[28].

REFERENCES

1 **World Bank.** International Comparison Program database. Available from: URL: https://data.worldbank.org

2 **Heimbach JK**, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. *Hepatology* 2018; **67**: 358-380 [PMID: 28130846 DOI: 10.1002/hep.29086]

3 **Méndez-Sánchez N,** Ridruejo E, Alves de Mattos A, Chávez-Tapia NC, Zapata R, Paraná R, Mastai R, Strauss E, Guevara-Casallas LG, Daruich J, Gadano A, Parise ER, Uribe M, Aguilar-Olivos NE, Dagher L, Ferraz-Neto BH, Valdés-Sánchez M, Sánchez-Avila JF. Latin American Association for the Study of the Liver clinical practice guidelines: management of hepatocellular carcinoma. *Ann Hepatol* 2014;**13** Suppl 1: S4–S40 [PMID 24998696]

4 **European Association for the Study of the Liver.** European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. *J Hepatol* 2018; **69**: 182-236 [PMID: 29628281 DOI: 10.1016/j.jhep.2018.03.019]

5 **Omata M**, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK, Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int* 2017; **11**: 317-370 [PMID: 28620797 DOI: 10.1007/s12072-017-9799-9]

6 **Piñero F**, Pages J, Marciano S, Fernández N, Silva J, Anders M, Zerega A, Ridruejo E, Ameigeiras B, D'Amico C, Gaite L, Bermúdez C, Cobos M, Rosales C, Romero G, McCormack L, Reggiardo V, Colombato L, Gadano A, Silva M. Fatty liver disease, an emerging etiology of hepatocellular carcinoma in Argentina. *World J Hepatol* 2018; **10**: 41-50 [PMID: 29399277 DOI: 10.4254/wjh.v10.i1.41]

7 **Piñero F**, Costa P, Boteon YL, Duque SH, Marciano S, Anders M, Varón A, Zerega A, Poniachik J, Soza A, Padilla Machaca M, Menéndez J, Zapata R, Vilatoba M, Muñoz L, Maraschio M, Podestá LG, McCormack L, Gadano A, Boin ISFF, García P, Silva M; Latin American Liver Research, Education, Awareness Network (LALREAN). A changing etiologic scenario in liver transplantation for hepatocellular carcinoma in a multicenter cohort study from Latin America. *Clin Res Hepatol Gastroenterol* 2018; Epub ahead of print [PMID: 29773419 DOI: 10.1016/j.clinre.2018.03.014]

8 **Fassio E**, Díaz S, Santa C, Reig ME, Martínez Artola Y, Alves de Mattos A, Míguez C, Galizzi J, Zapata R, Ridruejo E, de Souza FC, Hernández N, Pinchuk L; Multicenter Group for Study of Hepatocarcinoma in Latin America; Asociación Latinoamericana para el Estudio del Hígado (ALEH). Etiology of hepatocellular carcinoma in Latin America: a prospective, multicenter, international study. *Ann Hepatol* 2010; **9**: 63-69 [PMID: 20332549]

9 **Paranaguá-Vezozzo DC**, Ono SK, Alvarado-Mora MV, Farias AQ, Cunha-Silva M, França JI, Alves VA, Sherman M, Carrilho FJ. Epidemiology of HCC in Brazil: incidence and risk factors in a ten-year cohort. *Ann Hepatol* 2014; **13**: 386-393 [PMID: 24927609]

10 **Piñero F**, Marciano S, Anders M, Orozco F, Zerega A, Cabrera CR, Baña MT, Gil O, Andriani O, de Santibañes E, McCormack L, Gadano A, Silva M. Screening for liver cancer during transplant waiting list: a multicenter study from South America. *Eur J Gastroenterol Hepatol* 2015; **27**: 355-360 [PMID: 25563142 DOI: 10.1097/MEG.0000000000000272]

11 **Appel-da-Silva MC**, Miozzo SA, Dossin IA, Tovo CV, Branco F, de Mattos AA. Incidence of hepatocellular carcinoma in outpatients with cirrhosis in Brazil: A 10-year retrospective cohort study. *World J Gastroenterol* 2016; **22**: 10219-10225 [PMID: 28028370 DOI: 10.3748/wjg.v22.i46.10219]

12 **Debes JD**, Chan AJ, Balderramo D, Kikuchi L, Gonzalez Ballerga E, Prieto JE, Tapias M, Idrovo V, Davalos MB, Cairo F, Barreyro FJ, Paredes S, Hernandez N, Avendaño K, Diaz Ferrer J, Yang JD, Carrera E, Garcia JA, Mattos AZ, Hirsch BS, Gonçalves PT, Carrilho FJ, Roberts LR. Hepatocellular carcinoma in South America: Evaluation of risk factors, demographics and therapy. *Liver Int* 2018; **38**: 136-143 [PMID: 28640517 DOI: 10.1111/liv.13502]

13 **McCormack L**, Gadano A, Lendoire J, Imventarza O, Andriani O, Gil O, Toselli L, Bisigniano L, de Santibañes E. Model for end-stage liver disease-based allocation system for liver transplantation in Argentina: does it work outside the United States? *HPB* (Oxford) 2010; **12**: 456-464 [PMID: 20815854 DOI: 10.1111/j.1477-2574.2010.00199.x]

14 **Cejas NG**, Villamil FG, Lendoire JC, Tagliafichi V, Lopez A, Krogh DH, Soratti CA, Bisigniano L. Improved waiting-list outcomes in Argentina after the adoption of a model for end-stage liver disease-based liver allocation policy. *Liver Transpl* 2013; **19**: 711-720 [PMID: 23775946 DOI: 10.1002/lt.23665]

15 **Piñero F**, Tisi Baña M, de Ataide EC, Hoyos Duque S, Marciano S, Varón A, Anders M, Zerega A, Menéndez J, Zapata R, Muñoz L, Padilla Machaca M, Soza A, McCormack L, Poniachik J, Podestá LG, Gadano A, Boin IS, Duvoux C, Silva M; Latin American Liver

Research, Education and Awareness Network (LALREAN). Liver transplantation for hepatocellular carcinoma: evaluation of the alpha-fetoprotein model in a multicenter cohort from Latin America. *Liver Int* 2016; **36**: 1657-1667 [PMID: 27169841 DOI: 10.1111/liv.13159]

16 **Ayuso C**, Rimola J, Vilana R, Burrel M, Darnell A, García-Criado Á, Bianchi L, Belmonte E, Caparroz C, Barrufet M, Bruix J, Brú C. Diagnosis and staging of hepatocellular carcinoma (HCC): current guidelines. *Eur J Radiol* 2018; **101**: 72-81 [PMID: 29571804 DOI: 10.1016/j.ejrad.2018.01.025]

17 **Mitchell DG**, Bruix J, Sherman M, Sirlin CB. LI-RADS (Liver Imaging Reporting and Data System): summary, discussion, and consensus of the LI-RADS Management Working Group and future directions. *Hepatology* 2015; **61**: 1056-1065 [PMID: 25041904 DOI: 10.1002/hep.27304]

18 **Forner A**, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018; **391**: 1301-1314 [PMID: 29307467 DOI: 10.1016/S0140-6736(18)30010-2]

19 **Park JW**, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, Sherman M. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. *Liver Int* 2015; **35**: 2155-2166 [PMID: 25752327 DOI: 10.1111/liv.12818]

20 **Leoni S**, Piscaglia F, Serio I, Terzi E, Pettinari I, Croci L, Marinelli S, Benevento F, Golfieri R, Bolondi L. Adherence to AASLD guidelines for the treatment of hepatocellular carcinoma in clinical practice: experience of the Bologna Liver Oncology Group. *Dig Liver Dis* 2014; **46**: 549-555 [PMID: 24630947 DOI: 10.1016/j.dld.2014.02.012]

21 **Gashin L**, Tapper E, Babalola A, Lai KC, Miksad R, Malik R, Cohen E. Determinants and outcomes of adherence to recommendations from a multidisciplinary tumour conference for hepatocellular carcinoma. *HPB* (Oxford) 2014; **16**: 1009-1015 [PMID: 24888730 DOI: 10.1111/hpb.12280]

22 **Kim KM**, Sinn DH, Jung SH, Gwak GY, Paik YH, Choi MS, Lee JH, Koh KC, Paik SW. The recommended treatment algorithms of the BCLC and HKLC staging systems: does following these always improve survival rates for HCC patients? *Liver Int* 2016; **36**: 1490-1497 [PMID: 26936471 DOI: 10.1111/liv.13107]

23 **Wallace MC**, Huang Y, Preen DB, Garas G, Adams LA, MacQuillan G, Tibballs J, Ferguson J, Samuelson S, Jeffrey GP. HKLC Triages More Hepatocellular Carcinoma Patients to Curative Therapies Compared to BCLC and Is Associated with Better Survival. *Dig Dis Sci* 2017; **62**: 2182-2192 [PMID: 28547649 DOI: 10.1007/s10620-017-4622-y]

24 **Guarino M**, Tortora R, de Stefano G, Coppola C, Morisco F, Salomone Megna A, Izzo F, Nardone G, Piai G, Adinolfi LE, D' Adamo G, Gaeta GB, Messina V, Francica G, De Girolamo V, Coppola N, Persico M, Di Costanzo GG; Progetto Epatocarcinoma Campania Group. Adherence to Barcelona Clinic Liver Cancer guidelines in field practice: Results of Progetto Epatocarcinoma Campania. *J Gastroenterol Hepatol* 2018; **33**: 1123-1130 [PMID: 28994145 DOI: 10.1111/jgh.14013]

25 **Kikuchi L**, Chagas AL, Alencar RSSM, Tani C, Diniz MA, D'Albuquerque LAC, Carrilho FJ. Adherence to BCLC recommendations for the treatment of hepatocellular carcinoma: impact on survival according to stage. *Clinics* (Sao Paulo) 2017; **72**: 454-460 [PMID: 28954003 DOI: 10.6061/clinics/2017(08)01]

26 **Piñero F**, Marciano S, Fernández N, Silva J, Zambelo Y, Cobos M, Zerega A, Ridruejo E, Miguez C, Ameigeiras B, D'Amico C, Gaite L, Coronel M, Bermúdez C, Rosales C, Romero G, McCormack L, Reggiardo V, Colombato L, Gadano A, Rubinstein F, Silva M; Argentinean Association for the Study of Liver Diseases (A.A.E.E.H). Adherence to Barcelona Clinic Liver Cancer therapeutic algorithm for hepatocellular carcinoma in the daily practice: a multicenter cohort study from Argentina. *Eur J Gastroenterol Hepatol* 2018; **30**: 376-383 [PMID: 29509603 DOI: 10.1097/MEG.0000000000001049]

27 **Bruix J**, Reig M, Sherman M. Evidence-Based Diagnosis, Staging, and Treatment of Patients With Hepatocellular Carcinoma. *Gastroenterology* 2016; **150**: 835-853 [PMID: 26795574 DOI: 10.1053/j.gastro.2015.12.041]

28 **Mendizabal M**, Silva MO. Developing multicenter consortia in liver disease in Latin America: Challenges and opportunities. *Liver Transpl* 2017; **23**: 1210-1215 [PMID: 28590520 DOI: 10.1002/lt.24793]

P- Reviewer: Jang BK, Marcos M **S- Editor**: Ma RY **L- Editor**: A **E- Editor**: Huang Y

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4230-4242

DOI: 10.3748/wjg.v24.i37.4230

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

New prognostic biomarkers of mortality in patients undergoing liver transplantation for hepatocellular carcinoma

Leonardo Lorente

Leonardo Lorente, Intensive Care Unit, Hospital Universitario de Canarias, Santa Cruz de Tenerife 38320, Spain

ORCID number: Leonardo Lorente (0000-0003-4902-4065).

Author contributions: Lorente L drafted the manuscript.

Conflict-of-interest statement: The author has no financial or other conflicts of interest to declare related to the submitted manuscript.

Manuscript source: Invited manuscript

Correspondence to: Leonardo Lorente, MD, PhD, Attending Doctor, Medical Assistant, Intensive Care Unit, Hospital Universitario de Canarias, Ofra, s/n, Santa Cruz de Tenerife 38320, Spain. lorentemartin@msn.com
Telephone: +34-922-678000
Fax: +34-922-647112

Received: May 25, 2018
Peer-review started: May 25, 2018
First decision: July 4, 2018
Revised: August 18, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

The outcome prediction of hepatocellular carcinoma (HCC) patients undergoing liver transplantation (LT) was classically established using various macromorphological factors and serum alpha-fetoprotein levels prior to LT. However, other biomarkers have recently been reported to be associated with the prognosis of HCC patients undergoing to LT. This review summarizes clinical data on these new biomarkers. High blood levels of malondialdehyde, total antioxidant capacity, caspase-cleaved cytokeratin-18, soluble CD40 ligand, substance P, C-reactive protein, and vascular endothelial growth factor, increased neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in blood, high peripheral blood expression of human telomerase reverse transcriptase messenger ribonucleic acid, and high HCC expression of dickkopf-1 have recently been associated with decreased survival rates. In addition, high blood levels of des-gamma-carboxy prothrombin, and high HCC expression of glypican-3, E-cadherin and beta-catenin have been associated with increased HCC recurrence. Additional research is necessary to establish the prognostic role of these biomarkers in HCC prior to LT. Furthermore, some of these biomarkers are also interesting because their potential modulation could help to create new research lines for improving the outcomes of those patients.

Key words: Liver transplantation; Hepatocellular carcinoma; Biomarkers; Outcome; Survival; Recurrence; Genomic

Core tip: The outcome of liver transplantation (LT) for hepatocellular carcinoma (HCC) patients are generally predicted using various macromorphological factors and serum alpha-fetoprotein levels prior to LT. However, other biomarkers have recently been reported to be associated with the prognosis of HCC patients undergoing LT. Furthermore, some of these biomarkers are also interesting because their potential modulation could help to create new research lines for improving

the outcomes of those patients. This review summarizes clinical data on those new biomarkers.

Lorente L. New prognostic biomarkers of mortality in patients undergoing liver transplantation for hepatocellular carcinoma. *World J Gastroenterol* 2018; 24(37): 4230-4242 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4230.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4230

INTRODUCTION

Hepatocellular carcinoma (HCC), the most frequent primary liver malignancy, is one of the most common malignancies and causes of cancer-related deaths[1-3]. In liver transplantation (LT), the primary tumor is removed, and liver failure is treated. Therefore, LT is considered the treatment of choice for some HCC patients[1-11].

Various macromorphological factors assessed prior to LT have been classically used to predict the outcome of HCC patients undergoing LT. These factors include the tumor size, tumor number, degree of differentiation, hepatic microvascular invasion, hepatic macrovascular invasion, being outside the Milan criteria and infiltration[1-11].

However, establishing biomarkers to be assessed prior to LT could strengthen the predictions of prognoses for HCC patients undergoing LT. Currently, the most commonly studied biomarker are the serum alphafetoprotein levels[1-11]. However, other biomarkers have recently been reported to be associated with the prognosis of HCC patients undergoing to LT. This review summarizes clinical data on these new biomarkers.

BIOMARKERS

Malondialdehyde

Oxidative stress can lead to membrane lipid peroxidation, which creates many end products, including malondialdehyde, which is a low molecular weight aldehyde that is produced during the degradation of cellular membrane phospholipids. It is formed when free radicals attack polyunsaturated fatty acids. Malondialdehyde can be released into the extracellular space, and it can ultimately reach the bloodstream. Therefore, it has been used as a circulating biomarker of lipid oxidation[12,13].

Some studies have reported higher levels of serum malondialdehyde in HCC patients[14-16] and patients with chronic liver disease than in healthy controls[17,18]. Additionally, studies have reported that the tumoral tissue of HCC patients has higher malondialdehyde concentrations than non-tumoral tissue[19]. Studies have also found higher free radical intensity in the erythrocytes of HCC patients than in healthy controls[20], higher serum concentrations of reactive oxygen metabolites in HCC patients who exhibit recurrence after curative treatment by radiofrequency ablation or surgical resection[21], and higher circulating lipid peroxide levels prior to LT in patients who do not survive LT than in survivors[22].

A study by our team reported, for the first time, that serum malondialdehyde levels prior to LT were higher in non-surviving patients than in patients who survived for one year after LT. We also found an association between serum malondialdehyde levels in HCC patients prior to LT and their survival at one year after LT[23]. These findings are consistent with those from other studies that have reported an association between circulating malondialdehyde levels and mortality in patients with sepsis[24], traumatic brain injuries[25], brain infarctions[26] and spontaneous intracerebral hemorrhaging[27].

Total antioxidant capacity

The production of reactive oxygen species (ROS) is balanced by the production of antioxidant defenses, and the analysis of total antioxidant capacity (TAC) could provide a global information in respect to the antioxidant status[28].

Some studies have found lower circulating TAC levels in LT patients than in healthy controls[18], and lower circulating TAC levels in HCC patients than in healthy controls[14,15].

A study by our team was the first to find that serum TAC levels prior to LT were lower in non-surviving than in surviving patients during the first one year after LT[29]. Besides, we found that there is an association between low serum TAC levels in HCC patients prior to LT and their survival at one year after LT. In addition, we found a negative association between serum levels of TAC and malondialdehyde; thus, patients with lower serum TAC levels showed higher lipid peroxidation.

I think that those findings could suggest that it is possible that non-survivor LT patients remains during the first one year after LT with low serum TAC levels and high serum malondialdehyde levels (due to a higher lipid peroxidation because the high ROS production is not balanced by an insufficient antioxidant capacity) compared to survivor patients.

Antioxidant agents have been shown to reduce malondialdehyde concentrations in animal models of sepsis[30] and brain trauma[31] as well as in clinical trials involving septic newborns[32], acute ischemic stroke[33] and traumatic brain injuries[34]. Additionally, in a clinical trial of traumatic brain injuries[34], the administration of antioxidant agents reduced mortality rate. Thus, since non-surviving HCC patients showed higher serum malondialdehyde levels prior to LT than surviving patients, it could be interesting to explore the benefit of the administration of antioxidant agents to HCC patients undergoing LT. Antioxidant treatment could potentially improve their prognoses, especially for patients with a higher oxidative state.

Caspase-cleaved cytokeratin-18

Apoptosis, which leads to active and programmed cell

elimination, is increased in liver diseases[35-37]. Two main pathways exist (extrinsic and intrinsic) for cell death by apoptosis. The apoptotic extrinsic pathway is initiated when the tumor necrosis factor receptor superfamily (TNFRSF) is activated by its ligand (TNFSF). This leads to the formation of a death signal that activates caspase-8 and ultimately activates caspase-3. The intrinsic apoptotic pathway is activated *via* oxygen free radicals, interleukin (IL)-1, IL-6 and nitric oxide. These factors release cytochromes from the mitochondria to the cytosol, which activates caspase-3. Thus, both apoptotic pathways ultimately activate caspase 3, which leads to cell death.

Cytokeratin-18 is the main protein found in the intermediate filaments of the liver and is present in most parenchymal and epithelial cells. During hepatocyte apoptosis, cytokeratin-18 is cleaved by caspases and can be released into the bloodstream as caspase-cleaved cytokeratin (CCCK)-18[35-39], which can be detected using M30 monoclonal antibodies[40,41].

Some studies have reported higher circulating CCCK-18 levels in patients with tumoral diseases than in healthy controls[42,43] and in patients with tumoral diseases that had a poor evolution[44-48]. Additionally, HCC patients have higher circulating CCCK-18 levels than healthy controls[49,50] or cirrhotic patients[51,52]. Studies have reported an association between serum CCCK-18 levels and mortality in HCC patients[53].

A study by our team found, for the first time, that serum CCCK-18 levels prior to LT were higher in non-surviving patients than in patients who survived for one year after LT. Additionally, an association was found between serum CCCK-18 levels in HCC patients prior to LT and their survival for one year after LT[54]. These findings are consistent with the results of other studies that have shown that circulating CCCK-18 levels are associated with the prognosis of patients with various tumoral diseases[44-48], HCC[53], sepsis[55], traumatic brain injury[56] and cerebral artery infarction[57]. Additionally, circulating CCCK-18 levels have been associated with metastasis[45], serum AFP levels[46,54] and tumor size[47,48].

Soluble CD40 ligand

Soluble CD40 ligand (sCD40L) is a member in the TNFSF of proteins. It has proinflammatory and prothrombotic effects when bound to its receptor, CD40, which is also a member of the TNFRSF[58-65]. CD40L is mainly found in platelets and activated T-lymphocytes, although it is also present in smooth muscle cells, endothelial cells, microglia, monocytes, and B cells. When CD40L is cleaved, it is released into circulation and is present in its soluble form, sCD40L[58-65].

Some studies have reported higher circulating sCD40L levels in patients with ischemic stroke[66-69], acute coronary syndrome[70,71], and sepsis[72,73] than in healthy subjects. Additionally, high circulating sCD40L levels are associated with a poor prognosis in patients with ischemic stroke[69], acute coronary syndrome[74], sepsis[72,73] and traumatic brain injuries[75]. Patients with chronic hepatitis C virus infection[76], cirrhosis[77], and non-alcoholic fatty liver disease have been shown to exhibit higher circulating sCD40L levels than control subjects[78]. Furthermore, high circulating sCD40L levels are associated with a poor prognosis in HCC patients[79].

A study by our team was the first to report that serum sCD40L levels prior to LT were higher in patients who did not survive for one year after transplantation than in the surviving patients, and an association was also found between serum sCD4L levels in HCC patients prior to LT and survival for one year after LT[80]. These findings are consistent with the results of other studies reporting an association between circulating sCD40L levels and mortality in patients with cerebral infarction[69], acute coronary syndrome[74], sepsis[72,73] and traumatic brain injuries[75].

Circulating sCD40L levels could play a role in patients receiving LT for HCC by their proinflammatory[81,82] and procoagulant[83-88] effects. The proinflammatory effects of sCD40L may be due to an increase in the expression of proinflammatory mediators such as IL-1, IL-6, IL-12, TNF-alpha and interferon-gamma[81,82]. The procoagulant effects of sCD40L may be due to the induction of tissue factor expression[83-86], reduced expression of thrombomodulin[85,86] and its binding to glycoprotein Ⅱb/Ⅲa platelet receptor[87,88]. These proinflammatory and procoagulant effects could potentially favor the development of vascular thrombosis and organ dysfunction, ultimately resulting in patient death.

The statin administration has been associated with a reduction of circulating sCD40L levels in patients with coronary artery disease[89-91] and an improvement in the prognosis of patients with ischemic stroke[92] and infections[93-96]. Therefore, as non-surviving HCC patients showed higher serum sCD40L levels prior to LT than patients who survived for one year after LT, it could be interesting to explore the benefit of administering sCD40L modulators to HCC patients who are undergoing to LT to improve their prognosis, especially for patients with higher sCD40L levels.

Substance P

Substance P is a member of the tachykinin family, which is distributed by the central and peripheral nervous, respiratory and urinary systems and by the gut. Tachykinins may play a role in nociceptive responses, inflammation, vasodilation and plasma protein extravasation[97-99].

Circulating substance P levels are elevated in patients with liver diseases compared to control subjects[100-106] and in patients with severe liver diseases[104-106].

A study by our team was the first to report that serum levels of substance P prior to LT were higher in patients who did not survive for one year after LT than in surviving patients. The study also found an association between serum levels of substance P in HCC patients prior to LT and mortality within one year after LT[107].

These findings are consistent with the results of other studies that have reported an association between circulating serum P levels and mortality in patients with traumatic brain injuries[108] or ischemic stroke[109].

Substance P plays a role in the inflammatory response by producing inflammatory cytokines such as IL-1, IL-6 and TNF-alpha[110-114]. Various agents that reduce substance P activity have been identified in animal models of ischemic stroke[115-117] and traumatic brain injury[118,119]. These agents have been associated with a reduction in the inflammation process and edema. HCC patients who did not survive for one year after LT showed higher serum substance P levels prior to LT than surviving patients. Therefore, it could be interesting to explore the benefit of administering agents to control substance P activity to HCC patients undergoing LT, especially in patients with high circulating substance P levels.

Neutrophil to lymphocyte ratio and platelet to lymphocyte ratio

The blood neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) have both been used as biomarkers to evaluate systemic inflammatory responses. A meta-analysis published in 2017 by Zheng *et al*[120] analyzed the association between the NLR and PLR in the blood of HCC patients prior to receiving different treatments and its overall survival and HCC recurrence. The treatments included curative resection, transarterial chemoembolization (TACE), radiofrequency ablation (RFA), LT and chemotherapy. The authors examined the associations between the NLR and all treatments combined and individually. They found an association between a high NLR and both of the outcomes (poor overall survival and HCC recurrence) for all types of treatment. They also reported an association between a high NLR and survival when specifically analyzing LT. However, no association was found between the NLR and HCC recurrence when specifically analyzing LT. The authors also found an association between a high PLR and poor outcomes for all treatments combined and when analyzing LT specifically.

C-reactive protein

C-reactive protein (CRP) is synthesized in the liver by hepatocytes in response to factors released by macrophages and adipocytes during inflammation and afterwards is released to blood; thus, blood levels of CRP increase in response to inflammation. Elevated blood CRP levels are associated in multivariate analyses with an increase in the risk of HCC recurrence and decreased survival in patients undergoing LT for HCC[121,122], overall in patients with HCC beyond the Milan criteria[121].

Des-gamma-carboxy prothrombin or protein induced by vitamin K absence or antagonist Ⅱ (PIVKA-Ⅱ)

Des-gamma-carboxy prothrombin (DCP) is a nonfunctional prothrombin form produced by the liver. The normal liver converts the glutamic acid residues in the N-terminal portion of prothrombin by carboxylation in gamma-carboxyglutamic acid residues before its release into the peripheral blood. In many of HCC cells, the vitamin K dependent carboxylase that produces this carboxylation is absent; thus, an abnormal prothrombin is secreted. Several studies have found in multivariate analyses that high blood DCP levels are associated with a higher risk of HCC recurrence in HCC patients who undergo LT[123-127].

Glypican-3

Glypican (GPC)-3 is a member of the glypican protein family, which plays a role in regulating cell division and growth. One study reported that the protein expression of GPC-3 in HCC tissue samples prior to LT was associated with a higher rate of HCC recurrence after LT[128]; in addition, there was found that GPC-3 was expressed in 68% of HCC tissues, but not in adjacent non-tumoral tissues and in tissues of liver controls. Another study found that serum GPC-3 levels were higher in HCC patients than in cirrhosis patients[129]; however, the study did not examine the prognostic role of serum GPC-3 in HCC patients prior to LT.

Human telomerase reverse transcriptase messenger ribonucleic acid

Human telomerase reverse transcriptase messenger ribonucleic acid (h-TERT mRNA) is a ribonucleoprotein polymerase that maintains telomere ends in chromosomes. High h-TERT mRNA expression in the peripheral blood of HCC patients who undergo LT has been associated with decreased survival and increased HCC recurrence[130,131]; however, in another study, h-TERT mRNA concentrations in the peripheral blood were not associated with HCC recurrence after LT[132]. Therefore, additional research is necessary to determine the prognostic role of h-TERT mRNA expression in the peripheral blood of HCC patients prior to LT.

Matrix metalloproteinase-9

Matrix metalloproteinase (MMP)-9 is a member of the matrix metalloproteinases (MMPs), which are involved in degradation and remodeling of the extracellular matrix. MMPs play a role in physiological functions such as morphogenesis, tissue remodeling and the menstrual cycle. They are also involved in various diseases such as arthritis, tumors, atherosclerosis and sepsis. The activity of MMPs is regulated by several tissue inhibitor of matrix metalloproteinases (TIMPs).

Contradictory results have been found with regard to MMP-9. Patients who undergo LT for HCC and have high MMP-9 expression in the tumor have exhibited an unfavorable rates of overall survival and HCC recurrence[133,134]. Another study in patients undergoing LT due to HCC or for cirrhosis without HCC found that high serum MMP-9 levels at one week after LT were associated

with a higher rate of LT rejection[135]. However, one study also reported that high serum MMP-9 levels and low serum TIMP-1 levels in HCC patients receiving different treatments (curative resection, TACE, thermoablation, and LT) were associated with a higher survival rate, although the study did not specifically examine patients receiving LT because the sample size for that group was small[136]. Our group has previously reported a lower survival rate in patients with cerebral artery infarction[137], traumatic brain injury[138] and sepsis[139,140] who have high serum TIMP-1 levels than in patients who have low TIMP-1 levels. Therefore, additional research is necessary to establish the prognostic role of MMP-9 expression in the peripheral blood of HCC patients prior to LT.

E-cadherin

E-cadherin is a member of the cadherin family of proteins, which are cell adhesion molecules that participate in the formation of junctions between cells. One study found that high serum levels of soluble E-cadherin were associated with increased recurrence of HCC after a curative resection[141]. Another study of HCC patients who underwent LT found that E-cadherin expression in the liver was associated with HCC recurrence after LT[142].

Beta-catenin

Beta-catenin is a member of the catenin family of proteins, which also constitute a group of cell adhesion molecules that are involved in the formation of bonds between cells. A study of HCC patients who underwent LT found that beta-catenin expression in the liver was associated with HCC recurrence after LT[142]. However, in other recently published study, no association was found between beta-catenin expression in the liver prior to LT and the survival of HCC patients[143]. Therefore, additional research is necessary to establish the prognostic role of beta-catenin expression in the liver in HCC patients prior to LT.

AFP

AFP is a glycoprotein that is produced by the yolk sac and the fetal liver during fetal development. It is the most abundant plasma protein in the human fetus. Increased values are found in newborns (values gradually decrease to normal over the first year of life), pregnant women (values return to normal after delivery), and patients with various AFP-producing tumors such as HCC and tumors of the ovary and testis.

The blood AFP level is the most extensively studied biomarker in HCC patients undergoing LT. Elevated blood AFP levels are associated with decreased survival[144] as well as an increase in HCC recurrence[145] in patients undergoing LT for HCC.

A review of 13 observational studies published in 2012 involving 12,159 patients who underwent LT for HCC examined the role of pre-LT circulating AFP levels in predicting survival and HCC recurrence[144]. Nine of the 13 studies reported data about pre-LT serum AFP levels and survival. Only four studies reported absolute serum AFP values for all included patients, and the other studies used varying cut-off points for serum AFP levels. This heterogeneity precluded pooling of the data for a valid meta-analysis. The majority of the studies concluded that a high pre-LT serum AFP level was an independent predictor of death following LT for HCC. These studies also suggested that serum AFP levels higher than 1000 ng/mL may predict poorer survival. Ten of the 13 studies reported data on HCC recurrence and pre-LT serum AFP values. All of these studies concluded that high AFP levels were associated with increased HCC recurrence following LT for HCC. The authors were unable to perform a meta-analysis on this research question due to the heterogeneity in the data reported by the studies. Additionally, some of the studies included in the review found that pre-LT serum AFP levels were correlated with vascular invasion and poor differentiation of HCC.

A review and meta-analysis published in 2016 examined the prognostic role of biomarkers in HCC recurrence in patients who underwent LT for HCC[145]. The review included 49 studies with a total of 13693 patients that reported data on pre-LT serum AFP levels and HCC recurrence. However, the studies had 88% heterogeneity due to their use of varying definitions and cut-off values for AFP. Therefore, it was not possible to conduct a valid meta-analysis for this topic. However, a meta-analysis was performed using 17 of the studies with different cut-off values for pre-LT serum AFP levels, but the meta-analysis required a cut-off value higher than 400 ng/mL. In this analysis, an association was found between elevated pre-LT serum AFP levels and the risk of HCC recurrence (HR = 2.69; 95%CI: 2.08-3.47), with a heterogeneity of 46%.

Dickkopf-1

In several studies have been found higher circulating Dickkopf-1 (DKK1) levels in HCC patients than in healthy subjects[146-149] or than in patients with liver cirrosis without HCC[150,151]. In addition, in a meta-analysis published in 2014 including 4 studies[152] and in other recently published study[153] was found that higher DKK1 expression levels in HCC patients were associated with lower survival. Besides, in one study was found that higher DKK1 expression is associated with lower survival and higher recurrence in HCC patients after LT[154].

Vascular endothelial growth factor

In a meta-analysis of 11 studies was found that high serum Vascular endothelial growth factor (VEGF) leves in HCC patients were associated with lower survival[155]. In addition, in one study was found that high plasma VEGF levels in HCC patients previously to LT were associated with HCC recurrence and survival[156].

Caspase-1

Pyroptosis is a form of programmed cell death, which is dependent of caspase-1. In some studies has been

Table 1 New prognostic biomarkers in patients undergoing liver transplantation for hepatocellular carcinoma

Biomarker	Alteration	Outcome	Ref.
Malondialdehyde	High circulating levels	Lower survival	[23]
Total antioxidant capacity	High circulating levels	Lower survival	[29]
Caspase-cleaved cytokeratin-18	High circulating levels	Lower survival	[54]
Soluble CD40 ligand	High circulating levels	Lower survival	[80]
Substance P	High circulating levels	Lower survival	[107]
Neutrophil to lymphocyte ratio	High circulating ratio	Lower survival	[120]
Platelet to lymphocyte ratio	High circulating ratio	Lower survival	[120]
C-reactive protein	High circulating levels	Lower survival	[121,122]
Des-gamma-carboxy prothrombin	High circulating levels	Higher recurrence	[123-127]
Glypican-3	High HCC expression	Higher recurrence	[128]
H-TERT mRNA	High peripheral blood expression	Lower survival	[130,131]
E-cadherin	High HCC expression	Higher recurrence	[142]
Beta-catenin	High HCC expression	Higher recurrence	[142]
Dickkopf-1	High HCC expression	Lower survival	[154]
Vascular endothelial growth factor	High circulating levels	Lower survival	[156]

HCC: Hepatocellular carcinoma; H-TERT mRNA: Human telomerase reverse transcriptase messenger ribonucleic acid.

found lower caspase-1 expression in HCC tissues[157,158]. In a study was determined caspase-1 expression in HCC patients (from HCC tissues and adjacent normal tissues) and in hepatocyte cell lines[157]. There was found a significant decrease in caspase-1 expression in HCC tissues compared to adjacent normal tissues and hepatocyte cell lines. Besides, the use of berberine increased the expression of caspase-1, decreased cell number, and increased cell swelling in hepatocyte cell lines; and the use of the caspase-1 inhibitor Ac-YVAD-CMK attenuated the effects of berberine.

However, in one study has been found that liver tissue of patients infected with hepatitis C virus (HCV) showed caspase-1-mediated pyroptosis[159]. Besides, in other study of patients with resection of HCC was found lower survival in patients with high of caspase-1 expression in normal tissues[160].

Angiopoietin-2

Angiopoietin-2 is a protein that is involved in angiogenesis and inflammation[161]. In a recently published study of chronic HCV patients treated with direct acting antivirals (DAA) was found that angiopoietin-2 in liver tissue was related with the risk of HCC recurrence or de novo ocurrence.

Another interesting finding of that study was that patients with HCC recurrence or de novo ocurrence had significantly higher portal pressure than patients never developing HCC[162]; and in previous studies was found that portal hypertension was associated with poor prognosis in patients undergoing to LT[163] or with HCC[164,165].

The risk of HCC occurrence or recurrence following DAA remains unclear due to that the results of different studies are contradictories. In a review published in 2017 including 10 studies was found in meta-analyses a higher incidence of HCC occurrence and HCC recurrence with the administration of DAA[166]. However, in meta-regression analyses after adjusting for study follow-up and age, DAA therapy was not associated with higher HCC de novo ocurrence and neither with HCC recurrence. In the study by Faillaci *et al*[162] was found that the use of DAA was associated with de novo HCC, and that this risk is higher in patients with higher angiopoietin-2 expression.

Genomic

The Cancer Genome Atlas (TCGA) Research Network published in 2017 the genomic characterization of HCC[167]. There were analyzed 363 HCC cases by whole-genome sequencing and DNA copy number, and 196 HCC cases by DNA methylation, mRNA, miRNA, and proteomic expression. In total, 12136 genes had non-silent mutations, and 26 genes were determined to be significantly mutated genes. Of these 26 genes, 18 were reported in at least one previous HCC genome sequencing study and 8 were not previously associated with HCC. Whithin of know mutated genes, the most included TERTpromoter (51%), TP53 (31%), CTNNB1 (27%), ALB (13%), APOB (10%), ARID1A (7%), AXIN1 (8%), ARID2 (5%), BAP1(5%), KEAP1 (5%), RB1 (4%), and NFE2L2 (3%). There were identified 8 novel mutated gene with a low frequence (2%-3% of HCC patients), including LTZR1, EEF1A1, AZIN1, RP1L 1, GPATCH4, CREB3L3, AHCTF1, and HIST1H1C. In addition, other two mutated genes previously associated with other cancer types were associated with HCC in this study, F3B1 and SMARCA4. Besides, integrative clustering of datasets of DNA copy number, DNA methylation, mRNA expression and miRNA expression could define three HCC subtypes (iClust 1 to 3), and iClust1 subtype had a poor prognosis. In addition, the analysis of these mutations and pathways provide potential directions for future potential therapeutic in HCC patients by the use of inhibitors of WNT, MDM4, MET, VEGFA, MCL1, IDH1, TERT. Thus, this genome-wide characterization has been very important in improving our knowledge about mutated genes associated with HCC, prognostic gene signatures and potential treatments[168].

CONCLUSION

Various macromorphological factors measured prior to LT have been classically used to estimate the outcomes of HCC patients undergoing LT. Additionally, the determination of some valid biomarkers prior to LT could help predict the prognoses of HCC patients undergoing LT. The most frequently examined biomarker is the serum AFP level. Recently, an association was reported between decreased survival rates and high blood levels of malondialdehyde, TAC, CCCK-18, sCD40L, substance P, CRP, and VEGF, NLR and PLR in blood, high peripheral blood expression of h-TERT mRNA, and high HCC expression of DKK1. In addition, an association has been found between increased HCC recurrence and high blood levels of Des-gamma-carboxy prothrombin, and high HCC expression of GPC-3, E-cadherin and beta-catenin. Additional research is necessary to establish the prognostic role of these biomarkers for HCC prior to LT. Furthermore, some of these biomarkers are also interesting because their potential modulation could help to create new research lines for improving the outcomes of those patients. Those new biomarkers are summarized on Table 1.

REFERENCES

1 **Bodzin AS**, Busuttil RW. Hepatocellular carcinoma: Advances in diagnosis, management, and long term outcome. *World J Hepatol* 2015; **7**: 1157-1167 [PMID: 26019732 DOI: 10.4254/wjh.v7.i9.1157]

2 **Guerrero-Misas M**, Rodríguez-Perálvarez M, De la Mata M. Strategies to improve outcome of patients with hepatocellular carcinoma receiving a liver transplantation. *World J Hepatol* 2015; **7**: 649-661 [PMID: 25866602 DOI: 10.4254/wjh.v7.i4.649]

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

4 **Slotta JE**, Kollmar O, Ellenrieder V, Ghadimi BM, Homayounfar K. Hepatocellular carcinoma: Surgeon's view on latest findings and future perspectives. *World J Hepatol* 2015; **7**: 1168-1183 [PMID: 26019733 DOI: 10.4254/wjh.v7.i9.1168]

5 **Gomaa AI**, Waked I. Recent advances in multidisciplinary management of hepatocellular carcinoma. *World J Hepatol* 2015; **7**: 673-687 [PMID: 25866604 DOI: 10.4254/wjh.v7.i4.673]

6 **Clark T**, Maximin S, Meier J, Pokharel S, Bhargava P. Hepatocellular Carcinoma: Review of Epidemiology, Screening, Imaging Diagnosis, Response Assessment, and Treatment. *Curr Probl Diagn Radiol* 2015; **44**: 479-486 [PMID: 25979220 DOI: 10.1067/j.cpradiol.2015.04.004]

7 **Toyoda H**, Kumada T, Tada T, Sone Y, Kaneoka Y, Maeda A. Tumor Markers for Hepatocellular Carcinoma: Simple and Significant Predictors of Outcome in Patients with HCC. *Liver Cancer* 2015; **4**: 126-136 [PMID: 26020034 DOI: 10.1159/000367735]

8 **Cescon M**, Bertuzzo VR, Ercolani G, Ravaioli M, Odaldi F, Pinna AD. Liver transplantation for hepatocellular carcinoma: role of inflammatory and immunological state on recurrence and prognosis. *World J Gastroenterol* 2013; **19**: 9174-9182 [PMID: 24409045 DOI: 10.3748/wjg.v19.i48.9174]

9 **Lee HW**, Suh KS. Expansion of the criteria for living donor liver transplantation for hepatocellular carcinoma. *Curr Opin Organ Transplant* 2016; **21**: 231-237 [PMID: 26918880 DOI: 10.1097/MOT.0000000000000294]

10 **Cillo U**, Giuliani T, Polacco M, Herrero Manley LM, Crivellari G, Vitale A. Prediction of hepatocellular carcinoma biological behavior in patient selection for liver transplantation. *World J Gastroenterol* 2016; **22**: 232-252 [PMID: 26755873 DOI: 10.3748/wjg.v22.i1.232]

11 **Romano M**, De Francesco F, Pirozzi G, Gringeri E, Boetto R, Di Domenico M, Zavan B, Ferraro GA, Cillo U. Expression of cancer stem cell biomarkers as a tool for a correct therapeutic approach to hepatocellular carcinoma. *Oncoscience* 2015; **2**: 443-456 [PMID: 26097877]

12 **Draper HH**, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 1990; **186**: 421-431 [PMID: 2233309]

13 **Dalle-Donne I**, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem* 2006; **52**: 601-623 [PMID: 16484333 DOI: 10.1373/clinchem.2005.061408]

14 **Yahya RS**, Ghanem OH, Foyouh AA, Atwa M, Enany SA. Role of interleukin-8 and oxidative stress in patients with hepatocellular carcinoma. *Clin Lab* 2013; **59**: 969-976 [PMID: 24273918]

15 **Zhao J**, Zhao Y, Wang H, Gu X, Ji J, Gao C. Association between metabolic abnormalities and HBV related hepatocelluar carcinoma in Chinese: a cross-sectional study. *Nutr J* 2011; **10**: 49 [PMID: 21569630 DOI: 10.1186/1475-2891-10-49]

16 **Tsai SM**, Lin SK, Lee KT, Hsiao JK, Huang JC, Wu SH, Ma H, Wu SH, Tsai LY. Evaluation of redox statuses in patients with hepatitis B virus-associated hepatocellular carcinoma. *Ann Clin Biochem* 2009; **46**: 394-400 [PMID: 19641006 DOI: 10.1258/acb.2009.009029]

17 **Trevisani F**, Caraceni P, Simoncini M, Micati M, Domenicali M, Dazzani F, Zambruni A, Stefanelli C, Grazi G, Nardo B, Guarnieri C, Bernardi M. Evidence of oxidative imbalance in long-term liver transplant patients. *Dig Liver Dis* 2002; **34**: 279-284 [PMID: 12038812]

18 **Thorat VN**, Suryakar AN, Naik P, Tiwale BM. Total antioxidant capacity and lipid peroxidation in liver transplantation. *Indian J Clin Biochem* 2009; **24**: 102-104 [PMID: 23105816 DOI: 10.1007/s12291-009-0018-x]

19 **Czeczot H**, Scibior D, Skrzycki M, Podsiad M. Glutathione and GSH-dependent enzymes in patients with liver cirrhosis and hepatocellular carcinoma. *Acta Biochim Pol* 2006; **53**: 237-242 [PMID: 16404476]

20 **Váli L**, Hahn O, Kupcsulik P, Drahos A, Sárváry E, Szentmihályi K, Pallai Z, Kurucz T, Sípos P, Blázovics A. Oxidative stress with altered element content and decreased ATP level of erythrocytes in hepatocellular carcinoma and colorectal liver metastases. *Eur J Gastroenterol Hepatol* 2008; **20**: 393-398 [PMID: 18403940 DOI: 10.1097/MEG.0b013e3282f495c7]

21 **Suzuki Y**, Imai K, Takai K, Hanai T, Hayashi H, Naiki T, Nishigaki Y, Tomita E, Shimizu M, Moriwaki H. Hepatocellular carcinoma patients with increased oxidative stress levels are prone to recurrence after curative treatment: a prospective case series study using the d-ROM test. *J Cancer Res Clin Oncol* 2013; **139**: 845-852 [PMID: 23411687 DOI: 10.1007/s00432-013-1389-1]

22 **Liu C**, Zhou XS, Geng QM. [Evaluation oxygen free radicals related index before liver transplantation to forejudge prognosis]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* 2003; **15**: 560-562 [PMID: 12971857]

23 **Lorente L**, Rodriguez ST, Sanz P, Abreu-González P, Díaz D, Moreno AM, Borja E, Martín MM, Jiménez A, Barrera MA. Association between Pre-Transplant Serum Malondialdehyde Levels and Survival One Year after Liver Transplantation for Hepatocellular Carcinoma. *Int J Mol Sci* 2016; **17**: 500 [PMID: 27058525 DOI: 10.3390/ijms17040500]

24 **Lorente L**, Martín MM, Abreu-González P, Domínguez-Rodriguez A, Labarta L, Díaz C, Solé-Violán J, Ferreres J, Cabrera J, Igeño JC, Jiménez A. Sustained high serum malondialdehyde levels are associated with severity and mortality in septic patients. *Crit Care* 2013; **17**: R290 [PMID: 24326199 DOI: 10.1186/cc13155]

25 **Lorente L**, Martín MM, Abreu-González P, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Lorenzo JM, Molina I, Jiménez A.

Association between serum malondialdehyde levels and mortality in patients with severe brain trauma injury. *J Neurotrauma* 2015; **32**: 1-6 [PMID: 25054973 DOI: 10.1089/neu.2014.3456]
26 **Lorente L**, Martín MM, Abreu-González P, Ramos L, Argueso M, Solé-Violán J, Riaño-Ruiz M, Jiménez A. Serum malondialdehyde levels in patients with malignant middle cerebral artery infarction are associated with mortality. *PLoS One* 2015; **10**: e0125893 [PMID: 25933254 DOI: 10.1371/journal.pone.0125893]
27 **Lorente L**, Martín MM, Abreu-González P, Sabatel R, Ramos L, Argueso M, Solé-Violán J, Riaño-Ruiz M, Jiménez A, García-Marín V. Serum Malondialdehyde Levels and Mortality in Patients with Spontaneous Intracerebral Hemorrhage. *World Neurosurg* 2018; **113**: e542-e547 [PMID: 29477698 DOI: 10.1016/j.wneu.2018.02.085]
28 **Ghiselli A**, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic Biol Med* 2000; **29**: 1106-1114 [PMID: 11121717]
29 **Lorente L**, Rodriguez ST, Sanz P, Pérez-Cejas A, Abreu-González P, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Cerro P, Barrera MA. Serum total antioxidant capacity prior to liver transplantation for hepatocellular carcinoma is associated with 1-year liver transplantation survival. *J Int Med Res* 2018; **46**: 2641-2649 [PMID: 29911482 DOI: 10.1177/0300060518768150]
30 **Lowes DA**, Webster NR, Murphy MP, Galley HF. Antioxidants that protect mitochondria reduce interleukin-6 and oxidative stress, improve mitochondrial function, and reduce biochemical markers of organ dysfunction in a rat model of acute sepsis. *Br J Anaesth* 2013; **110**: 472-480 [PMID: 23381720 DOI: 10.1093/bja/aes577]
31 **Horáková L**, Ondrejicková O, Bachratá K, Vajdová M. Preventive effect of several antioxidants after oxidative stress on rat brain homogenates. *Gen Physiol Biophys* 2000; **19**: 195-205 [PMID: 11156442]
32 **Gitto E**, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P, Cordaro S, Corona G, Trimarchi G, Barberi I. Effects of melatonin treatment in septic newborns. *Pediatr Res* 2001; **50**: 756-760 [PMID: 11726736 DOI: 10.1203/00006450-200112000-00021]
33 **Ullegaddi R**, Powers HJ, Gariballa SE. Antioxidant supplementation enhances antioxidant capacity and mitigates oxidative damage following acute ischaemic stroke. *Eur J Clin Nutr* 2005; **59**: 1367-1373 [PMID: 16091766 DOI: 10.1038/sj.ejcn.1602248]
34 **Saniova B**, Drobny M, Lehotsky J, Sulaj M, Schudichova J. Biochemical and clinical improvement of cytotoxic state by amantadine sulphate. *Cell Mol Neurobiol* 2006; **26**: 1475-1482 [PMID: 16710757 DOI: 10.1007/s10571-006-9033-0]
35 **Luedde T**, Kaplowitz N, Schwabe RF. Cell death and cell death responses in liver disease: mechanisms and clinical relevance. *Gastroenterology* 2014; **147**: 765-783.e4 [PMID: 25046161 DOI: 10.1053/j.gastro.2014.07.018]
36 **Canbay A**, Feldstein A, Kronenberger B, Schulze-Osthoff K, Bantel H. [Cytokeratin 18 as marker for non-invasive diagnosis and prognosis of acute and chronic liver diseases]. *Z Gastroenterol* 2014; **52**: 290-295 [PMID: 24622872 DOI: 10.1055/s-0033-1356138]
37 **Yilmaz Y**. Systematic review: caspase-cleaved fragments of cytokeratin 18 - the promises and challenges of a biomarker for chronic liver disease. *Aliment Pharmacol Ther* 2009; **30**: 1103-1109 [PMID: 19769633 DOI: 10.1111/j.1365-2036.2009.04148.x]
38 **Chu PG**, Weiss LM. Keratin expression in human tissues and neoplasms. *Histopathology* 2002; **40**: 403-439 [PMID: 12010363]
39 **Caulín C**, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol* 1997; **138**: 1379-1394 [PMID: 9298992]
40 **Leers MP**, Kölgen W, Björklund V, Bergman T, Tribbick G, Persson B, Björklund P, Ramaekers FC, Björklund B, Nap M, Jörnvall H, Schutte B. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* 1999; **187**: 567-572 [PMID: 10398123 DOI: 10.1002/(SICI)1096-9896(199904)187:53.0.CO;2-J]
41 **Hägg M**, Bivén K, Ueno T, Rydlander L, Björklund P, Wiman KG, Shoshan M, Linder S. A novel high-through-put assay for screening of pro-apoptotic drugs. *Invest New Drugs* 2002; **20**: 253-259 [PMID: 12201488]
42 **Oyama K**, Fushida S, Kinoshita J, Okamoto K, Makino I, Nakamura K, Hayashi H, Inokuchi M, Nakagawara H, Tajima H, Fujita H, Takamura H, Ninomiya I, Kitagawa H, Fujimura T, Ohta T. Serum cytokeratin 18 as a biomarker for gastric cancer. *Clin Exp Med* 2013; **13**: 289-295 [PMID: 22825587 DOI: 10.1007/s10238-012-0202-9]
43 **Ueno T**, Toi M, Bivén K, Bando H, Ogawa T, Linder S. Measurement of an apoptotic product in the sera of breast cancer patients. *Eur J Cancer* 2003; **39**: 769-774 [PMID: 12651202]
44 **Ulukaya E**, Yilmaztepe A, Akgoz S, Linder S, Karadag M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. *Lung Cancer* 2007; **56**: 399-404 [PMID: 17316892 DOI: 10.1016/j.lungcan.2007.01.015]
45 **Yaman E**, Coskun U, Sancak B, Buyukberber S, Ozturk B, Benekli M. Serum M30 levels are associated with survival in advanced gastric carcinoma patients. *Int Immunopharmacol* 2010; **10**: 719-722 [PMID: 20362077 DOI: 10.1016/j.intimp.2010.03.013]
46 **de Haas EC**, di Pietro A, Simpson KL, Meijer C, Suurmeijer AJ, Lancashire LJ, Cummings J, de Jong S, de Vries EG, Dive C, Gietema JA. Clinical evaluation of M30 and M65 ELISA cell death assays as circulating biomarkers in a drug-sensitive tumor, testicular cancer. *Neoplasia* 2008; **10**: 1041-1048 [PMID: 18813353]
47 **Koelink PJ**, Lamers CB, Hommes DW, Verspaget HW. Circulating cell death products predict clinical outcome of colorectal cancer patients. *BMC Cancer* 2009; **9**: 88 [PMID: 19302716 DOI: 10.1186/1471-2407-9-88]
48 **Wu YX**, Wang JH, Wang H, Yang XY. [Study on expression of Ki-67, early apoptotic protein M30 in endometrial carcinoma and their correlation with prognosis]. *Zhonghua Bing Li Xue Za Zhi* 2003; **32**: 314-318 [PMID: 14514374]
49 **Gonzalez-Quintela A**, Mallo N, Mella C, Campos J, Perez LF, Lopez-Rodriguez R, Tome S, Otero E. Serum levels of cytokeratin-18 (tissue polypeptide-specific antigen) in liver diseases. *Liver Int* 2006; **26**: 1217-1224 [PMID: 17105587 DOI: 10.1111/j.1478-3231.2006.01380.x]
50 **Yagmur E**, Trautwein C, Leers MP, Gressner AM, Tacke F. Elevated apoptosis-associated cytokeratin 18 fragments (CK18Asp386) in serum of patients with chronic liver diseases indicate hepatic and biliary inflammation. *Clin Biochem* 2007; **40**: 651-655 [PMID: 17306787 DOI: 10.1016/j.clinbiochem.2006.12.010]
51 **Godin C**, Louandre C, Bodeau S, Diouf M, Saidak Z, Conte MA, Chauffert B, Barbare JC, Barget N, Trinchet JC, Ganne N, Galmiche A. Biomarkers of apoptosis and necrosis in patients with hepatocellular carcinoma treated with sorafenib. *Anticancer Res* 2015; **35**: 1803-1808 [PMID: 25750346]
52 **Waidmann O**, Köberle V, Bettinger D, Trojan J, Zeuzem S, Schultheiß M, Kronenberger B, Piiper A. Diagnostic and prognostic significance of cell death and macrophage activation markers in patients with hepatocellular carcinoma. *J Hepatol* 2013; **59**: 769-779 [PMID: 23792028 DOI: 10.1016/j.jhep.2013.06.008]
53 **Morris KL**, Tugwood JD, Khoja L, Lancashire M, Sloane R, Burt D, Shenjere P, Zhou C, Hodgson C, Ohtomo T, Katoh A, Ishiguro T, Valle JW, Dive C. Circulating biomarkers in hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2014; **74**: 323-332 [PMID: 24923562 DOI: 10.1007/s00280-014-2508-7]
54 **Lorente L**, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Barrera MA. Prognostic Value of Serum Caspase-Cleaved Cytokeratin-18 Levels before Liver Transplantation for One-Year Survival of Patients with Hepatocellular Carcinoma. *Int J Mol Sci* 2016; **17** [PMID: 27618033 DOI: 10.3390/ijms17091524]
55 **Lorente L**, Martín MM, González-Rivero AF, Ferreres J, Solé-Violán J, Labarta L, Díaz C, Jiménez A, Borreguero-León JM. Serum levels of caspase-cleaved cytokeratin-18 and mortality are

associated in severe septic patients: pilot study. *PLoS One* 2014; **9**: e109618 [PMID: 25290885 DOI: 10.1371/journal.pone.0109618]
56 **Lorente L**, Martín MM, González-Rivero AF, Argueso M, Ramos L, Solé-Violán J, Cáceres JJ, Jiménez A, Borreguero-León JM. Serum levels of caspase-cleaved cytokeratin-18 in patients with severe traumatic brain injury are associated with mortality: a pilot study. *PLoS One* 2015; **10**: e0121739 [PMID: 25822281 DOI: 10.1371/journal.pone.0121739]
57 **Lorente L**, Martín MM, Pérez-Cejas A, Ramos L, Argueso M, Solé-Violán J, Cáceres JJ, Jiménez A, García-Marín V. High serum levels of caspase-cleaved cytokeratin-18 are associated with malignant middle cerebral artery infarction patient mortality. *BMC Neurol* 2018; **18**: 32 [PMID: 29573748 DOI: 10.1186/s12883-018-1038-z]
58 **Antoniades C**, Bakogiannis C, Tousoulis D, Antonopoulos AS, Stefanadis C. The CD40/CD40 ligand system: linking inflammation with atherothrombosis. *J Am Coll Cardiol* 2009; **54**: 669-677 [PMID: 19679244 DOI: 10.1016/j.jacc.2009.03.076]
59 **Ferroni P**, Santilli F, Guadagni F, Basili S, Davì G. Contribution of platelet-derived CD40 ligand to inflammation, thrombosis and neoangiogenesis. *Curr Med Chem* 2007; **14**: 2170-2180 [PMID: 17691955]
60 **Aukrust P**, Damas JK, Solum NO. Soluble CD40 ligand and platelets: self-perpetuating pathogenic loop in thrombosis and inflammation? *J Am Coll Cardiol* 2004; **43**: 2326-2328 [PMID: 15193701 DOI: 10.1016/j.jacc.2004.03.023]
61 **Anand SX**, Viles-Gonzalez JF, Badimon JJ, Cavusoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombotic disease. *Thromb Haemost* 2003; **90**: 377-384 [PMID: 12958605 DOI: 10.1160/TH03-05-0268]
62 **Zhang B**, Wu T, Chen M, Zhou Y, Yi D, Guo R. The CD40/CD40L system: a new therapeutic target for disease. *Immunol Lett* 2013; **153**: 58-61 [PMID: 23892087 DOI: 10.1016/j.imlet.2013.07.005]
63 **Zhang T**, Pierson RN 3rd, Azimzadeh AM. Update on CD40 and CD154 blockade in transplant models. *Immunotherapy* 2015; **7**: 899-911 [PMID: 26268734 DOI: 10.2217/IMT.15.54]
64 **Pinelli DF**, Ford ML. Novel insights into anti-CD40/CD154 immunotherapy in transplant tolerance. *Immunotherapy* 2015; **7**: 399-410 [PMID: 25917630 DOI: 10.2217/imt.15.1]
65 **Aloui C**, Prigent A, Sut C, Tariket S, Hamzeh-Cognasse H, Pozzetto B, Richard Y, Cognasse F, Laradi S, Garraud O. The signaling role of CD40 ligand in platelet biology and in platelet component transfusion. *Int J Mol Sci* 2014; **15**: 22342-22364 [PMID: 25479079 DOI: 10.3390/ijms151222342]
66 **Mao DJ**, Guo RY, Tang YC, Zang YH. [Expression of sCD40L in peripheral blood and NF-κBp65 in PBMC of patients with acute progressive cerebral infarction]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2011; **27**: 177-179 [PMID: 21315050]
67 **Tuttolomondo A**, Di Raimondo D, Di Sciacca R, Casuccio A, Bivona G, Bellia C, Barreca L, Serio A, D'Aguanno G, Ciaccio M, Licata G, Pinto A. Fetuin-A and CD40 L plasma levels in acute ischemic stroke: differences in relation to TOAST subtype and correlation with clinical and laboratory variables. *Atherosclerosis* 2010; **208**: 290-296 [PMID: 19709661 DOI: 10.1016/j.atherosclerosis.2009.07.032]
68 **Davì G**, Tuttolomondo A, Santilli F, Basili S, Ferrante E, Di Raimondo D, Pinto A, Licata G. CD40 ligand and MCP-1 as predictors of cardiovascular events in diabetic patients with stroke. *J Atheroscler Thromb* 2009; **16**: 707-713 [PMID: 19755790]
69 **Lorente L**, Martín MM, González-Rivero AF, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Jiménez A, Borreguero-León JM. Association between Serum Soluble CD154 Levels and Mortality in Patients with Malignant Middle Cerebral Artery Infarction. *Int J Mol Sci* 2015; **16**: 12147-12158 [PMID: 26030673 DOI: 10.3390/ijms160612147]
70 **Aukrust P**, Müller F, Ueland T, Berget T, Aaser E, Brunsvig A, Solum NO, Forfang K, Frøland SS, Gullestad L. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina. Possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. *Circulation* 1999; **100**: 614-620 [PMID: 10441098]
71 **Varo N**, de Lemos JA, Libby P, Morrow DA, Murphy SA, Nuzzo R, Gibson CM, Cannon CP, Braunwald E, Schönbeck U. Soluble CD40L: risk prediction after acute coronary syndromes. *Circulation* 2003; **108**: 1049-1052 [PMID: 12912804 DOI: 10.1161/01.CIR.0000088521.04017.13]
72 **Lorente L**, Martín MM, Varo N, Borreguero-León JM, Solé-Violán J, Blanquer J, Labarta L, Díaz C, Jiménez A, Pastor E, Belmonte F, Orbe J, Rodríguez JA, Gómez-Melini E, Ferrer-Agüero JM, Ferreres J, Llimiñana MC, Páramo JA. Association between serum soluble CD40 ligand levels and mortality in patients with severe sepsis. *Crit Care* 2011; **15**: R97 [PMID: 21406105 DOI: 10.1186/cc10104]
73 **Martínez de Lizarrondo S**, Roncal C, Calvayrac O, Rodríguez C, Varo N, Purroy A, Lorente L, Rodríguez JA, Doeuvre L, Hervás-Stubbs S, Angles-Cano E, Páramo JA, Martínez-González J, Orbe J. Synergistic effect of thrombin and CD40 ligand on endothelial matrix metalloproteinase-10 expression and microparticle generation in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 2012; **32**: 1477-1487 [PMID: 22492089 DOI: 10.1161/ATVBAHA.112.248773]
74 **Dominguez-Rodriguez A**, Abreu-Gonzalez P, Garcia-Gonzalez MJ, Kaski JC. Soluble CD40 ligand:interleukin-10 ratio predicts in-hospital adverse events in patients with ST-segment elevation myocardial infarction. *Thromb Res* 2007; **121**: 293-299 [PMID: 17521712 DOI: 10.1016/j.thromres.2007.04.007]
75 **Lorente L**, Martín MM, González-Rivero AF, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Serrano N, Rodríguez ST, Jiménez A, Borreguero-León JM. Serum soluble CD40 Ligand levels are associated with severity and mortality of brain trauma injury patients. *Thromb Res* 2014; **134**: 832-836 [PMID: 25123332 DOI: 10.1016/j.thromres.2014.07.034]
76 **Lapiński TW**, Pogorzelska J, Grzeszczuk A, Swiderska M, Kowalczuk O, Nikliński J, Flisiak R. The importance of sCD40 and sCD40L concentration in patients with chronic HCV infection and HIV co-infection. *Przegl Epidemiol* 2014; **68**: 11-15, 105-108 [PMID: 25004625]
77 **Basili S**, Raparelli V, Riggio O, Merli M, Carnevale R, Angelico F, Tellan G, Pignatelli P, Violi F; CALC Group. NADPH oxidase-mediated platelet isoprostane over-production in cirrhotic patients: implication for platelet activation. *Liver Int* 2011; **31**: 1533-1540 [PMID: 22093328 DOI: 10.1111/j.1478-3231.2011.02617.x]
78 **Sookoian S**, Castaño GO, Burgueño AL, Rosselli MS, Gianotti TF, Mallardi P, Martino JS, Pirola CJ. Circulating levels and hepatic expression of molecular mediators of atherosclerosis in nonalcoholic fatty liver disease. *Atherosclerosis* 2010; **209**: 585-591 [PMID: 19896127 DOI: 10.1016/j.atherosclerosis.2009.10.011]
79 **Eltaher SM**, El-Gil R, Fouad N, Mitwali R, El-Kholy H. Evaluation of serum levels and significance of soluble CD40 ligand in screening patients with hepatitis C virus-related hepatocellular carcinoma. *East Mediterr Health J* 2016; **22**: 603-610 [PMID: 27834442]
80 **Lorente L**, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Barrera MA. High serum soluble CD40L levels previously to liver transplantation in patients with hepatocellular carcinoma are associated with mortality at one year. *J Crit Care* 2018; **43**: 316-320 [PMID: 29020665 DOI: 10.1016/j.jcrc.2017.09.032]
81 **Noelle RJ**, Roy M, Shepherd DM, Stamenkovic I, Ledbetter JA, Aruffo A. A 39-kDa protein on activated helper T cells binds CD40 and transduces the signal for cognate activation of B cells. *Proc Natl Acad Sci USA* 1992; **89**: 6550-6554 [PMID: 1378631]
82 **Mach F**, Schönbeck U, Sukhova GK, Bourcier T, Bonnefoy JY, Pober JS, Libby P. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc Natl Acad Sci USA* 1997; **94**: 1931-1936 [PMID: 9050882]
83 **Zhou L**, Stordeur P, de Lavareille A, Thielemans K, Capel P,

Goldman M, Pradier O. CD40 engagement on endothelial cells promotes tissue factor-dependent procoagulant activity. *Thromb Haemost* 1998; **79**: 1025-1028 [PMID: 9609241]
84 **Hezi-Yamit A**, Wong PW, Bien-Ly N, Komuves LG, Prasad KS, Phillips DR, Sinha U. Synergistic induction of tissue factor by coagulation factor Xa and TNF: evidence for involvement of negative regulatory signaling cascades. *Proc Natl Acad Sci USA* 2005; **102**: 12077-12082 [PMID: 16105945 DOI: 10.1073/pnas.0504526102]
85 **Miller DL**, Yaron R, Yellin MJ. CD40L-CD40 interactions regulate endothelial cell surface tissue factor and thrombomodulin expression. *J Leukoc Biol* 1998; **63**: 373-379 [PMID: 9500526]
86 **Slupsky JR**, Kalbas M, Willuweit A, Henn V, Kroczek RA, Müller-Berghaus G. Activated platelets induce tissue factor expression on human umbilical vein endothelial cells by ligation of CD40. *Thromb Haemost* 1998; **80**: 1008-1014 [PMID: 9869175]
87 **Prasad KS**, Andre P, He M, Bao M, Manganello J, Phillips DR. Soluble CD40 ligand induces beta3 integrin tyrosine phosphorylation and triggers platelet activation by outside-in signaling. *Proc Natl Acad Sci USA* 2003; **100**: 12367-12371 [PMID: 14519852 DOI: 10.1073/pnas.2032886100]
88 **André P**, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR, Wagner DD. CD40L stabilizes arterial thrombi by a beta3 integrin--dependent mechanism. *Nat Med* 2002; **8**: 247-252 [PMID: 11875495 DOI: 10.1038/nm0302-247]
89 **Li J**, Zhao SP, Peng DQ, Xu ZM, Zhou HN. Early effect of pravastatin on serum soluble CD40L, matrix metalloproteinase-9, and C-reactive protein in patients with acute myocardial infarction. *Clin Chem* 2004; **50**: 1696-1699 [PMID: 15265816 DOI: 10.1373/clinchem.2003.030940]
90 **Hamdan R**, Hajj F, Kadry Z, Kassab R, Salame E, Aboujaoude S, Azar R, Badaoui G. Benefit and tolerability of the coadministration of ezetimibe and atorvastatin in acute coronary syndrome patients. *J Med Liban* 2011; **59**: 65-69 [PMID: 21834489]
91 **Han SH**, Koh KK, Quon MJ, Lee Y, Shin EK. The effects of simvastatin, losartan, and combined therapy on soluble CD40 ligand in hypercholesterolemic, hypertensive patients. *Atherosclerosis* 2007; **190**: 205-211 [PMID: 16500662 DOI: 10.1016/j.atherosclerosis.2006.01.021]
92 **Ní Chróinín D**, Asplund K, Åsberg S, Callaly E, Cuadrado-Godia E, Díez-Tejedor E, Di Napoli M, Engelter ST, Furie KL, Giannopoulos S, Gotto AM Jr, Hannon N, Jonsson F, Kapral MK, Martí-Fàbregas J, Martínez-Sánchez P, Milionis HJ, Montaner J, Muscari A, Pikija S, Probstfield J, Rost NS, Thrift AG, Vemmos K, Kelly PJ. Statin therapy and outcome after ischemic stroke: systematic review and meta-analysis of observational studies and randomized trials. *Stroke* 2013; **44**: 448-456 [PMID: 23287777 DOI: 10.1161/STROKEAHA.112.668277]
93 **Tleyjeh IM**, Kashour T, Hakim FA, Zimmerman VA, Erwin PJ, Sutton AJ, Ibrahim T. Statins for the prevention and treatment of infections: a systematic review and meta-analysis. *Arch Intern Med* 2009; **169**: 1658-1667 [PMID: 19822822 DOI: 10.1001/archinternmed.2009.286]
94 **Thomsen RW**, Riis A, Kornum JB, Christensen S, Johnsen SP, Sørensen HT. Preadmission use of statins and outcomes after hospitalization with pneumonia: population-based cohort study of 29,900 patients. *Arch Intern Med* 2008; **168**: 2081-2087 [PMID: 18955636 DOI: 10.1001/archinte.168.19.2081]
95 **Hsu J**, Andes DR, Knasinski V, Pirsch J, Safdar N. Statins are associated with improved outcomes of bloodstream infection in solid-organ transplant recipients. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 1343-1351 [PMID: 19669657 DOI: 10.1007/s10096-009-0787-4]
96 **Dobesh PP**, Klepser DG, McGuire TR, Morgan CW, Olsen KM. Reduction in mortality associated with statin therapy in patients with severe sepsis. *Pharmacotherapy* 2009; **29**: 621-630 [PMID: 19476415 DOI: 10.1592/phco.29.6.621]
97 **Almeida TA**, Rojo J, Nieto PM, Pinto FM, Hernandez M, Martín JD, Candenas ML. Tachykinins and tachykinin receptors: structure and activity relationships. *Curr Med Chem* 2004; **11**: 2045-2081 [PMID: 15279567]
98 **Pennefather JN**, Lecci A, Candenas ML, Patak E, Pinto FM, Maggi CA. Tachykinins and tachykinin receptors: a growing family. *Life Sci* 2004; **74**: 1445-1463 [PMID: 14729395]
99 **Satake H**, Kawada T. Overview of the primary structure, tissue-distribution, and functions of tachykinins and their receptors. *Curr Drug Targets* 2006; **7**: 963-974 [PMID: 16918325]
100 **Trivedi M**, Bergasa NV. Serum concentrations of substance P in cholestasis. *Ann Hepatol* 2010; **9**: 177-180 [PMID: 20526012]
101 **El-Raziky MS**, Gohar N, El-Raziky M. Study of substance P, renin and aldosterone in chronic liver disease in Egyptian children. *J Trop Pediatr* 2005; **51**: 320-323 [PMID: 15980023 DOI: 10.1093/tropej/fmi060]
102 **Li CP**, Lee FY, Hwang SJ, Chang FY, Lin HC, Lu RH, Hou MC, Chu CJ, Chan CC, Luo JC, Lee SD. Role of substance P in the pathogenesis of spider angiomas in patients with nonalcoholic liver cirrhosis. *Am J Gastroenterol* 1999; **94**: 502-507 [PMID: 10022654 DOI: 10.1111/j.1572-0241.1999.883_l.x]
103 **Uemura M**, Tsujii T, Kikuchi E, Fukui H, Tsukamoto N, Matsumura M, Fujimoto M, Koizumi M, Takaya A, Kojima H, Ishii Y, Okamoto S. Increased plasma levels of substance P and disturbed water excretion in patients with liver cirrhosis. *Scand J Gastroenterol* 1998; **33**: 860-866 [PMID: 9754735]
104 **Lee FY**, Lin HC, Tsai YT, Chang FY, Lu RH, Hou MC, Li CP, Chu CJ, Wang SS, Lee SD. Plasma substance P levels in patients with liver cirrhosis: relationship to systemic and portal hemodynamics. *Am J Gastroenterol* 1997; **92**: 2080-2084 [PMID: 9362197]
105 **Fernández-Rodriguez CM**, Prieto J, Quiroga J, Zozoya JM, Andrade A, Núñez M, Sangro B, Penas J. Plasma levels of substance P in liver cirrhosis: relationship to the activation of vasopressor systems and urinary sodium excretion. *Hepatology* 1995; **21**: 35-40 [PMID: 7528711]
106 **Hörtnagl H**, Singer EA, Lenz K, Kleinberger G, Lochs H. Substance P is markedly increased in plasma of patients with hepatic coma. *Lancet* 1984; **1**: 480-483 [PMID: 6199630]
107 **Lorente L**, Rodriguez ST, Sanz P, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Cerro P, Barrera MA. Patients with high serum substance P levels previously to liver transplantation for hepatocellular carcinoma have higher risk of one-year liver transplantation mortality. *Oncotarget* 2018; **9**: 21552-21559 [PMID: 29765559 DOI: 10.18632/oncotarget.25097]
108 **Lorente L**, Martín MM, Almeida T, Hernández M, Ramos L, Argueso M, Cáceres JJ, Solé-Violán J, Jiménez A. Serum substance P levels are associated with severity and mortality in patients with severe traumatic brain injury. *Crit Care* 2015; **19**: 192 [PMID: 25928056 DOI: 10.1186/s13054-015-0911-z]
109 **Lorente L**, Martín MM, Almeida T, Pérez-Cejas A, Ramos L, Argueso M, Riaño-Ruiz M, Solé-Violán J, Hernández M. Serum Levels of Substance P and Mortality in Patients with a Severe Acute Ischemic Stroke. *Int J Mol Sci* 2016; **17** [PMID: 27338372 DOI: 10.3390/ijms170609]
110 **Lotz M**, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 1988; **241**: 1218-1221 [PMID: 2457950]
111 **Laurenzi MA**, Persson MA, Dalsgaard CJ, Haegerstrand A. The neuropeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells are preferentially influenced by the neuropeptide. *Scand J Immunol* 1990; **31**: 529-533 [PMID: 1692157]
112 **Ansel JC**, Brown JR, Payan DG, Brown MA. Substance P selectively activates TNF-alpha gene expression in murine mast cells. *J Immunol* 1993; **150**: 4478-4485 [PMID: 7683320]
113 **Rameshwar P**, Gascón P. Substance P (SP) mediates production of stem cell factor and interleukin-1 in bone marrow stroma: potential autoregulatory role for these cytokines in SP receptor expression and induction. *Blood* 1995; **86**: 482-490 [PMID: 7541664]
114 **Yamaguchi M**, Kojima T, Kanekawa M, Aihara N, Nogimura A, Kasai K. Neuropeptides stimulate production of interleukin-1 beta, interleukin-6, and tumor necrosis factor-alpha in human dental

pulp cells. *Inflamm Res* 2004; **53**: 199-204 [PMID: 15105969 DOI: 10.1007/s00011-003-1243-z]
115 **Turner RJ**, Helps SC, Thornton E, Vink R. A substance P antagonist improves outcome when administered 4 h after onset of ischaemic stroke. *Brain Res* 2011; **1393**: 84-90 [PMID: 21466790 DOI: 10.1016/j.brainres.2011.03.066]
116 **Turner RJ**, Vink R. NK1 tachykinin receptor treatment is superior to capsaicin pre-treatment in improving functional outcome following acute ischemic stroke. *Neuropeptides* 2014; **48**: 267-272 [PMID: 25151181 DOI: 10.1016/j.npep.2014.07.002]
117 **Yu Z,** Cheng G, Huang X, Li K, Cao X. Neurokinin-1 receptor antagonist SR140333: A novel type of drug to treat cerebral ischemia. *Neuroreport* 1997; **8**: 2117-2119
118 **Gabrielian L**, Helps SC, Thornton E, Turner RJ, Leonard AV, Vink R. Substance P antagonists as a novel intervention for brain edema and raised intracranial pressure. *Acta Neurochir Suppl* 2013; **118**: 201-204 [PMID: 23564132 DOI: 10.1007/978-3-7091-1434-6_37]
119 **Donkin JJ**, Cernak I, Blumbergs PC, Vink R. A substance P antagonist reduces axonal injury and improves neurologic outcome when administered up to 12 hours after traumatic brain injury. *J Neurotrauma* 2011; **28**: 217-224 [PMID: 21175297 DOI: 10.1089/neu.2010.1632]
120 **Zheng J**, Cai J, Li H, Zeng K, He L, Fu H, Zhang J, Chen L, Yao J, Zhang Y, Yang Y. Neutrophil to Lymphocyte Ratio and Platelet to Lymphocyte Ratio as Prognostic Predictors for Hepatocellular Carcinoma Patients with Various Treatments: a Meta-Analysis and Systematic Review. *Cell Physiol Biochem* 2017; **44**: 967-981 [PMID: 29179180 DOI: 10.1159/000485396]
121 **An HJ**, Jang JW, Bae SH, Choi JY, Yoon SK, Lee MA, You YK, Kim DG, Jung ES. Serum C-reactive protein is a useful biomarker for predicting outcomes after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2012; **18**: 1406-1414 [PMID: 22821639 DOI: 10.1002/lt.23512]
122 **Mori S**, Choi Y, Park MS, Kim H, Hong G, Yi NJ, Lee KW, Suh KS. Usefulness of preoperative C-reactive protein and alpha-fetoprotein levels for prognostication of patients with hepatocellular carcinoma after living donor liver transplantation. *Hepatogastroenterology* 2014; **61**: 2353-2358 [PMID: 25699382]
123 **Harimoto N**, Yoshizumi T, Shimagaki T, Nagatsu A, Motomura T, Harada N, Okabe H, Itoh S, Ikegami T, Uchiyama H, Soejima Y, Maehara Y. Inflammation-based Prognostic Score in Patients with Living Donor Liver Transplantation for Hepatocellular Carcinoma. *Anticancer Res* 2016; **36**: 5537-5542 [PMID: 27798927 DOI: 10.21873/anticanres.11137]
124 **Fujiki M**, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, Uemoto S. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. *Am J Transplant* 2009; **9**: 2362-2371 [PMID: 19656125 DOI: 10.1111/j.1600-6143.2009.02783.x]
125 **Taketomi A**, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, Harada N, Yamashita Y, Sugimachi K, Kayashima H, Iguchi T, Maehara Y. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Transplantation* 2009; **87**: 531-537 [PMID: 19307789 DOI: 10.1097/TP.0b013e3181943bee]
126 **Shindoh J**, Sugawara Y, Nagata R, Kaneko J, Tamura S, Aoki T, Sakamoto Y, Hasegawa K, Tanaka T, Kokudo N. Evaluation methods for pretransplant oncologic markers and their prognostic impacts in patient undergoing living donor liver transplantation for hepatocellular carcinoma. *Transpl Int* 2014; **27**: 391-398 [PMID: 24472068 DOI: 10.1111/tri.12274]
127 **Lee JH**, Cho Y, Kim HY, Cho EJ, Lee DH, Yu SJ, Lee JW, Yi NJ, Lee KW, Kim SH, Kim JM, Joh JW, Teperman LW, Park JS, Kim YJ, Suh KS, Yoon JH. Serum Tumor Markers Provide Refined Prognostication in Selecting Liver Transplantation Candidate for Hepatocellular Carcinoma Patients Beyond the Milan Criteria. *Ann Surg* 2016; **263**: 842-850 [PMID: 26779979 DOI: 10.1097/SLA.0000000000001578]
128 **Wang YL**, Zhu ZJ, Teng DH, Yao Z, Gao W, Shen ZY. Glypican-3 expression and its relationship with recurrence of HCC after liver transplantation. *World J Gastroenterol* 2012; **18**: 2408-2414 [PMID: 22654434 DOI: 10.3748/wjg.v18.i19.2408]
129 **Jing JS**, Ye W, Jiang YK, Ma J, Zhu MQ, Ma JM, Zhou H, Yu LQ, Yang YF, Wang SC. The Value of GPC3 and GP73 in Clinical Diagnosis of Hepatocellular Carcinoma. *Clin Lab* 2017; **63**: 1903-1909 [PMID: 29226636]
130 **Oya H**, Sato Y, Yamamoto S, Nakatsuka H, Kobayashi T, Hara Y, Waguri N, Suda T, Aoyagi Y, Hatakeyama K. Comparison between human-telomerase reverse transcriptase mRNA and alpha-fetoprotein mRNA as a predictive value for recurrence of hepatocellular carcinoma in living donor liver transplantation. *Transplant Proc* 2006; **38**: 3636-3639 [PMID: 17175353 DOI: 10.1016/j.transproceed.2006.10.172]
131 **Sato Y**, Yamamoto S, Oya H, Nakatsuka H, Kobayashi T, Takeishi T, Hirano K, Hara Y, Watanabe T, Waguri N, Suda T, Ichida T, Aoyagi Y, Hatakeyama K. Preoperative human-telomerase reverse transcriptase mRNA in peripheral blood and tumor recurrence in living-related liver transplantation for hepatocellular carcinoma. *Hepatogastroenterology* 2005; **52**: 1325-1328 [PMID: 16201066]
132 **Kim YD**, Hwang S, Lee YJ, Kim KH, Ahn CS, Park KM, Moon DB, Ha TY, Song GW, Jung DH, Park SR, Hong HN, Lee SG. Preoperative peripheral blood human telomerase reverse transcriptase mRNA concentration is not a prognostic factor for resection of hepatocellular carcinoma. *Hepatogastroenterology* 2012; **59**: 1512-1515 [PMID: 22683968 DOI: 10.5754/hge10342]
133 **Nart D**, Yaman B, Yilmaz F, Zeytunlu M, Karasu Z, Kiliç M. Expression of matrix metalloproteinase-9 in predicting prognosis of hepatocellular carcinoma after liver transplantation. *Liver Transpl* 2010; **16**: 621-630 [PMID: 20440771 DOI: 10.1002/lt.22028]
134 **Zhang Q**, Chen X, Zhou J, Zhang L, Zhao Q, Chen G, Xu J, Qian F, Chen Z. CD147, MMP-2, MMP-9 and MVD-CD34 are significant predictors of recurrence after liver transplantation in hepatocellular carcinoma patients. *Cancer Biol Ther* 2006; **5**: 808-814 [PMID: 16775432]
135 **Kuyvenhoven JP**, Verspaget HW, Gao Q, Ringers J, Smit VT, Lamers CB, van Hoek B. Assessment of serum matrix metalloproteinases MMP-2 and MMP-9 after human liver transplantation: increased serum MMP-9 level in acute rejection. *Transplantation* 2004; **77**: 1646-1652 [PMID: 15201662]
136 **Lempinen M**, Lyytinen I, Nordin A, Tervahartiala T, Mäkisalo H, Sorsa T, Isoniemi H. Prognostic value of serum MMP-8, -9 and TIMP-1 in patients with hepatocellular carcinoma. *Ann Med* 2013; **45**: 482-487 [PMID: 23962148 DOI: 10.3109/07853890.2013.823779]
137 **Lorente L**, Martín MM, Ramos L, Cáceres JJ, Solé-Violán J, Argueso M, Jiménez A, Borreguero-León JM, Orbe J, Rodríguez JA, Páramo JA. Serum tissue inhibitor of matrix metalloproteinase-1 levels are associated with mortality in patients with malignant middle cerebral artery infarction. *BMC Neurol* 2015; **15**: 111 [PMID: 26162891 DOI: 10.1186/s12883-015-0364-7]
138 **Lorente L**, Martín MM, López P, Ramos L, Blanquer J, Cáceres JJ, Solé-Violán J, Solera J, Cabrera J, Argueso M, Ortiz R, Mora ML, Lubillo S, Jiménez A, Borreguero-León JM, González A, Orbe J, Rodríguez JA, Páramo JA. Association between serum tissue inhibitor of matrix metalloproteinase-1 levels and mortality in patients with severe brain trauma injury. *PLoS One* 2014; **9**: e94370 [PMID: 24728097 DOI: 10.1371/journal.pone.0094370]
139 **Lorente L**, Martín MM, Solé-Violán J, Blanquer J, Labarta L, Díaz C, Borreguero-León JM, Orbe J, Rodríguez JA, Jiménez A, Páramo JA. Association of sepsis-related mortality with early increase of TIMP-1/MMP-9 ratio. *PLoS One* 2014; **9**: e94318 [PMID: 24727739 DOI: 10.1371/journal.pone.0094318]
140 **Lorente L**, Martín MM, Labarta L, Díaz C, Solé-Violán J, Blanquer J, Orbe J, Rodríguez JA, Jiménez A, Borreguero-León JM, Belmonte F, Medina JC, Llimiñana MC, Ferrer-Agüero JM, Ferreres J, Mora ML, Lubillo S, Sánchez M, Barrios Y, Sierra A, Páramo JA. Matrix metalloproteinase-9, -10, and tissue inhibitor

of matrix metalloproteinases-1 blood levels as biomarkers of severity and mortality in sepsis. *Crit Care* 2009; **13**: R158 [PMID: 19799791 DOI: 10.1186/cc8115]

141 **Soyama A**, Eguchi S, Takatsuki M, Kawashita Y, Hidaka M, Tokai H, Nagayoshi S, Mochizuki S, Matsumoto S, Hamasaki K, Tajima Y, Kanematsu T. Significance of the serum level of soluble E-cadherin in patients with HCC. *Hepatogastroenterology* 2008; **55**: 1390-1393 [PMID: 18795696]

142 **Fiorentino M**, Altimari A, Ravaioli M, Gruppioni E, Gabusi E, Corti B, Vivarelli M, Bringuier PP, Scoazec JY, Grigioni WF, D' Errico-Grigioni A. Predictive value of biological markers for hepatocellular carcinoma patients treated with orthotopic liver transplantation. *Clin Cancer Res* 2004; **10**: 1789-1795 [PMID: 15014033]

143 **Ataide EC**, Perales SR, Silva MG, Filho FC, Sparapani AC, Latuf Filho PF, Stucchi RSB, Vassallo J, Escanhoela CAF, Boin IFSF. Immunoexpression of Heat Shock Protein 70, Glypican 3, Glutamine Synthetase, and Beta-Catenin in Hepatocellular Carcinoma After Liver Transplantation: Association Between Positive Glypican 3 and Beta-Catenin With the Presence of Larger Nodules. *Transplant Proc* 2017; **49**: 858-862 [PMID: 28457411 DOI: 10.1016/j.transproceed.2017.01.048]

144 **Hakeem AR**, Young RS, Marangoni G, Lodge JP, Prasad KR. Systematic review: the prognostic role of alpha-fetoprotein following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2012; **35**: 987-999 [PMID: 22429190 DOI: 10.1111/j.1365-2036.2012.05060.x]

145 **Pommergaard HC**, Burcharth J, Rosenberg J, Rasmussen A. Serologic and molecular biomarkers for recurrence of hepatocellular carcinoma after liver transplantation: A systematic review and meta-analysis. *Transplant Rev* (Orlando) 2016; **30**: 171-177 [PMID: 27118303 DOI: 10.1016/j.trre.2016.03.001]

146 **Shen Q**, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, Wang N, Niu Y, Wu Z, Zhou J, Qiu SJ, Shi YH, Yu B, Tang N, Chu W, Wang M, Wu J, Zhang Z, Yang S, Gu J, Wang H, Qin W. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. *Lancet Oncol* 2012; **13**: 817-826 [PMID: 22738799 DOI: 10.1016/S1470-2045(12)70233-4]

147 **Erdal H**, Gül Utku Ö, Karatay E, Çelik B, Elbeg Ş, Doğan İ. Combination of DKK1 and AFP improves diagnostic accuracy of hepatocellular carcinoma compared with either marker alone. *Turk J Gastroenterol* 2016; **27**: 375-381 [PMID: 27458854 DOI: 10.5152/tjg.2016.15523]

148 **Kim SU**, Park JH, Kim HS, Lee JM, Lee HG, Kim H, Choi SH, Baek S, Kim BK, Park JY, Kim DY, Ahn SH, Lee JD, Han KH. Serum Dickkopf-1 as a Biomarker for the Diagnosis of Hepatocellular Carcinoma. *Yonsei Med J* 2015; **56**: 1296-1306 [PMID: 26256972 DOI: 10.3349/ymj.2015.56.5.1296]

149 **Ge T**, Shen Q, Wang N, Zhang Y, Ge Z, Chu W, Lv X, Zhao F, Zhao W, Fan J, Qin W. Diagnostic values of alpha-fetoprotein, dickkopf-1, and osteopontin for hepatocellular carcinoma. *Med Oncol* 2015; **32**: 59 [PMID: 25652109 DOI: 10.1007/s12032-014-0367-z]

150 **Fouad YM**, Mohamed HI, Kamal EM, Rasek MA. Clinical significance and diagnostic value of serum dickkopf-1 in patients with hepatocellular carcinoma. *Scand J Gastroenterol* 2016; **51**: 1133-1137 [PMID: 27161933 DOI: 10.3109/00365521.2016.1172337]

151 **Jang ES**, Jeong SH, Kim JW, Choi YS, Leissner P, Brechot C. Diagnostic Performance of Alpha-Fetoprotein, Protein Induced by Vitamin K Absence, Osteopontin, Dickkopf-1 and Its Combinations for Hepatocellular Carcinoma. *PLoS One* 2016; **11**: e0151069 [PMID: 26986465 DOI: 10.1371/journal.pone.0151069]

152 **Liu Y**, Tang W, Xie L, Wang J, Deng Y, Peng Q, Zhai L, Li S, Qin X. Prognostic significance of dickkopf-1 overexpression in solid tumors: a meta-analysis. *Tumour Biol* 2014; **35**: 3145-3154 [PMID: 24258111 DOI: 10.1007/s13277-013-1411-x]

153 **Sakabe T**, Azumi J, Umekita Y, Toriguchi K, Hatano E, Hirooka Y, Shiota G. Expression of Cancer Stem Cell-associated DKK1 mRNA Serves as Prognostic Marker for Hepatocellular Carcinoma. *Anticancer Res* 2017; **37**: 4881-4888 [PMID: 28870909 DOI: 10.21873/anticanres.11897]

154 **Huang Y**, Yang X, Zhao F, Shen Q, Wang Z, Lv X, Hu B, Yu B, Fan J, Qin W. Overexpression of Dickkopf-1 predicts poor prognosis for patients with hepatocellular carcinoma after orthotopic liver transplantation by promoting cancer metastasis and recurrence. *Med Oncol* 2014; **31**: 966 [PMID: 24878698 DOI: 10.1007/s12032-014-0966-8]

155 **Zhan P**, Qian Q, Yu LK. Serum VEGF level is associated with the outcome of patients with hepatocellular carcinoma: a meta-analysis. *Hepatobiliary Surg Nutr* 2013; **2**: 209-215 [PMID: 24570945 DOI: 10.3978/j.issn.2304-3881.2013.06.07]

156 **Zhang W**, Kim R, Quintini C, Hashimoto K, Fujiki M, Diago T, Eghtesad B, Miller C, Fung J, Tan A, Menon KV, Aucejo F. Prognostic role of plasma vascular endothelial growth factor in patients with hepatocellular carcinoma undergoing liver transplantation. *Liver Transpl* 2015; **21**: 101-111 [PMID: 25283528 DOI: 10.1002/lt.24013]

157 **Chu Q**, Jiang Y, Zhang W, Xu C, Du W, Tuguzbaeva G, Qin Y, Li A, Zhang L, Sun G, Cai Y, Feng Q, Li G, Li Y, Du Z, Bai Y, Yang B. Pyroptosis is involved in the pathogenesis of human hepatocellular carcinoma. *Oncotarget* 2016; **7**: 84658-84665 [PMID: 27705930 DOI: 10.18632/oncotarget.12384]

158 **Wan L**, Yuan X, Liu M, Xue B. miRNA-223-3p regulates NLRP3 to promote apoptosis and inhibit proliferation of hep3B cells. *Exp Ther Med* 2018; **15**: 2429-2435 [PMID: 29467847 DOI: 10.3892/etm.2017.5667]

159 **Kofahi HM**, Taylor NG, Hirasawa K, Grant MD, Russell RS. Hepatitis C Virus Infection of Cultured Human Hepatoma Cells Causes Apoptosis and Pyroptosis in Both Infected and Bystander Cells. *Sci Rep* 2016; **6**: 37433 [PMID: 27974850 DOI: 10.1038/srep37433]

160 **Sonohara F**, Inokawa Y, Kanda M, Nishikawa Y, Yamada S, Fujii T, Sugimoto H, Kodera Y, Nomoto S. Association of Inflammasome Components in Background Liver with Poor Prognosis After Curatively-resected Hepatocellular Carcinoma. *Anticancer Res* 2017; **37**: 293-300 [PMID: 28011505 DOI: 10.21873/anticanres.11320]

161 **Scholz A**, Plate KH, Reiss Y. Angiopoietin-2: a multifaceted cytokine that functions in both angiogenesis and inflammation. *Ann N Y Acad Sci* 2015; **1347**: 45-51 [PMID: 25773744 DOI: 10.1111/nyas.12726]

162 **Faillaci F**, Marzi L, Critelli R, Milosa F, Schepis F, Turola E, Andreani S, Vandelli G, Bernabucci V, Lei B, D'Ambrosio F, Bristot L, Cavalletto L, Chemello L, Sighinolfi P, Manni P, Maiorana A, Caporali C, Bianchini M, Marsico M, Turco L, de Maria N, Del Buono M, Todesca P, di Lena L, Romagnoli D, Magistri P, di Benedetto F, Bruno S, Taliani G, Giannelli G, Martinez-Chantar ML, Villa E. Liver Angiopoietin-2 is a key predictor of de novo or recurrent hepatocellular cancer after HCV direct-acting antivirals. *Hepatology* 2018; **68**: 1010-1024 [PMID: 29604220 DOI: 10.1002/hep.29911]

163 **Ogura Y**, Hori T, Uemoto S. Intentional portal pressure control is key to improving the outcome of living donor liver transplantation: the Kyoto University Hospital experience. *Clin Transpl* 2008; 143-149 [PMID: 19708453]

164 **Hidaka M**, Takatsuki M, Soyama A, Tanaka T, Muraoka I, Hara T, Kuroki T, Kanematsu T, Eguchi S. Intraoperative portal venous pressure and long-term outcome after curative resection for hepatocellular carcinoma. *Br J Surg* 2012; **99**: 1284-1289 [PMID: 22864890 DOI: 10.1002/bjs.8861]

165 **Hernandez-Gea V**, Turon F, Berzigotti A, Villanueva A. Management of small hepatocellular carcinoma in cirrhosis: focus on portal hypertension. *World J Gastroenterol* 2013; **19**: 1193-1199 [PMID: 23482437 DOI: 10.3748/wjg.v19.i8.1193]

166 **Waziry R**, Hajarizadeh B, Grebely J, Amin J, Law M, Danta M, George J, Dore GJ. Hepatocellular carcinoma risk following direct-acting antiviral HCV therapy: A systematic review, meta-analyses, and meta-regression. *J Hepatol* 2017; **67**: 1204-1212 [PMID: 28802876 DOI: 10.1016/j.jhep.2017.07.025]

167 **Cancer Genome Atlas Research Network.** Cancer Genome Atlas Research Network. Comprehensive and Integrative Genomic Characterization of Hepatocellular Carcinoma. *Cell* 2017; **169**: 1327-1341.e23 [PMID: 28622513 DOI: 10.1016/j.cell.2017.05.046]

168 **Chiang DY**, Villanueva A. Progress towards molecular patient stratification of hepatocellular carcinoma: Lost in translation? *J Hepatol* 2017; **67**: 893-895 [PMID: 28837834 DOI: 10.1016/j.jhep.2017.07.031]

P- Reviewer: Gorrell MD, Wang K, Zhu X **S- Editor**: Wang XJ
L- Editor: A **E- Editor**: Huang Y

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4243-4253

DOI: 10.3748/wjg.v24.i37.4243

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

MINIREVIEWS

Colonoscopy attachments for the detection of precancerous lesions during colonoscopy: A review of the literature

Paraskevas Gkolfakis, Georgios Tziatzios, Eleftherios Spartalis, Ioannis S Papanikolaou, Konstantinos Triantafyllou

Paraskevas Gkolfakis, Georgios Tziatzios, Ioannis S Papanikolaou, Konstantinos Triantafyllou, Hepato-gastroenterology Unit, Second Department of Internal Medicine -Propaedeutic, Research Institute and Diabetes Center, Medical School, National and Kapodistrian University of Athens, "Attikon" University General Hospital, Athens 12462, Greece

Eleftherios Spartalis, Laboratory of Experimental Surgery and Surgical Research, National and Kapodistrian University of Athens, Athens 12462, Greece

ORCID number: Paraskevas Gkolfakis (0000-0002-9677-4013); Georgios Tziatzios (0000-0002-2945-6007); Eleftherios Spartalis (0000-0003-4451-8074); Ioannis S Papanikolaou (0000-0002-7368-6168); Konstantinos Triantafyllou (0000-0002-5183-9426).

Author contributions: Triantafyllou K conceived the idea, revised and finally approved the manuscript; Gkolfakis P and Tziatzios G searched the literature and drafted the manuscript; Papanikolaou IS and Spartalis E revised and finally approved the manuscript.

Conflict-of-interest statement: The authors declare no conflict of interest related to this publication.

Manuscript source: Invited manuscript

Correspondence to: Konstantinos Triantafyllou, MD, PhD, Associate Professor, Hepato-gastroenterology Unit, Second Department of Internal Medicine - Propaedeutic, Research Institute and Diabetes Center, Medical School, National and Kapodistrian University of Athens, "Attikon" University General Hospital, Rimini 1, Athens 12462, Greece. ktriant@med.uoa.gr

Telephone: +30-210-5832087
Fax: +30-210-58326454

Received: June 11, 2018
Peer-review started: June 12, 2018
First decision: August 1, 2018
Revised: August 6, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

Although colonoscopy has been proven effective in reducing the incidence of colorectal cancer through the detection and removal of precancerous lesions, it remains an imperfect examination, as it can fail in detecting up to almost one fourth of existing adenomas. Among reasons accounting for such failures, is the inability to meticulously visualize the colonic mucosa located either proximal to haustral folds or anatomic curves, including the hepatic and splenic flexures. In order to overcome these limitations, various colonoscope attachments aiming to improve mucosal visualization have been developed. All of them - transparent cap, Endocuff, Endocuff Vision and Endorings - are simply mounted onto the distal tip of the scope. In this review article, we introduce the rationale of their development, present their mode of action and discuss in detail the effect of their implementation in the detection of lesions during colonoscopy.

Key words: Adenoma detection rate; Adenoma miss rate; Colonoscopy; Cup; Endocuff; Endocuff Vision; Endorings

Core tip: Colonoscopy is the modality of choice for the detection and removal of precancerous lesions. However,

almost one fourth of adenomas can be lost during conventional colonoscopy. Their location proximal to the colonic folds or in proximity to anatomic flexures is one of the reasons for this particular detection failure. To overcome this caveat, various single-use devices mounted onto the tip of the scope have been developed. They facilitate lesions' detection by manipulating and flattening the haustral folds. In this Minireview we present the development of these devices (Cap, Endocuff, Endocuff Vision and Endorings) and their effectiveness in improving detection rates of lesions during colonoscopy.

Gkolfakis P, Tziatzios G, Spartalis E, Papanikolaou IS, Triantafyllou K. Colonoscopy attachments for the detection of precancerous lesions during colonoscopy: A review of the literature. *World J Gastroenterol* 2018; 24(37): 4243-4253 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4243.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4243

INTRODUCTION

Colorectal cancer (CRC) is the second most lethal cancer among common cancers and more than 140200 new CRC cases are expected to be diagnosed in the United States by the end of 2018[1]. Colonoscopy has been proven efficient for both diagnosis and screening of colorectal cancer. It allows the detection and consequent removal of adenomas, the most well-known precancerous lesions, preventing CRC-associated death[2]. Adenoma detection rate (ADR)-the percentage of colonoscopies with at least one adenoma- has been associated with both decreased risk of interval CRC (*i.e.*, CRC that is diagnosed in the meantime between a screening colonoscopy and the next recommended surveillance examination) incidence and death[3-5]. Thus, ADR has been established as the core quality indicator for colonoscopy[6]. However, colonoscopy stands far from being the perfect examination. Back-to-back studies have shown that endoscopists fail to detect almost 25% of existing polyps and adenomas[7,8]. These miss rates are higher in the right colon, where a variant of precancerous lesions (the sessile serrated adenomas) that does not follow the classic adenoma-carcinoma pathway of carcinogenesis occurs more frequently[9,10]. To a great extent, missed lesions like these have been held responsible for the aforementioned interval cancers[11]. Inadequate bowel preparation, lack of physician's expertise, inability to accurately visualize the colonic mucosa located proximal to the haustral folds or in proximity to anatomic flexures have been listed among the main reasons such lesions can be missed during a colonoscopy[12]. Lately, several devices-ranging from complex endoscopic systems to simple plastic attachments- have been developed, in an attempt to address this problem[13]. They promise to flatten the mucosa during scope withdrawal and facilitate maneuverability around anatomic flexures offering meticulous mucosal visualization and detection of "hidden" lesions. In this review we aim to present the rationale that led to the development of these detachable devices, their evolution through time, their main mode of action and technical characteristics, as well as their impact on various patient-related colonoscopy outcomes. A comprehensive review of English literature published in MEDLINE until May 2018 was conducted. We aimed to identify high quality studies (randomized controlled trials and meta-analyses) using the following key words: "Cap", "Cap-assisted colonoscopy", "Endocuff", "Endocuff-Vision", "Endocuff-assisted colonoscopy" and "Endorings". Apart from ADR the following measures were assessed: polyp detection rate (PDR), *i.e.*, the percentage of colonoscopies with at least one polyp, mean number of adenomas detected per colonoscopy (MAC), adenoma miss rate-the percentage of adenomas missed by the index examination and detected by the tandem colonoscopy- and advanced ADR (the percentage of colonoscopies with at least one advanced adenoma).

THE CAP

The transparent cap is a simple, single-use device made of thermoplastic elastomer (Figure 1). It was initially designed to facilitate endoscopic mucosectomy, since it enables an optimal field of view by maintaining an appropriate distance between the endoscope tip and the intervention site[14]. Originally launched by Olympus (Olympus America Inc., Center Valley, PA, United States), it is available in various sizes, in order to accommodate all types of endoscopes. Its rounded edge prevents tissue damage during contact, while its side hole allows fluid draining. Endoscope functions such as suction and air insufflation remain undisturbed. The distal end of the cap protrudes from the scope's tip (protruding length ranges from 2 mm to 7 mm). Its basic characteristics are outlined in Table 1. It is the protruding edge that allows manipulation and flattening of the colonic folds in the field of view. During the last 10 years numerous randomized control trials[15-27] have been conducted to evaluate the usefulness of cap-assisted colonoscopy (CAC) in improving colonoscopy outcomes, including potential augmentation of the detection of precancerous lesions. Beyond ADR and PDR, additional outcomes such as cecal intubation rate and cecal intubation time were assessed as well.

Kondo *et al*[15] evaluated colonoscopy with two types of caps (2 mm-short or 4 mm-transparent) *vs* conventional colonoscopy without a cap. More than 200 patients undergoing colonoscopy for various indications were randomized in each of the three groups. The use of the transparent 4mm cap was associated with decreased cecal intubation time compared to the 2 mm-short cap and the control (11.5 min *vs* 13.5 min *vs* 15 min; $P = 0.008$). At the same time, PDR was significantly increased in the transparent cap group (49.3%) compared to the controls (39.1%; $P = 0.04$)[15]. In another Japanese randomized controlled trial (RCT) with 592 patients, CAC with a 2 mm

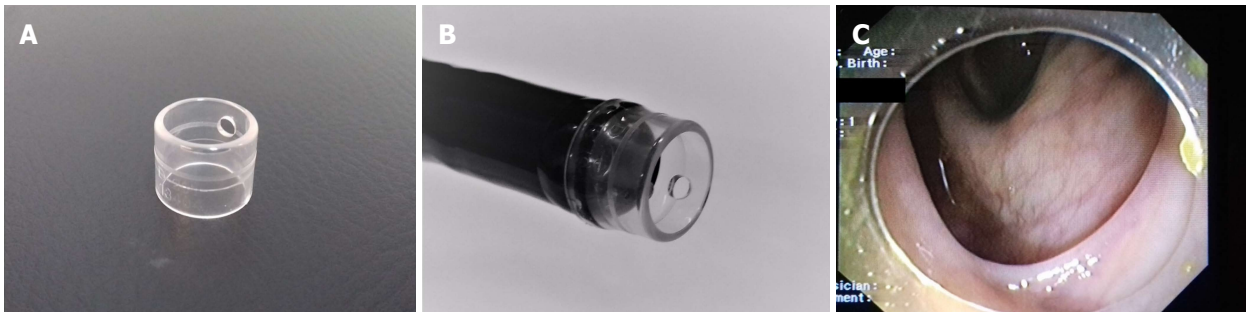

Figure 1 Cap (A) mounted on the tip of the scope (B) and the endoscopic view (C) (photos from the authors' archive).

short cap was also related to a shorter cecal intubation rate, but without any difference in the rate of polyp detection[17]. Horiuchi *et al*[16] randomized 60 patients diagnosed with colonic adenomas to repeat colonoscopy in three months, with or without a 7 mm cap; all lesions were removed during the second examination. Cap-assisted colonoscopy detected 20% more adenomas compared to a 4% increase in adenoma detection without the cap[16]. Moreover, a small back-to-back RCT of 67 screening/surveillance patients demonstrated a reduced adenoma miss rate associated to cap-assisted colonoscopy compared to conventional colonoscopy (21% *vs* 33%; $P = 0.04$)[19].

In terms of ADR and MAC, evidence remains controversial. A study from Japan[21], evaluating the efficacy of autofluorescence imaging with a transparent cap in a cancer referral center, found that CAC leads to an increased ADR (62% *vs* 56%; $P = 0.023$) compared to conventional white-light imaging. A few years later, in the first USA. study, Rastogi *et al*[23] randomized 420 screening/surveillance individuals (210 in each group) to undergo either CAC using a 4 mm transparent cap or a conventional examination. Investigators concluded that CAC not only shortened the cecal intubation time (3.3 min *vs* 4 min; $P < 0.001$), but also increased ADR (69% *vs* 56%; $P = 0.009$) and MAC (2.3 *vs* 1.4; $P < 0.001$) compared to colonoscopy without the cap[23]. In a study that randomized 1113 patients with various indications to undergo either CAC (4 mm cap) or conventional colonoscopy[24], cecal intubation was faster in the CAC arm (4.9 min *vs* 5.8 min; $P < 0.001$), but both arms had similar ADR (42% *vs* 40%; $P = 0.452$) and MAC (0.89 *vs* 0.82; $P = 0.432$)[24]. It is of great interest that among the 10 participating endoscopists the effect of CAC in terms of ADR ranged from a 15% decrease to a 20% increase[24]. Looking at individual endoscopists' performance, the authors concluded that CAC may be beneficial especially for endoscopists who spend more time during scope withdrawal since the cap may further enhance their already meticulous examination[24]. Recently, Othman *et al*[26] showed that CAC compared to conventional colonoscopy is related to an increased advanced ADR - the detection rate of advanced adenomas- (9.9% *vs* 4.6%; $P = 0.049$) and detection of more polyps larger than 9mm (9.5% *vs* 3.7%; $P = 0.026$)[26]. However, in this RCT of 440 screening/surveillance participants no difference between the two groups in terms of ADR and PDR was found[26].

In two RCTs[20,22], 400 individuals of mixed indications[20] and 1380 screening participants[22] were allocated either to CAC or conventional examination. A 4-mm cap was used in both studies. The first study[20] did not show any benefit of CAC in terms of PDR (32.8% *vs* 31.3%; $P = 0.75$) and cecal intubation time (9.9 min *vs* 10.3 min; $P = 0.21$), while in the second study[22] CAC was associated with a shorter intubation time (7.7 min *vs* 8.9 min; $P < 0.001$), but ADR (29% *vs* 29%; $P = 0.96$) and MAC (0.52 *vs* 0.50; $P = 0.83$) did not differ between the two groups.

Two studies involving endoscopy trainees provided similar results; trainees had a higher cecal intubation rate (CIR) and reached the cecum faster using the cap[27,28]. However, CAC did not improve trainees' detection rates (ADR and advanced ADR)[27,28].

Paradoxically, in a large RCT (1000 patients recruited) from Hong Kong, ADR was lower in the cap-assisted arm compared to the standard one (30.5% *vs* 37.5%; $P = 0.018$), but there was no difference regarding advanced lesions[18]. Shorter withdrawal times and inadequate bowel preparation in the CAC arm were postulated by the authors as potential explanations for this finding[18]. In accordance with previous results, cecal intubation time was shorter in the cap arm (6 min *vs* 7.2 min; $P < 0.001$) with no difference in the CIR[18].

Data from meta-analyses

Seven meta-analyses[29-35] that attempt to summarize the role of CAC in improving colonoscopy outcomes have been published so far (Table 2). Despite their different designs and inclusion criteria, one can figure out a couple of mutual conclusions. Four[30,31,34,35] out of five meta-analyses reporting on cecal intubation time, conclude that CAC significantly shortens it (mean difference ranging from -0.93 min to -0.64 min), while three of them[30,34,35] did not show any increase of CIR associated to CAC. Moreover, six[29-32,34,35] and four[30,32,34,35] meta-analyses examined PDR and ADR, respectively. The majority of these[29-31,34] link CAC to a higher PDR. On the contrary, none of the relevant meta-analyses showed a benefit in terms of ADR with CAC[29,30,32,34,35]. However, in the most

Table 1 Add-on devices' main characteristics

	Cap	Endocuff	Endocuff Vision	Endorings
Manufacturer	Olympus, Centre Valley, Pennsylvania	Arc Medical Leeds, United Kingdom	Norgine Pharmaceuticals Ltd, Uxbridge, United Kingdom	EndoAid, Caesarea, Israel
Launched in market	1993	2011	2016	2015
Short description	Transparent, single-use distal attachment with side hole for draining of fluid	Single-use, soft, radiopaque, 2 cm long cylindrical sleeve with flexible projections arranged in 2 rows of 8, emerging from gaps on the shaft of the device	Single-use, device with single row of 8 flexible 15 mm spikes	Single-use device composed of 2 layers of flexible, soft circular rings, placed on a cylindrical cuff
Material	Thermoplastic elastomer	Core: Non-latex, biocompatible polymer; Projections: thermoplastic elastomer	Latex free, polypropylene	Silicone
Dimensions	Outer diameter ranging from 13.9-16.1 mm according to each type of cap	Finger projections: proximal 8.15 mm, distal 5mm; core length: 23.8 mm; diameter: 16.1, 16.7, 17.2, and 18.5 mm (hairs folded back) and 32.6, 33.1, 33.6, and 34.8 mm (hairs opened out)	Diameter: 16.1, 16.7, 17.2, and 18.5mm (spikes folded back) and 39.07, 39.07, 39.07, and 39.66 mm (spikes opened out)	22-50 mm diameter
Mode of action	Protruding cap manipulates and flattens haustral folds to inspect the mucosa on the proximal side of the fold maintaining optimal field of view	Hinged projections flatten and spread mucosa and folds	Hinged projections flatten and improve visibility behind the colon folds	Sequential rings stretches out the folds of the colon during withdrawal for a clear view
Interfere with view of field	Edge of the hood comes into the vision field of the colonoscope, but lesions can be seen through the transparent wall	No interference of vision	No interference with vision	No interference with vision
Compatible scopes	Adult, pediatric: Ten different sizes, to fit all scopes	Adult, pediatric: 4 color-coded sizes (purple, orange, green and blue) to fit all scopes	Adult, pediatric: 4 color-coded sizes (purple, orange, green and blue) to fit all scopes	Scope Distal End Diameter [mm]; Adult colonoscope 12.8-14.5 mm; Slim Adult colonoscope 11.5-13.0 mm
Advantages	Resection of wider areas; Suction and insufflation of air unaffected	Folds movement provides a dynamic picture - even the smallest polyps can be identified; Centers the scope in the middle of the lumen preventing sudden slip back and "red-out"; Projections allow traction to avoid sudden slippage around turns and flexures, improving scope's stability; Helps perform EMR	Delivers more tip control without compromising intubation - improving loop management; Early and controlled view of the upstream surface of large folds - no need for repeated intubation; Prevents sudden slip back and red out; Optimizes tip position during therapy and polyp retrieval	Maintains position during loop reduction, decreases slippage, anchoring during endoscopic therapy; Maintains identical depth and breadth of scope's viewing field; Minimal resistance on insertion; Easy ileum intubation
Disadvantages	Interfere with the field of view	Petechial marks on colon; Potential dislodgement; Larger model more effective than smaller; Ileum intubation may be difficult	Potential dislodgement	Ileum intubation may be difficult

recent meta-analysis that included 23 RCTs and almost 13000 participants[34], sensitivity analysis showed that the exclusion of one large study[18]-in which the quality of bowel preparation was significantly worse in the CAC arm- not only eliminated the existing heterogeneity, but also altered the synthetic outcome direction, showing a significant benefit of CAC *vs* conventional colonoscopy regarding ADR [OR (95%CI): 1.17 (1.04-1.33)][34]. Finally, one meta-analysis[33] has evaluated the effect of CAC on the ADR of the right colon. Pooled data from 4 studies (2546 and 2547 patients in the CAC and the conventional arms, respectively) associated CAC with an increased right colon ADR [OR (95%CI): 1.49 (1.08-2.05)] compared to conventional colonoscopy[33].

THE ENDOCUFF

The first generation Endocuff

Endocuff (Arc Medical Design, Leeds, United Kingdom) is a single-use soft, radiopaque device that consists of a cylindrical polypropylene core and 2 rows of flexible thermoplastic elastomer-made projections. Each row counts 8 projections that emerge from gaps on the shaft of the device (Figure 2A and B). It is available in 4 different color-coded sizes to fit all scopes and its technical characteristics are presented in Table 1. Its designers were inspired through the practical difficulties that occur during a conventional colonoscopy, including the scope slipping back, difficulties in tip stabilization

Table 2 Meta-analyses evaluating the effect of accessories on colonoscopy outcomes

Author (yr)	Device *vs* comparator	Included Studies (*n*)	Included studies' design	Patients (*n*)	ADR	PDR	MAC	CIR	CIT
Westwood 2012	CAC *vs* CC	12 (9 FP, 3 AB)	RCTs	6185	NR	[a]OR (95%CI): 1.13 (1.02-1.26)	NR	[a]OR (95%CI): 1.36 (1.06-1.74)	MD (95%CI): 0.04 (-0.03 to 0.12) min
Ng 2012	CAC *vs* CC	16 (13 FP, 3 AB)	RCTs	8991	RR (95%CI): 1.04 (0.90-1.19)	[a]RR (95%CI): 1.08 (1.00-1.17)	NR	RR (95%CI): 1.00 (0.90-1.02)	[a]MD (95%CI): -0.64 (-1.19 to -0.10) min
He 2012	CAC *vs* CC	19 (14 FP, 5 AB)	RCTs	9235	NR	[a]OR (95%CI): 1.12 (1.02-1.22)	NR	[a]OR (95%CI): 1.36 (1.13-1.64)	[a]MD (95%CI): -0.65 (-0.85 to −0.44) min
Omata 2014	CAC *vs* CC	10 (10 FP)	RCTs	5219	RR (95%CI): 1.07 (0.94-1.23)	RR (95%CI): 1.00 (0.86-1.16)	NR	NR	NR
Desai 2017	CAC *vs* CC	4 (4 FP)	2 RCTs; 2 retrospective	5093	[a,1]OR (95%CI): 1.49 (1.08-2.05)	NR	NR	NR	NR
Mir 2017	CAC *vs* CC	23 (18 FP, 5 AB)	RCTs	12947	OR (95%CI): 1.11 (0.95-1.30)	[a]OR (95%CI): 1.17 (1.06-1.29)	NR	OR (95%CI): 1.32 (0.94-1.87)	[a]MD (95%CI): -0.82 (-1.20 to -0.44) min
Chin 2016	[2]EAC *vs* CC	9 (4FP, 5 AB)	4 RCTs; 1 prospective observational; 4 retrospective	5624	[a]OR (95%CI): 1.49 (1.23-1.80)	NR	NR	OR (95%CI): 1.26 (0.70-2.27)	NR
Williet 2018	[2]EAC *vs* CC	12 (7 FP, 5 AB)	RCTs	8376	[a]RR (95%CI): 1.20 (1.06-1.36)	[a]RR (95%CI): 1.20 (1.06-1.36)	MD (95%CI): 0.11 (-0.17-0.38)	RR (95%CI): 0.99 (0.97- 1.00)	MD (95%CI): -0.57 (-1.43 to 0.28) min
[3]Facciorusso 2017	CAC *vs* CC	14 (14 FP)	RCTs	8306	RR (95%CI): 1.07 (0.96-1.19)	RR (95%CI): 1.08 (0.99-1.18)	NR	RR (95%CI): 1.00 (1.00- 1.01)	[a]MD (95%CI): -0.68 (-1.11 to -0.24) min
	[2]EAC *vs* CC	9 (4FP, 5 AB)	RCTs	7072	[a]RR (95%CI): 1.21 (1.03-1.41)	[a]RR (95%CI): 1.22 (1.07-1.40)	NR	RR (95%CI): 1.00 (0.98- 1.01)	[a]MD (95%CI): -0.93 (-1.55 to -0.30) min
	Endorings *vs* CC	1 (1 FP)	RCTs	116	RR (95%CI): 1.70 (0.86-3.36)	RR (95%CI): 1.68 (0.94-2.99)	NR	NR	MD (95%CI): 0.90 (-1.47 to 3.27) min

[1]refers to right colon ADR; [2]refers to both first generation Endocuff and Endocuff Vision; [3]network meta-analysis. [a]Statistical significant. ADR: Adenoma detection rate; AMR: Adenoma miss rate; PDR: Polyp detection rate; MAC: Mean adenomas detected per colonoscopy; CIR: Cecal intubation rate; CIT: Cecal intubation time; CAC: Cap-assisted colonoscopy; CC: Conventional colonoscopy; EAC: Endocuff-assisted colonoscopy; FP: Full paper; AB: Abstract; RCT: Randomized controlled trial; NR: Not reported; OR: Odds ratio; 95%CI: 95% confidence intervals; RR: Relative risk; MD: Mean difference.

Figure 2 Endocuff (A) mounted on the tip of the scope (B) and the endoscopic view of the hinged projections during the withdrawal phase (C) (photos from the authors' archive).

and inability to inspect the mucosa located behind folds, to mention a few. Endocuff was launched in 2012 and its use was reported for the first time in a small retrospective feasibility study where it facilitated endoscopic access for complex polypectomy and scar assessment in the sigmoid colon[36]. The projections move independently from another in a passive way when in contact with the mucosa and during withdrawal, they extend radially manipulating colonic folds away from the field of view, allowing a more meticulous mucosa inspection (Figure 2C). Moreover, the device stabilizes the scope in the middle of the lumen and allows traction against sudden slippage around flexures. Moreover, the examiner's visibility is not affected, since the device does

not extend beyond the tip of the scope and thus does not interfere with suction, flushing or the working channel.

There are enough data regarding the effect of Endocuff on colonoscopy outcomes, since seven RCTs of parallel[37-43] and one of tandem[44] design have been published. The first German studies[37,38]-each recruiting almost 500 patients who underwent colonoscopy for various indications (screening included)-showed a significant benefit of Endocuff-assisted colonoscopy (EAC) compared to the conventional one regarding ADR (35.4% *vs* 20.7%, $P < 0.0001$ and 36% *vs* 28%, $P = 0.043$, respectively)[37,38]. Similar results regarding PDR were also achieved (55.4% *vs* 38.4%, $P < 0.0001$ and 56% *vs* 42%, $P = 0.001$, respectively), while only the second study[38] detected a difference in the mean number of adenomas detected per colonoscopy [2 (IQR: 1-3) *vs* 1 (IQR: 1-2), $P = 0.002$]. Regarding polyp location, both studies identified a superiority of EAC for the detection of polyps located in the sigmoid and the cecum. Moreover, no major adverse events related to EAC were reported and there were no differences between overall procedure and withdrawal times[37,38].

Similar positive results associated to Endocuff use were reported from Japan[39] (477 patients, mixed indications for colonoscopy) and Mexico[40] (337 screening individuals), where two single-centre RCTs demonstrated increase of ADR (55.2% *vs* 39.2%, $P = 0.0002$ and 22.4% *vs* 13.5%, $P = 0.02$, respectively), PDR (61.9% *vs* 49.2%, $P = 0.003$ and 29.9% *vs* 16%, $P = 0.002$, respectively) and MAC (1.11 *vs* 0.66, $P < 0.01$ and 0.29 *vs* 0.22, $P = 0.04$) in the device arms. An Italian single-centre study by De Palma *et al*[41] enrolled 288 patients with mixed indications and reported that EAC increased ADR by 3.3% (29.6% *vs* 26.3%) compared to the conventional colonoscopy. However, use of Endocuff was associated with mucosal erosions in 7 (2.5%) cases, with one of them needing to be treated with adrenaline solution injection at the site of bleeding[41].

Additionally, in a recently published 4-arm multicenter parallel-group study comparing Endocuff, Endorings, FUSE and conventional colonoscopy, 299 and 295 patients underwent Endocuff-assisted and conventional high definition colonoscopy, respectively[42]. EAC performed significantly better compared to conventional colonoscopy in terms of ADR (64% *vs* 56%, $P = 0.003$), PDR (83% *vs* 77%, $P = 0.001$) and MAC (1.82 ± 2.58 *vs* 1.53 ± 2.33, $P = 0.014$)[42]. However, Endocuff did not enhance the detection rate of sessile serrated polyps (11% *vs* 12%, $P = 0.047$)[42]. There were no differences between the mean insertion time (354 s ± 216 s *vs* 422 s ± 319 s) for Endocuff-assisted and conventional colonoscopy respectively and no adverse events were reported[42]. On the contrary, a benefit regarding sessile serrated adenoma/polyp detection was shown in a retrospective veterans' study[45] which included almost 500 participants: Endocuff detected 50 sessile serrated adenomas/polyps compared to 8 detected by conventional colonoscopy (detection rate 15% *vs* 3%, $P < 0.0001$).

So far, the largest parallel RCT[43] failed to confirm the positive results reported in the abovementioned studies. In this multicentre study from the Netherlands[43] more than 1000 patients of various indications were randomized to undergo either Endocuff-assisted or conventional colonoscopy; MAC and ADR consisted the primary outcomes. ADR was the same in both groups (52%, $P = 0.92$), whereas the higher number of adenomas per patient in the Endocuff group (1.36 ± 2.10 *vs* 1.17 ± 1.65) did not reach statistical significance ($P = 0.08$). Interestingly, detection rates did not differ either according to indication or between academic and non-academic centres[43]. Cecal intubation time was significantly shorter in the Endocuff arm [median (IQR) 7 min (5-10) *vs* 8.3 min (6-12), $P < 0.001$] and there were no Endocuff-associated adverse events[43].

Finally, a multicentre back-to-back study[44] assessed Endocuff in terms of adenoma miss rates. Two hundred patients (86.5% were screening and surveillance cases) were randomized (1:1) to undergo either initial EAC followed by a conventional one or vice versa[44]. EAC was associated with lower adenoma miss rates, both overall and in the proximal colon compared to conventional colonoscopy (14.7% *vs* 38.4% and 10.4% *vs* 38.9%, respectively)[44]. It is worthy to note that all examinations were performed by endoscopists with an historical ADR > 35%, suggesting that the device could enhance detection ability even of experienced and skilled endoscopists. Despite the fact that there were no serious adverse events, in three index Endocuff examinations cecal intubation failed to be achieved, compared to none with the conventional scope ($P = 0.08$)[44].

The Endocuff Vision

Despite its revolutionary design, Endocuff was associated with a couple of drawbacks (mucosal erosions and difficulties in terminal ileum intubation) that paved to way for its descendant, namely Endocuff Vision (Norgine Pharmaceuticals Ltd, Uxbridge, United Kingdom). This single-use device is made of a polypropylene cylinder and a single row of 8-longer than in the first generation Endocuff-thermoplastic elastomer-made "spikes" (Figure 3). There are 4 different sizes with respective colors to fit in all scopes ranging from pediatric to adult ones (Table 1). Endocuff Vision is also mounted onto the tip of the scope before insertion and its "spikes" fold around the scope while it advances in the colon due to a hinge at the base of each spike that thins progressively. On the other hand, the "spikes" evert during withdrawal (Figure 3). This leads to an early and controlled view of the upstream surface of the large colonic folds in the right colon and prevents sudden scope slip-back. Moreover, when in the sigmoid colon, the device facilitates the opening of contracted folds, permitting a clearer view of the in-between mucosa. Similar to the first generation Endocuff it optimizes the tip's position during endoscopically applied therapy (*e.g.*, polypectomy).

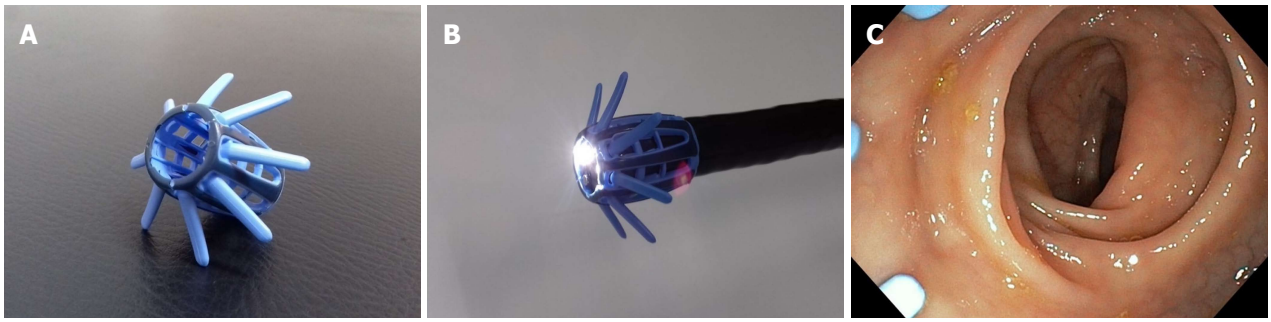

Figure 3 Endocuff-Vision (A), illustration (B) and endoscopic view (C) of the opened-out projections during the withdrawal phase (photos from the authors' archive).

Endocuff Vision has been evaluated only in two parallel multicenter RCTs from the United Kingdom[46,47]. The "ADENOMA" study[46] recruited 1772 adult patients (45% screening). Of them, 884 underwent conventional colonoscopy and 886 Endocuff Vision-assisted colonoscopy. ADR was significantly higher with EAC compared to conventional colonoscopy (40.9% *vs* 36.2%, $P = 0.02$). The benefit of Endocuff Vision was even higher in patients participating in the screening program, where ADR was 61.5% for EAC compared to 50.9% ($P < 0.001$) for the conventional colonoscopy arm. Similar results in favor of EAC were also reported regarding PDR (54.1% *vs* 48%; $P = 0.005$ and 73.9% *vs* 63.3%; $P < 0.001$ for the whole and the screening cohorts, respectively). Of note, EAC showed a statistically significant increase in the detection rate in the left colon (26.1% *vs* 2.2%; $P = 0.03$), of small (10.6% *vs* 7.7%; $P = 0.02$) and of diminutive adenomas (34.6% *vs* 30.8%; $P = 0.04$). It should be underlined that in this study[46] EAC detected significantly more cancers both in the whole cohort (4.1% *vs* 2.3%; $P = 0.02$) as well as in the screening participants (6.6% *vs* 3.7%; $P = 0.03$). Moreover, median insertion time was shorter with Endocuff Vision compared to conventional colonoscopy (8 min *vs* 9 min; $P = 0.001$). The investigators did not report any adverse event related to use of Endocuff Vision; however the device had to be removed in 4.1% of the cases mostly due to acute angulation in a fixed sigmoid colon.

On the contrary, the "E-cap" study failed to show any benefit in terms of ADR (60.9% *vs* 63%, $P = 0.85$), PDR (70.3% *vs* 69.8%, $P = 0.93$) and MAC (1.3 ± 1.8 *vs* 1.4 ± 1.5, $P = 0.54$)[47]. This single center study had PDR as the primary endpoint. Only patients attending the national screening program with a positive FOBT test were enrolled and all four participating endoscopists had an extremely high pre-study ADR (58.9%)[47]. All these reasons may attribute to the lack of any significant benefit deriving from application of Endocuff Vision and should be considered in the design of future "real-life" studies, which should possibly include both endoscopists with an average or even a low ADR and patients with various indications for colonoscopy.

Finally, a pilot evaluation study[48] demonstrated that Endocuff Vision was associated with an improvement in endoscopists' performance measured as increased ADR, increased MAC and decreased insertion time. In this non-randomized study[48], the investigators performed 410 screening colonoscopies in three periods (137 pre-Endocuff, 136 using Endocuff Vision and 137 post-Endocuff). Overall, an increase in ADR (16%, $P < 0.03$) and MAC (83%, $P = 0.007$) was noted between the pre-Endocuff and the Endocuff period; this benefit was maintained in the post-Endocuff period, where the device was not available. A potential explanation could be that during the Endocuff period the endoscopists had the chance to comprehend their flaws during the withdrawal phase, look for adenomas in more detail and improve their skills[49]. Interestingly, insertion time was statistically lower during the Endocuff period compared to pre- and post-Endocuff one (7 min *vs* 8 min, $P = 0.002$ and 7 min *vs* 9 min, $P = 0.002$, respectively)[48]; no adverse events were reported[48].

Data from meta-analyses

To date, three meta-analyses attempting to summarize the impact of Endocuff devices on colonoscopy outcomes have already been published[35,50,51] (Table 2).

The earliest one[50] meta-analyzed data from three published papers and six studies presented as abstracts, four of which with a prospective and five with a retrospective design. Eight studies ($n = 4387$) of mixed populations reported on ADR, which was measured to be higher for the Endocuff group [OR (95%CI): 1.49 (1.23-1.80), $I^2 = 50\%$][50]. In this pooled analysis, 27 patients (2.3%) in the Endocuff group experienced superficial mucosal lacerations[50].

A recently published meta-analysis updated these data by including only RCTs (7 published and 5 presented as abstracts)[51]. Regarding ADR, data from more than 8370 patients demonstrated a benefit of EAC compared to conventional colonoscopy [RR (95%CI): 1.20 (1.06-1.36), $P = 0.003$, $I^2 = 79\%$]. Of interest, this benefit was lower in the subgroup of studies with a mean conventional arm ADR > 45% [RR (95%CI): 1.01 (0.93-1.09), $P = 0.087$, $I^2 = 0$], while it was maximized in the subgroup of studies with a respective ADR lower than 35% [RR (95%CI): 1.51 (1.35-1.69), $P < 0.001$, $I^2 = 43\%$]. These data imply a potential ancillary role of

Figure 4 Endorings (A) mounted on the tip of the scope (B) and illustration of rings stretching during withdrawal phase (C) (photos courtesy of Endoaid).

the device especially for lower detectors[51]. Furthermore, a numerical higher MAC was detected in the Endocuff-assisted colonoscopy group, but this difference did not reach statistical significance [mean difference (95%CI): 0.11(-0.17 to 0.38][51]. Mean insertion times did not differ between the two groups and 4% of the Endocuff patients experienced adverse events (exclusively minor lacerations)[51]. This meta-analysis reported on additional outcomes such as advanced ADR and right colon ADR, with no difference detected between the two groups [RR (95%CI): 0.93 (0.76-1.13), P = 0.47 and RR (95%CI): 1.36 (0.80-2.34), P = 0.26, respectively]. However, the small number of studies included in the analysis regarding these outcomes warrants caution when attempting to generalize the respective results.

Finally, similar results were shown in a network meta-analysis investigating the comparative efficacy of distal attachments in increasing detection rates during colonoscopy[35]. The mixed effect estimate (including both pairwise and indirect treatment effects) supported that ADR increased significantly with EAC compared to the conventional examination [RR (95%CI): 1.21 (1.03-1.41)][35]. Interestingly and contrary to the meta-analysis from Williet *et al*[51] this network meta-analysis[35] calculated a very modest benefit of Endocuff regarding low (baseline ADR 10%) detectors [anticipated ADR (95%CI): 11 (10-12)%] compared to a more considerable effect [anticipated ADR (95%CI): 48 (14-56)%] on ADR of high detectors (baseline ADR 40%).

THE ENDORINGS

EndoRings (EndoAid Ltd., Caesarea, Israel) is a single-use scope attachment consisting of 2 layers of flexible, soft circular silicon rings placed on a cylindrical cuff (Figure 4A and B). Endorings fit on scopes of an outer diameter ranging from 12.8 mm to 14.5 mm and two sizes for adult and slim adult scopes are available (Table 1). The flexible rings deflect to the opposite direction during scope manipulation. In that way scope insertion is not affected -minimal resistance may be noted- as the rings fold at the side of the scope's shaft without projecting beyond the distal end of the scope. During withdrawal, the two rings deploy with the proximal-most circular ring creating a wider lumen fenestration by stretching the mucosa and colonic folds (Figure 4C) assisting the detection of otherwise "hidden" lesions. Moreover, the device maintains identical depth and width of scope viewing by stabilizing the scope, maintaining position during loop reduction, deceasing slippage and finally by anchoring during application of endoscopic therapy. Terminal ileum intubation is reported not to be limited by use of the device. Endorings has been evaluated in two RCTs[42,52]. In the first one, a multicentre back-to-back study[52], 116 patients of mixed indications were randomized to undergo initial examination using the Endorings followed by conventional colonoscopy or vice versa. Applying Endorings on the tip of the scope was associated with a statistically significant lower adenoma miss rate compared to conventional colonoscopy (10.4% *vs* 48.3%, P < 0.001). A similar benefit was also noted for polyp miss rates (9.1% *vs* 52.8%, P < 0.001). Endorings significantly decreased adenoma miss rates both in the proximal (10.6% *vs* 58.1%, P < 0.001) and the distal colon (10% *vs* 37%, P < 0.001). Cecal intubation time was shorter with conventional colonoscopy compared to EndoRings-assisted colonoscopy (8.4 min *vs* 9.3 min), but this difference did not achieve statistical significance.

In the aforementioned 4-arm parallel group multicenter study[42], 295 and 295 patients were allocated in the in Endorings and the conventional colonoscopy arms respectively. ADR did not differ between the two groups (57% *vs* 56%). Moreover, in one of the participating centers, insertion time was significantly increased when Endorings was used (251 s *vs* 170 s, P = 0.003), but this finding was not uniform for all participating centers (overall time to cecum: 263 s *vs* 319 s, no statistical difference). Of note, the device was not able to pass the sigmoid colon in 6 patients, thus authors commented that the larger the diameter of Endorings, the greater the difficulty of scope insertion.

CONCLUSION

Detecting and removing precancerous lesions remains the mainstay of screening colonoscopy. In this review, data on how 4 different colonoscope attachments influence detection rates were presented. The transparent

cap was the first device launched in the market. CAC achieves a better orientation during insertion by pushing away the folded mucosa and reduces the time needed to reach the cecum. During scope withdrawal, the cap opens and manipulates colonic folds, revealing otherwise obscured lesions[23]. CAC has been validated extensively and pooled data from numerous studies have linked it with a significant increase in polyp detection[34]. Taking into account its simplicity in use, it can be an interesting and accessible solution for colonoscopy outcome improvement.

Endocuff and Endocuff Vision - two similar detachable attachments - have been commercialized during the last years. They are fitted at the tip of the scope and enhance mucosa visualization through their opened-out projections that flatten the colonic folds. Data from studies conclude that their application increases ADR[35,50,51]. Furthermore, they can prove to be beneficial for both low adenoma detectors[51] (increase in ADR) as well as in high ones[44] (decrease in miss rates). Moreover, Endocuff and Endocuff Vision are safe, user-friendly and affordable devices. Taking this into account, Endocuff-assisted colonoscopy should be considered as a pivotal part of the physicians' arsenal in their effort to diminish missed precancerous lesions during colonoscopy.

Endorings is the last device that was presented. It functions in a -more or less- similar way to EAC. Its double-layered rings stretch the mucosa and optimize visualization. While significantly decreased adenoma miss rates have been reported[52], this device has been validated in only two studies[42,52] and more data are definitely needed to reach safe and generalizable conclusions regarding its effectiveness.

An issue that remains to be highlighted is the efficacy of these devices in terms of screening colonoscopy. To the best of our knowledge, no study has been conducted in an exclusively screening population yet. Thus, conclusions are extrapolated only from studies enrolling mixed populations (screening, symptomatic, surveillance). Although promising, more robust data are definitely needed in order to systematically assess the performance of these mechanical novelties overall and particularly in specific populations. Moreover, it is still unknown whether all levels of endoscopists are to benefit from their implementation. Finally, optimizing several useful and simultaneously low-cost existing alternatives (*e.g.*, water-aided colonoscopy, second observer, dynamic position change) may be as effective as these add-on devices in improving colonoscopy and patient outcomes. All the aforementioned reasons probably prevent these devices from being integrated into current everyday clinical practice worldwide.

In conclusion, technological advancements offer physicians various choices to improve their performance during colonoscopy. Undoubtedly, they can be considered an adjuvant approach to improve colonoscopy outcomes as they are feasible, easy-to-use, affordable and effective. At the same time, these devices should not be considered a panacea and physicians should always pay attention to other well-established quality measures such as high level education, adequate bowel preparation, and adequate withdrawal time to ensure that they offer their patients optimal clinical services.

REFERENCES

1 SEER Cancer Stat Facts: Colorectal Cancer. 2018
2 **Zauber AG**, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegooijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish JF, Stewart ET, Waye JD. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* 2012; **366**: 687-696 [PMID: 22356322 DOI: 10.1056/NEJMoa1100370]
3 **Corley DA**, Jensen CD, Marks AR, Zhao WK, Lee JK, Doubeni CA, Zauber AG, de Boer J, Fireman BH, Schottinger JE, Quinn VP, Ghai NR, Levin TR, Quesenberry CP. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014; **370**: 1298-1306 [PMID: 24693890 DOI: 10.1056/NEJMoa1309086]
4 **Kaminski MF**, Regula J, Kraszewska E, Polkowski M, Wojciechowska U, Didkowska J, Zwierko M, Rupinski M, Nowacki MP, Butruk E. Quality indicators for colonoscopy and the risk of interval cancer. *N Engl J Med* 2010; **362**: 1795-1803 [PMID: 20463339 DOI: 10.1056/NEJMoa0907667]
5 **Kaminski MF**, Wieszczy P, Rupinski M, Wojciechowska U, Didkowska J, Kraszewska E, Kobiela J, Franczyk R, Rupinska M, Kocot B, Chaber-Ciopinska A, Pachlewski J, Polkowski M, Regula J. Increased Rate of Adenoma Detection Associates With Reduced Risk of Colorectal Cancer and Death. *Gastroenterology* 2017; **153**: 98-105 [PMID: 28428142 DOI: 10.1053/j.gastro.2017.04.006]
6 **Kaminski MF**, Thomas-Gibson S, Bugajski M, Bretthauer M, Rees CJ, Dekker E, Hoff G, Jover R, Suchanek S, Ferlitsch M, Anderson J, Roesch T, Hultcranz R, Racz I, Kuipers EJ, Garborg K, East JE, Rupinski M, Seip B, Bennett C, Senore C, Minozzi S, Bisschops R, Domagk D, Valori R, Spada C, Hassan C, Dinis-Ribeiro M, Rutter MD. Performance measures for lower gastrointestinal endoscopy: a European Society of Gastrointestinal Endoscopy (ESGE) Quality Improvement Initiative. *Endoscopy* 2017; **49**: 378-397 [PMID: 28268235 DOI: 10.1055/s-0043-103411]
7 **Heresbach D**, Barrioz T, Lapalus MG, Coumaros D, Bauret P, Potier P, Sautereau D, Boustière C, Grimaud JC, Barthélémy C, Sée J, Serraj I, D'Halluin PN, Branger B, Ponchon T. Miss rate for colorectal neoplastic polyps: a prospective multicenter study of back-to-back video colonoscopies. *Endoscopy* 2008; **40**: 284-290 [PMID: 18389446 DOI: 10.1055/s-2007-995618]
8 **Leufkens AM**, van Oijen MG, Vleggaar FP, Siersema PD. Factors influencing the miss rate of polyps in a back-to-back colonoscopy study. *Endoscopy* 2012; **44**: 470-475 [PMID: 22441756 DOI: 10.1055/s-0031-1291666]
9 **Rex DK**, Cutler CS, Lemmel GT, Rahmani EY, Clark DW, Helper DJ, Lehman GA, Mark DG. Colonoscopic miss rates of adenomas determined by back-to-back colonoscopies. *Gastroenterology* 1997; **112**: 24-28 [PMID: 8978338 DOI: 10.1016/S0016-5085(97)70214-2]
10 **Lee J**, Park SW, Kim YS, Lee KJ, Sung H, Song PH, Yoon WJ, Moon JS. Risk factors of missed colorectal lesions after colonoscopy. *Medicine* (Baltimore) 2017; **96**: e7468 [PMID: 28682916 DOI: 10.1097/MD.0000000000007468]
11 **Robertson DJ**, Lieberman DA, Winawer SJ, Ahnen DJ, Baron JA, Schatzkin A, Cross AJ, Zauber AG, Church TR, Lance P, Greenberg ER, Martínez ME. Colorectal cancers soon after colonoscopy: a pooled multicohort analysis. *Gut* 2014; **63**: 949-956 [PMID: 23793224 DOI: 10.1136/gutjnl-2012-303796]
12 **Xiang L**, Zhan Q, Zhao XH, Wang YD, An SL, Xu YZ, Li AM, Gong W, Bai Y, Zhi FC, Liu SD. Risk factors associated with missed colorectal flat adenoma: a multicenter retrospective tandem colonoscopy study. *World J Gastroenterol* 2014; **20**: 10927-10937 [PMID: 25152596 DOI: 10.3748/wjg.v20.i31.10927]
13 **Gkolfakis P**, Tziatzios G, Dimitriadis GD, Triantafyllou K. New endoscopes and add-on devices to improve colonoscopy performance. *World J Gastroenterol* 2017; **23**: 3784-3796 [PMID: 28638218 DOI:

10.3748/wjg.v23.i21.3784]
14 **Tada M**, Inoue H, Yabata E, Okabe S, Endo M. Colonic mucosal resection using a transparent cap-fitted endoscope. *Gastrointest Endosc* 1996; **44**: 63-65 [PMID: 8836719 DOI: 10.1016/S0016-5107(96)70231-6]
15 **Kondo S**, Yamaji Y, Watabe H, Yamada A, Sugimoto T, Ohta M, Ogura K, Okamoto M, Yoshida H, Kawabe T, Omata M. A randomized controlled trial evaluating the usefulness of a transparent hood attached to the tip of the colonoscope. *Am J Gastroenterol* 2007; **102**: 75-81 [PMID: 17100978 DOI: 10.1111/j.1572-0241.2006.00897.x]
16 **Horiuchi A**, Nakayama Y. Improved colorectal adenoma detection with a transparent retractable extension device. *Am J Gastroenterol* 2008; **103**: 341-345 [PMID: 18076740 DOI: 10.1111/j.1572-0241.2007.01555.x]
17 **Harada Y**, Hirasawa D, Fujita N, Noda Y, Kobayashi G, Ishida K, Yonechi M, Ito K, Suzuki T, Sugawara T, Horaguchi J, Takasawa O, Obana T, Oohira T, Onochi K, Kanno Y, Kuroha M, Iwai W. Impact of a transparent hood on the performance of total colonoscopy: a randomized controlled trial. *Gastrointest Endosc* 2009; **69**: 637-644 [PMID: 19251004 DOI: 10.1016/j.gie.2008.08.029]
18 **Lee YT**, Lai LH, Hui AJ, Wong VW, Ching JY, Wong GL, Wu JC, Chan HL, Leung WK, Lau JY, Sung JJ, Chan FK. Efficacy of cap-assisted colonoscopy in comparison with regular colonoscopy: a randomized controlled trial. *Am J Gastroenterol* 2009; **104**: 41-46 [PMID: 19098847 DOI: 10.1038/ajg.2008.56]
19 **Hewett DG**, Rex DK. Cap-fitted colonoscopy: a randomized, tandem colonoscopy study of adenoma miss rates. *Gastrointest Endosc* 2010; **72**: 775-781 [PMID: 20579648 DOI: 10.1016/j.gie.2010.04.030]
20 **Tee HP**, Corte C, Al-Ghamdi H, Prakoso E, Darke J, Chettiar R, Rahman W, Davison S, Griffin SP, Selby WS, Kaffes AJ. Prospective randomized controlled trial evaluating cap-assisted colonoscopy vs standard colonoscopy. *World J Gastroenterol* 2010; **16**: 3905-3910 [PMID: 20712051 DOI: 10.3748/wjg.v16.i31.3905]
21 **Takeuchi Y**, Inoue T, Hanaoka N, Higashino K, Iishi H, Chatani R, Hanafusa M, Kizu T, Ishihara R, Tatsuta M, Shimokawa T, Uedo N. Autofluorescence imaging with a transparent hood for detection of colorectal neoplasms: a prospective, randomized trial. *Gastrointest Endosc* 2010; **72**: 1006-1013 [PMID: 21034901 DOI: 10.1016/j.gie.2010.06.055]
22 **de Wijkerslooth TR**, Stoop EM, Bossuyt PM, Mathus-Vliegen EM, Dees J, Tytgat KM, van Leerdam ME, Fockens P, Kuipers EJ, Dekker E. Adenoma detection with cap-assisted colonoscopy versus regular colonoscopy: a randomised controlled trial. *Gut* 2012; **61**: 1426-1434 [PMID: 22187070 DOI: 10.1136/gutjnl-2011-301327]
23 **Rastogi A**, Bansal A, Rao DS, Gupta N, Wani SB, Shipe T, Gaddam S, Singh V, Sharma P. Higher adenoma detection rates with cap-assisted colonoscopy: a randomised controlled trial. *Gut* 2012; **61**: 402-408 [PMID: 21997547 DOI: 10.1136/gutjnl-2011-300187]
24 **Pohl H**, Bensen SP, Toor A, Gordon SR, Levy LC, Berk B, Anderson PB, Anderson JC, Rothstein RI, MacKenzie TA, Robertson DJ. Cap-assisted colonoscopy and detection of Adenomatous Polyps (CAP) study: a randomized trial. *Endoscopy* 2015; **47**: 891-897 [PMID: 26126162 DOI: 10.1055/s-0034-1392261]
25 **Huang RX**, Xiao ZL, Li F, Ji DN, Zhou J, Xiang P, Bao ZJ. Black hood assisted colonoscopy for detection of colorectal polyps: a prospective randomized controlled study. *Eur Rev Med Pharmacol Sci* 2016; **20**: 3266-3272 [PMID: 27467003]
26 **Othman MO**, Zhang D, Elhanafi S, Eloliby M, Davis B, Guererro R, Alvarado L, Sanchez L, Dwivedi A, Zuckerman MJ. Cap-Assisted Colonoscopy Increases Detection of Advanced Adenomas and Polyps. *Am J Med Sci* 2017; **353**: 367-373 [PMID: 28317624 DOI: 10.1016/j.amjms.2017.01.015]
27 **Tang Z**, Zhang DS, Thrift AP, Patel KK. Impact of cap-assisted colonoscopy on the learning curve and quality in colonoscopy: a randomized controlled trial. *Gastrointest Endosc* 2018; **87**: 723-732.e3 [PMID: 28648577 DOI: 10.1016/j.gie.2017.06.011]
28 **Park SM**, Lee SH, Shin KY, Heo J, Sung SH, Park SH, Choi SY, Lee DW, Park HG, Lee HS, Jeon SW, Kim SK, Jung MK. The cap-assisted technique enhances colonoscopy training: prospective randomized study of six trainees. *Surg Endosc* 2012; **26**: 2939-2943 [PMID: 22538693 DOI: 10.1007/s00464-012-2288-2]
29 **Westwood DA**, Alexakis N, Connor SJ. Transparent cap-assisted colonoscopy versus standard adult colonoscopy: a systematic review and meta-analysis. *Dis Colon Rectum* 2012; **55**: 218-225 [PMID: 22228167 DOI: 10.1097/DCR.0b013e31823461ef]
30 **Ng SC**, Tsoi KK, Hirai HW, Lee YT, Wu JC, Sung JJ, Chan FK, Lau JY. The efficacy of cap-assisted colonoscopy in polyp detection and cecal intubation: a meta-analysis of randomized controlled trials. *Am J Gastroenterol* 2012; **107**: 1165-1173 [PMID: 22664471 DOI: 10.1038/ajg.2012.135]
31 **He Q**, Li JD, An SL, Liu SD, Xiao B, Zhang YL, Jiang B, Bai Y, Zhi FC. Cap-assisted colonoscopy versus conventional colonoscopy: systematic review and meta-analysis. *Int J Colorectal Dis* 2013; **28**: 279-281 [PMID: 22460306 DOI: 10.1007/s00384-012-1466-y]
32 **Omata F**, Ohde S, Deshpande GA, Kobayashi D, Masuda K, Fukui T. Image-enhanced, chromo, and cap-assisted colonoscopy for improving adenoma/neoplasia detection rate: a systematic review and meta-analysis. *Scand J Gastroenterol* 2014; **49**: 222-237 [PMID: 24328858 DOI: 10.3109/00365521.2013.863964]
33 **Desai M**, Sanchez-Yague A, Choudhary A, Pervez A, Gupta N, Vennalaganti P, Vennelaganti S, Fugazza A, Repici A, Hassan C, Sharma P. Impact of cap-assisted colonoscopy on detection of proximal colon adenomas: systematic review and meta-analysis. *Gastrointest Endosc* 2017; **86**: 274-281.e3 [PMID: 28365356 DOI: 10.1016/j.gie.2017.03.1524]
34 **Mir FA**, Boumitri C, Ashraf I, Matteson-Kome ML, Nguyen DL, Puli SR, Bechtold ML. Cap-assisted colonoscopy versus standard colonoscopy: is the cap beneficial? A meta-analysis of randomized controlled trials. *Ann Gastroenterol* 2017; **30**: 640-648 [PMID: 29118558 DOI: 10.20524/aog.2017.0180]
35 **Facciorusso A**, Del Prete V, Buccino RV, Della Valle N, Nacchiero MC, Monica F, Cannizzaro R, Muscatiello N. Comparative Efficacy of Colonoscope Distal Attachment Devices in Increasing Rates of Adenoma Detection: A Network Meta-analysis. *Clin Gastroenterol Hepatol* 2018; **16**: 1209-1219.e9 [PMID: 29133257 DOI: 10.1016/j.cgh.2017.11.007]
36 **Tsiamoulos ZP**, Saunders BP. A new accessory, endoscopic cuff, improves colonoscopic access for complex polyp resection and scar assessment in the sigmoid colon (with video). *Gastrointest Endosc* 2012; **76**: 1242-1245 [PMID: 23164515 DOI: 10.1016/j.gie.2012.08.019]
37 **Floer M**, Biecker E, Fitzlaff R, Röming H, Ameis D, Heinecke A, Kunsch S, Ellenrieder V, Ströbel P, Schepke M, Meister T. Higher adenoma detection rates with endocuff-assisted colonoscopy-a randomized controlled multicenter trial. *PLoS One* 2014; **9**: e114267 [PMID: 25470133 DOI: 10.1371/journal.pone.0114267]
38 **Biecker E**, Floer M, Heinecke A, Ströbel P, Böhme R, Schepke M, Meister T. Novel endocuff-assisted colonoscopy significantly increases the polyp detection rate: a randomized controlled trial. *J Clin Gastroenterol* 2015; **49**: 413-418 [PMID: 24921209 DOI: 10.1097/MCG.0000000000000166]
39 **Wada Y**, Fukuda M, Ohtsuka K, Watanabe M, Fukuma Y, Wada Y, Wada M. Efficacy of Endocuff-assisted colonoscopy in the detection of colorectal polyps. *Endosc Int Open* 2018; **6**: E425-E431 [PMID: 29607395 DOI: 10.1055/s-0044-101142]
40 **González-Fernández C**, García-Rangel D, Aguilar-Olivos NE, Barreto-Zúñiga R, Romano-Munive AF, Grajales-Figueroa G, Zamora-Nava LE, Téllez-Avila FI. Higher adenoma detection rate with the endocuff: a randomized trial. *Endoscopy* 2017; **49**: 1061-1068 [PMID: 28898920 DOI: 10.1055/s-0043-117879]
41 **De Palma GD**, Giglio MC, Bruzzese D, Gennarelli N, Maione F, Siciliano S, Manzo B, Cassese G, Luglio G. Cap cuff-assisted colonoscopy versus standard colonoscopy for adenoma detection: a randomized back-to-back study. *Gastrointest Endosc* 2018; **87**: 232-240 [PMID: 28082115 DOI: 10.1016/j.gie.2016.12.027]
42 **Rex DK**, Repici A, Gross SA, Hassan C, Ponugoti PL, Garcia JR, Broadley HM, Thygesen JC, Sullivan AW, Tippins WW, Main SA, Eckert GJ, Vemulapalli KC. High-definition colonoscopy versus Endocuff versus EndoRings versus full-spectrum endoscopy for adenoma detection at colonoscopy: a multicenter randomized trial.

Gastrointest Endosc 2018; **88**: 335-344.e2 [PMID: 29530353 DOI: 10.1016/j.gie.2018.02.043]

43 **van Doorn SC**, van der Vlugt M, Depla A, Wientjes CA, Mallant-Hent RC, Siersema PD, Tytgat K, Tuynman H, Kuiken SD, Houben G, Stokkers P, Moons L, Bossuyt P, Fockens P, Mundt MW, Dekker E. Adenoma detection with Endocuff colonoscopy versus conventional colonoscopy: a multicentre randomised controlled trial. *Gut* 2017; **66**: 438-445 [PMID: 26674360 DOI: 10.1136/gutjnl-2015-310097]

44 **Triantafyllou K**, Polymeros D, Apostolopoulos P, Lopes Brandao C, Gkolfakis P, Repici A, Papanikolaou IS, Dinis-Ribeiro M, Alexandrakis G, Hassan C. Endocuff-assisted colonoscopy is associated with a lower adenoma miss rate: a multicenter randomized tandem study. *Endoscopy* 2017; **49**: 1051-1060 [PMID: 28763808 DOI: 10.1055/s-0043-114412]

45 **Baek MD**, Jackson CS, Lunn J, Nguyen C, Shah NK, Serrao S, Juma D, Strong RM. Endocuff assisted colonoscopy significantly increases sessile serrated adenoma detection in veterans. *J Gastrointest Oncol* 2017; **8**: 636-642 [PMID: 28890813 DOI: 10.21037/jgo.2017.03.07]

46 **Ngu WS**, Bevan R, Tsiamoulos ZP, Bassett P, Hoare Z, Rutter MD, Clifford G, Totton N, Lee TJ, Ramadas A, Silcock JG, Painter J, Neilson LJ, Saunders BP, Rees CJ. Improved adenoma detection with Endocuff Vision: the ADENOMA randomised controlled trial. *Gut* 2018; Epub ahead of print [PMID: 29363535]

47 **Bhattacharyya R**, Chedgy F, Kandiah K, Fogg C, Higgins B, Haysom-Newport B, Gadeke L, Thursby-Pelham F, Ellis R, Goggin P, Longcroft-Wheaton G, Bhandari P. Endocuff-assisted vs. standard colonoscopy in the fecal occult blood test-based UK Bowel Cancer Screening Programme (E-cap study): a randomized trial. *Endoscopy* 2017; **49**: 1043-1050 [PMID: 28614895 DOI: 10.1055/s-0043-111718]

48 **Tsiamoulos ZP**, Misra R, Rameshshanker R, Elliott TR, Beintaris I, Thomas-Gibson S, Haycock A, Suzuki N, Rees C, Saunders BP. Impact of a new distal attachment on colonoscopy performance in an academic screening center. *Gastrointest Endosc* 2018; **87**: 280-287 [PMID: 28412271 DOI: 10.1016/j.gie.2017.04.001]

49 **Bugajski M**, Kaminski MF. Devices for adenoma detection rate: Holy Grail or training tool? *Gastrointest Endosc* 2018; **87**: 241-242 [PMID: 29241852 DOI: 10.1016/j.gie.2017.07.041]

50 **Chin M**, Karnes W, Jamal MM, Lee JG, Lee R, Samarasena J, Bechtold ML, Nguyen DL. Use of the Endocuff during routine colonoscopy examination improves adenoma detection: A meta-analysis. *World J Gastroenterol* 2016; **22**: 9642-9649 [PMID: 27920485 DOI: 10.3748/wjg.v22.i43.9642]

51 **Williet N**, Tournier Q, Vernet C, Dumas O, Rinaldi L, Roblin X, Phelip JM, Pioche M. Effect of Endocuff-assisted colonoscopy on adenoma detection rate: meta-analysis of randomized controlled trials. *Endoscopy* 2018; **50**: 846-860 [PMID: 29698990 DOI: 10.1055/a-0577-3500]

52 **Dik VK**, Gralnek IM, Segol O, Suissa A, Belderbos TD, Moons LM, Segev M, Domanov S, Rex DK, Siersema PD. Multicenter, randomized, tandem evaluation of EndoRings colonoscopy--results of the CLEVER study. *Endoscopy* 2015; **47**: 1151-1158 [PMID: 26220283 DOI: 10.1055/s-0034-1392421]

P- Reviewer: Arya V, Qayed E, Rerknimitr R **S- Editor**: Wang XJ
L- Editor: A **E- Editor**: Yin SY

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4254-4262

DOI: 10.3748/wjg.v24.i37.4254

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

VSL#3 can prevent ulcerative colitis-associated carcinogenesis in mice

Chun-Sai-Er Wang, Wen-Bin Li, Hong-Ying Wang, Yi-Ming Ma, Xin-Hua Zhao, Hong Yang, Jia-Ming Qian, Jing-Nan Li

Chun-Sai-Er Wang, Wen-Bin Li, Hong Yang, Jia-Ming Qian, Jing-Nan Li, Department of Gastroenterology, PUMC Hospital, CAMS and PUMC, Beijing 100730, China

Hong-Ying Wang, Yi-Ming Ma, Xin-Hua Zhao, National Cancer Center/Cancer Hospital, CAMS and PUMC, Beijing 100021, China

ORCID number: Chun-Sai-Er Wang (0000-0002-4410-0834); Wen-Bin Li (0000-0003-0003-9768); Hong-Ying Wang (0000-0001-8443-3839); Yi-Ming Ma (0000-0002-4654-5642); Xin-Hua Zhao (0000-0002-4523-9416); Hong Yang (0000-0002-9475-1256); Jia-Ming Qian (0000-0001-6570-9262); Jing-Nan Li (0000-0002-9578-2580).

Author contributions: Wang CS and Li WB are the co-first author; Li JN conceived and designed the experiments; Wang CS and Li WB performed the experiments, and analyzed and interpreted the data; Wang CS and Li JN drafted the paper and revised it critically for important intellectual content; Wang HY, Ma YM, Zhao XH, Yang H, and Qian JM offered help during the experiments; all authors approved the final version of the manuscript.

Supported by the National Natural Science Foundation of China, No. 81370500 and No. 81770559.

Institutional animal care and use committee statement: All animal experiments were conducted in accordance with the recommendations of the Animal Care Ethics and Use Committee of Peking Union Medical College Hospital and approved by the same Committee (XHDW-2015-0032).

Conflict-of-interest statement: To the best of our knowledge, no conflict of interest exists.

Data sharing statement: No additional data are available.

ARRIVE guidelines statement: The ARRIVE Guidelines have been adopted.

Manuscript source: Unsolicited manuscript

Correspondence to: Jing-Nan Li, MD, Doctor, Department of Gastroenterology, PUMC Hospital, CAMS and PUMC, No.1 Shuai Fu Yuan, Beijing 100730, China. lijn2008@126.com
Telephone: +86-10-69155019
Fax: +86-10-69155017

Received: May 27, 2018
Peer-review started: May 27, 2018
First decision: July 6, 2018
Revised: August 7, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

AIM

To investigate the effects of VSL#3 on tumor formation, and fecal and intestinal mucosal microbiota in azoxymethane/dextran sulfate sodium (AOM/DSS) induced mice model.

METHODS

C57BL/6 mice were administered AOM/DSS to develop the ulcerative colitis (UC) carcinogenesis model. Mice were treated with 5-ASA (75 mg/kg/d), VSL#3 (1.5×10^9 CFU/d), or 5-ASA combined with VSL#3 by gavage from the day of AOM injection for three months (five days/week). The tumor load was compared in

each group, and tumor necrosis factor (TNF-α) and interleukin (IL)-6 levels were evaluated in colon tissue. The stool and intestinal mucosa samples were collected to analyze the differences in the intestinal microbiota by 16s rDNA sequencing method.

RESULTS

VSL#3 significantly reduced the tumor load in AOM/DSS-induced mice model and decreased the level of TNF-α and IL-6 in colon tissue. The model group had a lower level of *Lactobacillus* and higher level of *Oscillibacter* and *Lachnoclostridium* in fecal microbiota than the control group. After the intervention with 5-ASA and VSL#3, *Bacillus* and *Lactococcus* were increased, while *Lachnoclostridium* and *Oscillibacter* were reduced. 5-ASA combined with VSL#3 increased the *Lactobacillus* and decreased the *Oscillibacter*. The intestinal mucosal microbiota analysis showed a lower level of *Bifidobacterium* and *Ruminococcaceae*_UCG-014 and higher level of *Alloprevotella* in the model group as compared to the control group. After supplementation with VSL#3, *Bifidobacterium* was increased. 5-ASA combined with VSL#3 increased the level of both *Lachnoclostridium* and *Bifidobacterium*.

CONCLUSION

VSL#3 can prevent UC-associated carcinogenesis in mice, reduce the colonic mucosal inflammation levels, and rebalance the fecal and mucosal intestinal microbiota.

Key words: Tumor necrosis factor-α; VSL#3; Ulcerative colitis carcinogenesis; Interleukin-6; Intestinal microbiota

Core tip: Microbiota and chronic inflammation play an important role in the process of ulcerative colitis (UC)-associated carcinogenesis. Our study found VSL#3 could effectively prevent UC-associated carcinogenesis in azoxymethane/dextran sulfate sodium induced mice and decrease the level of tumor necrosis factor-α and IL-6 in colon tissue. The intestinal microbiota dysbiosis exists in UC-associated carcinogenesis. Supplementary VSL#3 is beneficial for rebalancing the fecal and mucosal intestinal microbiota. Based on the data presented here, VSL#3 may be a potential therapeutic agent for UC-associated carcinogenesis prevention.

Wang CS, Li WB, Wang HY, Ma YM, Zhao XH, Yang H, Qian JM, Li JN. VSL#3 can prevent ulcerative colitis-associated carcinogenesis in mice. *World J Gastroenterol* 2018; 24(37): 4254-4262 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4254.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4254

INTRODUCTION

Recently, the incidence of ulcerative colitis (UC) has shown an upward trend, leading to increased clinical attention on UC-associated carcinogenesis. A recent meta-analysis encompassing eight population-based cohort studies reported a 1.6% prevalence of colorectal cancer (CRC) in patients with UC, and the rate of CRC was 2.4-fold higher than that in the general population[1]. Moreover, the existing treatment for UC is not satisfactory for the prevention of carcinogenesis, involving several risks and side effects with long-term usage. Thus, finding new treatment regimens are essential.

Although the etiology of UC is yet to be elucidated, several studies have indicated that the host intestinal microbiota triggers an immune response that is requisite for the onset of the disease[2]. Microbiota also plays a major role in promoting UC-associated carcinogenesis. It downregulates the host immune response, improves the epithelial barrier function, and increases the mucus production[3]. Previous studies demonstrated that in the sterile intestinal environment, *i.e.*, the lack of intestinal microbiota, a significant reduction in carcinogenic mutations and intestinal tumor formation was observed[4]. Chronic inflammation plays a crucial role in UC-associated tumorigenesis *via* cellular DNA damage, telomere shortening, and senescence[5]. Previous studies demonstrated that probiotics exert a superior therapeutic effect on inflammation and UC[6]. VSL#3 is a mixture of *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis*, and *Streptococcus salivarius*[7]. It proved to be beneficial in the treatment of UC, including remission and relief of the relapse in mild to moderate disease[8-10]. Thus, we speculated that probiotic treatment or adjuvant treatment of UC could prevent carcinogenesis. One study demonstrated that VSL#3 can inhibit UC-associated carcinogenesis in a mouse model[11]. However, the mechanism underlying the VSL#3 treatment of UC carcinogenesis is yet to be elucidated.

Therefore, in the present study, VSL#3 was selected to investigate the effect of prevention on UC-associated carcinogenesis and the differences between fecal and mucosal microbiota were analyzed to gain a theoretical insight for the prevention of UC-associated carcinogenesis.

MATERIALS AND METHODS

Animals

Eight week old C57BL/6 male mice were purchased from the Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China), housed under 12 h light/dark cycle conditions (temperature 22 ± 1 ℃, humidity 40%-60%) in the National Cancer Center/Cancer Hospital animal facilities, and fed a standard diet for the duration of the study. All animal experiments were conducted in accordance with the recommendations of the Animal Care Ethics and Use Committee of Peking Union Medical College Hospital and approved by the same Committee

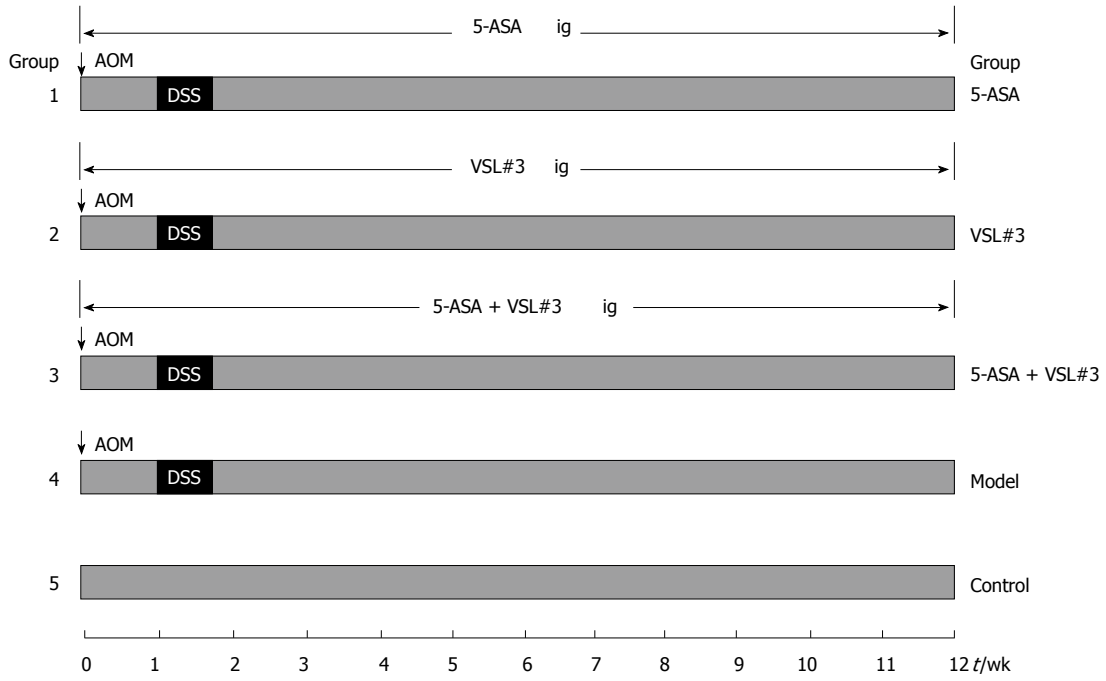

Figure 1 Experimental protocol for ulcerative colitis-associated carcinogenesis model and treatment.

(XHDW-2015-0032).

Development of UC-associated carcinogenesis model and in vivo treatment

All mice (n = 90) were initially housed together (5 animals/cage) for adaption one week before randomization into five experimental groups: control (no induction of UC-associated carcinogenesis, n = 10), model (no treatment) (n = 20), 5-ASA treatment (n = 20), VSL#3 treatment (n = 20), and 5-ASA + VSL#3 treatment (n = 20). In order to establish the UC-associated carcinogenesis model, mice were injected with 12.5 mg/kg body weight (BW) Azoxymethane (AOM) intraperitoneally, and after one week, 2.5% dextran sulfate sodium (DSS) (Mpbio, Solon, OH, United States) was added to their drinking water for five days, followed by ten weeks and two days of regular drinking water. This modeling method was based on a method described previously with some changes[12]. The three treatment groups, including 5-ASA, VSL#3, and 5-ASA + VSL#3 were gavaged 5-ASA (75 mg/kg BW, QD, Ferring Pharmaceuticals Ltd, solubilized in drinking water), VSL#3 (1.5×10^9 CFU/mice, QD, Sigma-Tau Pharmaceuticals Ltd, solubilized in drinking water), and 5-ASA + VSL#3 (75 mg/kg BW + 1.5×10^9 CFU/mice, QD) from the day of AOM injection. The control and model group were not subjected to gavage (Figure 1).

Specimen collection

The mice were sacrificed by the 12th week *via* transcardiac perfusion, and colon tissues were removed. The colons were slit longitudinally along the main axis and washed with 0.9% saline. The long and short diameter of each tumor was measured using sliding calipers, and the total tumor load of each colon was calculated (sum of the product of long and short diameter of each tumor). Subsequently, the whole colon was divided into four sections. The section near the anus washed with 0.9% saline to remove the non-adherent bacteria were flash-frozen in liquid nitrogen and stored at -80 ℃ for subsequent microbiota analysis. The remaining sections were used for enzyme-linked immunosorbent assays (ELISA) and histopathological examinations. A stool sample was collected just before AOM injection and sacrifice. A total of six mice were randomly selected from each group, and their stool and intestinal mucosa samples were sent to Allwegene (Beijing, China) for analyzing the differences in intestinal microbiota by 16S rDNA sequencing method.

Fecal DNA extraction and pyrosequencing

Microbial genomic DNA was isolated using a QIAamp DNA Micro Kit according to the manufacturer's instructions. The final quantity and quality of the DNA were assessed at 260 nm and 280 nm using an ultraviolet spectrophotometer and stored at -20 ℃ before further analysis. The V3-V4 hypervariable regions of the 16S rDNA gene were subjected to high-throughput sequencing by Allwegene using the Illumina Miseq PE300 sequencing platform (Illumina Inc., CA, United States).

ELISA for tumor necrosis factor-α and interleukin-6 in colon mucosa

The levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 in the colon mucosa were measured using commercial mouse TNF-α and IL-6 ELISA Kits (eBioscience, United States), according to the manu-

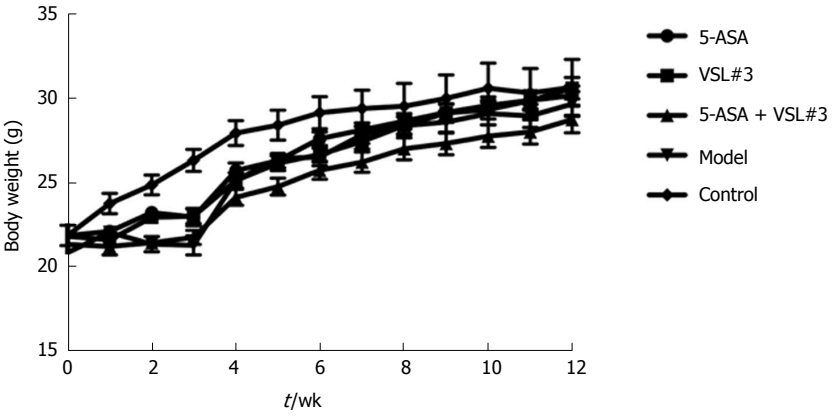

Figure 2 Body weight in each group.

facturer's protocols. The absorbance was measured at 450 nm. The results were expressed as pg/mg tissue. A total of eight mice were selected randomly from each group for ELISA.

Statistical analysis

Data are presented as mean ± SE. All statistical analyses were performed using GraphPad Prism Software Version 6.0 (GraphPad Software Inc., La Jolla, CA, United States). Statistical differences between experimental variants were assessed by two-tailed independent *t*-test, and data from more than two groups were analyzed by one-way ANOVA. Anosim and metastats analysis were used for microbiota analysis. $P < 0.05$ was considered statistically significant.

RESULTS

General health of mice in each group

As shown in Figure 2, compared to the control mice, the body weight loss was significantly higher in mice treated with azoxymethane/dextran sulfate sodium (AOM/DSS) after day 10 of DSS administration, which was accompanied by colitis symptoms, such as loose and bloody stool and dim body hair, fatigue, and less movement. These symptoms were alleviated when the mice received ordinary drinking water. In week 9, some mice treated with AOM/DSS presented bloody stool again, as well as, anal prolapse in week 10. However, no apparent weight loss was observed in the control mice, and no significant differences were detected among the five groups at the end of week 12.

Establishment of UC-associated carcinogenesis mice model

The mice were sacrificed by week 12, and the colorectal tumors were observed in the model and treatment groups (5-ASA, VSL#3, and 5-ASA + VSL#3). Strikingly, the tumor was primarily localized in the distal two-thirds of the colon. Anal tumor fusion and ring growth at the end of the rectum were observed in mice with anal prolapse (Figure 3). The pathological analysis showed mucosal carcinoma or high-grade intraepithelial neoplasia in mice treated with AOM/DSS. They were manifested with colonic gland structure disorder, large nuclei, deep staining, and nucleoplasmic ratio imbalance (Figure 4).

Effects of VSL#3 on UC-associated carcinogenesis

Treatment with AOM and DSS led to 100% (19/19, one mouse died during the experiment due to fighting) incidence of colonic neoplasms in the model group with the mean tumor load of 0.97 ± 0.19 cm. 5-ASA and VSL#3 administration significantly reduced both the tumor formation rate and the tumor load (Table 1 and Figure 5). Furthermore, no colonic tumor was detected in the control group.

Colonic TNF-α and IL-6 level comparison

As illustrated in Figure 6 and Tables 2 and 3, the levels of colonic tissue TNF-α and IL-6 in the model group were significantly higher than that in the control group. The increased levels of these inflammatory factors induced by AOM/DSS were attenuated by 5-ASA and VSL#3 treatment.

VSL#3 treatment alters the composition of fecal microbiota in AOM/DSS treated mice

In order to characterize the diversity of fecal-associated community in UC-associated carcinogenesis, we used Chao 1 and the observed species indexes, as well as the Shannon and Simpson indexes. No significant difference was detected in the diversity and composition of fecal microbiota in each group at the beginning of the experiment. After the 12-wk experiment, although no statistically significant difference was detected in the diversity among groups, the microbiota composition was altered considerably. The change in the composition of fecal microbiota induced by AOM/DSS administration was characterized by a decrease in *Lactobacillus* coupled with an increase in *Oscillibacter* and *Lachnoclostridium* as indicated by metastats analysis ($P < 0.05$). Both 5-ASA and VSL#3 supplementation was associated with a significant increase in *Bacillus* and *Lactococcus* and a decrease in *Oscillibacter* and

Table 1 Tumor formation rate and tumor load in each group

Group	*n*	Tumor formation rate (%)	Tumor load (cm)	*P* value (*vs* model group)
5-ASA	20	65.0 (13/20)	0.43 ± 0.14	0.0269
VSL#3	20	65.0 (13/20)	0.25 ± 0.07	0.0009
5-ASA + VSL#3	19	63.2 (12/19)	0.46 ± 0.11	0.0261
Model	19	100.0 (19/19)	0.97 ± 0.19	-
Control	10	0	0	-

Table 2 Level of tumor necrosis factor-α in colon tissue in each group

Group	*n*	TNF-α (pg/mg tissue)	*P* value (*vs* model group)
5-ASA	8	14.66 ± 0.72	< 0.05
VSL#3	8	25.89 ± 5.25	< 0.05
5-ASA + VSL#3	8	21.33 ± 4.55	< 0.05
Model	8	68.38 ± 18.73	-
Control	8	10.49 ± 0.30	< 0.01

TNF-α: Tumor necrosis factor.

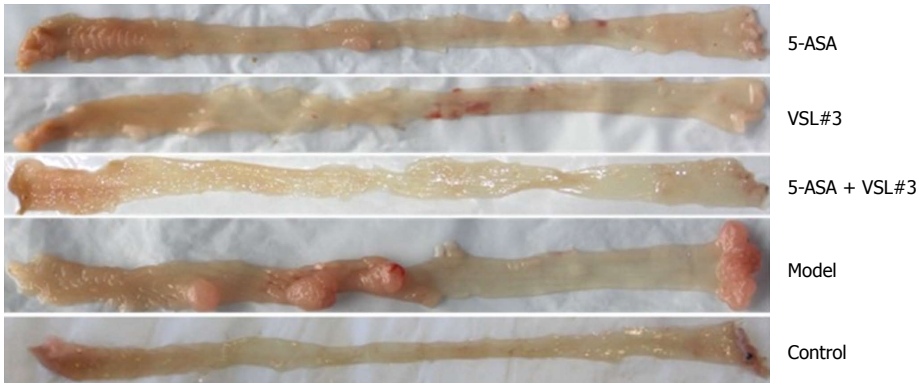

Figure 3 Representative image of colonic tumor in each group that was examined under naked eye.

Lachnoclostridium as compared to the model group ($P < 0.05$). 5-ASA combined with VSL#3 increased the level of *Lactobacillus* and decreased that of *Oscillibacter* ($P < 0.05$) (Table 4).

VSL#3 treatment alters the composition of mucosal microbiota in AOM/DSS treated mice

For the mucosal microbiota, no difference was observed in the community diversity among the groups after the 12-wk experiment. However, the distinct shift in the microbiota composition was observed by PCA and Anosim analysis ($R > 0$, $P < 0.05$). Further investigation into the discrete bacterial taxa revealed that *Ruminococcaceae* UCG-014 and *Bifidobacterium* decreased, while *Alloprevotella* increased in the model group compared to the control group. After supplementation with VSL#3, *Bifidobacterium* was increased. Although 5-ASA alone did not alter the mucosal microbiota, the combination with VSL#3 increased *Lachnoclostridium* and *Bifidobacterium* in the mucosa (Table 5).

DISCUSSION

The current study found that the rate of tumor formation and tumor load decreased after VSL#3 treatment compared to the model group, while the levels of TNF-α and IL-6 in the colon tissue in the model group were significantly higher than the control group. After the 12 wk treatment of VSL#3, the increase in TNF-α and IL-6 caused by AOM/DSS declined significantly. These findings were consistent with that of previous studies[11,13,14]. The major risk of long-term chronic inflammation is tumor occurrence[2]. Thus, we speculated that VSL#3 could prevent UC carcinogenesis by inhibiting the inflammatory response.

Herein, we found differences between the fecal and mucosal microbiota. In the case of fecal microbiota, the model group mice possessed less *Lactobacillus* and more *Oscillibacter* and *Lachnoclostridium* as compared to the control group. Previous studies have shown that *Lactobacillus bulgaricus* can reduce colitis[15], and *Lactobacillus rhamnosus* can effectively maintain UC remission[16]. *Oscillibacter* and *Lachnoclostridium* are newly discovered genera with respect to digestive diseases. In the case of mucosal microbiota, the level of the genus UCG-014 of Ruminococcaceae and Bifidobacterium decreased, while that of *Alloprevotella* increased in the model group as compared to the control

Table 3 Level of interleukin-6 in colon tissue in each group

Group	*n*	IL-6 (pg/mg tissue)	*P* value (*vs* model group)
5-ASA	8	28.19 ± 6.80	< 0.0001
VSL#3	8	99.71 ± 31.14	< 0.0500
5-ASA+VSL#3	8	81.43 ± 26.98	< 0.0100
Model	8	254.20 ± 32.49	-
Control	8	25.47 ± 5.50	< 0.0001

IL-6: Interleukin 6.

Table 4 Comparison of fecal microbiota (abundance)

Genus	Control (%)	Model (%)	5-ASA (%)	VSL#3 (%)	5-ASA + VSL#3 (%)
Lactobacillus	4.77	3.26[1]	2.65	4.06	9.86[4]
Oscillibacter	0.64	1.30[1]	0.54[2]	0.52[3]	0.79[4]
Lachnoclostridium	0.45	1.19[1]	0.48[2]	0.39[3]	1.08
Bacillus	1.01	0.98	24.00[2]	23.34[3]	0.66
Lactococcus	2.30	2.29	8.58[2]	7.86[3]	1.59

[1]$P < 0.05$ between the model and control groups; [2]$P < 0.05$ between the model and 5-ASA groups; [3]$P < 0.05$ between the model and VSL#3 groups; [4]$P < 0.05$ between the model and 5-ASA + VSL#3 groups.

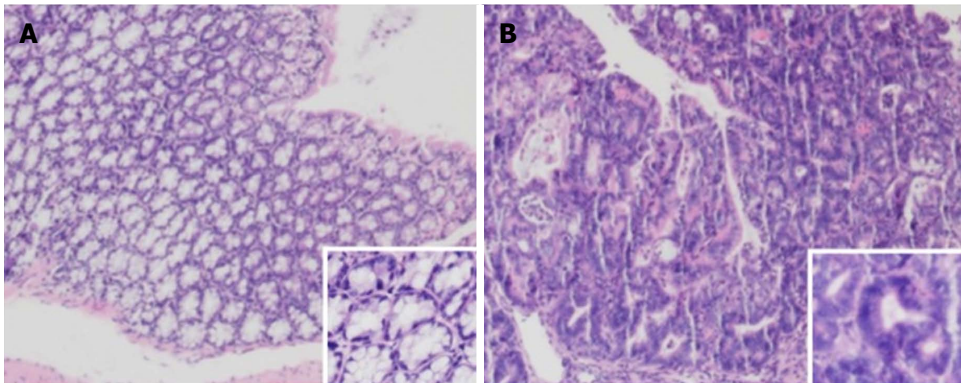

Figure 4 Representative image of hematoxylin-eosin staining of colon tissue examined under a microscope (40 × and 100 ×). A: Control group, the colonic mucosa glands were normal in the control group, the structure was regular, and the opening was good; B: Model group, the colonic gland structure presented disorder, large nuclei, deep staining, and nucleoplasmic ratio imbalance.

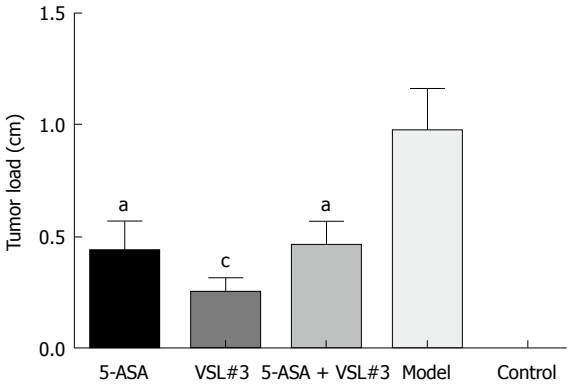

Figure 5 Tumor load in each group. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

group. Some genus of *Ruminococcaceae* can consume hydrogen to produce acetate, which is subsequently used by *Roseburia* to produce butyrate that is not only the main source of energy for intestinal epithelial cells but can also inhibit the signaling pathway of proinflammatory cytokines[17]. *Bifidobacterium* can produce bacteriocin and organic acids against pathogens on intestinal mucosal invasion[18]. It regulates the intestinal mucosal immunity and prevents the colonization of pathogens. The role of *Alloprevotella* is not yet clarified as it is not reported frequently in the digestive disease. Therefore, we hypothesize that dysbiosis occurs during UC-associated carcinogenesis, which reduces the beneficial types and increases the detrimental types.

Previous studies have shown that supplementation of probiotics can balance the intestinal microbiota of UC patients[6], which led us to speculate that supplementation of probiotics can also balance the intestinal microbiota of UC-associated carcinogenesis. The current study demonstrated that *Bacillus* and *Lactococcus* were increased, while *Oscillibacter* and *Lachnoclostridium* were decreased in the feces following VSL#3 treatment as compared to the model group. Some species of *Bacillus* and *Lactococcus* are widely used as probiotics. For example, *Bacillus subtilis* can significantly reduce

Table 5 Comparison of mucosal microbiota (abundance)

Genus	Control (%)	Model (%)	5-ASA (%)	VSL#3 (%)	5-ASA + VSL#3 (%)
Alloprevotella	0.26	1.57[1]	1.16	0.95	1.22
*Ruminococcaceae*_UCG-014	6.63	1.49[1]	1.64	1.50	1.15
Bifidobacterium	3.45	0.24[1]	0.19	3.34[2]	1.90[3]
Lachnoclostridium	0.24	0.40	2.05	0.25	2.03[3]

[1]$P < 0.05$ between the model and the control groups; [2]$P < 0.05$ between the model and the VSL#3 groups; [3]$P < 0.05$ between the model and the 5-ASA + VSL#3 groups.

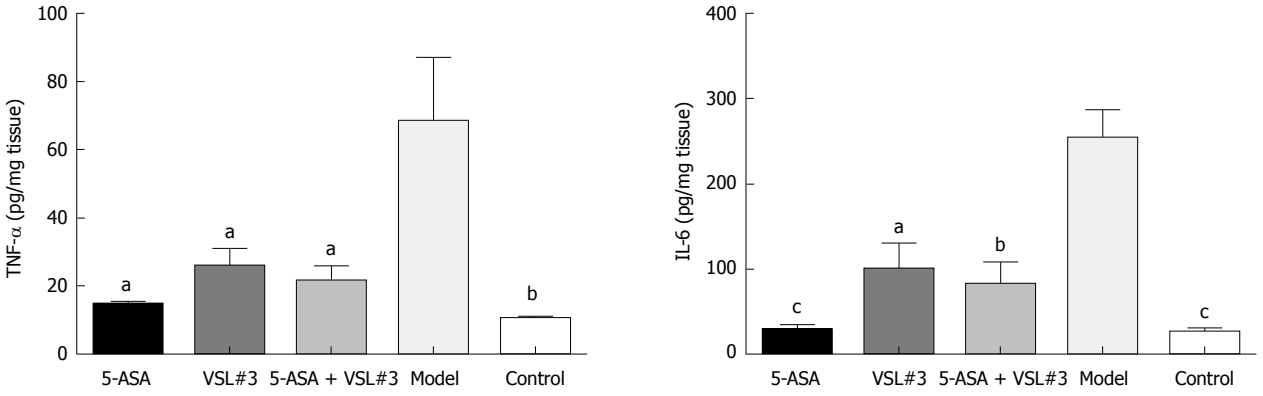

Figure 6 Colonic tumor necrosis factor-α and interleukin-6 levels in different groups. [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$. TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6.

DSS-induced colonic mucosal injury and inflammatory factors in mice and improve the levels of short-chain fatty acids[19]. *Lactococcus lactis* exerts a protective effect on DSS-induced colitis model mice[20].

Furthermore, *Bifidobacterium* increased in the mucosa after VSL#3 supplementation, thereby suggesting that VSL#3 supplementation, following the onset of AOM/DSS-induced colitis, promotes a healthy gastrointestinal bacterial community. Interestingly, VSL#3 is composed of eight strains, including one *Streptococcus*, three *Bifidobacterium*, and four *Lactobacillus*. However, none of the above strains increased significantly in the fecal intestinal microbiota after three-month gavage, suggesting that the positive effect of probiotics on the intestinal microbiota of the host is by regulating the proportion of beneficial and harmful bacteria.

For the differences between fecal and mucosal microbiota, we make the following explanation. There are three kinds of *Bifidobacterium* in VSL#3, and *Bifidobacterium* increased in mucosal microbiota but not in fecal. This phenomenon indicated that *Bifidobacterium* is easily colonized in the mucosa. Conversely, *Bacillus* and *Lactococcus* increased in fecal microbiota after VSL#3 intervention but not in the mucosa, indicating that *Bacillus* and *Lactococcus* can colonize easily in the feces. Strikingly, the four types of *Lactobacillus* in VSL#3 did not increase either in the fecal or mucosal microbiota, thereby suggesting that the intestinal environment of UC-associated carcinogenesis is not optimal for the growth of *Lactobacillus*. Only in the 5-ASA + VSL#3 group, the increase in *Lactobacillus* was observed in feces, which might be attributed to the low luminal pH. However, these hypotheses necessitate further studies for substantiation.

5-ASA is the first-line treatment for mild-to-moderate UC, and studies have found that 5-ASA $\geqslant$ 1.2 g/d could reduce the risk of carcinogenesis in patients with mild-to-moderate UC[21]. Thus, considering the clinical significance, we designed the 5-ASA monotherapy group and the 5-ASA + VSL#3 group. Interestingly, the change in the fecal microbiota in the 5-ASA group was similar to that in the VSL#3 monotherapy group. The potential mechanisms regulating the microbiota by 5-ASA are as follows: (1) Change in the colonic luminal pH: 5-ASA is released in the colon and translated into acetylsalicylic acid, which in turn, can decrease the luminal pH[22]. Low luminal pH is optimal for the growth of *Bifidobacteria* and *Lactobacilli*[23]; (2) improvement in the anoxia environment: 5-ASA can inhibit the production of chemotactic eicosanoids and cyclooxygenase 2 (COX2), which induces anoxia and can inactivate the oxygen-derived free radicals, improving the anoxia situation, which might affect the composition of intestinal microbiota[22]; and (3) 5-ASA can downregulate the expression of genes that are involved in bacterial metabolism, invasiveness, and antibiotic/stress resistance[24].

Nevertheless, the present study has some limitations. Herein, we only observed the phenomenon of gut microbiota changes while the specific role of flora is yet to be explored. Our future *in vitro* studies would focus on the underlying mechanisms.

In conclusion, the current study demonstrated that VSL#3 prevented UC-associated carcinogenesis in the AOM/DSS-induced mice model and decreased the level of

TNF-α and IL-6 in colon tissue. The intestinal microbiota dysbiosis was exhibited in UC-associated carcinogenesis mice. Supplementary VSL#3 was beneficial for a balanced fecal and mucosal microbiota in UC-associated carcinogenesis mice. Taken together, VSL#3 may serve as a potential therapeutic agent for the prevention of UC-associated carcinogenesis. Ongoing studies in our group are focused on the underlying mechanisms.

ARTICLE HIGHLIGHTS

Research background

Recently, an upward trend has been observed in the incidence of ulcerative colitis (UC) leading to increased clinical attention on UC-associated carcinogenesis.

Research motivation

Existing treatment for UC in the prevention of carcinogenesis involves several risks and side effects with long-term usage. Finding new treatment regimens are essential.

Research objectives

To investigate the effects of VSL#3 on tumor formation, and fecal and intestinal mucosal microbiota in the azoxymethane/dextran sulfate sodium (AOM/DSS) induced mice model.

Research methods

C57BL/6 mice were administered AOM/DSS to develop the UC-associated carcinogenesis model. The treatment group was gavaged with 5-ASA (75 mg/kg/d), VSL#3 (1.5×10^9 CFU/d), and 5-ASA + VSL#3 from the day of AOM injection for three months (five days/week). The tumor load was compared in each group, and tumor necrosis factor (TNF-α) and interleukin (IL)-6 levels evaluated in colon tissue. The stool and intestinal mucosa samples were collected to analyze the differences in the intestinal microbiota by 16s rDNA sequencing.

Research results

VSL#3 significantly reduced the tumor load in the AOM/DSS-induced mice model, and decreased the level of TNF-α and IL-6 in colon tissue. The model group had a lower level of *Lactobacillus* and higher level of *Oscillibacter* and *Lachnoclostridium* in fecal microbiota than the control group (UC-associated carcinogenesis not induced). *Bacillus* and *Lactococcus* were increased after the intervention with 5-ASA and VSL#3, while *Lachnoclostridium* and *Oscillibacter* were reduced. 5-ASA + VSL#3 increased the *Lactobacillus* and decreased the *Oscillibacter*. The intestinal mucosal microbiota analysis showed a lower level of *Bifidobacterium* and *Ruminococcaceae*_UCG-014 and higher level of *Alloprevotella* in the model group compared to the control group. *Bifidobacterium* was increased after supplementation with VSL#3. 5-ASA + VSL#3 increased the level of both *Lachnoclostridium* and *Bifidobacterium*.

Research conclusions

In mice, VSL#3 can prevent UC-associated carcinogenesis, reduce the colonic mucosal inflammation levels, and is beneficial for rebalancing the fecal and mucosal intestinal microbiota.

Research perspectives

VSL#3 may be a potential therapeutic agent for UC-associated carcinogenesis prevention based on the data presented here.

ACKNOWLEDGMENTS

We thank the staff at the National Cancer Center/Cancer Hospital of the Chinese Academy of Medical Sciences and Peking Union Medical College.

REFERENCES

1 **Jess T**, Rungoe C, Peyrin-Biroulet L. Risk of colorectal cancer in patients with ulcerative colitis: a meta-analysis of population-based cohort studies. *Clin Gastroenterol Hepatol* 2012; **10**: 639-645 [PMID: 22289873 DOI: 10.1016/j.cgh.2012.01.010]
2 **Uronis JM**, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLoS One* 2009; **4**: e6026 [PMID: 19551144 DOI: 10.1371/journal.pone.0006026]
3 **Derikx LA**, Dieleman LA, Hoentjen F. Probiotics and prebiotics in ulcerative colitis. *Best Pract Res Clin Gastroenterol* 2016; **30**: 55-71 [PMID: 27048897 DOI: 10.1016/j.bpg.2016.02.005]
4 **Rakoff-Nahoum S**, Medzhitov R. Role of toll-like receptors in tissue repair and tumorigenesis. *Biochemistry* (Mosc) 2008; **73**: 555-561 [PMID: 18605980]
5 **Risques RA**, Lai LA, Himmetoglu C, Ebaee A, Li L, Feng Z, Bronner MP, Al-Lahham B, Kowdley KV, Lindor KD, Rabinovitch PS, Brentnall TA. Ulcerative colitis-associated colorectal cancer arises in a field of short telomeres, senescence, and inflammation. *Cancer Res* 2011; **71**: 1669-1679 [PMID: 21363920 DOI: 10.1158/0008-5472.CAN-10-1966]
6 **Verna EC**, Lucak S. Use of probiotics in gastrointestinal disorders: what to recommend? *Therap Adv Gastroenterol* 2010; **3**: 307-319 [PMID: 21180611 DOI: 10.1177/1756283X10373814]
7 **Liang J**, Sha SM, Wu KC. Role of the intestinal microbiota and fecal transplantation in inflammatory bowel diseases. *J Dig Dis* 2014; **15**: 641-646 [PMID: 25389085 DOI: 10.1111/1751-2980.12211]
8 **Shen J**, Zuo ZX, Mao AP. Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. *Inflamm Bowel Dis* 2014; **20**: 21-35 [PMID: 24280877 DOI: 10.1097/01.MIB.0000437495.30052.be]
9 **Sood A**, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, Tandon RK. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009; **7**: 1202-1209, 1209.e1 [PMID: 19631292 DOI: 10.1016/j.cgh.2009.07.016]
10 **Tursi A**, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, Forti G, Morini S, Hassan C, Pistoia MA, Modeo ME, Rodino' S, D'Amico T, Sebkova L, Sacca' N, Di Giulio E, Luzza F, Imeneo M, Larussa T, Di Rosa S, Annese V, Danese S, Gasbarrini A. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2010; **105**: 2218-2227 [PMID: 20517305 DOI: 10.1038/ajg.2010.218]
11 **Talero E**, Bolivar S, Ávila-Román J, Alcaide A, Fiorucci S, Motilva V. Inhibition of chronic ulcerative colitis-associated adenocarcinoma development in mice by VSL#3. *Inflamm Bowel Dis* 2015; **21**: 1027-1037 [PMID: 25793324 DOI: 10.1097/MIB.0000000000000346]
12 **Tanaka T**, Kohno H, Suzuki R, Yamada Y, Sugie S, Mori H. A novel inflammation-related mouse colon carcinogenesis model induced by azoxymethane and dextran sodium sulfate. *Cancer Sci* 2003; **94**: 965-973 [PMID: 14611673]
13 **Bromberg J**, Wang TC. Inflammation and cancer: IL-6 and STAT3 complete the link. *Cancer Cell* 2009; **15**: 79-80 [PMID: 19185839 DOI: 10.1016/j.ccr.2009.01.009]
14 **Bassaganya-Riera J**, Viladomiu M, Pedragosa M, De Simone C, Hontecillas R. Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. *PLoS One* 2012; **7**: e34676 [PMID: 22511958 DOI: 10.1371/journal.pone.0034676]
15 **Takamura T**, Harama D, Fukumoto S, Nakamura Y, Shimokawa N, Ishimaru K, Ikegami S, Makino S, Kitamura M, Nakao A. Lactobacillus bulgaricus OLL1181 activates the aryl hydrocarbon receptor pathway and inhibits colitis. *Immunol Cell Biol* 2011; **89**:

817-822 [PMID: 21321579 DOI: 10.1038/icb.2010.165]
16 **Zocco MA**, dal Verme LZ, Cremonini F, Piscaglia AC, Nista EC, Candelli M, Novi M, Rigante D, Cazzato IA, Ojetti V, Armuzzi A, Gasbarrini G, Gasbarrini A. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1567-1574 [PMID: 16696804 DOI: 10.1111/j.1365-2036.2006.02927.x]
17 **Morgan XC**, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, Xavier RJ, Huttenhower C. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012; **13**: R79 [PMID: 23013615 DOI: 10.1186/gb-2012-13-9-r79]
18 **Zhiwei L**, Xuequn W, tiantian L. The clinical significance of intestinal flora changes in patients with ulcerative colitis. *Chin J Gastroenterol Hepatol* 2016; **25**: 554-556
19 **Zhang HL**, Li WS, Xu DN, Zheng WW, Liu Y, Chen J, Qiu ZB, Dorfman RG, Zhang J, Liu J. Mucosa-reparing and microbiota-balancing therapeutic effect of Bacillus subtilis alleviates dextrate sulfate sodium-induced ulcerative colitis in mice. *Exp Ther Med* 2016; **12**: 2554-2562 [PMID: 27698758 DOI: 10.3892/etm.2016.3686]
20 **Berlec A**, Perše M, Ravnikar M, Lunder M, Erman A, Cerar A, Štrukelj B. Dextran sulphate sodium colitis in C57BL/6J mice is alleviated by Lactococcus lactis and worsened by the neutralization of Tumor necrosis Factor α. *Int Immunopharmacol* 2017; **43**: 219-226 [PMID: 28039805 DOI: 10.1016/j.intimp.2016.12.027]
21 **Zhao LN**, Li JY, Yu T, Chen GC, Yuan YH, Chen QK. 5-Aminosalicylates reduce the risk of colorectal neoplasia in patients with ulcerative colitis: an updated meta-analysis. *PLoS One* 2014; **9**: e94208 [PMID: 24710620 DOI: 10.1371/journal.pone.0094208]
22 **Xue L**, Huang Z, Zhou X, Chen W. The possible effects of mesalazine on the intestinal microbiota. *Aliment Pharmacol Ther* 2012; **36**: 813-814 [PMID: 22984958 DOI: 10.1111/apt.12034]
23 **Kerr BJ**, Weber TE, Ziemer CJ, Spence C, Cotta MA, Whitehead TR. Effect of dietary inorganic sulfur level on growth performance, fecal composition, and measures of inflammation and sulfate-reducing bacteria in the intestine of growing pigs. *J Anim Sci* 2011; **89**: 426-437 [PMID: 20952529 DOI: 10.2527/jas.2010-3228]
24 **Kaufman J**, Griffiths TA, Surette MG, Ness S, Rioux KP. Effects of mesalamine (5-aminosalicylic acid) on bacterial gene expression. *Inflamm Bowel Dis* 2009; **15**: 985-996 [PMID: 19202572 DOI: 10.1002/ibd.20876]

P- Reviewer: Gardlik R, Suzuki H **S- Editor**: Wang XJ
L- Editor: Filipodia **E- Editor**: Huang Y

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4263-4271

DOI: 10.3748/wjg.v24.i37.4263

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Basic Study

Potential involvement of heat shock proteins in pancreatic-duodenal homeobox-1-mediated effects on the genesis of gastric cancer: A 2D gel-based proteomic study

Juan Ma, Bei-Bei Wang, Xiao-Yan Ma, Wei-Ping Deng, Li-Shu Xu, Wei-Hong Sha

Juan Ma, Bei-Bei Wang, Wei-Ping Deng, Li-Shu Xu, Wei-Hong Sha, Department of Gastroenterology and Hepatology, Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangdong Geriatrics Institute, Guangzhou 510080, Guangdong Province, China

Xiao-Yan Ma, Forensic Identification Institute, Key Laboratory of Reproduction and Genetics of Guangdong Higher Education Institutes, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou 510150, Guangdong Province, China

ORCID number: Juan Ma (0000-0003-1256-4914); Bei-Bei Wang (0000-0002-7950-3641); Xiao-Yan Ma (0000-0001-6101-1318); Wei-Ping Deng (0000-0003-0624-9846); Li-Shu Xu (0000-0002-2629-2684); Wei-Hong Sha (0000-0001-6615-422X).

Author contributions: Ma J and Wang BB contributed equally to this work; Ma J and Sha WH conceived the study; Ma J and Ma XY performed the research; Wang BB and Deng WP analyzed the data; Ma J wrote this manuscript; Xu LS revised this manuscript; Sha WH supervised the report; all authors gave intellectual input to the study and approved the final version of the manuscript.

Supported by Guangdong Natural Science Fund, No. 2016A030313765; and Guangdong medical scientific research fund, No. A2017070 and No. A2017122.

Institutional review board statement: The institutional review board at the Guangdong General Hospital approved the study protocol. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict-of-interest statement: The authors declare that they have no conflict of interest.

Data sharing statement: No additional data are available.

Manuscript source: Unsolicited manuscript

Correspondence to: Wei-Hong Sha, MD, PhD, Chief Doctor, Professor, Department of Gastroenterology and Hepatology, Guangdong General Hospital (Guangdong Academy of Medical Sciences), Guangdong Geriatrics Institute, 106 Zhongshan Er Road, Guangzhou 510080, Guangdong Province, China. shaweihong@gdghospital.com.cn
Telephone: +86-20-83827812
Fax: +86-20-83827812

Received: May 30, 2018
Peer-review started: May 30, 2018
First decision: July 18, 2018
Revised: August 9, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

AIM

To identify functional proteins involved in pancreatic-duodenal homeobox-1 (PDX1)-mediated effects on gastric carcinogenesis.

METHODS

A PDX1-overexpressed model was established by transfecting gastric cancer cell line SGC7901 with pcDNA3.1(+)-PDX1 vector (SGC-PDX1). Transfection with empty pcDNA3.1 vector (SGC-pcDNA) served as control. Comparative protein profiles of the two groups

were analyzed by two-dimensional electrophoresis based-proteomics (2DE gel-based proteomics). The differential proteins identified by 2DE were further validated by qRT-PCR and immunoblotting. Finally, co-immunoprecipitation was used to determine any direct interactions between PDX1 and the differential proteins.

RESULTS

2DE gel proteomics identified seven differential proteins in SGC-PDX1 when compared with those in SGC-pcDNA. These included four heat shock proteins (HSPs; HSP70p1B, HSP70p8, HSP60, HSP27) and three other proteins (ER60, laminin receptor 1, similar to epsilon isoform of 14-3-3 protein). Immunoblotting validated the expression of the HSPs (HSP70, HSP60, HSP27). Furthermore, their expressions were lowered to 80%, 20% and 24%, respectively, in SGC-PDX1, while PDX1 exhibited a 9-fold increase, compared to SGC-pcDNA. However, qRT-PCR analysis revealed that mRNA levels of the *HSP*s were increased in SGC-PDX1, suggesting that the expression of the *HSP*s was post-translationally regulated by the PDX1 protein. Finally, co-immunoprecipitation failed to identify any direct interaction between PDX1 and HSP70 proteins.

CONCLUSION

This study demonstrates the potential involvement of HSPs in PDX1-mediated effects on the genesis of gastric cancer.

Key words: Pancreatic-duodenal homeobox-1; Heat shock proteins; Gastric cancer; Proteomics; Two-dimensional electrophoresis

Core tip: Using a pcDNA3.1(+)-pancreatic-duodenal homeobox-1 (PDX1) vector, a PDX1-overexpressed model was built. Seven differential proteins were identified in SGC-PDX1 by two-dimensional electrophoresis gel proteomics compared with those in SGC-pcDNA. Four heat shock proteins were identified and confirmed by immunoblotting. qRT-PCR analysis further revealed that the expression of the *HSP*s was post-translationally regulated by the PDX1 protein. This study suggests the potential involvement of HSPs in PDX1 mediated effects on gastric carcinogenesis.

Ma J, Wang BB, Ma XY, Deng WP, Xu LS, Sha WH. Potential involvement of heat shock proteins in pancreatic-duodenal homeobox-1-mediated effects on the genesis of gastric cancer: A 2D gel-based proteomic study. *World J Gastroenterol* 2018; 24(37): 4263-4271 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4263.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4263

INTRODUCTION

Homeobox genes greatly contribute to the pattern formation of embryos by encoding many homeodomain transcriptional regulators[1,2]. The human gene of pancreatic-duodenal homeobox-1 (PDX1) is located at chromosomal locus 13q12.1[3]. In adults, PDX1 is expressed in Brunner's glands of the duodenum, pancreatic β cells and gastric pyloric gland cells. As one of the key homeodomain transcription regulators, PDX1 plays an important role in the development of the digestive system, including antrum, duodenum, and pancreas[4,5]. PDX1 is also a biomarker of pancreatic stem cells and regulates normal islet function[6]. In addition, PDX1 is expressed in the distal stomach and is involved in the secretion of hormones, such as somatostatin, serotonin, and gastrin[4]. Dysregulation of PDX1 may lead to pancreatic and gastric carcinogenesis. For example, overexpression of PDX1 was shown to be associated with the development of pancreatic cancer as it promoted proliferation, invasion, and colony formation in cancer cells[7]. Moreover, we earlier reported downregulation of PDX1 in gastric cancer, which suggests its potential role as a tumor suppressor. Further, overexpression of PDX1 induced apoptosis and inhibited proliferation, clone formation, and migration of gastric cancer cells. In addition, stable transfection with PDX1 was shown to suppress development of gastric cancer *in vivo*[8]. We have also shown that silencing of PDX1 in gastric cancer is likely caused by promoter hypermethylation and histone hypoacetylation[9]. However, the downstream mechanism by which PDX1 mediates gastric tumorigenesis remains elusive.

Proteins are the fundamental molecules that perform cellular functions. Changes in the protein expression profile under pathological conditions may reflect potential pathogenic mechanisms. Proteomic approaches represent a powerful tool to explore the underlying mechanism of tumorigenesis by characterizing the cellular events related to tumor development, angiogenesis, and progression. A number of proteomic approaches have been used to investigate the pathogenesis of gastric cancer[10-12]. Among these, 2D gel electrophoresis is a conventional approach and has been the principal step in the development of proteomics. Subsequent to 2D gel electrophoresis, protein expression profiles have been elucidated by computational image analysis and identified by mass spectrometry[13]. Although a number of different proteomics techniques have evolved in recent years, 2DE-based proteomics is still widely used to identify cancer-associated proteins[14].

To clarify the role of downstream mediators of PDX1 in gastric tumorigenesis, we established a PDX1-overexpressed gastric cancer cell model by transfection. After validation of the PDX1 overexpressed model, we used a 2D gel-based proteomic approach to determine the differentially expressed proteins as compared to that in the empty vector transfected gastric cancer cells. The expressions of identified candidate proteins were further assessed by real-time qRT-PCR and/or immunoblotting. Finally, co-immunoprecipitation was used to examine whether a differential protein, HSP70 has direct inter-

actions with PDX1 protein. Our results will greatly extend our understanding of the mechanisms of PDX1 mediated effects on gastric tumorigenesis.

MATERIALS AND METHODS

Cell culture

Human gastric cancer cell line SGC7901 was cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 μg/mL penicillin. The cells were maintained in a constant temperature incubator at 37 ℃ and 5% CO_2. The cells were harvested and passaged as required.

Cell transfection and protein sample preparation

The vector of pcDNA3.1(+)-PDX1 was established according to the methods previously published[15]. The SGC7901 cells were cultured in 6-well plates and transfected with pcDNA3.1(+)-PDX1 vector (SGC-PDX1) or pcDNA3.1(+) control vector (SGC-pcDNA) using liposome transfection reagent (Lipofectamine™ 2000 Transfection Reagent, ThermoFisher Scientific, United States). After six hours, fresh complete medium was used to replace the transfection medium and the cells of each group were aliquoted in triplicate. After 24-h incubation, the transfected SGC7901 cells were harvested for protein extraction. Initially, the culture plates were placed on ice, followed by addition of 250 μL lysis buffer for 10 min. The lysed cells were scraped off and sonicated for 10 min. Subsequently, the cell lysates were centrifuged at 20000 g for 30 min, and the supernatant containing cell proteins was collected. The whole cell lysate proteins were purified using a Cleanup kit (ProteoPrep® Total Extraction Sample Kit, Sigma-Aldrich, United States) to remove salt and lipid impurities. Protein concentration was determined by bicinchoninic acid (BCA) method. Finally, 600 μg protein from each sample was used for 2D gel electrophoresis.

2D gel electrophoresis, image analysis, and mass spectroscopy

2D gel electrophoresis was performed according to the method described elsewhere[16]. The first-dimensional isoelectric focusing of protein sample was conducted on Immobiline™ pH 3-10 IPG linear strips (Amersham, Pharmacia Biotech Inc.), and focused by an IPGphor electrophoresis system (Ettan IPGphor Isoelectric Focusing System, Amersham Biosciences, United States) by following the manufacturer's instructions. During second dimensional separation, focused strips were subjected onto the top of 12.5% SDS-PAGE gradient gels and overlaid with 1% agarose gel buffer. The gels were run under 20 ℃ at a speed of 15 mA/gel in stacking gel and 30 mA/gel in resolving gel until bromophenol blue front was within 0.5-1 cm of the gel bottom. The 2D gels were then stained with silver and scanned with UMax Powerlook 2110XL (GE Amersham). Image analysis was conducted using Image Master 2D platinum 5.0 software (Amersham pharmcia, Biotech) to identify differentially expressed protein profiles.

The differential protein spots between the two groups were excised using an automated Spot Handling Workstation (Amersham pharmcia, Biotech) and were subjected to discoloration and dehydration. The proteins were digested by trypsin and the peptides were obtained using peptide extraction buffer. The peptide suspension was vacuum-dried and resuspended in D/W. The resuspended peptide extracts were then spotted on a matrix-assisted laser-desorption ionization (MALDI) target. MALDI-MS analysis was conducted through a MALDI-time-of-flight (MALDI-TOF) mass spectrometer (Applied Biosystems, United States) and peptide mass mapping was performed by searching the NCBInr database.

qRT-PCR analysis

Total RNA from the cells was isolated using a Mini-RNease RNA extract kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA was reverse-transcribed from total RNA using a Thermoscript RT-PCR system (Gibco BRL, Gaithersburg, MD, United States). Applied Biosystems Sequence Detection System 7900 (Applied Biosystems, United States) was used to perform the qRT-PCR analysis. The reaction was performed using 10 μL mixture of 300 ng cDNA templates, 500 nmol of each primer and Power SYBR GREEN PCR Master Mix (Applied Biosystems, United States) as previously reported[8]. The generated melting curves and CT values were used to calculate the copy numbers of *PDX1* or *HSP*s mRNA. Online tool was utilized to design the PCR primers and the corresponding sequences, which are shown in Table 1.

Immunoblotting

As described in our previous study[8], the whole cell lysate protein was mixed with SDS-PAGE sample buffer and boiled for five minutes. Prepared protein samples were separated by SDS-PAGE electrophoresis and electrotransferred onto polyvinylidene fluoride membranes. After blocking, the membranes were blotted with primary antibodies against PDX1, HSP27, HSP60, and HSP70 (Santa Cruz Biotechnology). The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies and visualized using an enhanced chemiluminescence system (Amersham, Piscataway, NJ, United States).

Co-immunoprecipitation

SGC7901 cells were co-transfected with both pcDNA3.1(+)-PDX1 and pcDNA3.1(+)-HSP70 plasmids and cultured for 24 h. The cells were subsequently washed with PBS and lysed using lysis buffer (1 mmol/L phenylmethylsulfonyl fluoride, 5 mmol/L 2-mercaptoethanol, 2 mmol/L $MgCl_2$, 20 mmol/L HEPES, 150 mmol/L NaCl, 10 μg/mL leupeptin and 10 μg/mL aprotinin). The lysates were transferred onto protein A beads (Thermo Fisher Scientific) and incubated overnight with primary antibodies for PDX1 or HSP70 (Santa Cruz Biotechnology) at 4 ℃.

Table 1 Primer sequences of pancreatic-duodenal homeobox-1 (*PDX1*) and heat shock protein (*HSP*) genes used in the RT-PCR analysis

Gene name	Forward	Reverse	Product size (bp)
PDX1	5′-ATCTCCCCATACGAAGTGCC-3′	5′-CGTGAGCTTTGGTGGATTTCAT-3′	92
GAPDH	5′-ATGGGGAAGGTGAAGGTCG-3′	5′-GGGGTCATTGATGGCAACAATA-3	108
HSPA2	5′-CACCACCTATTCGTGCGTC-3′	5′-TTTCCGTCCAATCAGCCTCTT-3′	196
HSPA6	5′-CAAGGTGCGCGTATGCTAC-3′	5′-GCTCATTGATGATCCGCAACAC-3′	224
HSPA8	5′-GGAGGTGGCACTTTTGATGTG-3′	5′-CAAGCAGTACGGAGGCGTCT-3′	200
HSPA1B	5′-TTTGAGGGCATCGACTTCTACA-3	5′-CCAGGACCAGGTCGTGAATC-3′	148
HSPA1L	5′-CTACTGCCAAGGGAATCGCC-3′	5′GCCGATCAGACGTTTAGCATC-3′	227
HSP27	5′-GGACGAGCATGGCTACATCT-3′	5′-CTTTACTTGGCGGCAGTCTC-3′	237
HSP60	5′-CACCGTAAGCCTTTGGTCAT-3′	5′-CCCTCTTCTCCAAACACTGC-3′	188

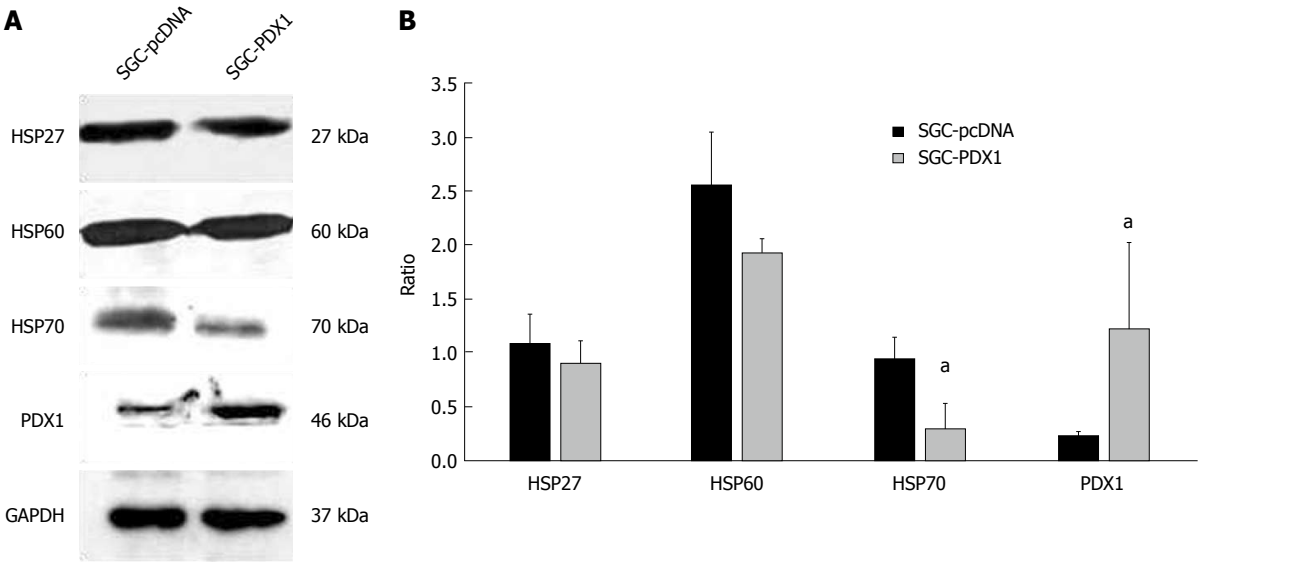

Figure 1 Pancreatic-duodenal homeobox-1 overexpression regulated the expression of heat shock proteins in gastric cancer SGC7901 cells. A: Immunoblotting analysis confirmed the enhanced expression of PDX1 protein after PDX1-pcDNA transfection in SGC7901 cells. It also revealed that PDX1 overexpression could downregulate the expression of HSP27, HSP60, and HSP70 proteins in SGC-PDX1 cells; B: Quantitative analysis of the results of immunoblotting indicated that HSP70 protein was significantly decreased in SGC-PDX1 cells. $^{a}P < 0.05$.

The beads were washed twice using lysis buffer to remove unbound proteins. To elute the bound proteins, the beads were then resuspended in sample buffer and boiled at 95 ℃ for three minutes. The collected unbound and bound proteins were stored at -80 ℃ for further use during immunoblotting.

Statistical analysis

Data are presented as mean ± SD, and the differences in continuous variables were assessed by Mann-Whitney *U* test (Student *t* test). *P*-values < 0.05 were considered statistically significant and all statistical tests were two-tailed.

RESULTS

Establishment of PDX1 overexpressed model using gastric cell line

To study the downstream mechanisms of PDX1 in gastric carcinogenesis, we established a PDX1 overexpressed model of gastric cancer cell line SGC7901 using a vector of pcDNA3.1(+)-PDX1 (SGC-PDX1). The whole cell lysate proteins were extracted and subjected to immunoblotting to confirm the establishment of PDX1 overexpressed model. As shown in Figure 1A, the expression of PDX1 in pSGC-PDX1 cells was significantly increased compared to that in the SGC-pcDNA cells. These results indicated the successful establishment of a PDX1 overexpressed model, which was further used for 2D gel-based proteomic analysis.

Differentially expressed protein profiles in PDX1 overexpressed model revealed by 2D gel-based proteomics

The comparative protein profile of PDX1 overexpressed model and control group was determined using 2D PAGE coupled with MALDI-TOF-MS. The 2-DE maps were displayed between a pH range of 3-10. Figure 2A and 2B shows the representative 2-DE maps of SGC-PDX1 and SGC-pcDNA. Compared with SGC-pcDNA control cells, seven proteins were found to be downregulated about two-fold in SGC-PDX1 cells (Figure 2C). The seven

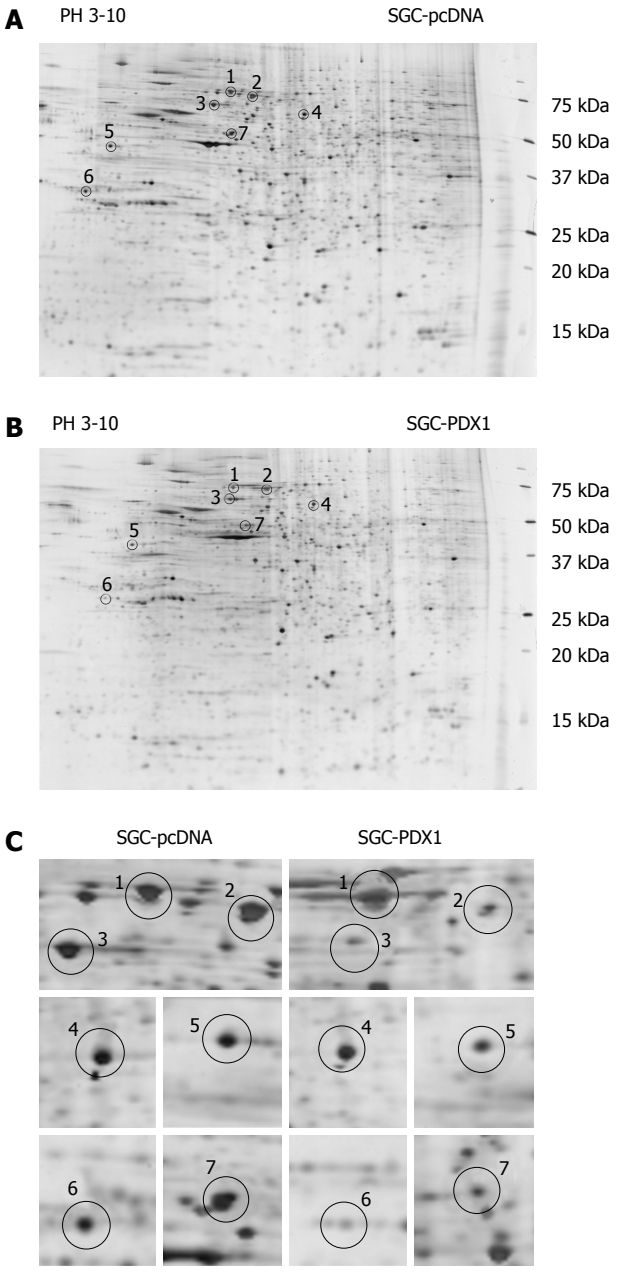

Figure 2 Two-dimensional electrophoresis-gel images of proteins from SGC7901 with pcDNA3.1(+)-pancreatic-duodenal homeobox-1 (PDX1) vector and SGC7901-pcDNA cells. A and B: Representative 2DE-gel images of SGC-PDX1 and SGC-pcDNA groups. The differentially expressed proteins are indicated by circles; C: Close-up images of the differential protein spots between SGC-PDX1 and SGC-pcDNA groups. 2DE: 2-dimensional electrophoresis.

differentially expressed protein spots were excised from replicate 2D-gels and the corresponding peptides were obtained by proteolytic digestion. Extracted peptides were subjected to MALDI-TOF-MS analysis and then the database search was performed. The seven differentially expressed proteins were identified as, heat shock protein (70 kDa) and its isoforms, heat shock protein (60 kDa), heat shock protein (27 kDa), glucose regulated protein (58 kDa), laminin receptor 1, epsilon isoform of 14-3-3 protein. The detailed information of the seven differentially expressed proteins is shown in Table 2.

Validation of differentially expressed proteins by qRT-PCR and immunoblotting

Through literature search and review, we found that the heat shock proteins (HSPs) were shown to play an important role in the regulation of tumorigenesis, including proliferation, invasion, and colony formation of cancer cells[17-19]. This finding suggested that the HSPs might also be involved in gastric carcinogenesis because they also showed differential protein expressions in SGC-PDX1 as revealed by 2D gel-based proteomics. Therefore, we selected the heat shock proteins for further evaluation.

SGC7901 cells were maintained under the same culture conditions and transfected with corresponding vectors as mentioned earlier. Total RNA and whole cell proteins were extracted for qRT-PCR and immunoblotting, respectively. The expression of *PDX1* mRNA was significantly upregulated in SGC-PDX1 cells as compared to that in the control group (Figure 3). This indicated the successful transfection of SGC7901 cells with the *PDX1* gene. In addition, the mRNA levels of the *HSP*70 isoforms (*HSPA1B*, *HSPA1L*, *HSPA2*, *HSPA6*), *HSP27*, and *HSP60* were found to have increased after *PDX1* overexpression (Figure 3) in SGC7901 cells. Immunoblotting confirmed that the expression of the PDX1 protein was significantly increased in SGC-PDX1 cells. Moreover, the expression of HSP70, HSP27, and HSP60 were downregulated in SGC-PDX1 cells (reduced to 80%, 24%, and 20%, respectively), which was consistent with the proteomic results (Figure 1). However, these results demonstrated the opposite patterns of mRNA and protein expression of HSP70, HSP27, and HSP60 in SGC-PDX1 cells, which suggests that the expressions of these proteins were post-translationally regulated in SGC-PDX1 cells.

Interaction between PDX1 and HSP70 evaluated by co-immunoprecipitation

Results of immunoblotting showed that the difference in the expression of HSP70 between the two groups was much higher than that for the other two HSP proteins, which emphasized the significance of the HSP70 protein in PDX1-mediated effects on gastric tumorigenesis. Therefore, we further evaluated whether PDX1 and HSP70 proteins have direct interactions. SGC7901 cells were co-transfected with both pcDNA3.1(+)-PDX1 and pcDNA3.1(+)-HSP70 plasmids and cultured for 24 h. The whole cell lysate protein of co-transfected SGC7901 cells were then subjected to co-immunoprecipitation. As illustrated in Figure 4A, both PDX1 and HSP70 proteins were identified in the cell lysate proteins of co-transfected SGC7901 cells, which confirmed the input of PDX1 and HSP70 proteins in co-immunoprecipitation assay. The whole cell lysate proteins were then subjected to co-immunoprecipitation assay using precipitating antibodies against PDX1 and HSP70. The corresponding immunoprecipitated proteins were further detected by immunoblotting using primary antibodies against HSP70 and PDX1. Interestingly, no significant binding between PDX1 and HSP70 proteins was observed (Figure

Table 2 Differential protein spots between SGC7901 with pcDNA3.1(+)-pancreatic-duodenal homeobox-1 (PDX1) vector and SGC7901-pcDNA cells analyzed by matrix-assisted laser desorption ionization-time of flight-mass spectrometry

Spot No.	Rank protein name	Accession No.	Protein score	Protein score CI%	Protein MW
S1	Heat shock 70 kDa protein 8 isoform 2 (homo sapiens)	gi 24234686	595	100	53598.4
	HSPA8 protein (homo sapiens)	gi 48257068	546	100	54804.2
	Heat shock 70 kDa protein 2 (homo sapiens)	gi 3287 9973	290	100	70263.0
	Heat shock 70 kDa protein 6 (HSP70B') (homo sapiens)	gi 55960611	81	100	71440.4
S2	DNAK-type molecular chaperone HSPA1L-human	gi 2119712	559	100	70110.0
	HSPA1A protein (homo sapiens)	gi 14414588	558	100	70294.1
	Heat shock 10 kDa protein 1-like (homo sapiens)	gi 55961919	372	100	70730.5
	Heat shock 70 kDa protein 1-like (homo sapiens)	gi 21759781	361	100	70748.4
	Heat shock 70 kDa protein 1B (homo sapiens)	gi 55962554	296	100	52199.8
S3	Chaperonin 60 (Hsp60) (homo sapiens)	gi 6996447	375	100	61187.4
S4	ER-60 protein (homo sapiens)	gi 2245365	223	100	57146.9
	Glucose regulated protein 58 kDa (Bos taurus)	gi 27805905	113	100	57293.0
S5	Ribosomal protein SA (laminin receptor 1) (homo sapiens)	gi 47125390	204	100	32933.5
S6	PREDICTED: Similar to epsilon isoform of 14-3-3 protein	gi 57091321	53	37.851	29326.5
S7	Heat shock 70 kDa protein 1B (homo sapiens)	gi 55962554	67	97.468	52199.8

4B), which implied that there was no direct interaction between PDX1 and HSP70 proteins.

DISCUSSION

Gastric cancer is a leading cause of cancer-associated mortality across the world[20]. Despite years of extensive research, the molecular mechanisms involved in the pathogenesis of gastric cancer are not completely understood. Recent advances in proteomics display its great potential for use in cancer diagnosis, prognostic assessment, and to understand the molecular mechanism of carcinogenesis. Proteomic approaches have been widely applied in research on gastric cancer[21-23]. Liu *et al*[24] used 2D gel-based proteomics to compare the differential serum protein profiles between gastric cancer patients and healthy controls, and identified several serum biomarkers for gastric cancer. Poon *et al*[22] also demonstrated that an exclusive serum proteomic fingerprint, identified by SELDI-based proteomics, can be used for noninvasive diagnosis of gastric cancer. In addition, many prognostic biomarkers of gastric cancer were identified by proteomic approaches, such as S100P[25] and S100A9[25] proteins. Chen *et al*[26] utilized a quantitative proteomic technique to identify the differentially expressed proteins in metastatic gastric cancer cells as compared to that in noninvasive gastric cancer cells. After downstream validation, they found that vimentin and galectin-1 were potential markers of gastric cancer metastasis and suggested their involvement in cell-cell and cell-ECM adhesion interactions.

PDX1 is a member of the homeobox family and plays an important role in the development of embryonic digestive system. A previous study demonstrated that PDX1 knockout mice showed abnormal growth of gastroduodenal junction, which increased the difficulty in emptying gastric contents and ultimately led to gastric retention[27]. Aberrant expression of the *PDX1* gene has been associated with carcinogenesis. PDX1 was shown to function as a tumor promoter by enhancing the proliferation, invasion[28], and induction of acinar-to-ductal metaplasia[29] in pancreatic cancer. Hence, there is a growing interest to develop a novel therapy for pancreatic cancer by targeting PDX1. Interestingly, PDX1 was also found to be associated with the tumorigenesis of gastric cancer. Both Faller *et al*[30] and Sakai *et al*[31] have reported abnormal expression of PDX1 protein in pseudo-pyloric glandular metaplasia. In a previous study, we found that PDX1 protein was downregulated in *Helicobacter pylori* infection, incisural antralisation, and intestinal metaplasia[32]. We further identified significantly decreased *PDX1* mRNA and PDX1 protein expression in gastric cancer cells. Downregulation of PDX1 in gastric cancer tissues might be caused by promoter hypermethylation and histone hypoacetylation[9]. We also observed that the transient overexpression of the *PDX1* gene could inhibit proliferation and induce apoptosis of gastric cancer cells. Moreover, stable overexpression of the *PDX1* gene suppressed colony formation, wound healing, migration of gastric cancer cells, and decreased tumor incidence in nude mice; these findings imply that PDX1 may act as a tumor suppressor in the context of gastric cancer[8].

In the present study, we sought to clarify the downstream mediators of PDX1-mediated effects on gastric cancer tumorigenesis. We used 2D gel-based proteomics to identify differentially expressed proteins between PDX1-overexpressed gastric cancer cells and the control empty vector transfected gastric cells. Seven differentially expressed proteins were identified by proteomic analysis, which included three heat shock proteins. The three HSPs proteins, HSP70, HSP60, and HSP27, were significantly decreased in gastric cells after *PDX1* overexpression. The results of immunoblotting analysis were consistent with those of proteomics analysis. However, the mRNA levels of *HSP70*, *HSP60*, and *HSP27* were significantly increased after *PDX1* overexpression. These results indicated that overexpression of PDX1

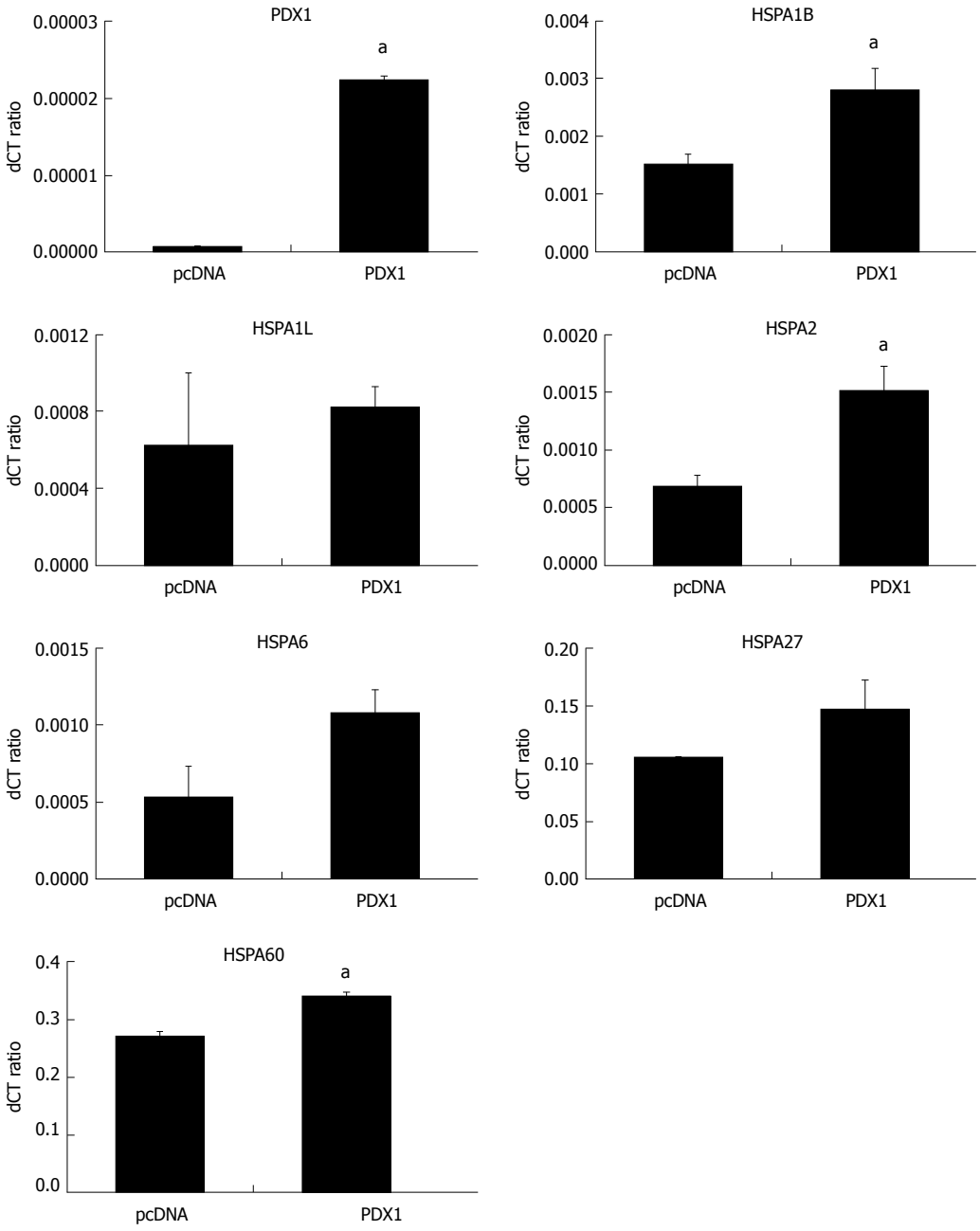

Figure 3 Comparisons of mRNA levels of the heat shock proteins in SGC7901 with pcDNA3.1(+)-pancreatic-duodenal homeobox-1 (PDX1) vector and SGC7901-pcDNA cells. The mRNA expressions of HSPs in SGC-PDX1 and SGC-pcDNA cells were compared by qRT-PCR. The relative mRNA levels of two of HSP70 isoforms (*HSPA1B and HSPA2*) and *HSP60* were significantly increased in the SGC-PDX1 group, while no significant differences were identified for the other HSP genes. ($^{a}P < 0.05$). HSPs: Heat shock proteins.

could downregulate HSP70, HSP60, and HSP27 proteins through post-translational regulation pathways, such as mRNA degradation, glycosylation, degradation, or phosphorylation.

HSPs are a group of chaperone proteins that play dual roles in tumorigenesis[33]. HSP70, HSP60, and HSP27 proteins were reported to be upregulated in gastric cancer and associated with tumor progression and poor prognosis[34-36]. Our immunoblotting analysis revealed that HSP70 proteins showed the greatest differential expression in PDX1-overexpressed gastric cells, which emphasized its significance in PDX1 mediated effects on gastric cancer tumorigenesis. Besides, HSP70 protein was shown to serve as a tumor promoter in gastric cancer by reducing apoptosis[37,38]. Therefore, PDX1 might affect the apoptosis of gastric cancer cells by regulating the expression of HSP70. To further evaluate the interaction between PDX1 and HSP70, we performed a co-immunoprecipitation assay. However, no direct interactions could be found between the two proteins, which indicates that there may be some intermediate mediators that link PDX1 and HSP70 proteins in the regulation of gastric cancer tumorigenesis. Hence, further studies are warranted to elucidate the relationship

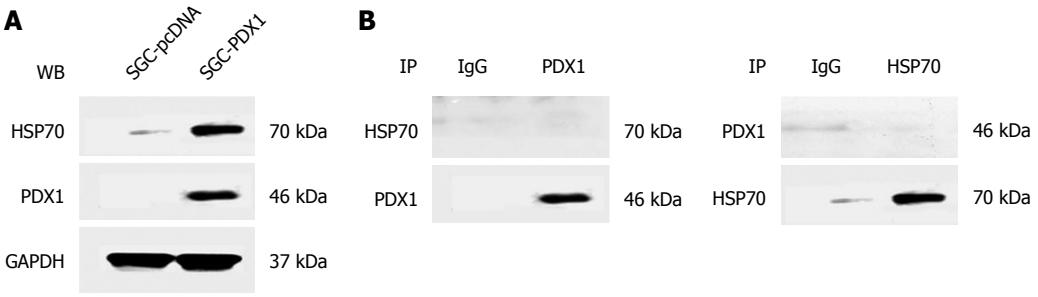

Figure 4 Results of co-immunoprecipitation analysis showing no direct interaction of pancreatic-duodenal homeobox-1 (PDX1) and HSP70 in SGC7901 with pcDNA3.1(+)-PDX1 cells. A: Both PDX1 and HSP70 proteins were detected in the whole cell lysate proteins of co-transfected SGC7901 cells, ensuring the input of PDX1 and HSP70 proteins in the co-immunoprecipitation experiment; B: The immunoprecipitated proteins obtained by precipitating antibodies against PDX1 or HSP70 were immunoblotted by HSP70 or PDX1, respectively. No direct interactions between PDX1 and HSP70 were detected.

between PDX1 and HSP70, along with a detailed mechanism regarding their collaboration to regulate gastric tumorigenesis.

In conclusion, our study showed that proteomics is a powerful tool to study the molecular mechanisms involved in the genesis of gastric cancer. In addition, the inhibition of PDX1 in gastric cancers may contribute to the upregulation of HSPs, especially HSP70.

ARTICLE HIGHLIGHTS

Research background

As one of the homeobox genes that play critical roles in the pattern formation of embryos, pancreatic-duodenal homeobox-1 (PDX1) is widely expressed in Brunner's glands of the duodenum, pancreatic β cells, and gastric pyloric gland cells. PDX1 plays a key role in the development of the digestive system, including antrum, duodenum, and pancreas. Downregulation of PDX1 has been observed in gastric cancer, which suggests its potential role in gastric tumorigenesis. Nevertheless, the downstream mechanisms that mediate the effect of PDX1 on gastric tumorigenesis are still poorly understood.

Research motivation

Although PDX1 has been found to be involved in gastric tumorigenesis, its downstream regulating mechanism is still unclear.

Research objectives

To clarify the differential protein profile in PDX1-overexpressed gastric cell line and explore functional proteins involved in PDX1-mediated effects on gastric tumorigenesis.

Research methods

A PDX1-overexpressed model was established using gastric cancer cell line SGC7901 (SGC-PDX1). As a method that is still widely used to identify cancer-associated proteins, 2DE-based proteomics was applied to determine the differential protein profile between SGC-PDX1 and SGC-pcDNA. The differential proteins were then subjected to qRT-PCR and immunoblotting for further confirmation. Finally, direct interactions between PDX1 and the identified differential proteins were evaluated by co-immunoprecipitation.

Research results

Seven proteins were found to be differentially expressed in SGC-PDX1 using 2-DE proteomics. Immunoblotting confirmed that the three differential HSPs (HSP70, HSP60, HSP27) were downregulated in SGC-PDX1. However, qRT-PCR analysis identified increased *HSP* mRNA in SGC-PDX1, which indicates that PDX1 may post-translationally regulate the expression of the *HSP*s. Further study is warranted to elucidate the relationship of HPS70 and PDX1, as co-immunoprecipitation did not identify direct interaction between them.

Research conclusions

HSPs are involved in PDX1-mediated effects on the genesis of gastric cancer and the interaction between HSP70 and PDX1 is indirect.

Research perspectives

This study demonstrates the involvement of HSPs in PDX1-mediated effects on gastric tumorigenesis. Further study is warranted to elucidate the downstream regulating mechanism of PDX1 on HSPs.

ACKNOWLEDGMENTS

HSP70 plasmid was kindly provided by Dr. BCY Wong, Hong Kong University, Hong Kong.

REFERENCES

1 **Melton DA**. Pattern formation during animal development. *Science* 1991; **252**: 234-241 [PMID: 1672778 DOI: 10.1126/science.1672778]
2 **Alappat S**, Zhang ZY, Chen YP. Msx homeobox gene family and craniofacial development. *Cell Res* 2003; **13**: 429-442 [PMID: 14728799 DOI: 10.1038/sj.cr.7290185]
3 **Brooke NM**, Garcia-Fernàndez J, Holland PW. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. *Nature* 1998; **392**: 920-922 [PMID: 9582071 DOI: 10.1038/31933]
4 **Larsson LI**, Madsen OD, Serup P, Jonsson J, Edlund H. Pancreatic-duodenal homeobox 1 -role in gastric endocrine patterning. *Mech Dev* 1996; **60**: 175-184 [PMID: 9025070 DOI: 10.1016/S0925-4773(96)00609-0]
5 **Offield MF**, Jetton TL, Labosky PA, Ray M, Stein RW, Magnuson MA, Hogan BL, Wright CV. PDX-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* 1996; **122**: 983-995 [PMID: 8631275]
6 **Edlund H**. Pancreatic organogenesis--developmental mechanisms and implications for therapy. *Nat Rev Genet* 2002; **3**: 524-532 [PMID: 12094230 DOI: 10.1038/nrg841]
7 **Liu T**, Gou SM, Wang CY, Wu HS, Xiong JX, Zhou F. Pancreas duodenal homeobox-1 expression and significance in pancreatic cancer. *World J Gastroenterol* 2007; **13**: 2615-2618 [PMID: 17552012 DOI: 10.3748/wjg.v13.i18.2615]
8 **Ma J**, Chen M, Wang J, Xia HH, Zhu S, Liang Y, Gu Q, Qiao L, Dai Y, Zou B, Li Z, Zhang Y, Lan H, Wong BC. Pancreatic duodenal homeobox-1 (PDX1) functions as a tumor suppressor in gastric cancer. *Carcinogenesis* 2008; **29**: 1327-1333 [PMID: 18477649 DOI: 10.1093/carcin/bgn112]
9 **Ma J**, Wang JD, Zhang WJ, Zou B, Chen WJ, Lam CS, Chen MH, Pang R, Tan VP, Hung IF, Lan HY, Wang QY, Wong BC. Promoter hypermethylation and histone hypoacetylation contribute to pancreatic-duodenal homeobox 1 silencing in gastric cancer. *Carcinogenesis* 2010; **31**: 1552-1560 [PMID: 20622005 DOI: 10.1093/carcin/bgq140]
10 **Hu HD**, Ye F, Zhang DZ, Hu P, Ren H, Li SL. iTRAQ quantitative

analysis of multidrug resistance mechanisms in human gastric cancer cells. *J Biomed Biotechnol* 2010; **2010**: 571343 [PMID: 20625496 DOI: 10.1155/2010/571343]
11 **Lin LL**, Huang HC, Juan HF. Discovery of biomarkers for gastric cancer: a proteomics approach. *J Proteomics* 2012; **75**: 3081-3097 [PMID: 22498886 DOI: 10.1016/j.jprot.2012.03.046]
12 **Chen J**, Kähne T, Röcken C, Götze T, Yu J, Sung JJ, Chen M, Hu P, Malfertheiner P, Ebert MP. Proteome analysis of gastric cancer metastasis by two-dimensional gel electrophoresis and matrix assisted laser desorption/ionization-mass spectrometry for identification of metastasis-related proteins. *J Proteome Res* 2004; **3**: 1009-1016 [PMID: 15473690 DOI: 10.1021/pr049916l]
13 **Gygi SP**, Corthals GL, Zhang Y, Rochon Y, Aebersold R. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc Natl Acad Sci USA* 2000; **97**: 9390-9395 [PMID: 10920198 DOI: 10.1073/pnas.160270797]
14 **Rogowska-Wrzesinska A**, Le Bihan MC, Thaysen-Andersen M, Roepstorff P. 2D gels still have a niche in proteomics. *J Proteomics* 2013; **88**: 4-13 [PMID: 23353020 DOI: 10.1016/j.jprot.2013.01.010]
15 **Ma J**, Liu QH, Sha WH, Wang QY. The construction and expression of the PDX1 vector (in Chinese). *Guangdong Medical Journal* 2010; **31**: 3030-3031
16 **Görg A**, Weiss W, Dunn MJ. Current two-dimensional electrophoresis technology for proteomics. *Proteomics* 2004; **4**: 3665-3685 [PMID: 15543535 DOI: 10.1002/pmic.200401031]
17 **Arata S**, Hamaguchi S, Nose K. Inhibition of colony formation of NIH 3T3 cells by the expression of the small molecular weight heat shock protein HSP27: involvement of its phosphorylation and aggregation at the C-terminal region. *J Cell Physiol* 1997; **170**: 19-26 [PMID: 9012781 DOI: 10.1002/(SICI)1097-4652(199701)170:1<19::AID-JCP3>3.0.CO;2-O]
18 **Yang Y**, Rao R, Shen J, Tang Y, Fiskus W, Nechtman J, Atadja P, Bhalla K. Role of acetylation and extracellular location of heat shock protein 90alpha in tumor cell invasion. *Cancer Res* 2008; **68**: 4833-4842 [PMID: 18559531 DOI: 10.1158/0008-5472.CAN-08-0644]
19 **Park JW**, Yeh MW, Wong MG, Lobo M, Hyun WC, Duh QY, Clark OH. The heat shock protein 90-binding geldanamycin inhibits cancer cell proliferation, down-regulates oncoproteins, and inhibits epidermal growth factor-induced invasion in thyroid cancer cell lines. *J Clin Endocrinol Metab* 2003; **88**: 3346-3353 [PMID: 12843186 DOI: 10.1210/jc.2002-020340]
20 **Wadhwa R**, Song S, Lee JS, Yao Y, Wei Q, Ajani JA. Gastric cancer-molecular and clinical dimensions. *Nat Rev Clin Oncol* 2013; **10**: 643-655 [PMID: 24061039 DOI: 10.1038/nrclinonc.2013.170]
21 **Ryu JW**, Kim HJ, Lee YS, Myong NH, Hwang CH, Lee GS, Yom HC. The proteomics approach to find biomarkers in gastric cancer. *J Korean Med Sci* 2003; **18**: 505-509 [PMID: 12923326 DOI: 10.3346/jkms.2003.18.4.505]
22 **Poon TC**, Sung JJ, Chow SM, Ng EK, Yu AC, Chu ES, Hui AM, Leung WK. Diagnosis of gastric cancer by serum proteomic fingerprinting. *Gastroenterology* 2006; **130**: 1858-1864 [PMID: 16697748 DOI: 10.1053/j.gastro.2006.02.011]
23 **Bai Z**, Ye Y, Liang B, Xu F, Zhang H, Zhang Y, Peng J, Shen D, Cui Z, Zhang Z, Wang S. Proteomics-based identification of a group of apoptosis-related proteins and biomarkers in gastric cancer. *Int J Oncol* 2011; **38**: 375-383 [PMID: 21165559 DOI: 10.3892/ijo.2010.873]
24 **Liu W**, Liu B, Cai Q, Li J, Chen X, Zhu Z. Proteomic identification of serum biomarkers for gastric cancer using multi-dimensional liquid chromatography and 2D differential gel electrophoresis. *Clin Chim Acta* 2012; **413**: 1098-1106 [PMID: 22446497 DOI: 10.1016/j.cca.2012.03.003]
25 **Jia SQ**, Niu ZJ, Zhang LH, Zhong XY, Shi T, Du H, Zhang GG, Hu Y, Su XL, Ji JF. Identification of prognosis-related proteins in advanced gastric cancer by mass spectrometry-based comparative proteomics. *J Cancer Res Clin Oncol* 2009; **135**: 403-411 [PMID: 18830628 DOI: 10.1007/s00432-008-0474-3]
26 **Chen YR**, Juan HF, Huang HC, Huang HH, Lee YJ, Liao MY, Tseng CW, Lin LL, Chen JY, Wang MJ, Chen JH, Chen YJ. Quantitative proteomic and genomic profiling reveals metastasis-related protein expression patterns in gastric cancer cells. *J Proteome Res* 2006; **5**: 2727-2742 [PMID: 17022644 DOI: 10.1021/pr060212g]
27 **Gu G**, Dubauskaite J, Melton DA. Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* 2002; **129**: 2447-2457 [PMID: 11973276]
28 **Liu S**, Ballian N, Belaguli NS, Patel S, Li M, Templeton NS, Gingras MC, Gibbs R, Fisher W, Brunicardi FC. PDX-1 acts as a potential molecular target for treatment of human pancreatic cancer. *Pancreas* 2008; **37**: 210-220 [PMID: 18665085 DOI: 10.1097/MPA.0b013e31816a4a33]
29 **Miyatsuka T**, Kaneto H, Shiraiwa T, Matsuoka TA, Yamamoto K, Kato K, Nakamura Y, Akira S, Takeda K, Kajimoto Y, Yamasaki Y, Sandgren EP, Kawaguchi Y, Wright CV, Fujitani Y. Persistent expression of PDX-1 in the pancreas causes acinar-to-ductal metaplasia through Stat3 activation. *Genes Dev* 2006; **20**: 1435-1440 [PMID: 16751181 DOI: 10.1101/gad.1412806]
30 **Faller G**, Kirchner T. Immunological and morphogenic basis of gastric mucosa atrophy and metaplasia. *Virchows Arch* 2005; **446**: 1-9 [PMID: 15583929 DOI: 10.1007/s00428-004-1157-3]
31 **Sakai H**, Eishi Y, Li XL, Akiyama Y, Miyake S, Takizawa T, Konishi N, Tatematsu M, Koike M, Yuasa Y. PDX1 homeobox protein expression in pseudopyloric glands and gastric carcinomas. *Gut* 2004; **53**: 323-330 [PMID: 14960508 DOI: 10.1136/gut.2003.026609]
32 **Zhu S**, Xia HH, Yang Y, Ma J, Chen M, Hu P, Gu Q, Liang Y, Lin H, Wong BC. Alterations of gastric homeoprotein expression in Helicobacter pylori infection, incisural antralisation, and intestinal metaplasia. *Dig Dis Sci* 2009; **54**: 996-1002 [PMID: 18754095 DOI: 10.1007/s10620-008-0459-8]
33 **Calderwood SK**, Khaleque MA, Sawyer DB, Ciocca DR. Heat shock proteins in cancer: chaperones of tumorigenesis. *Trends Biochem Sci* 2006; **31**: 164-172 [PMID: 16483782 DOI: 10.1016/j.tibs.2006.01.006]
34 **Li XS**, Xu Q, Fu XY, Luo WS. Heat shock protein 60 overexpression is associated with the progression and prognosis in gastric cancer. *PLoS One* 2014; **9**: e107507 [PMID: 25207654 DOI: 10.1371/journal.pone.0107507]
35 **Ge H**, He X, Guo L, Yang X. Clinicopathological significance of HSP27 in gastric cancer: a meta-analysis. *Onco Targets Ther* 2017; **10**: 4543-4551 [PMID: 28979146 DOI: 10.2147/OTT.S146590]
36 **Bodoor K**, Jalboush SA, Matalka I, Abu-Sheikha A, Waq RA, Ebwaini H, Abu-Awad A, Fayyad L, Al-Arjat J, Haddad Y. Heat Shock Protein Association with Clinico-Pathological Characteristics of Gastric Cancer in Jordan : HSP70 is Predictive of Poor Prognosis. *Asian Pac J Cancer Prev* 2016; **17**: 3929-3937 [PMID: 27644641]
37 **Xiang TX**, Li Y, Jiang Z, Huang AL, Luo C, Zhan B, Wang PL, Tao XH. RNA interference-mediated silencing of the Hsp70 gene inhibits human gastric cancer cell growth and induces apoptosis in vitro and in vivo. *Tumori* 2008; **94**: 539-550 [PMID: 18822691 DOI: 10.1177/030089160809400416]
38 **Arora N**, Alsaied O, Dauer P, Majumder K, Modi S, Giri B, Dudeja V, Banerjee S, Von Hoff D, Saluja A. Downregulation of Sp1 by Minnelide leads to decrease in HSP70 and decrease in tumor burden of gastric cancer. *PLoS One* 2017; **12**: e0171827 [PMID: 28192510 DOI: 10.1371/journal.pone.0171827]

P- Reviewer: Aurello P, Christodoulidis G, Matowicka-Karna J
S- Editor: Wang XJ **L- Editor**: Filipodia **E- Editor**: Yin SY

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4272-4280

DOI: 10.3748/wjg.v24.i37.4272

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Case Control Study

Evaluation of elastography combined with serological indexes for hepatic fibrosis in patients with chronic hepatitis B

Bin Xu, Ning-Ming Zhou, Wei-Tian Cao, Xiao-Jing Li

Bin Xu, Ning-Ming Zhou, Wei-Tian Cao, Department of ultrasound, Fudan University affiliated Shanghai fifth people's hospital, Shanghai 200240, China

Xiao-Jing Li, Department of pathology, Fudan University affiliated Shanghai fifth people's hospital, Shanghai 200240, China

ORCID number: Bin Xu (0000-0002-9184-107X); Ning-Ming Zhou (0000-0001-8424-7825); Wei-Tian Cao (0000-0002-4880-7589); Xiao-Jing Li (0000-0001-6370-9146).

Author contributions: Xu B designed research; Xu B, Cao WT, Li XJ performed research; Zhou NM contributed new reagents or analytic tools; Li XJ analyzed data; Xu B wrote the paper.

Supported by the Natural Science Research Project of Minhang District, No. 2013MHZ003.

Institutional review board statement: The study was approved by the ethics committee of Fudan University affiliated Shanghai fifth people's hospital.

Informed consent statement: All patients gave informed consent.

Conflict-of-interest statement: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Data sharing statement: Data are available from the corresponding author at zhouningming@5thhospital.com.

STROBE Statement: The STROBE Statement have been adopted.

Manuscript source: Unsolicited manuscript

Correspondence to: Ning-Ming Zhou, MD, Chief Doctor, Department of ultrasound, Fudan University affiliated Shanghai fifth people's hospital, 128 Ruili road, Minhang district, Shanghai 200240, China. zhouningming@5thhospital.com
Telephone: +86-21-24289356
Fax: +86-21-24289356

Received: June 22, 2018
Peer-review started: June 22, 2018
First decision: July 25, 2018
Revised: August 6, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

AIM

To investigate the value of ultrasound elastography combined with serological indexes in diagnosing liver fibrosis and assessing its severity.

METHODS

A total of 338 chronic hepatitis B (CHB) patients were divided into a disease group (patients with hepatic fibrosis) and control group (subjects without hepatic fibrosis). The disease group was further divided into S1-S4 according to the degree of fibrosis. Independent risk factors for hepatic fibrosis were analyzed using multivariate logistic regression. The diagnostic values of hepatic fibrosis from different indicators were compared using receiver operating characteristic (ROC) curves. The combination of elastography and serological indexes was explored to assess the severity of hepatic fibrosis.

RESULTS

The multivariate logistic regression analysis results revealed that shear wave velocity (SWV), hyaluronic acid (HA), type Ⅳ collagen (CⅣ) and aspartate aminotransferase-to-platelet ratio index (APRI) significantly affected the occurrence of hepatic fibrosis. The ROC curve revealed that the accuracy of the diagnosis of hepatic fibrosis for SWV and HA were 87.3% and 84.8%, respectively. The accuracy of SWV combined with HA was 88.9%. The multiple linear regression analysis revealed that SWV, aspartate aminotransferase (AST)/alanine aminotransferase (ALT), HA, CⅣ, APRI and fibrosis index based on the 4 factor (FIB-4) were screened as statistically significant independent factors. The established regression equation was: Fibrosis level = -4.046 + 1.024 × SWV + 1.170 × AST/ALT + 0.011 × HA + 0.020 × CⅣ + 0.719 × APRI + 0.379 × FIB-4.

CONCLUSION

SWV combined with serological indexes can improve the accuracy of diagnosis for CHB hepatic fibrosis. Serum indexes can help diagnose the degree of hepatic fibrosis.

Key words: Elastography; Serology; Hepatic fibrosis; Non-invasive diagnosis

Core tip: Hepatic fibrosis affects the physiological function of the liver. The current assessment method for the degree of hepatic fibrosis is still unreliable. This study found that the shear wave velocity of ultrasound elastography can improve the accuracy of the diagnosis of hepatic fibrosis. Its combination with serological indicators (aspartate aminotransferase/alanine aminotransferase, hyaluronic acid, type Ⅳ collagen, aspartate aminotransferase-to-platelet ratio index and fibrosis index based on the 4 factor) can further help in the clinical assessment of the degree of hepatic fibrosis.

Xu B, Zhou NM, Cao WT, Li XJ. Evaluation of elastography combined with serological indexes for hepatic fibrosis in patients with chronic hepatitis B. *World J Gastroenterol* 2018; 24(37): 4272-4280 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4272.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4272

INTRODUCTION

Hepatic fibrosis is a pathological change caused by chronic liver injury, which in turn affects the physiological function of the liver[1-4]. Pathological examination is the gold standard for the diagnosis of hepatic fibrosis, which enables a definitive diagnosis of hepatic fibrosis[5-8]. However, pathological examination mainly relies on biopsy. Biopsy is a kind of invasive examination with the drawbacks of poor reproducibility and sampling errors. Therefore, non-invasive diagnostic methods that seek repeatable measurements have presently become research hotspots. At present, serological indexes are the main clinical methods to assess hepatic fibrosis, although the accuracy needs to be improved[9-11]. The latest research has shown that ultrasound elastography can measure the hardness of liver tissue to determine the degree of hepatic fibrosis with features of non-invasiveness, simplicity, speed and repeatability[12,13]. However, its diagnostic accuracy is not high, and the accuracy of different studies are different[14,15]. Hence, we still need to explore the diagnostic methods of hepatic fibrosis, as well as search for a reliable method to assess the degree of hepatic fibrosis. The investigators therefore collected patients who were admitted to our hospital with chronic hepatitis B (CHB) as subjects in the present study. Their final pathological results were used as a basis for the diagnosis of hepatic fibrosis, and the serological indexes and ultrasound elastography data of these patients were analyzed. The aim of the present study was to search for an optimal method for the combined diagnosis of hepatic fibrosis, and establish an optimal non-invasive assessment model for the severity of hepatic fibrosis.

MATERIALS AND METHODS

Research object

A total of 338 CHB patients were randomly enrolled in our hospital from January 2015 to June 2017. Among these patients, 200 patients were male and 138 patients were female. Inclusion criteria: (1) Patients who underwent liver biopsy; and (2) patients who received ultrasound elastography and serological detection before the biopsy. Exclusion criteria: (1) Patients combined with other types of liver disease; (2) patients with severe heart, liver and kidney insufficiency, coma, or puncture site infection; and (3) patients associated with liver cancer, immune system disease, or active bleeding and other diseases. These patients were divided into two groups according to the presence of hepatic fibrosis *via* biopsy: disease group (patients with hepatic fibrosis) and control group (subjects without hepatic fibrosis). The disease group was further divided into four subgroups, according to the degree of fibrosis: S1, S2, S3 and S4. All patients or their families provided a signed informed consent. The present study met the requirements of the hospital ethics committee and received their approval.

Research methods

Detection of hepatitis B hepatic fibrosis *via* acoustic radiation force impulse: The acoustic radiation force impulse (ARFI) test was performed using an ACOUSON S2000 Color Ultrasound Scanner (Siemens). (1) The patient underwent fasting and was placed in the left lateral decubitus position, with the right-hand on the head. Then, the right hepatic tissue of the liver was detected; (2) the elastic sampling frame was placed perpendicular to the

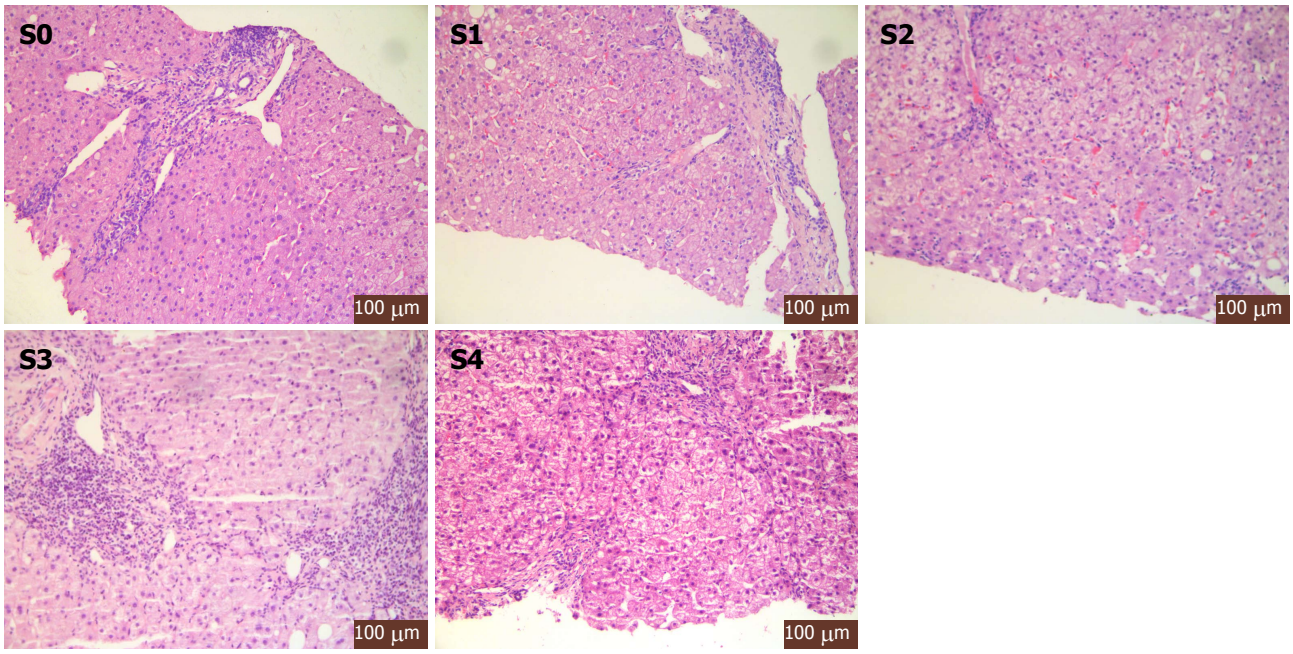

Figure 1 Histopathology of different pathological stages of hepatic fibrosis. S0 phase: No fibrosis; S1 phase: Fibrous enlargement in the portal area, but no fibrillary septum formation; S2 phase: Fibrosis enlargement in the portal area, a few fibrous septae formed; S3 stage: Most fibrous septae formed but no hardened nodules; S4 stage: Liver cirrhosis.

liver surface, and then placed in the liver parenchyma at approximately 4 cm away from the probe surface in order to avoid the surrounding blood vessels. The patient was instructed to hold their breath; and (3) the update key was pressed, a high-intensity low-frequency pulse wave was launched, the transverse shear wave velocity (SWV) was received in m/s, and the value was recorded. The measurement was repeated three times, and the SWV value was taken as the SWV value of the liver parenchyma.

Serological examination: On the next day of admission, 3 mL of fasting venous blood was collected in the morning. Alanine aminotransferase (ALT), aspartate transaminase (AST), blood platelet (PLT), total bilirubin (TBIL), hyaluronic acid (HA) levels, laminin (LN), type Ⅳ collagen (CⅣ), and type Ⅲ procollagen (PⅢNP) were measured. Serum ALT, AST, PLT and TBIL were detected using an automatic biochemical analyzer, while HA, LN, CⅣ and PⅢNP were measured by photochemiluminescence. APRI score: the AST and platelet (PLT) ratio index (aspartate aminotransferase-to-platelet ratio index, APRI). APRI = [(AST/ULN) × 100/PLT (10^9/L)][16]. FIB-4 index: FIB-4 = (age × AST) ÷ (platelet ×√ALT).

Liver biopsy: Liver tissue biopsies were simultaneously performed with ultrasound elastography and serological tests. The subjects were placed in the supine position, the preoperative ultrasound was localized, and the liver puncture was performed under ultrasound guidance. The puncture gun was an automatic biopsy gun obtained from Bard Inc. (United States), with a 16 G disposable biopsy. The needle biopsy was performed in the ARFI sampling frame area. Liver biopsy was conducted with routine disinfection, which was covered with a towel, and local anesthesia with 5% lidocaine was given to avoid the visible pipeline in the liver. A tissue length of 1-2 cm was removed. The degree of hepatic fibrosis in patients with CHB was determined based on histological staging criteria, according to the "Guidelines for the Prevention and Treatment of Chronic Hepatitis B (2015 Update version)". In particular, S0 phase refers to patients with no fibrosis, the S1 phase refers to patients with enlarged fibrosis in the portal area but no fibril septum formation, the S2 phase refers to patient with a fibrous enlargement in the portal area and minimal fibrillary septae formation, the S3 phase refers to patients with the most fibrillary septae formed but without hardened nodules, and the S4 phase refers to patients with cirrhosis (Figure 1).

Statistical analysis

Statistical analysis was performed using SPSS 19.0 and MedCalc software. Measurement data were expressed as mean ± SD. The *t*-test was used for comparisons between the two groups. The rate of adoption of count data was expressed using a Chi-square test to compare the two groups. Independent risk factors of fibrotic liver were analyzed by multivariate logistic regression analysis, while ROC curve analysis was conducted to determine the accuracy in the diagnosis of hepatic fibrosis. Spearman correlation analysis was used to compare the degree of hepatic fibrosis with serological markers and elastography. Multiple linear regression was used to establish a hepatic fibrosis assessment model and determine its degree of fit. $P < 0.05$ was considered

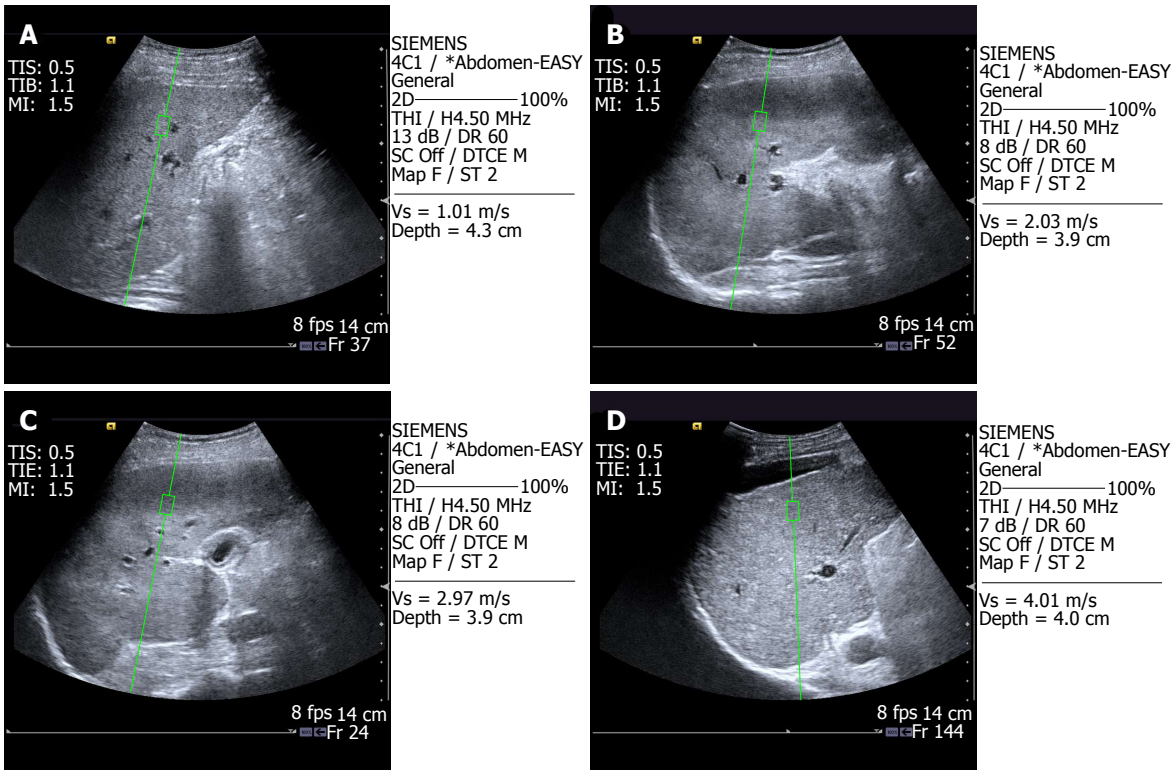

Figure 2 Image of hepatic fibrosis assessed by acoustic radiation force impulse to assess liver tissue elasticity. A: Normal liver tissue, SWV = 1.01 m/s; B: Mild hepatic fibrosis, SWV = 2.03 m/s; C: Moderate hepatic fibrosis, SWV = 2.97 m/s; D: Severe hepatic fibrosis, SWV = 4.01m/s. ARFI: Acoustic radiation force impulse.

Table 1 Comparison of clinical data in the two groups

	Hepatic fibrosis group (n = 245)	Control group (n = 93)	t/χ^2 value	P value
Sex (male/%)	136/55.51	45/48.39	1.375	0.241
Age	40.31 ± 10.47	37.82 ± 13.72	1.785	0.075
SWV (m/s)	3.02 ± 0.80	1.52 ± 0.62	16.310	0.000
ALT (U/L)	52.23 ± 26.02	46.87 ± 87.45	1.487	0.138
AST (U/L)	41.12 ± 12.72	36.14 ± 14.52	1.607	0.109
AST/ALT	0.93 ± 0.41	0.74 ± 0.23	4.221	0.000
PLT (10^9/L)	184.02 ± 02.21	192.37 ± 37.42	1.220	0.223
TBIL (μmol/L)	19.83 ± 9.83	18.75 ± 8.72	1.570	0.117
HA (μg/L)	113.24 ± 24.78	63.23 ± 23.54	15.748	0.000
LN (μg/L)	36.21 ± 21.34	34.21 ± 10.28	1.485	0.139
CⅣ (μg/L)	33.28 ± 28.20	20.89 ± 9.56	4.144	0.000
PⅢNP (μg/L)	36.29 ± 29.45	32.87 ± 2.32	1.100	0.272
APRI	0.85 ± 0.61	0.62 ± 0.52	3.219	0.001
FIB-4	1.63 ± 0.89	1.17 ± 0.62	4.578	0.000

SWV: Shear wave velocity; ALT: Alanine aminotransferase; AST: Aspartate transaminase; PLT: Blood platelet; TBIL: Total bilirubin; HA: Hyaluronic acid; LN: Laminin; CⅣ: Type Ⅳ collagen; PⅢNP: Type Ⅲ procollagen; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis index based on the 4 factor.

statistically significant.

RESULTS

Comparison of clinical data

A total of 338 patients were enrolled in the present study. Among these patients, 93 subjects were assigned to the control group, while 245 patients were assigned to the disease group. Among the patients in the disease group, 72 patients were in the S1 phase, 65 patients were in the S2 phase, 58 patients were in the S3 phase, and 50 patients were in the S4 phase. Furthermore, among the 245 patients in the disease group, 62 patients had mild hepatic fibrosis, 176 patients had moderate hepatic fibrosis, and seven patients had severe hepatic fibrosis (Figure 2). The serological indexes, such as AST/ALT, HA, CⅣ, APRI and FIB-4, were significantly greater in the disease group than in the control group, and the differences were statistically significant (P < 0.05). For the elastography, SWV was significantly greater in the disease group than in the control group, and the difference was statistically significant (P < 0.05). The remaining indicators were similar between the two groups, and the difference was not statistically significant (P > 0.05) (Table 1).

Multivariate analysis of hepatic fibrosis

Indicators with significant differences (SWV, AST/ALT, HA, CⅣ, APRI and FIB-4) were used as independent variables. The occurrence of fibrosis was a dependent variable, and a multivariate logistic regression analysis was conducted. These results revealed that SWV, HA, CⅣ and APRI had a significant effect on hepatic fibrosis (P < 0.05). According to the OR value, the sequence was SWV, HA, APRI and CⅣ (Table 2).

Diagnosis of different indicators in hepatic fibrosis

The ROC curve for the diagnosis of hepatic fibrosis by

Table 2 Binary logistic regression analysis of risk factors associated with hepatic fibrosis

Influencing factors	B	SE	Wald	OR	95%CI	*P* value
SWV	0.931	0.325	5.024	2.537	1.342-4.797	0.025
AST/ALT	0.561	0.286	2.765	1.752	0.896-3.069	0.056
HA	0.838	0.127	5.352	2.311	1.802-2.964	0.021
CⅣ	0.466	0.183	4.042	1.593	1.113-2.280	0.046
APRI	0.719	0.312	4.642	2.053	1.114-3.784	0.037
FIB-4	0.433	0.287	2.973	1.542	0.879-2.706	0.063

SWV: Shear wave velocity; ALT: Alanine aminotransferase; AST: Aspartate transaminase; HA: Hyaluronic acid; CⅣ: Type Ⅳ collagen; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis index based on the 4 factor.

Table 3 Diagnostic efficacy of various indicators in the diagnosis of hepatic fibrosis

Index	AUC	Best diagnostic point	Sensitivity, %	Specificity, %
SWV	0.873	1.66 m/s	86.90	88.20
AST/ALT	0.803	0.920	55.90	95.70
HA	0.848	87.79 μg/L	91.00	79.60
CⅣ	0.784	30.36 μg/L	52.70	98.90
APRI	0.789	0.787	57.60	86.00
FIB-4	0.797	1.157	80.00	65.60

SWV: Shear wave velocity; ALT: Alanine aminotransferase; AST: Aspartate transaminase; HA: Hyaluronic acid; CⅣ: Type Ⅳ collagen; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis index based on the 4 factor.

Table 4 Correlation between serological data and the degree of hepatic fibrosis

Index	r	*P* value
SWV (m/s)	0.767	0.000
HA (μg/L)	0.711	0.000
AST/ALT	0.684	0.000
CⅣ (μg/L)	0.681	0.000
APRI	0.634	0.000
FIB-4	0.702	0.000

SWV: Shear wave velocity; HA: Hyaluronic acid; ALT: Alanine aminotransferase; AST: Aspartate transaminase; CⅣ: Type Ⅳ collagen; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis index based on the 4 factor.

each index is illustrated in Figure 3. The area under the curve (AUC) for hepatic fibrosis diagnosed by SWV was the highest (0.873), followed by HA (0.848). The remaining AUC rankings were as follows: AST/ALT, APRI, FIB-4, and CⅣ (Figure 3). The combined diagnosis of SWV and HA with the highest AUC indicated that diagnostic accuracy was further improved with an AUC of 0.889 (sensitivity: 95.92% and specificity: 72.04%) (Table 3 and Figure 4).

Association of serological markers with elastography and hepatic fibrosis

Spearman correlation analysis revealed that hepatic fibrosis was positively correlated with SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 levels. The R values were 0.767, 0.684, 0.711, 0.681, 0.634 and 0.702, respectively, and the difference was statistically significant (all $P < 0.05$) (Table 4). The statistically significant indicators in the correlation analysis were included in the multiple linear regression analysis. The results revealed that SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 were selected as statistically significant independent factors, and the constant analysis was statistically significant. The following regression equation was established: degree of fibrosis = -4.046 + 1.024 × SWV + 1.170 × AST/ALT + 0.011 × HA + 0.020 × CⅣ + 0.719 × APRI + 0.379 × FIB-4 (Table 5).

DISCUSSION

CHB is one of the most common causes of liver-related diseases[17-21], which can gradually develop into hepatic fibrosis, cirrhosis and liver cancer[22-30]. At present, hepatic fibrosis remains a reversible process. Its early diagnosis, as well as its timely and effective treatment, can delay or avoid the development of irreversible cirrhosis stages. Developing an approach to simply and correctly evaluate the severity of hepatic fibrosis has become a clinical challenge that needs to be solved[31]. The literature revealed that liver pathology biopsy is the most important diagnostic basis for the diagnosis of hepatic fibrosis[32-34]. Although it is the "gold standard" for the diagnosis of hepatic fibrosis, it requires immense invasiveness and demonstrates poor reproducibility. Imaging and serological examination can reflect hepatic fibrosis. However, neither of them can be used as an independent diagnostic indicator. Elastography has been used to measure shear waves in liver tissues by ultrasound. The speed of ultrasound propagation is used to calculate the hardness of the liver and determine the degree of hepatic fibrosis. Changes in serological indexes reflect the progression of the disease in patients with hepatic fibrosis[35-44]. In this study, in order to search for non-invasive methods for the diagnosis of hepatic fibrosis, 245 patients with hepatic fibrosis and 93 subjects without hepatic fibrosis were used as observation subjects. The general data, elastography and serological indicators of these subjects were used to analyze the feasibility of ultrasound elastography combined with serological markers for the diagnosis of hepatic fibrosis and the degree of hepatic fibrosis.

The present study first analyzed the clinical data of these two groups. The results revealed that SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 were significantly greater in the disease group than in the control group. This suggests that SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 are the six indicators that can help in the clinical screening for patients with hepatic fibrosis, which is consistent with previous studies[45-50]. Subsequently, in the present study, a multivariate logistic regression analysis was performed on these indicators, which showed significant differences. These results revealed that SWV, HA, CⅣ and APRI

Table 5 Multiple linear regression analysis of the degree of hepatic fibrosis

	Non-standardized coefficient		Standard coefficient	*t*	Sig.
	B value	Standard error	*B* value		
Constant	-4.046	0.209	-	-19.365	0.000
SWV	1.024	0.148	0.2200	6.930	0.000
AST/ALT	1.170	0.190	0.3231	7.861	0.000
HA	0.011	0.005	0.2010	7.126	0.000
CⅣ	0.020	0.003	0.1980	5.749	0.000
APRI	0.719	0.102	0.1880	7.040	0.000
FIB-4	0.379	0.088	0.1490	4.304	0.000

SWV: Shear wave velocity; ALT: Alanine aminotransferase; AST: Aspartate transaminase; HA: Hyaluronic acid; CⅣ: Type Ⅳ collagen; APRI: Aspartate amino-transferase-to-platelet ratio index; FIB-4: Fibrosis index based on the 4 factor.

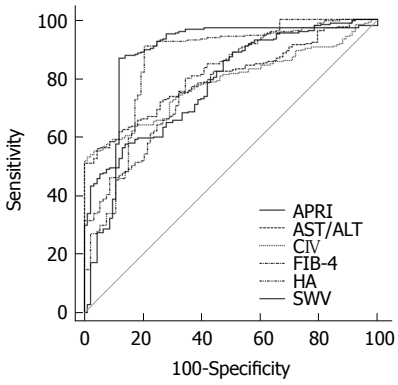

Figure 3 Receiver operating characteristic curve for the diagnosis of hepatic fibrosis based on different indicators. APRI: Aspartate amino-transferase-to-platelet ratio index; AST: Aspartate transaminase; ALT: Alanine aminotransferase; CⅣ: Type Ⅳ collagen; FIB-4: Fibrosis index based on the 4 factor; HA: Hyaluronic acid; SWV: Shear wave velocity.

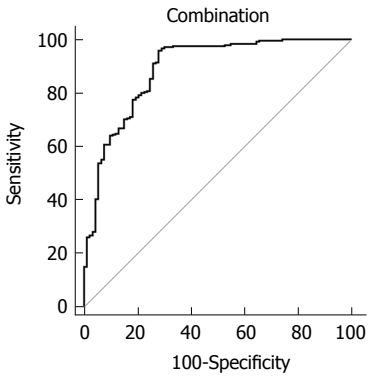

Figure 4 Receiver operating characteristic curve for the diagnosis of hepatic fibrosis based on different combined indicators.

had a significant effect on the development of hepatic fibrosis, suggesting that clinical attention should be given to patients with high levels of SWV, HA, CⅣ and APRI. In order to further explore the clinical significance of these indicators, an ROC curve analysis was performed. Among these four indicators, the maximum area under the ROC curve for SWV was 0.873, suggesting that SWV may be used as an ideal indicator for hepatic fibrosis screening. After these indicators were combined, it was noted that the accuracy of the diagnosis was further enhanced, suggesting that the clinical accuracy of hepatic fibrosis can be improved by combining SWV with serological indexes.

In order to fully explain the effects of SWV and serological indexes on hepatic fibrosis in patients with clinical hepatic fibrosis, correlation analyses and multiple linear regression analyses were performed. The results revealed that the degree of hepatic fibrosis and SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 were positively correlated. After multiple linear regression analysis, the results revealed that SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 were independent factors that affected the degree of hepatic fibrosis, and these were further established. Multiple linear regression equation: Degree of fibrosis = -4.046 + 1.024 × SWV + 1.170 × AST/ALT + 0.011 × HA + 0.020 × CⅣ + 0.719 × APRI + 0.379 × FIB-4. A non-invasive clinical tool was provided for assessing hepatic fibrosis. The SPSS software can be used in clinic to assess the extent of the hepatic fibrosis in a patient by entering the above parameters. Although the richness degree of data collected in the present study can be further improved, the present single-center study was not sufficient to fully guarantee the reliability of the study. Hence, the equation cannot be used as a clinical tool to predict lymph node metastasis. However, this method is worthy of further clinical validation and promotion. In addition, for serological indexes that can reflect the degree of hepatic fibrosis, further review of the literature is needed to explore the mechanism of the degree of fibrosis of the indicator response. This will allow us to obtain a deeper understanding of the significance of serological indexes in the diagnosis of hepatic fibrosis.

In summary, SWV can improve the accuracy of hepatic fibrosis diagnosis, and overcomes the invasive and poor reproducibility shortcomings associated with liver biopsy. At the same time, SWV in combination with serological indexes can further help in the clinical assessment of the extent of hepatic fibrosis in patients.

ARTICLE HIGHLIGHTS

Research background

Pathological examination is known to be the gold standard for diagnosing

liver fibrosis, as it enables a clear diagnosis of liver fibrosis grading. However, pathological examination is an invasive examination and cannot be used as a screening tool. At present, the degree of liver fibrosis is mainly evaluated by serological indicators in the clinic, however the accuracy is relatively low. With advances in technology, ultrasound elastography can be used to assess liver tissue stiffness, although the accuracy is not high. Therefore, it is necessary to explore reliable methods for diagnosing liver fibrosis and assessing the degree of liver fibrosis.

Research motivation

The motivation of this study is to find a more suitable method for the combined diagnosis of liver fibrosis and to establish an optimal non-invasive model for assessing the severity of liver fibrosis. This will provide a reference for non-invasive screening of liver fibrosis.

Research objectives

This study enrolled patients with chronic hepatitis B (CHB) as the research subjects. The aim of this study is to analyze serum markers and ultrasound elastography indicators for diagnosing liver fibrosis and liver fibrosis grading based on pathological results.

Research methods

According to the results of liver biopsy, 338 patients with CHB admitted to our hospital were divided into a diseased group and control group. The diseased group continued to be divided into four groups according to the degree of fibrosis. General data, shear wave velocity (SWV), and serological markers were compared between the two groups. Further independent risk factors for liver fibrosis in patients were analyzed by logistic regression. The accuracy of different indicators in diagnosing liver fibrosis was compared by receiver operating characteristic (ROC) curves. The correlation between different fiber levels and serum indicators or elastography indicators was analyzed. Finally, a multivariate linear regression was used to establish a mathematical model for assessing the severity of liver fibrosis with elastography combined with serological markers.

Research results

SWV, aspartate aminotransferase (AST)/alanine aminotransferase (ALT), hyaluronic acid (HA), type-Ⅳ collagen (CⅣ), aspartate aminotransferase-to-platelet ratio index (APRI) and fibrosis index based on the 4 factor (FIB-4) were significantly higher in the disease group than in the control group ($P < 0.05$). The multivariate logistic regression analysis results revealed that SWV, HA, CⅣ and APRI significantly affected the occurrence of hepatic fibrosis. The ROC curve revealed that the accuracy of the diagnosis of hepatic fibrosis for SWV and HA were 87.3% and 84.8%, respectively. The accuracy of SWV combined with HA was 88.9%. Spearman correlation analysis revealed that hepatic fibrosis was positively correlated with SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 levels. The R values were 0.767, 0.684, 0.711, 0.681, 0.634 and 0.702, respectively, and the difference was statistically significant (all $P < 0.05$). The multiple linear regression analysis revealed that SWV, AST/ALT, HA, CⅣ, APRI and FIB-4 were screened as statistically significant independent factors. The established model was: fibrosis level = -4.046 + 1.024 × SWV + 1.170 × AST/ALT + 0.011 × HA + 0.020 × CⅣ + 0.719 × APRI + 0.379 × FIB-4.

Research conclusions

SWV can non-invasively and effectively diagnose liver fibrosis. SWV combined with serological indicators can further improve the accuracy of diagnosing liver fibrosis. The multiple linear regression equation established by SWV combined with serological indicators is expected to be a non-invasive tool for assessing the degree of liver fibrosis.

Research perspectives

This study is a single-center study, and the sample size is limited and insufficient to fully guarantee the reliability of the study. Therefore, the equation we established cannot be used as an accurate tool for clinical prediction of lymph node metastasis, but it is worthy of further clinical validation and promotion. In addition, for serological indicators that can reflect the degree of liver fibrosis, we can further consult the literature to explore the mechanism of the degree of fibrosis. This would help us understand the diagnostic significance of serological markers with respect to the degree of liver fibrosis.

REFERENCES

1 **Friedman SL**. Liver fibrosis - from bench to bedside. *J Hepatol* 2003; **38** Suppl 1: S38-S53 [PMID: 12591185 DOI: 10.1016/S0168-8278(02)00429-4]

2 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074 DOI: 10.1172/JCI24282]

3 **Wallace K**, Burt AD, Wright MC. Liver fibrosis. *Biochem J* 2008; **411**: 1-18 [PMID: 18333835 DOI: 10.1042/BJ20071570]

4 **Tsukada S**, Parsons CJ, Rippe RA. Mechanisms of liver fibrosis. *Clin Chim Acta* 2006; **364**: 33-60 [PMID: 16139830 DOI: 10.1016/j.cca.2005.06.014]

5 **Lefkowitch JH**. Liver biopsy assessment in chronic hepatitis. *Arch Med Res* 2007; **38**: 634-643 [PMID: 17613355 DOI: 10.1016/j.arcmed.2006.08.005]

6 **Pinzani M**, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. *J Hepatol* 2005; **42** Suppl: S22-S36 [PMID: 15777570 DOI: 10.1016/j.jhep.2004.12.008]

7 **Theise ND**. Liver biopsy assessment in chronic viral hepatitis: a personal, practical approach. *Mod Pathol* 2007; **20** Suppl 1: S3-14 [PMID: 17486049 DOI: 10.1038/modpathol.3800693]

8 **Sun Y**, Zhou J, Wang L, Wu X, Chen Y, Piao H, Lu L, Jiang W, Xu Y, Feng B, Nan Y, Xie W, Chen G, Zheng H, Li H, Ding H, Liu H, Lv F, Shao C, Wang T, Ou X, Wang B, Chen S, Wee A, Theise ND, You H, Jia J. New classification of liver biopsy assessment for fibrosis in chronic hepatitis B patients before and after treatment. *Hepatology* 2017; **65**: 1438-1450 [PMID: 28027574 DOI: 10.1002/hep.29009]

9 **Lee HH**, Seo YS, Um SH, Won NH, Yoo H, Jung ES, Kwon YD, Park S, Keum B, Kim YS, Yim HJ, Jeen YT, Chun HJ, Kim CD, Ryu HS. Usefulness of non-invasive markers for predicting significant fibrosis in patients with chronic liver disease. *J Korean Med Sci* 2010; **25**: 67-74 [PMID: 20052350 DOI: 10.3346/jkms.2010.25.1.67]

10 **Imbert-Bismut F**, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T; MULTIVIRC Group. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069-1075 [PMID: 11297957 DOI: 10.1016/S0140-6736(00)04258-6]

11 **Bottero J**, Lacombe K, Guéchot J, Serfaty L, Miailhes P, Bonnard P, Wendum D, Molina JM, Lascoux-Combe C, Girard PM. Performance of 11 biomarkers for liver fibrosis assessment in HIV/HBV co-infected patients. *J Hepatol* 2009; **50**: 1074-1083 [PMID: 19398234 DOI: 10.1016/j.jhep.2009.01.022]

12 **Khallafi H**, Qureshi K. Imaging Based Methods of Liver Fibrosis Assessment in Viral Hepatitis: A Practical Approach. *Interdiscip Perspect Infect Dis* 2015; **2015**: 809289 [PMID: 26779260 DOI: 10.1155/2015/809289]

13 **Shima H**, Igarashi G, Wakisaka M, Hamano S, Nagae H, Koyama M, Kitagawa H. Noninvasive acoustic radiation force impulse (ARFI) elastography for assessing the severity of fibrosis in the post-operative patients with biliary atresia. *Pediatr Surg Int* 2012; **28**: 869-872 [PMID: 22864589 DOI: 10.1007/s00383-012-3140-4]

14 **Zeng J**, Liu GJ, Huang ZP, Zheng J, Wu T, Zheng RQ, Lu MD. Diagnostic accuracy of two-dimensional shear wave elastography for the non-invasive staging of hepatic fibrosis in chronic hepatitis B: a cohort study with internal validation. *Eur Radiol* 2014; **24**: 2572-2581 [PMID: 25027837 DOI: 10.1007/s00330-014-3292-9]

15 **Samir AE**, Dhyani M, Vij A, Bhan AK, Halpern EF, Méndez-Navarro J, Corey KE, Chung RT. Shear-wave elastography for the estimation of liver fibrosis in chronic liver disease: determining accuracy and ideal site for measurement. *Radiology* 2015; **274**: 888-896 [PMID: 25393946 DOI: 10.1148/radiol.14140839]

16 **Wai CT**, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518-526 [PMID: 12883497 DOI:

10.1053/jhep.2003.50346]
17 **Chu CM**. Natural history of chronic hepatitis B virus infection in adults with emphasis on the occurrence of cirrhosis and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2000; **15** Suppl: E25-E30 [PMID: 10921378 DOI: 10.1046/j.1440-1746.2000.02097.x]
18 **European Association For The Study Of The Liver.** EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]
19 **European Association for the Study of the Liver**. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. *J Hepatol* 2017; **67**: 370-398 [PMID: 28427875 DOI: 10.1016/j.jhep.2017.03.021]
20 **Papatheodoridis GV**, Manolakopoulos S. EASL clinical practice guidelines on the management of chronic hepatitis B: the need for liver biopsy. *J Hepatol* 2009; **51**: 226-227 [PMID: 19410321 DOI: 10.1016/j.jhep.2009.02.017]
21 **Yapali S**, Talaat N, Lok AS. Management of hepatitis B: our practice and how it relates to the guidelines. *Clin Gastroenterol Hepatol* 2014; **12**: 16-26 [PMID: 23660419 DOI: 10.1016/j.cgh.2013.04.036]
22 **Huang CF**, Sun CC, Zhao F, Zhang YD, Li DJ. miR-33a levels in hepatic and serum after chronic HBV-induced fibrosis. *J Gastroenterol* 2015; **50**: 480-490 [PMID: 25155445 DOI: 10.1007/s00535-014-0986-3]
23 **Alam MM**, Mahtab MA, Akbar SM, Kamal M, Rahman S. Hepatic necroinflammation and severe liver fibrosis in patients with chronic hepatitis B with undetectable HBV DNA and persistently normal alanine aminotransferase. *Bangladesh Med Res Counc Bull* 2014; **40**: 92-96 [PMID: 26402972 DOI: 10.3329/bmrcb.v40i3.25229]
24 **Chan HL**, Tsang SW, Leung NW, Tse CH, Hui Y, Tam JS, Chan FK, Sung JJ. Occult HBV infection in cryptogenic liver cirrhosis in an area with high prevalence of HBV infection. *Am J Gastroenterol* 2002; **97**: 1211-1215 [PMID: 12014730 DOI: 10.1111/j.1572-0241.2002.05706.x]
25 **Hu J**, Ludgate L. HIV-HBV and HIV-HCV coinfection and liver cancer development. *Cancer Treat Res* 2007; **133**: 241-252 [PMID: 17672044 DOI: 10.1007/978-0-387-46816-7_9]
26 **Morales-Romero J**, Vargas G, García-Román R. Occult HBV infection: a faceless enemy in liver cancer development. *Viruses* 2014; **6**: 1590-1611 [PMID: 24717680 DOI: 10.3390/v6041590]
27 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]
28 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ; REVEAL-HBV Study Group. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143 [PMID: 18695135 DOI: 10.1093/jnci/djn243]
29 **El-Serag HB**. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; **142**: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
30 **Liu CJ**, Kao JH. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. *J Chin Med Assoc* 2007; **70**: 141-145 [PMID: 17475593 DOI: 10.1016/S1726-4901(09)70346-6]
31 **Standish RA**, Cholongitas E, Dhillon A, Burroughs AK, Dhillon AP. An appraisal of the histopathological assessment of liver fibrosis. *Gut* 2006; **55**: 569-578 [PMID: 16531536 DOI: 10.1136/gut.2005.084475]
32 **Chevallier M**, Guerret S, Chossegros P, Gerard F, Grimaud JA. A histological semiquantitative scoring system for evaluation of hepatic fibrosis in needle liver biopsy specimens: comparison with morphometric studies. *Hepatology* 1994; **20**: 349-355 [PMID: 8045495 DOI: 10.1002/hep.1840200213]
33 **Mani H**, Kleiner DE. Liver biopsy findings in chronic hepatitis B. *Hepatology* 2009; **49**: S61-S71 [PMID: 19399798 DOI: 10.1002/hep.22930]
34 **Cholongitas E**, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006; **125**: 710-721 [PMID: 16707372 DOI: 10.1309/W3XC-NT4H-KFBN-2G0B]
35 **Serra C**, Grasso V, Conti F, Felicani C, Mazzotta E, Lenzi M, Verucchi G, D'errico A, Andreone P. A New Two-Dimensional Shear Wave Elastography for Noninvasive Assessment of Liver Fibrosis in Healthy Subjects and in Patients with Chronic Liver Disease. *Ultraschall Med* 2018; **39**: 432-439 [PMID: 29458217 DOI: 10.1055/s-0043-119356]
36 **Rockey DC**, Bissell DM. Noninvasive measures of liver fibrosis. *Hepatology* 2006; **43**: S113-S120 [PMID: 16447288 DOI: 10.1002/hep.21046]
37 **Friedrich-Rust M**, Koch C, Rentzsch A, Sarrazin C, Schwarz P, Herrmann E, Lindinger A, Sarrazin U, Poynard T, Schäfers HJ, Zeuzem S, Abdul-Khaliq H. Noninvasive assessment of liver fibrosis in patients with Fontan circulation using transient elastography and biochemical fibrosis markers. *J Thorac Cardiovasc Surg* 2008; **135**: 560-567 [PMID: 18329470 DOI: 10.1016/j.jtcvs.2007.09.039]
38 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]
39 **Zhang D**, Li P, Chen M, Liu L, Liu Y, Zhao Y, Wang R. Non-invasive assessment of liver fibrosis in patients with alcoholic liver disease using acoustic radiation force impulse elastography. *Abdom Imaging* 2015; **40**: 723-729 [PMID: 24811766 DOI: 10.1007/s00261-014-0154-5]
40 **Chen SH**, Li YF, Lai HC, Kao JT, Peng CY, Chuang PH, Su WP, Chiang IP. Noninvasive assessment of liver fibrosis via spleen stiffness measurement using acoustic radiation force impulse sonoelastography in patients with chronic hepatitis B or C. *J Viral Hepat* 2012; **19**: 654-663 [PMID: 22863270 DOI: 10.1111/j.1365-2893.2012.01588.x]
41 **Gangadharan B**, Antrobus R, Chittenden D, Rossa J, Bapat M, Klenerman P, Barnes E, Dwek RA, Zitzmann N. New approaches for biomarker discovery: the search for liver fibrosis markers in hepatitis C patients. *J Proteome Res* 2011; **10**: 2643-2650 [PMID: 21410221 DOI: 10.1021/pr101077c]
42 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N; Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695 [PMID: 15987917 DOI: 10.1056/NEJMoa043470]
43 **Bonnard P**, Sombié R, Lescure FX, Bougouma A, Guiard-Schmid JB, Poynard T, Calès P, Housset C, Callard P, Le Pendeven C, Drabo J, Carrat F, Pialoux G. Comparison of elastography, serum marker scores, and histology for the assessment of liver fibrosis in hepatitis B virus (HBV)-infected patients in Burkina Faso. *Am J Trop Med Hyg* 2010; **82**: 454-458 [PMID: 20207872 DOI: 10.4269/ajtmh.2010.09-0088]
44 **Moreno S**, García-Samaniego J, Moreno A, Ortega E, Pineda JA, del Romero J, Tural C, von Wichmann MA, Berenguer J, Castro A, Espacio R. Noninvasive diagnosis of liver fibrosis in patients with HIV infection and HCV/HBV co-infection. *J Viral Hepat* 2009; **16**: 249-258 [PMID: 19215579 DOI: 10.1111/j.1365-2893.2009.01088.x]
45 **Pinto J**, Matos H, Nobre S, Cipriano MA, Marques M, Pereira JM, Gonçalves I, Noruegas MJ. Comparison of acoustic radiation force impulse/serum noninvasive markers for fibrosis prediction in liver transplant. *J Pediatr Gastroenterol Nutr* 2014; **58**: 382-386 [PMID: 24164902 DOI: 10.1097/MPG.0000000000000226]
46 **Ala-Kokko L**, Pihlajaniemi T, Myers JC, Kivirikko KI, Savolainen ER. Gene expression of type I, Ⅲ and Ⅳ collagens in hepatic fibrosis induced by dimethylnitrosamine in the rat. *Biochem J* 1987; **244**: 75-79 [PMID: 3663119 DOI: 10.1042/bj2440075]
47 **Petersen JR**, Stevenson HL, Kasturi KS, Naniwadekar A, Parkes J, Cross R, Rosenberg WM, Xiao SY, Snyder N. Evaluation of the aspartate aminotransferase/platelet ratio index and enhanced liver fibrosis tests to detect significant fibrosis due to chronic hepatitis

C. *J Clin Gastroenterol* 2014; **48**: 370-376 [PMID: 24045284 DOI: 10.1097/MCG.0b013e3182a87e78]

48 **Vallet-Pichard A**, Mallet V, Nalpas B, Verkarre V, Nalpas A, Dhalluin-Venier V, Fontaine H, Pol S. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**: 32-36 [PMID: 17567829 DOI: 10.1002/hep.21669]

49 **Parés A**, Deulofeu R, Giménez A, Caballería L, Bruguera M, Caballería J, Ballesta AM, Rodés J. Serum hyaluronate reflects hepatic fibrogenesis in alcoholic liver disease and is useful as a marker of fibrosis. *Hepatology* 1996; **24**: 1399-1403 [PMID: 8938169 DOI: 10.1002/hep.510240615]

50 **Liu J**, Ji Y, Ai H, Ning B, Zhao J, Zhang Y, Dun G. Liver Shear-Wave Velocity and Serum Fibrosis Markers to Diagnose Hepatic Fibrosis in Patients with Chronic Viral Hepatitis B. *Korean J Radiol* 2016; **17**: 396-404 [PMID: 27134527 DOI: 10.3348/kjr.2016.17.3.396]

P- Reviewer: Apisarnthanarax S, Eun Sun K, Hayes MJ, Tomiyasu A, Ward J
S- Editor: Wang XJ **L- Editor**: Filipodia **E- Editor**: Bian YN

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4281-4290

DOI: 10.3748/wjg.v24.i37.4281

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Observational Study

Risk factors for liver disease among adults of Mexican descent in the United States and Mexico

Yvonne N Flores, Zuo-Feng Zhang, Roshan Bastani, Mei Leng, Catherine M Crespi, Paula Ramírez-Palacios, Heather Stevens, Jorge Salmerón

Yvonne N Flores, Paula Ramírez-Palacios, Jorge Salmerón, Unidad de Investigación Epidemiológica y en Servicios de Salud, Delegación Morelos, Instituto Mexicano del Seguro Social, Cuernavaca, Morelos 62000, México

Yvonne N Flores, Roshan Bastani, UCLA Department of Health Policy and Management and Kaiser Permanente Center for Health Equity, Fielding School of Public Health, Los Angeles, CA 90095, United States

Yvonne N Flores, Roshan Bastani, Catherine M Crespi, UCLA Cancer Prevention and Control Research Center, Fielding School of Public Health and Jonsson Comprehensive Cancer Center, Los Angeles, CA 90095, United States

Zuo-Feng Zhang, UCLA Department of Epidemiology, Fielding School of Public Health, Los Angeles, CA 90095, United States

Mei Leng, UCLA Division of General Internal Medicine and Health Services Research, Los Angeles, CA 90095, United States

Catherine M Crespi, UCLA Department of Biostatistics, Fielding School of Public Health, Los Angeles, CA 90095, United States

Heather Stevens, University of Washington, School of Medicine, Seattle, WA 98195, United States

Jorge Salmerón, Universidad Nacional Autónoma de México, Academic Epidemiology Research Unit, Avenida Universidad 3000, Ciudad Universitaria, Coyoacán, Mexico City 04510, México

Jorge Salmerón, Centro de Investigación en Salud Poblacional, Instituto Nacional de Salud Pública, Cuernavaca, Morelos 62100, México

ORCID number: Yvonne N Flores (0000-0002-0601-357X); Zuo-Feng Zhang (0000-0002-4669-3995); Roshan Bastani (0000-0001-6594-9231); Mei Leng (0000-0002-2758-7599); Catherine M Crespi (0000-0002-6150-2181); Paula Ramírez-Palacios (0000-0002-2586-4396); Heather Stevens (0000-0002-8261-2005); Jorge Salmerón (0000-0001-8654-2393).

Author contributions: Flores YN, Zhang ZF, Bastani R, Leng M, Crespi CM, Ramírez-Palacios P, Stevens H, Salmerón J contributed to the study design; statistical analysis; interpretation of the findings; writing the article and approval of the final draft.

Supported by the Programa de Investigación en Migracion y Salud (PIMSA), No. 2015-2106; the Instituto Mexicano del Seguro Social (IMSS), No. 2005/1/ I /093; and the Consejo Nacional de Ciencia y Tecnología (CONACYT), No. 26267M and No. SALUD-2011-01-161930; the NIH, No. UL1TR000124 to Crespi CM, and NIH/NCI No. K07CA197179 to Flores YN.

Institutional review board statement: Approval for both the Health Worker Cohort Study and this bi-national investigation was obtained from the Internal Review Boards of IMSS and UCLA.

Conflict-of-interest statement: All authors declare no potential conflicts of interest relevant to this article.

Data sharing statement: No additional data are available.

STROBE statement: The guidelines of the STROBE Statement have been adopted.

Manuscript source: Unsolicited manuscript

Correspondence to: Yvonne N Flores, PhD, Unidad de Investigación Epidemiológica y en Servicios de Salud, Delegación Morelos, Instituto Mexicano del Seguro Social, Blvd. Juárez #31, Colonia Centro, Cuernavaca, Morelos 62000, México. ynflores@ucla.edu
Telephone: +52-777-1001364

Received: June 20, 2018
Peer-review started: June 20, 2018
First decision: July 4, 2018
Revised: August 1, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

AIM

To compare the prevalence of chronic liver disease (CLD) risk factors in a representative sample of Mexican-Americans born in the United States (US) or Mexico, to a sample of adults in Mexico.

METHODS

Data for Mexican-Americans in the US were obtained from the 1999-2014 National Health and Nutrition Examination Survey (NHANES), which includes persons of Mexican origin living in the US (n = 4274). The NHANES sample was restricted to Mexican-American participants who were 20 years and older, born in the US or Mexico, not pregnant or breastfeeding, and with medical insurance. The data in Mexico were obtained from the 2004-2013 Health Worker Cohort Study in Cuernavaca, Mexico (n = 9485). The following known risk factors for liver disease/cancer were evaluated: elevated aminotransferase levels (elevated alanine aminotransferase was defined as > 40 IU/L for males and females; elevated aspartate aminotransferase was defined as > 40 IU/L for males and females), infection with hepatitis B or hepatitis C, metabolic syndrome, high total cholesterol, diabetes, obesity, abdominal obesity, and heavy alcohol use. The main independent variables for this study classified individuals by country of residence (*i.e.*, Mexico *vs* the US) and place of birth (*i.e.*, US-born *vs* Mexico-born). Regression analyses were used to investigate CLD risk factors.

RESULTS

After adjusting for socio-demographic characteristics, Mexican-American males were more likely to be obese, diabetic, heavy/binge drinkers or have abdominal obesity than males in Mexico. The adjusted multivariate results for females also indicate that Mexican-American females were significantly more likely to be obese, diabetic, be heavy/binge drinkers or have abdominal obesity than Mexican females. The prevalence ratios and prevalence differences mirror the multivariate analysis findings for the aforementioned risk factors, showing a greater risk among US-born as compared to Mexico-born Mexican-Americans.

CONCLUSION

In this study, Mexican-Americans in the US had more risk factors for CLD than their counterparts in Mexico. These findings can be used to design and implement more effective health promotion policies and programs to address the specific factors that put Mexicans at higher risk of developing CLD in both countries.

Key words: Liver disease; Risk factors; Health disparities; Mexico; Mexican Americans; Latinos

Core tip: United States (US) Latinos have greater morbidity and mortality from liver disease than non-Hispanic whites, and liver disease is the fifth leading cause of death in Mexico. Known risk factors for chronic liver disease include hepatitis B or C infection, heavy/binge drinking, obesity, diabetes, and metabolic syndrome. We found that Mexican-Americans in the US have a greater risk of obesity, diabetes and heavy/binge drinking than their counterparts in Mexico. The prevalence of heavy/binge drinking was alarmingly high among Mexican-Americans, with over 70% among males and over 50% among US-born females. Our results identify a high prevalence of specific risk factors that should be targeted to reduce the high rates of liver disease-related mortality in this population.

Flores YN, Zhang ZF, Bastani R, Leng M, Crespi CM, Ramírez-Palacios P, Stevens H, Salmerón J. Risk factors for liver disease among adults of Mexican descent in the United States and Mexico. *World J Gastroenterol* 2018; 24(37): 4281-4290 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4281.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4281

INTRODUCTION

Over 30 million people, or one in ten, are likely to have some form of chronic liver disease (CLD) in the United States (US). This number includes the approximately 850000 to 2.2 million cases of chronic hepatitis B (HBV), 3.5 million cases of chronic hepatitis C (HCV)[1], the estimated 30% of Americans who have non-alcoholic fatty liver disease (NAFLD), and the 10% with advanced fibrosis[2,3]. Also included are the nearly 20000 individuals who die from alcoholic liver disease in the US each year[4]. In 2017, there were an estimated 40000 incident cases of liver cancer and 30000 deaths from liver cancer[5]. New cases of liver cancer have more than tripled since 1980 and liver cancer mortality has increased by nearly 3% each year since 2000[5]. CLD is the 12th leading cause of general mortality in the US[4], the fifth among individuals between 45-54 years, and the sixth among 25-44 year olds and those aged 55-64 years[6].

Latinos in the US have disproportionately higher rates of CLD. Since 2002, CLD has consistently been the sixth leading cause of mortality for Latinos[7], and the third cause of death among Latino males ages 55-64[8]. They are twice as likely to have CLD and 1.7 times more likely to die from liver cancer than non-Hispanic

whites (whites)[9]. The prevalence of earlier stage liver disease, such as steatohepatitis, is also higher among Latinos (45%) than whites (33%) or blacks (24%)[10]. In Mexico, cirrhosis and other forms of CLD were the fourth leading cause of general mortality in 2016, and the third among males aged 35-65 years[11]. An estimated 3 million individuals are infected with HBV and 400000 to 1400000 people are infected with HCV[12]. In 2016, there were 38755 deaths due to CLD in Mexico and 14029 (36%) were attributed to alcoholic liver disease[11]. By 2050, an estimated 90% of cases of CLD in Mexico will be attributable to obesity and excessive alcohol consumption, as compared to other populations with high rates of CLD due to infection with HBV or HCV[13].

Although infection with HBV or HCV and heavy alcohol use are well known risk factors for CLD and liver cancer, a significant proportion of cases (15% to 50%) do not present with these risk factors[14]. Other risk factors for CLD include obesity and diabetes, and the proposed mechanism is through the development of NAFLD and non-alcoholic steatohepatitis (NASH)[15-18]. NAFLD is found in up to 80-90% of obese adults, in 30-50% of patients with diabetes, and in up to 90% of patients with hyperlipidemia[19]. In the US, the prevalence of NAFLD and NASH is highest among Latinos, followed by whites and blacks[10,15,20]. Rates of obesity, diabetes, and hyperlipidemia are also higher among Latinos than whites in the US[21-23]. In Mexico, over 70% of the population is overweight or obese, and this figure is predicted to rise to 90% by 2050[24]. Additionally, the prevalence of metabolic syndrome is estimated to be 40%[25], and in 2015, diabetes was the second cause of general mortality in Mexico, which has one of the highest incidence rates of diabetes in the world[11,26].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels are common clinical measures used to assess liver health. Elevated aminotransferase levels can indicate sudden or acute liver injury, or they can be persistently elevated, suggesting ongoing liver disease. The leading cause of mild aminotransferase levels is NAFLD, but other common causes include excessive alcohol consumption, medication-associated liver injury, infection with HVB or HCV, and hemochromatosis[27]. While not all individuals with elevated aminotransferase levels have liver damage or disease, these measures can be used to detect asymptomatic disease[27]. Several US studies report a higher prevalence of elevated ALT among Mexican-Americans (17.4%) than among whites (8.2%)[28-30].

The aim of this study was to compare the prevalence of known risk factors for CLD in a representative sample of Mexican-Americans, who were born in the US or Mexico, to a sample of adults in Mexico. We examined data from the 1999-2014 National Health and Nutrition Examination Survey (NHANES), which includes persons of Mexican origin living in the US, and data from the 2004-2013 Health Worker Cohort Study (HWCS) in Cuernavaca, Mexico. We hypothesized that Mexican-Americans in the US would have a higher prevalence of CLD risk factors than their counterparts in Mexico. This hypothesis is based on studies suggesting that immigrant Mexican-Americans have better health outcomes than more acculturated, US-born Mexican-Americans[31-33]. We tested this hypothesis by analyzing the independent association between country of residence (*i.e.*, Mexico *vs* the US) and place of birth, sex, and the following risk factors for CLD: Elevated ALT or AST levels, infection with HBV or HCV, metabolic syndrome, high cholesterol, diabetes, obesity, abdominal obesity, and heavy or binge drinking, while also controlling for potential confounders.

MATERIALS AND METHODS

Ethical considerations

Ethical approval for the HWCS and this bi-national study was granted from the Internal Review Boards of the Mexican Social Security Institute (IMSS) and the University of California, Los Angeles (UCLA). Informed consent was obtained from all the HWCS subjects prior to their participation in any study activities.

Data sources

This observational study used existing data from Mexican-Americans who participated in NHANES, a cross-sectional, representative, examination survey of the total civilian non-institutionalized population residing in the continental US and Hawaii. This continuous survey is conducted by the National Center for Health Statistics to assess and track the health and nutritional status of Americans over time. The survey collects health data through standardized questionnaires, physical examinations and a series of laboratory tests. The design of NHANES over-samples Mexican-Americans to allow for analyses of this subgroup. The 1999-2014 NHANES data includes a total of 3929 male and 4182 female Mexican-Americans, for a total sample size of 8111[34].

The data in Mexico came from the HWCS, a longitudinal study of workers and their immediate family members from two large health care institutions in Cuernavaca, Mexico: the IMSS and the National Institute of Public Health. Briefly, the HWCS collects information using physical examinations, self-reported questionnaires, and laboratory tests in order to prospectively evaluate risk factors and the incidence of chronic diseases, including heart disease, diabetes, and liver disease (CLD). From 2004 to 2006 (Wave 1), approximately 9000 health workers enrolled in the HWCS. During 2011 to 2013 (Wave 2), a total of 1855 participants were followed-up. Details about the design and methods of the HWCS are described elsewhere[35]. The clinical and anthropometric procedures that were used for the HWCS are comparable to those used for the NHANES surveys[36].

Study samples in the United States and Mexico

The 1999-2014 NHANES sample was restricted to Mexican-American participants who were 20 years and older, born in the US or Mexico, and had medical insurance. Females who were pregnant at the time of

data collection were excluded. The final NHANES sample consisted of 2097 males and 2177 females 20 years and older with completed questionnaire data. Of these individuals, 4075 also underwent physical examinations including laboratory studies, and of the individuals with laboratory studies, 1775 provided fasting blood samples.

The HWCS sample was limited to participants 20 years and older who reside and were born in Mexico, and had medical insurance. Of the 10035 participants in the HWCS sample with questionnaire and laboratory data, 193 females were excluded because they were pregnant or breastfeeding at the time of the survey. An additional 48 individuals were excluded because they were not born in Mexico, and 309 were excluded because they did not report a place of birth. The final HWCS sample consisted of 3010 males and 6475 females 20 years and older who reside and were born in Mexico, with completed questionnaire and laboratory data. The total study sample of 13798 individuals consisted of 9485 Mexican subjects who currently reside in Mexico, 2324 US-born Mexican-Americans who live in the US, and 1989 Mexican-Americans who were born in Mexico and now live in the US.

Definition of chronic liver disease risk factors

Elevated aminotransferase levels: Elevated ALT was defined as > 40 IU/L for males and females; elevated AST was defined as > 40 IU/L for males and females[29,30].

Hepatitis B or hepatitis C infection: HBV infection was identified by having a positive hepatitis B core antibody serology and a positive hepatitis B surface antigen serology. We identified an HCV infection by a positive antibody titer[36]. Individuals infected with HBV and HCV were combined into one category due to their small sample sizes.

Metabolic syndrome: We used the definition of metabolic syndrome from the Third Report of the National Cholesterol Education Program's Adult Treatment Panel Ⅲ (NCEP/ATPⅢ) criteria[37].

High Total Cholesterol: Following NCEP/ATPⅢ recommendations, high total cholesterol was defined as > 200 mg/dL for males and females[37].

Diabetes: Type 2 diabetes in males and females was defined as having one of the following: a plasma glucose level > 125 mg/dL after > 8 h of fasting, a medical history of diabetes (other than during pregnancy), currently taking medication for diabetes, or a random glucose test > 200 mg/dL[38].

Obesity: Subjects were categorized according to body mass index (BMI) following the recommendations of the National Heart, Lung and Blood Institute: Normal (BMI 18.5-24.9 kg/m^2), overweight (BMI 25.0-29.9 kg/m^2), and obese (BMI $\geqslant$ 30.0 kg/m^2)[39].

Abdominal obesity: Abdominal obesity was defined as a waist circumference > 102 cm for males and a waist circumference > 88 cm for females[40].

Alcohol consumption: Heavy drinking was defined as two to four drinks per day for females and three to four drinks per day for males, and binge drinking was defined as having five or more drinks at one time for both males and females[41].

Definition of independent variables

The main independent variables for this study classified individuals by country of residence (*i.e.*, Mexico *vs* the US) and place of birth (*i.e.*, US-born *vs* Mexico-born). The HWCS participants represent Mexicans who were born and currently live in Mexico. Individuals from the NHANES sample were further classified by birthplace (US-born *vs* Mexico-born). The following three groups were compared: (1) HWCS (Mexico resident, Mexico-born), (2) NHANES (US resident, Mexico-born), and (3) NHANES (US resident, US-born). Other independent variables included age, sex, marital status, and education level. Approximately 18% of the subjects in the HWCS sample had missing education data, which was imputed using a three-step procedure. There were no missing data for the other independent variables.

Statistical analysis

Descriptive analyses were performed to characterize the study population and examine the study variables. Chi-square tests were used to compare the socio-demographic characteristics of the study sample by country of birth/residence, which were stratified by sex. Age-adjusted means and prevalence rates were calculated for each CLD risk factor, which were stratified by sex and country of birth/residence. Separate multiple logistic regression models were estimated for males and females to evaluate the independent associations of each liver disease risk factor to country of birth/residence. Adjusted odds ratios with 95% confidence intervals (95%CI) are reported. Marginal standardization was used to calculate the predicted probability as well as the prevalence ratios and prevalence differences with their corresponding 95%CIs. This allowed us to compare the prevalence of CLD risk factors between the three groups. For all analyses, a two-sided *P*-value < 0.05 was considered statistically significant. The data analyses were conducted using SAS software, version 9.4 for Windows, and STATA 14.

RESULTS

Sample characteristics

The socio-demographic characteristics of the bi-national study sample are presented in Table 1. One third of the HWCS participants in Mexico are male, as compared to the US NHANES sample, which is 49% male. There are no differences between the US and Mexico samples in

Table 1 Socio-demographic characteristics of the study sample *n* (%)

	Male			*P* value[1]		Female			*P* value[1]	
	Mexico cohort (REF)	NHANES Mexico born	NHANES US born	Mexico born	US born	Mexico cohort (REF)	NHANES Mexico born	NHANES US born	Mexico born	US born
Total sample sizes	3010 (58.9)	1021 (20.0)	1076 (21.1)	0.925	0.897	6475 (68.3)	944 (10.9)	1233 (14.3)	0.923	0.845
Age (yr)										
20-44	1680 (55.8)	380 (58.6)	377 (59.8)			3357 (51.8)	356 (55.5)	460 (56.9)		
45-59	951 (31.6)	239 (25.6)	222 (22.7)			2031 (31.4)	196 (24.3)	256 (23.4)		
60+	379 (12.6)	402 (15.9)	477 (17.5)			1087 (16.8)	392 (20.2)	517 (19.8)		
Marital status				0.074	0.804				0.088	0.423
Never married/single	458 (15.2)	73 (9.8)	162 (22.4)			1489 (23.0)	81 (10.0)	169 (19.5)		
Married/living together	2390 (79.4)	843 (83.)	746 (65.2)			3701 (57.2)	580 (67.8)	697 (57.3)		
Divorced/separated/widowed	162 (5.4)	105 (6.6)	167 (12.4)			1285 (19.8)	283 (22.2)	367 (23.2)		
Education										
Less than high school	706 (23.5)	724 (61.7)	381 (24.8)	0.000	0.463	2073 (32.0)	660 (61.3)	436 (24.8)	0.002	0.705
High school graduate	574 (19.1)	136 (17.6)	246 (26.2)			1121 (17.3)	108 (14.6)	279 (23.6)		
More than high school	1730 (57.5)	160 (20.7)	447 (48.9)			3281 (50.7)	173 (24.1)	513 (51.5)		

[1]The Chi square test was used to determine differences between groups.

terms of age or marital status. Approximately half the total sample is between 20 to 44 years, nearly 30% is 45 to 59 years, and roughly 20% is 60 years or older. Most of the study subjects are married/living together (65%), almost 20% are never married/single, and the rest are divorced/separated/widowed. There are no differences in the levels of education observed between the HWCS participants in Mexico and the Mexican-Americans who were born in the US. Approximately half of the participants in both groups have an education beyond high school and less than a third did not finish high school. The only significant difference observed between the two samples is that over 60% of the Mexico-born NHANES participants did not graduate from high school, compared to 29% of the participants in Mexico.

Chronic liver disease risk factors

Table 2 reports the age-adjusted means and prevalence of CLD risk factors for males and females by country of birth/residence. Elevated ALT levels are more common among males (22%-27%) than females (8%-10%), with a mean ALT ranging from 35-36 IU/L among males and 23-25 IU/L among females. There are no differences in mean AST levels among males (range 28-30 IU/L), but a significantly higher mean AST is observed among the Mexico-born (23.6 IU/L) and US-born (25.9 IU/L) females, as compared to the mean AST of 22.2 IU/L found among females in Mexico. The prevalence of diabetes, obesity, abdominal obesity, and heavy/binge drinking is higher among the NHANES participants than among the HWCS subjects. Conversely, more HWCS participants are current smokers and have lower levels of HDL cholesterol and elevated triglycerides compared with their NHANES counterparts.

Mexican-American males born in Mexico have a lower rate of HBV or HCV infection (0.4%) than either US-born Mexican-Americans (3.0%) or the HWCS participants (2.6%). A greater proportion of Mexico-born Mexican-American males have high cholesterol (49%) compared with males in Mexico (40%) and US-born Mexican-Americans (39%). Males in Mexico are more likely to have elevated triglycerides (57.5%) compared with Mexico-born (35.4%) and US-born Mexican-Americans (41%). The prevalence of diabetes is significantly lower among males in Mexico (6%) when compared with the 11% and 16% rates among the Mexico- and US-born NHANES participants, respectively. The proportion of obese males in Mexico is 17%, compared to 30% and 45% among Mexico- and US-born males in the US, respectively. Males in Mexico have a lower prevalence of abdominal obesity (16%) than Mexico- and US-born Mexican-American males (36% and 49%, respectively), with mean waist circumferences of 91.4 cm, 98.7 cm and 103.1 cm, respectively. Heavy or binge drinking is more common among the Mexico- and US-born Mexican-American males (75% and 71%, respectively) compared with their counterparts in Mexico (38%).

Rates of HBV or HCV infection are lower among Mexico-born and US-born Mexican-American females (0.3% and 0.8%, respectively) when compared to females in Mexico (1.7%). A lower percentage of females in Mexico have high cholesterol (36%) compared to the Mexico-born (40.5%) and US-born (40.2%) Mexican-American females. US-born females in the US are less likely to have elevated triglycerides (23.8%) than Mexico-born females (31.9%) and females living in Mexico (34.5%). The prevalence of diabetes among the females in the Mexican sample is 7%, compared to 14% and 11% among Mexico- and US-born Mexican-American females, respectively. Obesity rates are also lower among females in Mexico than among Mexican-American females (19% *vs* 39% and 47%, respectively). Abdominal obesity among females in Mexico is 48%, compared to a prevalence of 68% among their Mexican-American counterparts. Rates of heavy or binge drinking are substantially higher among Mexican-American females born in Mexico or the US (34% and 54%, respectively), as compared to females in Mexico (10%)

Table 2 Age-adjusted means and prevalence of chronic liver disease risk factors

	Male			Female		
	Mexico cohort (REF)	NHANES Ⅶ Mexico-born	NHANES Ⅶ US-born	Mexico cohort (REF)	NHANES Ⅶ Mexico-born	NHANES Ⅶ US-born
Elevated ALT (> 40 IU/L)[1], %	26.7	22.1	25.2	8.7	8.3	10.2
ALT (IU/L), mean	35.9	35.0	36.0	23.0	23.7	25.3[a]
Elevated AST (> 40 IU/L)[2], %	11.2	8.4	12.2	5.2	4.6	5.2
AST (IU/L), mean	27.9	30.1	29.5	22.2	23.6[a]	25.9[b]
Hepatitis B or C, %	2.6	0.4[b]	3.0	1.7	0.3[b]	0.8[a]
Metabolic syndrome[3], %	27.5	23.5	33.4	30.8	28.6	27.6
Elevated total cholesterol (mg/dL)[4], %	39.8	48.8[b]	39.1	36.0	40.5[a]	40.2[a]
Total cholesterol (mg/dL), mean	193.6	199.1[a]	192.7	189.6	193.1[a]	193.6[a]
Low HDL cholesterol (HDL < 40)[5], %	62.9	35.4[b]	27.7[b]	50.1	14.1[b]	12.0[b]
HDL cholesterol (mg/dL), mean	37.6	45.1[b]	46.7[b]	40.7	52.7[b]	55.1[b]
LDL cholesterol (mg/dL), mean	116.5	124.1[b]	117.2	115.2	110.9	11.5[a]
Elevated triglycerides[6] (mg/dL), %	57.5	35.4[b]	41.0[b]	34.5	31.9	23.8[b]
Triglycerides (mg/dL), mean	202.4	150.0[b]	173.9[a]	143.5	132.0[a]	123.8[b]
Diabetic[7], %	6.3	11.0[a]	16.1[b]	6.9	14.4[b]	11.0[a]
Overweight (BMI ⩾ 25)[8], %	48.1	46.3	37.3[b]	37.2	31.1[a]	26.7[b]
Obesity (BMI ⩾ 30)[9], %	17.1	30.0[b]	45.0[b]	19.2	39.2[b]	46.6[b]
BMI (kg/m^2), mean	26.7	28.3[b]	30.1[b]	26.4	29.0[b]	30.2[b]
Abdominal obesity[10], %	15.8	36.0[b]	49.0[b]	48.3	68.3[b]	67.9[b]
Waist circumference (cm), mean	91.4	98.7[a]	103.1[b]	88.5	94.7[b]	97.2[b]
Heavy or binge drinker[11], %	38.0	75.1[b]	71.3[b]	9.6	33.8[b]	54.0[b]
Current smoker[12], %	36.9	21.6[b]	21.9[b]	20.8	8.2[b]	14.2[b]

[1]Elevated alanine aminotransferase (ALT) was defined as ALT > 40 IU/L for males and females; [2]Elevated alanine aminotransferase (AST) was defined as AST > 40 IU/L for males and females; [3]Metabolic syndrome was defined base on the Third Report of the National Cholesterol Education Program's Adult Treatment Panel Ⅲ criteria; [4]Elevated total cholesterol was defined as ⩾ 200 mg/dL; [5]Low High Density Lipoprotein-Cholesterol (HDL-C) was defined as < 40 mg/dL; [6]Elevated triglycerides was defined as ⩾ 150 mg/dL; [7]Diabetes was defined as having a plasma glucose level > 125 mg/dL after a more than 8 h fast, and/or a medical history of diabetes, and/or currently taking medication for diabetes, and/or a random glucose test >200 mg/dL; [8]Overweight was defined as having a body mass index (BMI) of ⩾ 25.0 kg/m^2; [9]Obesity was defined as having a BMI of ⩾ 30.0 kg/m^2; [10]Abdominal obesity was defined as having a waist circumference > 102 cm for males, and a waist circumference > 88 cm for females; [11]Heavy drinking was defined as two to four drinks per day for females and three to four drinks per day for males, and binge drinking was defined as having five or more drinks at one time for both males and females; [12]Current cigarette smoking was defined as having smoked at least 100 cigarettes and being a current smoker. [a]*P* value ⩽ 0.05; [b]*P* value ⩽ 0.001.

(Table 2).

Multivariate analyses and other effect measures

After controlling for age, marital status, and education level, the logistic regression results indicate that Mexico-born Mexican-American males are less likely to have HBV or HCV (OR: 0.2, 95%CI: 0.1-0.6), but are more likely to have high cholesterol (OR: 1.4, 95%CI: 1.1-1.8) than their counterparts in Mexico. US-born Mexican-American males are more likely to have metabolic syndrome (OR: 1.4, 95%CI: 1.1-1.9) and diabetes (OR: 3.0, 95%CI: 1.9-4.8) than males in Mexico. Regardless of where they were born, Mexican-American males are more likely to be obese, diabetic, have abdominal obesity, or be heavy/binge drinkers than Mexican males. The prevalence ratios and predicted probabilities confirm the results of our multivariate analyses and may provide more precise estimates of the increased risk of diabetes, obesity, abdominal obesity, and heavy/binge drinking observed among Mexican-American males, as compared with males in Mexico. The probability of having any of the aforementioned risk factors is significantly greater among US-born Mexican-Americans than among their Mexico-born counterparts (Table 3).

The adjusted multivariate results presented in Table 4 also indicate that Mexico-born Mexican-American females are significantly more likely to be diabetic (OR: 2.2, 95%CI: 1.4-3.4), obese (OR: 2.5, 95%CI: 1.8-3.5), have abdominal obesity (OR: 2.1, 95%CI: 1.2-3.6), or be heavy/binge drinkers (OR: 5.6, 95%CI: 4.2-7.3) than females in Mexico. The same is true for US-born Mexican-American females, who are also more likely to be diabetic (OR: 1.7, 95%CI: 1.2-2.6), obese (OR: 3.5, 95%CI: 2.5-4.9), have abdominal obesity (OR: 2.3, 95%CI: 1.4-3.8), or be heavy/binge drinkers (OR: 12.8, 95%CI: 10.0-16.3) than their counterparts in Mexico. The prevalence ratios and predicted probabilities mirror the multivariate analysis findings for the aforementioned risk factors, showing a greater risk among US-born as compared to Mexico-born Mexican-American females. However, the prevalence ratios indicate that Mexican-American females are significantly less likely to be infected with HBV or HCV than females in Mexico (Table 4).

Supplemental Tables 1 and 2 report the odds ratios and 95% confidence intervals for CLD risk factors among males and females, respectively, by country of birth/residence, age, marital status and education.

DISCUSSION

This epidemiological study is the first to compare the risk factors for CLD in a cohort of Mexican health workers with nationally representative samples of US- and Mexico-born Mexican-Americans living in the US. The findings of our

Table 3 Effect measures comparing prevalence of risk factors among males by country of residence/birth

	Elevated ALT or AST[1]	Hepatitis B or C	Metabolic syndrome[2]	High cholesterol	Diabetes[3]	Obesity[4]	Abdominal obesity[5]	Heavy/binge drinker[6]
Odds ratios								
HWCS (Mexico)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NHANES- Mexico-born	0.8 (0.5-1.2)	0.2 (0.1-0.6)[a]	0.8 (0.6-1.1)	1.4 (1.1-1.8)[a]	1.7 (1.0-2.8)	2.1 (1.7-2.6)[a]	3.3 (1.8-6.2)[a]	3.9 (3.2-4.7)[a]
NHANES- US-born	0.9 (0.6-1.4)	1.3 (0.7-2.4)	1.4 (1.1-1.9)[a]	0.9 (0.7-1.2)	3.0 (1.9-4.8)[a]	3.9 (3.1-4.9)[a]	5.4 (2.9-10.1)[a]	4.1 (3.4-5.1)[a]
Predicted probabilities								
HWCS (Mexico)	25.0	2.4	30.2	40.9	8.5	17.4	16.8	36.4
NHANES- Mexico-born	20.8	0.5	25.8	49.3	13.0	30.6	39.3	67.1
NHANES- US-born	24.0	3.2	38.0	39.6	20.4	44.7	51.1	68.5
Prevalence ratios (95%CI)								
NHANES- Mexico-born *vs* HWCS	0.8 (0.6, 1.1)	0.2 (0.0, 0.4)[a]	0.9 (0.7, 1.1)	1.2 (1.0, 1.4)[a]	1.5 (0.9, 2.2)[a]	1.8 (1.5, 2.0)[a]	2.3 (1.2, 3.5)[a]	1.8 (1.7, 2.0)[a]
NHANES- US-born *vs* HWCS	1.0 (0.7, 1.3)	1.3 (0.6, 2.0)	1.3 (1.0, 1.5)[a]	1.0 (0.8, 1.1)	2.4 (1.5, 3.3)[a]	2.6 (2.2, 2.9)[a]	3.0 (1.6, 4.5)[a]	1.9 (1.7, 2.0)[a]
Prevalence differences (95%CI)								
NHANES- Mexico-born *vs* HWCS	-4.2 (-11.5, 3.1)	-1.9 (-3.0, -0.9)[a]	-4.3 (-10.4, 1.7)	8.5 (2.4, 14.5)[a]	4.5 (0.0, 8.9)[a]	13.2 (9.0, 17.4)[a]	22.6 (13.2, 32.0)[a]	30.7 (26.4, 35.0)[a]
NHANES- US-born *vs* HWCS	-1.0 (-8.5, 6.5)	0.7 (-0.8, 2.3)	7.8 (1.4, 14.2)[a]	-1.3 (-6.9, 4.3)	11.8 (7.2, 16.5)[a]	27.3 (22.1, 32.4)[a]	34.4 (25.3, 43.5)[a]	32.1 (28.2, 36.0)[a]

Logistic regression models adjusted for age, marital status and education. Predicted probabilities, prevalence ratios and prevalence differences were produced using marginal standardization. [1]Elevated alanine aminotransferase (ALT) and elevated alanine aminotransferase (AST) were defined as > 40 IU/L; [2]Metabolic syndrome was defined base on the Third Report of the National Cholesterol Education Program's Adult Treatment Panel Ⅲ criteria; [3]Diabetes was defined as having a plasma glucose level > 125 mg/dL after a more than 8 h fast, a medical history of diabetes, currently taking medication for diabetes, and/or a random glucose test > 200 mg/dL; [4]Obesity was defined as having a body mass index (BMI) of ≥ 30.0 kg/m^2; [5]Abdominal obesity was defined as having a waist circumference > 102 cm for males, and a waist circumference > 88 cm for females; [6]Heavy drinking was defined as two to four drinks per day for females and three to four drinks per day for males, and binge drinking was defined as having five or more drinks at one time for both males and females. [a]P< 0.05 for testing the null hypothesis of no difference between groups.

bi-national study indicate that the HWCS participants in Mexico have fewer CLD risk factors than their counterparts in the US. Specifically, we found that Mexican-American males who were born in the US are more likely to be infected with HBV or HCV, have metabolic syndrome, diabetes, obesity, abdominal obesity, or being heavy/binge drinkers when compared to immigrant Mexican-American males or their counterparts in Mexico. Similar trends are observed among females, with US-born Mexican-American females having a greater probability of having elevated AST, obesity, abdominal obesity and heavy/binge drinking. Mexican-American females who were born in Mexico are more likely to have elevated total cholesterol or diabetes when compared to those born in the US or the HWCS participants in Mexico.

Our results are consistent with other studies that report high rates of obesity, diabetes, and excessive alcohol consumption among Mexicans in both countries[29,33,42-44]. The high prevalence of obesity (30%-47%), diabetes (11%-16%), as well as heavy/binge drinking (34%-75%) we found among Mexican-Americans in this binational study are of particular concern, and are likely contributing to the liver disease disparities observed among Latinos in the US. Additionally, having a combination of certain factors, such as obesity and excessive drinking, or diabetes and HCV, has been shown to increase the risk of elevated aminotransferase levels and liver cancer[45,46]. More studies are needed to evaluate how the accumulation of specific risk factors may be contributing to the increased risk of CLD among Mexican-Americans.

Latinos are the largest ethnic or racial minority in the US. In 2016, an estimated 57.5 million Americans identified as Hispanic or Latino, representing 17.8% of the US population[47]. By 2060, the number of Latinos is projected to increase to 119 million and make up 29% of the US population. Mexican-Americans are the largest group of Latinos in the US (63%)[47]. Identifying ways to prevent CLD in this rapidly growing population is very important. As the Mexican-American population continues to grow, the challenges to address the high rates of CLD in this group will also increase. A keener awareness and deeper understanding of CLD risk factors is needed to help policy makers anticipate how changes in immigration policy, coupled with health trends in Mexico, are likely to affect the health and health care needs of the growing number of Mexican-Americans in the US. We hope our findings can be used to develop health policy strategies and programs to prevent CLD by addressing the specific risk factors that affect Mexicans in both countries.

This study has some limitations. First, unlike NHANES,

Table 4 Effect measures comparing prevalence of risk factors among females by country of residence/birth

	Elevated ALT or AST[1]	Hepatitis B or C	Metabolic syndrome[2]	High cholesterol	Diabetes[3]	Obesity[4]	Abdominal obesity[5]	Heavy/binge drinker[6]
Odds ratios								
HWCS (Mexico)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
NHANES- Mexico-born	0.8 (0.5, 1.1)	0.3 (0.1, 1.8)	0.7 (0.5, 1.1)	1.2 (1.0, 1.5)	2.2 (1.4, 3.4)[a]	2.5 (1.8, 3.5)[a]	2.1 (1.2, 3.6)[a]	5.6 (4.2, 7.3)[a]
NHANES- US-born	1.0 (0.8, 1.3)	0.4 (0.2, 1.1)	0.8 (0.6, 1.2)	1.1 (0.9, 1.4)	1.7 (1.2, 2.6)[a]	3.5 (2.5, 4.9)[a]	2.3 (1.4, 3.8)[a]	12.8 (10.0, 16.3)[a]
Predicted probabilities								
HWCS (Mexico)	10.2	2.2	34.9	41.2	8.3	20.0	51.1	8.4
NHANES- Mexico-born	8.2	0.7	28.8	45.8	15.4	38.4	67.8	32.1
NHANES- US-born	10.6	1.0	31.2	43.3	12.9	46.3	69.6	50.3
Prevalence ratios (95%CI)								
NHANES- Mexico-born *vs* HWCS	0.8 (0.5, 2.1)	0.3 (0.0, 0.9)[a]	0.8 (0.6, 1.0)	1.1 (1.0, 1.2)	1.9 (1.2, 2.5)[a]	1.9 (1.4, 2.4)[a]	1.3 (1.0, 1.6)[a]	3.8 (3.0, 4.6)[a]
NHANES- US-born *vs* HWCS	1.0 (0.8, 1.2)	0.4 (0.0, 0.8)[a]	0.9 (0.7, 1.1)	1.1 (0.9, 1.2)	1.6 (1.1, 2.1)[a]	2.3 (1.7, 2.9)[a]	1.4 (1.0, 1.7)[a]	6.0 (4.9, 7.1)[a]
Prevalence differences (95%CI)								
NHANES- Mexico-born *vs* HWCS	-2.1 (-4.7, 0.6)	-1.5 (-2.8, -0.2)[a]	-6.1 (-13.0, 0.8)	4.6 (-0.7, 9.8)	7.1 (1.8, 12.4)[a]	18.4 (12.5, 24.3)[a]	16.8 (4.6, 29.0)[a]	23.7 (19.4, 28.0)[a]
NHANES- US-born *vs* HWCS	0.3 (-1.7, 2.3)	-1.2 (-2.7, 0.0)	-3.7 (-10.2, 2.7)	2.2 (-2.9, 7.3)	4.7 (1.3, 8.0)[a]	26.2 (20.5, 31.9)[a]	18.5 (6.5, 30.6)[a]	41.9 (38.0, 45.9)[a]

Logistic regression models adjusted for age, marital status and education. Predicted probabilities, prevalence ratios and prevalence differences were produced using marginal standardization. [1]Elevated alanine aminotransferase (ALT) and elevated alanine aminotransferase (AST) were defined as > 40 IU/L; [2]Metabolic syndrome was defined base on the Third Report of the National Cholesterol Education Program's Adult Treatment Panel Ⅲ criteria; [3]Diabetes was defined as having a plasma glucose level > 125 mg/dL after a more than 8 h fast, a medical history of diabetes, currently taking medication for diabetes, and/or a random glucose test > 200 mg/dL; [4]Obesity was defined as having a body mass index (BMI) of ≥ 30.0 kg/m^2; [5]Abdominal obesity was defined as having a waist circumference > 102 cm for males, and a waist circumference > 88 cm for females; [6]Heavy drinking was defined as two to four drinks per day for females and three to four drinks per day for males, and binge drinking was defined as having five or more drinks at one time for both males and females. [a]$P < 0.05$ for testing the null hypothesis of no difference between groups.

the HWCS is not a population-based study that is representative of the Mexican population. The HWCS participants are health workers who are younger, more educated, and predominantly female. However, to the best of our knowledge, the HWCS is the only longitudinal study in Mexico that includes ALT and AST measures as well as HBV and HCV results for a large number of Mexican adults, which is why we used the HWCS data for this binational study. In order to address this limitation, we compared some of the HWCS results to the findings of the 2012 Encuesta Nacional de Salud y Nutrición (ENSANUT)[48], a larger, population-based study that is representative of the Mexican population and can be considered a simplified version of NHANES. The prevalence of diabetes reported in the 2012 ENSANUT was 9.2%[48], while the prevalence of diabetes among the HWCS was 6.7%. Obesity was also higher among the male (26.8%) and female (37.5%) ENSANUT participants[48], as compared to the HWCS participants. The prevalence of heavy/binge drinking (42%) was also more common among the ENSANUT participants[48]. Nonetheless, even when compared to the ENSANUT results, the prevalence of these risk factors remains greater among the Mexican-Americans from the NHANES sample. Due to the limited generalizability of our study's results, they should be viewed as exploratory and preliminary.

Another limitation was our ability to control for confounding variables in the comparisons between Mexico and the US. To address this issue, all analyses were stratified by sex and controlled for age, marital status, and educational level in the regression analyses. We also limited the US sample to individuals with health insurance, since all the HWCS participants have health insurance. Our ability to control for potential confounders was restricted by the available data, and it is therefore possible that other unobserved differences between the two samples may be confounding our results.

In conclusion, the results of this bi-national study indicate that Mexican-Americans in the US have more risk factors for CLD than their counterparts in Mexico, and point to a critical need for prevention programs. Of particular concern are the high rates of heavy/binge drinking observed among Mexican-Americans. We hope these findings can be used by health professionals in Mexico and the US to tailor screening and prevention strategies to help reduce the risk of CLD among their patients. Our results could also be used to design and implement more effective health promotion programs to address the specific factors that put Mexicans at higher risk for developing CLD in both countries. These findings add to the relatively scarce literature on bi-national research, and provide preliminary data for future studies of migrant health in the US and Mexico. Other bi-national primary data collection projects with representative samples and comparable

demographic, socioeconomic and health status measures are needed to further investigate the growing problem of CLD among Mexicans in both countries. The results of this bi-national analysis indicate that Mexican-Americans in the US have more risk factors for CLD than their counterparts in Mexico. These results can be used to design and implement more effective health promotion programs and policies to address the specific factors that put Mexicans at higher risk of developing CLD in both countries. Our findings add to the relatively scarce literature on bi-national research, and provide preliminary data for future studies of migrant health in the US and Mexico. Other bi-national primary data collection projects with representative samples and comparable demographic, socioeconomic and health status measures are needed to further investigate the growing problem of CLD among Mexicans in both countries.

ARTICLE HIGHLIGHTS

Research background

United States (US) Latinos have greater morbidity and mortality from liver disease than non-Hispanic whites. Liver disease is the fifth leading cause of death in Mexico. In the US, Mexican-Americans have a greater risk of obesity, diabetes and heavy/binge drinking than in Mexico.

Research motivation

Over 30 million people are likely to have some form of chronic liver disease (CLD) in the US. CLD is the 12th leading cause of general mortality in the US.

Research objectives

To compare the prevalence of CLD risk factors in a representative sample of Mexican-Americans, born in the US or Mexico, to a sample of adults in Mexico.

Research methods

The main independent variables for this study classified individuals by country of residence and place of birth. Regression analyses were used to investigate CLD risk factors.

Research results

There is a greater risk among US-born *vs* Mexico-born Mexican-Americans.

Research conclusions

Mexican-Americans in the US had more risk factors for CLD.

Research perspectives

Our findings add to the relatively scarce literature on bi-national research, providing preliminary data for future studies of migrant health in the US and Mexico. Other bi-national primary data collection projects with representative samples and comparable demographic, socioeconomic and health status measures are needed to further investigate the growing problem of CLD among Mexicans in both countries.

REFERENCES

1 Surveillance for Viral Hepatitis-United States. 2017. Available from: URL: https://www.cdc.gov/hepatitis/statistics/2015surveillance/commentary.htm

2 **Le MH**, Devaki P, Ha NB, Jun DW, Te HS, Cheung RC, Nguyen MH. Prevalence of non-alcoholic fatty liver disease and risk factors for advanced fibrosis and mortality in the United States. *PLoS One* 2017; **12**: e0173499 [PMID: 28346543 DOI: 10.1371/journal.pone.0173499]

3 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

4 **Kochanek KD**, Murphy SL, Xu J, Tejada-Vera B. Deaths: Final Data for 2014. *Natl Vital Stat Rep* 2016; **65**: 1-122 [PMID: 27378572]

5 **American Cancer Society**. Key Statistics about Liver Cancer. 2017. Available from: URL: https://www.cancer.org/cancer/liver-cancer/about/what-is-key-statistics.html

6 Ten Leading Causes of Death by Age Group, United States, 2014. Available from: URL: https://www.cdc.gov/injury/wisqars/leadingcauses.html

7 **Heron M**. Deaths: Leading Causes for 2014. *Natl Vital Stat Rep* 2016; **65**: 1-96 [PMID: 27376998]

8 Leading Causes of Death in Males, 2014. 2017. Available from: URL: https://www.cdc.gov/men/lcod/2014/hispanic/index.htm

9 **US Department of Health and Human Services**. Office of Minority Health. Chronic Liver Disease and Hispanic Americans. 2015. Available from: URL: http://minorityhealth.hhs.gov/omh/browse.aspx?lvl=4lvlid=62

10 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

11 **Instituto Nacional de Estadística y Geografía (INEGI)**. Principales causas de mortalidad por residencia habitual, grupos de edad y sexo. 2016. Available from: URL: http://www.inegi.org.mx/est/contenidos/proyectos/registros/vitales/mortalidad/tabulados/ConsultaMortalidad.asp

12 **Panduro A**, Escobedo Meléndez G, Fierro NA, Ruiz Madrigal B, Zepeda-Carrillo EA, Román S. Epidemiology of viral hepatitis in Mexico. *Salud Publica Mex* 2011; **53** Suppl 1: S37-S45 [PMID: 21877071]

13 **Méndez-Sánchez N**, Villa AR, Chávez-Tapia NC, Ponciano-Rodriguez G, Almeda-Valdés P, González D, Uribe M. Trends in liver disease prevalence in Mexico from 2005 to 2050 through mortality data. *Ann Hepatol* 2005; **4**: 52-55 [PMID: 15798662]

14 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34 [PMID: 15508094 DOI: 10.1053/j.gastro.2004.09.013]

15 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-10 [PMID: 16540768]

16 **Ascha MS**, Hanouneh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology* 2010; **51**: 1972-1978 [PMID: 20209604 DOI: 10.1002/hep.23527]

17 **Davila JA**. Diabetes and hepatocellular carcinoma: what role does diabetes have in the presence of other known risk factors? *Am J Gastroenterol* 2010; **105**: 632-634 [PMID: 20203644 DOI: 10.1038/ajg.2009.715]

18 **Regimbeau JM**, Colombat M, Mognol P, Durand F, Abdalla E, Degott C, Degos F, Farges O, Belghiti J. Obesity and diabetes as a risk factor for hepatocellular carcinoma. *Liver Transpl* 2004; **10**: S69-S73 [PMID: 14762843 DOI: 10.1002/lt.20033]

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

20 **Weston SR**, Leyden W, Murphy R, Bass NM, Bell BP, Manos MM, Terrault NA. Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology* 2005; **41**: 372-379 [PMID: 15723436 DOI: 10.1002/hep.20554]

21 **Flegal KM**, Kruszon-Moran D, Carroll MD, Fryar CD, Ogden CL. Trends in Obesity Among Adults in the United States, 2005 to 2014. *JAMA* 2016; **315**: 2284-2291 [PMID: 27272580 DOI: 10.1001/jama.2016.6458]

22 National Diabetes Statistics Report, 2017. Estimates of Diabetes and Its Burden in the United States. Atlanta, GA: Centers for Disease

Control and Prevention, US Dept of Health and Human Services; 2017. Available from: URL: https://www.cdc.gov/diabetes/data/statistics-report/index.html

23 **Mozaffarian D**, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. *Circulation* 2015; **131**: e29-e322 [PMID: 25520374 DOI: 10.1161/CIR.0000000000000152]

24 **Gutiérrez JP,** Rivera-Dommarco J, Shamah-Levy T, Villalpando-Hernández S, Franco A, Cuevas-Nasu L, Romero-Martínez M, Hernández-Ávila M. Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales. Cuernavaca, México: Instituto Nacional de Salud Pública (MX), 2012. Available from: URL: https://ensanut.insp.mx/informes/ENSANUT2012ResultadosNacionales.pdf

25 **Rojas R**, Aguilar-Salinas CA, Jiménez-Corona A, Shamah-Levy T, Rauda J, Avila-Burgos L, Villalpando S, Ponce EL. Metabolic syndrome in Mexican adults: results from the National Health and Nutrition Survey 2006. *Salud Publica Mex* 2010; **52** Suppl 1: S11-S18 [PMID: 20585723 DOI: 10.1590/S0036-36342010000700004]

26 **González-Villalpando C**, Dávila-Cervantes CA, Zamora-Macorra M, Trejo-Valdivia B, González-Villalpando ME. Incidence of type 2 diabetes in Mexico: results of the Mexico City Diabetes Study after 18 years of follow-up. *Salud Publica Mex* 2014; **56**: 11-17 [PMID: 24912516 DOI: 10.21149/spm.v56i1.7318]

27 **Oh RC**, Hustead TR. Causes and evaluation of mildly elevated liver transaminase levels. *Am Fam Physician* 2011; **84**: 1003-1008 [PMID: 22046940]

28 **Tsai J**, Ford ES, Li C, Zhao G. Past and current alcohol consumption patterns and elevations in serum hepatic enzymes among US adults. *Addict Behav* 2012; **37**: 78-84 [PMID: 21975024 DOI: 10.1016/j.addbeh.2011.09.002]

29 **Flores YN**, Yee HF Jr, Leng M, Escarce JJ, Bastani R, Salmerón J, Morales LS. Risk factors for chronic liver disease in Blacks, Mexican Americans, and Whites in the United States: results from NHANES IV, 1999-2004. *Am J Gastroenterol* 2008; **103**: 2231-2238 [PMID: 18671818 DOI: 10.1111/j.1572-0241.2008.02022.x]

30 **Ioannou GN**, Boyko EJ, Lee SP. The prevalence and predictors of elevated serum aminotransferase activity in the United States in 1999-2002. *Am J Gastroenterol* 2006; **101**: 76-82 [PMID: 16405537 DOI: 10.1111/j.1572-0241.2005.00341.x]

31 **Abraído-Lanza AF**, Dohrenwend BP, Ng-Mak DS, Turner JB. The Latino mortality paradox: a test of the "salmon bias" and healthy migrant hypotheses. *Am J Public Health* 1999; **89**: 1543-1548 [PMID: 10511837 DOI: 10.2105/AJPH.89.10.1543]

32 **Rubalcava LN**, Teruel GM, Thomas D, Goldman N. The healthy migrant effect: new findings from the Mexican Family Life Survey. *Am J Public Health* 2008; **98**: 78-84 [PMID: 18048791 DOI: 10.2105/AJPH.2006.098418]

33 **Morales LS**, Flores YN, Leng M, Sportiche N, Gallegos-Carrillo K, Salmerón J. Risk factors for cardiovascular disease among Mexican-American adults in the United States and Mexico: a comparative study. *Salud Publica Mex* 2014; **56**: 197-205 [PMID: 25014426 DOI: 10.21149/spm.v56i2.7335]

34 National Health and Nutrition Examination Survey, 2013-2014. Available from: URL: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm

35 **Denova-Gutiérrez E**, Flores YN, Gallegos-Carrillo K, Ramírez-Palacios P, Rivera-Paredez B, Muñoz-Aguirre P, Velázquez-Cruz R, Torres-Ibarra L, Meneses-León J, Méndez-Hernández P, Hernández-López R, Salazar-Martínez E, Talavera JO, Tamayo J, Castañón S, Osuna-Ramírez I, León-Maldonado L, Flores M, Macías N, Antúnez D, Huitrón-Bravo G, Salmerón J. Health workers cohort study: methods and study design. *Salud Publica Mex* 2016; **58**: 708-716 [PMID: 28225947 DOI: 10.21149/spm.v58i6.8299]

36 **National Health and Nutrition Examination Survey (NHANES).** 2013-2014 MEC Laboratory Procedures Manual. Available from: URL: https://wwwn.cdc.gov/nchs/data/nhanes/2013-2014/manuals/2013_MEC_Laboratory_Procedures_Manual.pdf

37 Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel Ⅲ). *JAMA* 2001; **285**: 2486-2497 [PMID: 11368702 DOI: 10.1001/jama.285.19.2486]

38 American Diabetes Association. Screening for type 2 diabetes. *Diabetes Care* 2004; **27** Suppl 1: S11-S14 [PMID: 14693922 DOI: 10.2337/diacare.27.2007.S11]

39 Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. *Am J Clin Nutr* 1998; **68**: 899-917 [PMID: 9771869 DOI: 10.1093/ajcn/68.4.899]

40 **Sundquist J**, Winkleby MA, Pudaric S. Cardiovascular disease risk factors among older black, Mexican-American, and white women and men: an analysis of NHANES Ⅲ, 1988-1994. Third National Health and Nutrition Examination Survey. *J Am Geriatr Soc* 2001; **49**: 109-116 [PMID: 11207863 DOI: 10.1046/j.1532-5415.2001.49030.x]

41 **Jiles R**, Hughes E, Murphy W, Flowers N, McCracken M, Roberts H, Ochner M, Balluz L, Mokdad A, Elam-Evans L, Giles W. Surveillance for certain health behaviors among states and selected local areas--Behavioral Risk Factor Surveillance System, United States, 2003. *MMWR Surveill Summ* 2005; **54**: 1-116 [PMID: 16319816]

42 **Flores YN**, Lang CM, Salmerón J, Bastani R. Risk factors for liver disease and associated knowledge and practices among Mexican adults in the US and Mexico. *J Community Health* 2012; **37**: 403-411 [PMID: 21877109 DOI: 10.1007/s10900-011-9457-4]

43 **Vega WA**, Rodriguez MA, Gruskin E. Health disparities in the Latino population. *Epidemiol Rev* 2009; **31**: 99-112 [PMID: 19713270 DOI: 10.1093/epirev/mxp008]

44 **Islam N**, Flores YN, Ramirez P, Bastani R, Salmerón J. Hepatitis and liver disease knowledge and preventive practices among health workers in Mexico: a cross-sectional study. *Int J Public Health* 2014; **59**: 381-394 [PMID: 24097058 DOI: 10.1007/s00038-013-0515-9]

45 **Mahli A**, Hellerbrand C. Alcohol and Obesity: A Dangerous Association for Fatty Liver Disease. *Dig Dis* 2016; **34** Suppl 1: 32-39 [PMID: 27548267 DOI: 10.1159/000447279]

46 **Hammerstad SS**, Grock SF, Lee HJ, Hasham A, Sundaram N, Tomer Y. Diabetes and Hepatitis C: A Two-Way Association. *Front Endocrinol* (Lausanne) 2015; **6**: 134 [PMID: 26441826 DOI: 10.3389/fendo.2015.00134]

47 **United States Census Bureau.** Facts for Features: Hispanic Heritage Month 2017. Available from: URL: https://www.census.gov/newsroom/facts-for-features/2017/hispanic-heritage.html

48 **Encuesta Nacional de Salud y Nutrición.** Resultados Nacionales. 2012. Available from: URL: http://ensanut.insp.mx/informes/ENSANUT2012ResultadosNacionales.pdf

P- Reviewer: Abenavoli L, Trovato GM **S- Editor**: Wang XJ
L- Editor: Filipodia **E- Editor**: Bian YN

World Journal of Gastroenterology

Submit a Manuscript: http://www.f6publishing.com

World J Gastroenterol 2018 October 7; 24(37): 4291-4296

DOI: 10.3748/wjg.v24.i37.4291

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

Cerebral lipiodol embolism related to a vascular lake during chemoembolization in hepatocellular carcinoma: A case report and review of the literature

Hideki Ishimaru, Minoru Morikawa, Takayuki Sakugawa, Ichiro Sakamoto, Yasuhide Motoyoshi, Yohei Ikebe, Masataka Uetani

Hideki Ishimaru, Minoru Morikawa, Department of Radiology, Nagasaki University Hospital, Nagasaki 852-8501, Japan

Takayuki Sakugawa, Department of Radiology, University of the Ryukyus, Okinawa 903-0213, Japan

Ichiro Sakamoto, Department of Radiology, Nagasaki Harbor Medical Cancer, Nagasaki 850-8555, Japan

Yasuhide Motoyoshi, Department of Gastroenterology, Nagasaki Harbor Medical Cancer, Nagasaki 850-8555, Japan

Yohei Ikebe, Masataka Uetani, Department of Radiological Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8501, Japan

ORCID number: Hideki Ishimaru (0000-0002-7817-0507); Minoru Morikawa (0000-0003-3423-3008); Takayuki Sakugawa (0000-0003-3593-8739); Ichiro Sakamoto (0000-0002-4512-4950); Yasuhide Motoyoshi (0000-0002-7295-8758); Yohei Ikebe (0000-0002-4130-1446); Masataka Uetani (0000-0001-5922-0508).

Author contributions: Ishimaru H, Morikawa M, and Sakamoto I designed the report; Sakugawa T and Motoyoshi Y collected the patient's clinical data; Ishimaru H, Ikebe Y, and Uetani M wrote the paper.

Informed consent statement: Consent was obtained from relatives of the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest.

CARE Checklist (2013): The guidelines of the CARE Checklist (2013) were adopted.

Manuscript source: Unsolicited manuscript

Correspondence to: Minoru Morikawa, MD, Assistant Professor, Department of Radiology, Nagasaki University Hospital, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan. m-minoru@nagasaki-u.ac.jp
Telephone: +81-95-8197354
Fax: +81-95-8197357

Received: July 16, 2018
Peer-review started: July 16, 2018
First decision: August 1, 2018
Revised: August 4, 2018
Accepted: August 24, 2018
Article in press: August 24, 2018
Published online: October 7, 2018

Abstract

A male patient underwent conventional transcatheter chemoembolization for advanced recurrent hepatocellular carcinoma (HCC). Even after the injection of 7 mL of lipiodol followed by gelatin sponge particles, the flow of feeding arteries did not slow down. A repeat angiography revealed a newly developed vascular lake draining into systemic veins; however, embolization was continued without taking noticing of the vascular lake. The patient's level of consciousness deteriorated immediately after the procedure, and non-contrast computed tomography revealed pulmonary and cerebral lipiodol embolisms. The patient's level of consciousness gradually improved after 8 wk in intensive care. In this

case, a vascular lake emerged during chemoembolization and drained into systemic veins, offering a pathway carrying lipiodol to pulmonary vessels, the most likely cause of this serious complication. We should be aware that vascular lakes in HCC may drain into systemic veins and can cause intratumoral arteriovenous shunts.

Key words: Transcatheter arterial chemoembolization; Arteriovenous shunt; Hepatocellular carcinoma; Vascular lake; Cerebral embolism

Core tip: Vascular lakes that resemble extravasation within hepatocellular carcinomas occasionally emerge during chemoembolization. To date, the drainage routes from vascular lakes are not well understood. We present a patient with a recurrent large hepatocellular carcinoma in which a vascular lake emerged during conventional chemoembolization, draining into systemic veins and causing pulmonary and cerebral lipiodol embolism.

Ishimaru H, Morikawa M, Sakugawa T, Sakamoto I, Motoyoshi Y, Ikebe Y, Uetani M. Cerebral lipiodol embolism related to a vascular lake during chemoembolization in hepatocellular carcinoma: A case report and review of the literature. *World J Gastroenterol* 2018; 24(37): 4291-4296 Available from: URL: http://www.wjgnet.com/1007-9327/full/v24/i37/4291.htm DOI: http://dx.doi.org/10.3748/wjg.v24.i37.4291

INTRODUCTION

Transcatheter arterial chemoembolization (TACE) is utilized worldwide for the treatment of patients with unresectable hepatocellular carcinoma (HCC). Although various complications of TACE have been reported[1], cerebral lipiodol embolism (CLE) after TACE is very rare. To our knowledge, 27 cases have been reported in the English literature, and possible pathways for carrying lipiodol from HCCs to systemic arteries have been hypothesized[2-18]. This is the first report of CLE, in which the vascular lake phenomenon emerged during the TACE procedure and caused an intratumoral arteriovenous shunt, playing the most important role in its occurrence. We also discussed the mechanism of CLE and technical considerations to avoid this serious complication. We also provided a review of the literature.

CASE REPORT

A 63-year-old man with a large recurrent HCC replacing most of the lateral segment of the liver and expanding to the left diaphragm was admitted to our hospital for a second TACE. He had a history of type-B cirrhosis for 3 years. Eight months prior to admission, he had undergone TACE for the same lesion *via* the left hepatic artery (LHA), and the postprocedural course was uneventful. Laboratory tests before second TACE revealed a serum total bilirubin level of 1.4 mg/dL, serum albumin level of 3.0 mg/dL, and prothrombin activity level of 81%. Neither ascites nor hepatic encephalopathy were found, which corresponded to Child-Pugh class A. The α-fetoprotein level was 900 ng/mL. At angiography, the large HCC was supplied by the LHA and the left inferior phrenic artery (LIPA) (Figure 1A) without an apparent arteriovenous (AV) shunt. For arterial redistribution to convert multiple feeding arteries into a single arterial supply, distal branches of the LIPA supplying HCC were embolized initially. To avoid migration of embolic materials into pulmonary vessels, the LIPA was embolized with 0.6 mL of a 33% mixture of n-butyl cyanoacrylate (Histoacryl; B. Braun, Melsungen, Germany) and lipiodol (Lipiodol; Terumo Corporation, Tokyo, Japan) (Figure 1B). Subsequently, we infused 140 mg of miriplatin (Miripla; Dainippon Sumitomo Pharma, Osaka, Japan) suspended in 7 mL of lipiodol *via* the LHA (Figure 2A). This was followed by the injection of 1-mm-diameter gelatin sponge particles (Gelpart; Nippon Kayaku Co. Ltd., Tokyo, Japan). However, the flow of the LHA did not slow down. A repeated left hepatic angiography showed contrast material pooling associated with an aberrant intratumoral space newly developed during embolization, called the "vascular lake phenomenon" (Figure 2B). Late-phase imaging revealed that the pericardiacophrenic vein was the draining vein (Figure 2C), which was not recognized at the time of the procedure but rather retrospectively after remasking and pixel shifting were performed. Epirubicin (10 mg) (Farmorubicin; Kyowa Hakko, Tokyo, Japan) emulsified in 9 mL of lipiodol was additionally infused into the LHA, followed by the injection of a 2-mm block gelatin sponge (Spongel; Astellas, Tokyo, Japan) until the flow slowed down. Immediately after TACE, the patient became drowsy and disoriented. Unenhanced CT of the brain obtained 30 minutes after the procedure revealed multiple lesions of increased attenuation in the cerebral cortex, basal ganglia, thalami and cerebellum (Figure 3). Simultaneously, a CT of the chest revealed hyperattenuation at both lung bases (Figure 4). The patient's oxygen saturation was 88%, indicating hypoxia. The patient was monitored in our intensive care unit on mechanical ventilator support. Echocardiography revealed no atrial septal defect or other intracardiac shunts. The patient's level of consciousness gradually improved, but his limb weakness persisted. He was discharged 8 wk later requiring the use of a wheelchair and assistance to eat meals.

DISCUSSION

TACE has been widely accepted as an effective therapy for HCC. Lipiodol is the most common embolic material used in TACE; it is usually mixed with anticancer drugs dissolved in non-ionic contrast medium. Lipiodol can enter the microcirculation of the tumor and flow into the surrounding portal vein, which is the main drainage route from the hypervascular HCC[19]. However, lipiodol

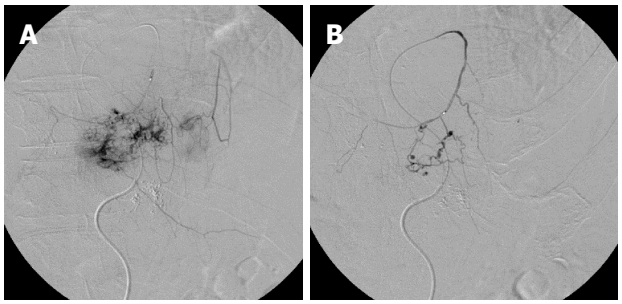

Figure 1 Angiography. A: Selective angiography of the left inferior phrenic artery shows feeding of the anterior part of the tumor without a shunt to the pulmonary vasculature; B: Angiography during glue embolization shows no influx into the pulmonary vasculature.

can reportedly flow into systemic circulation, causing pulmonary embolism and, rarely, cerebral embolism. We found 27 reports of CLE following TACE in 17 English studies (Table 1). To reach the cerebral arteries, lipiodol must pass through two pathways, one from the tumor to the pulmonary artery (PA) and the other from the pulmonary artery to the left atrium (LA). Lipiodol accumulation in the lung was identified simultaneously in 17 of 27 previously reported cases[2-4,6,8-10,14,16,18].

Localized pooling of the contrast medium emerges occasionally during chemoembolization using drug eluting beads (DEB-TACE), resembling extravasation within the tumor. This angiographic finding is known as the vascular lake phenomenon (VLP). As we reported herein, this phenomenon may also appear during chemoembolization using lipiodol, although it is difficult to distinguish a vascular lake from lipiodol accumulating in the tumor by fluoroscopy. Concerning the etiology of VLP during chemoembolization, Seki *et al*[20] speculated that rapid occlusion of most of the tumor vessels increased the pressure inside the fragile tumor microvasculature, causing disruption of the tumor architecture and a new blood space. VLP is thought to indicate a good local response in HCC patients undergoing DEB-TACE[20,21]. Nevertheless, the drainage routes from vascular lakes are unknown. To the best of our knowledge, this is the first report observing a vascular lake draining into systemic veins, resulting in an intratumoral AV shunt and affording lipiodol pathways to pulmonary vessels.

Intratumoral AV shunts can be the pathway to pulmonary vessels[5,7,10], and this was confirmed by angiography in only one CLE case[10]. On hepatic arteriography, the incidence of intratumoral AV shunts was reported to be 2.4%[22]. According to one study using technetium-99-m-labeled macroaggregated albumin[23], lung shunt fractions exceeding 10% were identified in 50 of 125 HCC patients (40%). The study also reported a direct correlation between angiographic tumor vascularity and lung shunt fractions[23]. The particles of lipiodol emulsion were less than 30 μm in size[24], within the range of the size of technetium-99-m-labeled macroaggregated albumin (10-60 μm in size). Lipiodol redistribution in the lungs may be more frequent than that previously thought.

TACE *via* IPA frequently results in pulmonary complications due to the existence of an AV shunt between the IPA and PA[25]. Some authors speculated that communication between the IPA and pulmonary vessels *via* adhesive pleurae or tumor invasion is the most likely pathway from the tumor to the PA[4,6]. In the present case, we performed IPA embolization using glue under DSA guidance, and the glue fragment never flowed into the pulmonary vessels.

In most previously reported cases of CLE, including the case described herein, a large dose of lipiodol was infused (Table 1). Cerebral and pulmonary lipiodol embolisms have been reported in patients receiving more than 20 mL of iodized oil after TACE of HCC[4,6,16,18]. According to Kishi *et al*[26], when lipiodol was infused into the dog's hepatic artery, the amount of lipiodol oil deposition in the lungs was proportional to the lipiodol dose infused. They also found lipiodol deposits in the brain and pancreas[26]. These findings suggested a dose-dependent circulation of oil droplets *via* hepatic sinusoids to pulmonary capillaries and then into the systemic circulation. It was suggested that the lipiodol dose should not exceed 15-20 mL to prevent the risk of an extrahepatic embolism[4,6]. Nevertheless, in 7 previous cases, CLE occurred when the lipiodol dose was < 15 mL[2,5,7,14,18]. The required lipiodol dose was determined by multiple factors, including the blood supply to the tumor, tumor size, catheter position and liver function reserve. When lipiodol goes through an intratumoral AV shunt, the dose of lipiodol required to accomplish HCC flow stasis increases. If there had not been an intratumoral AV shunt in the presented case, we could have accomplished flow stoppage with a lower dose of lipiodol.

CLE is thought to be associated with intrapulmonary or intracardiac shunts[4,7,10,16]. Evidence of an underlying intracardiac right-to-left shunt was proven by transesophageal echocardiogram in one previous case[7]; however, the pathway from the PA to the LA was not verified in most previously reported cases of CLE, including the present case. Wu *et al*[9] speculated that an intra-pulmonary arteriovenous shunt might appear during pulmonary lipiodol embolization due to increasing pulmonary artery pressure or hypoxia. Wu *et al*[10] added that communication between the systemic and pulmonary vessels might develop *via* adhesive pleurae or tumor invasion of the diaphragm, leading to a right-to-left shunt. In a case report of delayed CLE, Wu *et al*[8] concluded that the rapid flow of the feeding artery washed out the lipiodol, and the lipiodol deposited in the lungs was washed out again upon entering the systemic circulation. Since it has been verified that fat globules < 7 μm in diameter can pass directly through the pulmonary arteriolar network[2], lipiodol can enter the systemic circulation in the absence of a right-to-left shunt to cause cerebrovascular complications.

In summary, we presented a case in which a vascular lake draining into systemic veins caused a lipiodol cerebral embolism. As intratumoral AV shunts *via* vas-

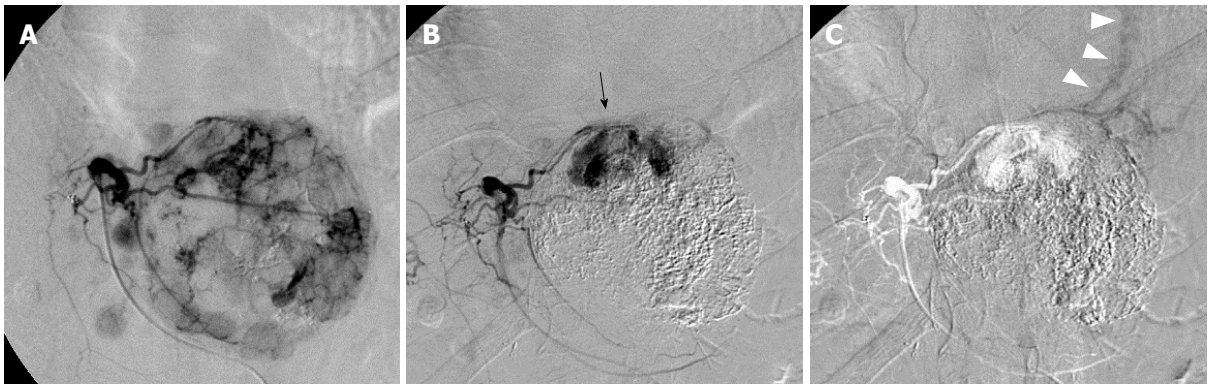

Figure 2 Infused 140 mg of miriplatin suspended in 7 mL of lipiodol *via* the left hepatic artery. A: Left hepatic angiography shows a large hypervascular tumor in the left hepatic lobe and intrahepatic metastases neighborhood without an intratumoral AV shunt; B: Repeated left hepatic angiography shows a vascular lake in the superior portion of the tumor (arrow) that developed after chemoembolization; C: Venous phase shows drainage into the pericardiacophrenic vein (arrow heads), which was unrecognized until remasking and pixel shifting were performed. AV: Arteriovenous.

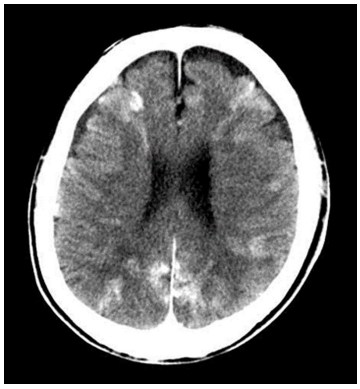

Figure 3 Non-contrast brain computed tomography scan obtained 30 min after chemoembolization shows multiple increased attenuated lesions in the bilateral cerebral hemisphere, consistent with lipiodol embolism.

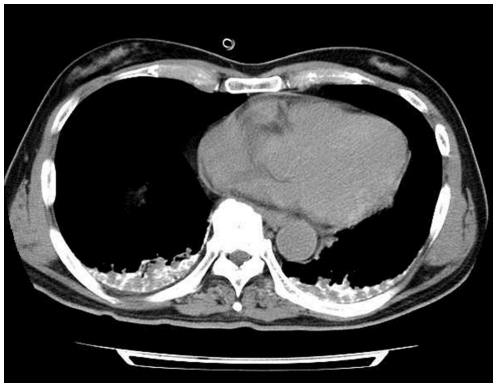

Figure 4 Chest computed tomography scan obtained 30 min after chemoembolization shows lipiodol deposition at both lung bases.

cular lakes may develop during chemoembolization, we recommend performing repeated angiography during TACE procedures, especially when obtaining a decreased blood flow is difficult. When an intratumoral AV shunt is identified by angiography, embolization of the feeding artery using coils or glue should be considered instead of an additional injection of lipiodol or embolic particles. We should try to prevent lipiodol accumulation in the lungs because the pathways from the PA to the LA cannot be blocked regardless of the mechanism by which they are developed.

ARTICLE HIGHLIGHTS

Case characteristics

A 63-year-old man with a large hepatocellular carcinoma underwent transcatheter arterial chemoembolization (TACE), but his level of consciousness deteriorated immediately after the procedure.

Clinical diagnosis

CT scan of the brain revealed multiple lesions of increased attenuation, and cerebral lipiodol embolism (CLE) was confirmed.

Differential diagnosis

There is no differential diagnosis.

Laboratory diagnosis

No specific finding was obtained by laboratory testing.

Imaging diagnosis

An angiography during TACE procedure revealed a newly developed vascular lake draining into systemic veins, which offered a pathway carrying lipiodol to pulmonary vessels and was the most likely cause of this serious complication.

Pathological diagnosis

No pathological examination was performed.

Treatment

The patient was treated in our intensive care unit.

Related reports

This is the first report of CLE, in which vascular lake phenomenon emerging during the procedure caused intratumoral arteriovenous shunt and played the most important role for its occurrence.

Term explanation

The term CLE describes cerebral lipiodol embolism.

Experiences and lessons

Arteriovenous shunt *via* vascular lake may develop during chemoembolization, repeated angiography during TACE procedures should be performed to prevent

Table 1 Characteristics of 28 cases of cerebral lipiodol embolism

Age	Sex	Tumor characteristics	Pulmonary involvement	Embolization *via* extrahepatic artery supply	Dose of lipiodol	AV shunt	RL shunt	Ref.
52	M	Advanced	+	-	35		ND	[2]
58	M		+		8		ND	[2]
56	M		+				ND	[2]
76	M	Large	+	IPA				[3]
81	F	Large	+	IPA	20		ND	[4]
70	F	Large			12	ND		[5]
62	F	15.0 cm	+	IPA	30	ND	ND	[6]
67	M			IPA	5		PFO	[7]
63	F			IPA	10		+	[7]
36	M	Huge	+		40	ND		[8]
51	F	Huge	+		40	ND		[9]
41	M	Multiple and PVTT	+		30	+	ND	[10]
71	M						ND	[11]
44	M	Large		BA				[12]
54	M	13.0 cm		IPA	30			[13]
66	M	11.5 cn	+	-	4		ND	[14]
62	M	16.0 cm					ND	[15]
52	M	18.0 cm	+	IPA	50	ND	Pulmonary AV shunt	[16]
66	F			IPA	20			[17]
39	M	8.0 cm		IPA	13	PV		[18]
51	M	13.0 cm	+	-	30	PV		[18]
73	F	19.0 cm	+	IPA	15	PV		[18]
67	F	6.0 cm	+	LGA	10			[18]
54	F	3.0 cm		IPA	90			[18]
63	M	14.0 cm	+	RSGA	50			[18]
52	M	17.0 cm	+	-	30			[18]
72	M	10.0 cm	+	-	20			[18]
63	M	9.0 cm	+	IPA	16	+	ND	Present case

ND: Not detected; IPA: Inferior phrenic artery; AV: Arteriovenous; RL: Right-to-left; PFO: Patent foramen ovale; PVTT: Portal vein tumor thrombus; BA: Bronchial artery; PV: Shunt to pulmonary vein; LGA: Left gastric artery; RSGA: Right superior gluteal artery.

CLE, especially when it is difficult to obtain a decrease of blood flow.

REFERENCES

1 **Sakamoto I**, Aso N, Nagaoki K, Matsuoka Y, Uetani M, Ashizawa K, Iwanaga S, Mori M, Morikawa M, Fukuda T, Hayashi K, Matsunaga N. Complications associated with transcatheter arterial embolization for hepatic tumors. *Radiographics* 1998; **18**: 605-619 [PMID: 9599386 DOI: 10.1148/radiographics.18.3.9599386]

2 **Yoo KM**, Yoo BG, Kim KS, Lee SU, Han BH. Cerebral lipiodol embolism during transcatheter arterial chemoembolization. *Neurology* 2004; **63**: 181-183 [PMID: 15249637 DOI: 10.1212/01.WNL.0000132645.23611.2D]

3 **Takao H**, Makita K, Doi I, Watanabe T. Cerebral lipiodol embolism after transcatheter arterial chemoembolization of hepatocellular carcinoma. *J Comput Assist Tomogr* 2005; **29**: 680-682 [PMID: 16163042 DOI: 10.1097/01.rct.0000165906.83160.7d]

4 **Wu RH**, Tzeng WS, Chang CM. Iodized oil embolization to brain following transcatheter arterial embolization of liver. *J Gastroenterol Hepatol* 2005; **20**: 1465-1467 [PMID: 16105143 DOI: 10.1111/j.1440-1746.2005.03412.x]

5 **Matsumoto K**, Nojiri J, Takase Y, Egashira Y, Azama S, Kato A, Kitahara K, Miyazaki K, Kudo S. Cerebral lipiodol embolism: a complication of transcatheter arterial chemoembolization for hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2007; **30**: 512-514 [PMID: 17171304 DOI: 10.1007/s00270-006-0092-x]

6 **Choi CS**, Kim KH, Seo GS, Cho EY, Oh HJ, Choi SC, Kim TH, Kim HC, Roh BS. Cerebral and pulmonary embolisms after transcatheter arterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2008; **14**: 4834-4837 [PMID: 18720550 DOI: 10.3748/wjg.14.4834]

7 **Kim JT**, Heo SH, Choi SM, Lee SH, Park MS, Kim BC, Kim Y, Kim MK, Cho KH. Cerebral embolism of iodized oil (lipiodol) after transcatheter arterial chemoembolization for hepatocellular carcinoma. *J Neuroimaging* 2009; **19**: 394-397 [PMID: 19496902 DOI: 10.1111/j.1552-6569.2009.00380.x]

8 **Wu JJ**, Chao M, Zhang GQ, Li B. Delayed cerebral lipiodol embolism after transcatheter arterial chemoembolization of hepatocellular carcinoma. *Chin Med J* (Engl) 2009; **122**: 878-880 [PMID: 19493407]

9 **Wu JJ**, Chao M, Zhang GQ, Li B, Dong F. Pulmonary and cerebral lipiodol embolism after transcatheter arterial chemoembolization [corrected] in hepatocellular carcinoma. *World J Gastroenterol* 2009; **15**: 633-635 [PMID: 19195069 DOI: 10.3748/wjg.15.633]

10 **Wu L**, Yang YF, Liang J, Shen SQ, Ge NJ, Wu MC. Cerebral lipiodol embolism following transcatheter arterial chemoembolization for hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 398-402 [PMID: 20082490 DOI: 10.3748/wjg.v16.i3.398]

11 **Karapanayiotides T**, Goulis J, Theodorou A, Anastasiou A, Georgiadis G, Ilonidis G. Lipiodol brain embolism during hepatic transcatheter arterial chemoembolization. *J Neurol* 2009; **256**: 1171-1173 [PMID: 19252772 DOI: 10.1007/s00415-009-5066-x]

12 **Lee CS**, Kim SJ, Choi JW, Choi CG, Lee DH. Cerebral lipiodol embolism proven by dual-energy computed tomography: a case report. *J Comput Assist Tomogr* 2010; **34**: 105-106 [PMID: 20118731 DOI: 10.1097/RCT.0b013e3181b382f8]

13 **Jia ZZ**, Tian F, Jiang GM. Cerebral lipiodol embolism after transarterial chemoembolization for hepatic carcinoma: a case report. *World J Gastroenterol* 2012; **18**: 4069-4070 [PMID: 22912560 DOI: 10.3748/wjg.v18.i30.4069]

14 **Zach V**, Rapaport B, Yoo JY, Goldfeder L, Weinberger J. Multiple

ischemic strokes after transcatheter arterial chemoembolization for hepatocellular carcinoma with a radiographic and pathological correlate. *J Stroke Cerebrovasc Dis* 2012; **21**: 217-224 [PMID: 21036627 DOI: 10.1016/j.jstrokecerebrovasdis.2010.08.001]

15 **Ishikawa T**, Kubota T, Abe H, Toduka Y, Horigome R, Watanabe Y, Kimura N, Honda H, Iwanaga A, Seki K, Honma T, Yoshida T, Sato M. Case of cerebral lipiodol embolism after repeated transcatheter arterial chemoembolization of hepatocellular carcinoma. *Hepatol Res* 2013; **43**: 1251-1252 [PMID: 24580681 DOI: 10.1111/hepr.12074]

16 **Bánsághi Z**, Kaposi PN, Lovas G, Szentmártoni G, Várallyay G, Bata P, Kalina I, Futácsi B, Bérczi V. Cerebral iodized lipid embolization via a pulmonary arteriovenous shunt: rare complication of transcatheter arterial embolization for hepatocellular carcinoma. *World J Surg Oncol* 2013; **11**: 122 [PMID: 23721061 DOI: 10.1186/1477-7819-11-122]

17 **Wan CC**, Liu KL. Cerebral Lipiodol embolism. *Liver Int* 2015; **35**: 673 [PMID: 24571486 DOI: 10.1111/liv.12511]

18 **Chu HJ**, Lee CW, Yeh SJ, Tsai LK, Tang SC, Jeng JS. Cerebral Lipiodol Embolism in Hepatocellular Carcinoma Patients Treated with Transarterial Embolization/Chemoembolization. *PLoS One* 2015; **10**: e0129367 [PMID: 26107693 DOI: 10.1371/journal.pone.0129367]

19 **Ueda K**, Matsui O, Kawamori Y, Nakanuma Y, Kadoya M, Yoshikawa J, Gabata T, Nonomura A, Takashima T. Hypervascular hepatocellular carcinoma: evaluation of hemodynamics with dynamic CT during hepatic arteriography. *Radiology* 1998; **206**: 161-166 [PMID: 9423667 DOI: 10.1148/radiology.206.1.9423667]

20 **Seki A**, Hori S, Shimono C. Management of vascular lake phenomenon on angiography during chemoembolization with superabsorbent polymer microspheres. *Jpn J Radiol* 2015; **33**: 741-748 [PMID: 26493810 DOI: 10.1007/s11604-015-0486-2]

21 **Cavalcante RN**, Nasser F, Motta-Leal-Filho JM, Affonso BB, Galastri FL, De Fina B, Garcia RG, Wolosker N. Occurrence of Vascular Lake Phenomenon as a Predictor of Improved Tumor Response in HCC Patients That Underwent DEB-TACE. *Cardiovasc Intervent Radiol* 2017; **40**: 1044-1051 [PMID: 28500458 DOI: 10.1007/s00270-017-1678-1]

22 **Ngan H**, Peh WC. Arteriovenous shunting in hepatocellular carcinoma: its prevalence and clinical significance. *Clin Radiol* 1997; **52**: 36-40 [PMID: 9022578 DOI: 10.1016/S0009-9260(97)80303-0]

23 **Leung WT**, Lau WY, Ho SK, Chan M, Leung NW, Lin J, Metreweli C, Johnson PJ, Li AK. Measuring lung shunting in hepatocellular carcinoma with intrahepatic-arterial technetium-99m macroaggregated albumin. *J Nucl Med* 1994; **35**: 70-73 [PMID: 8271063]

24 **Kanematsu M**. Transcatheter arterial chemoembolization therapy with epirubicin hydrochloride, mitomycin C-iohexol-Lipiodol emulsion (EMILE) for hepatocellular carcinoma. *J Gastroenterol* 1995; **30**: 215-223 [PMID: 7773353 DOI: 10.1007/BF02348668]

25 **Tajima T**, Honda H, Kuroiwa T, Yabuuchi H, Okafuji T, Yosimitsu K, Irie H, Aibe H, Masuda K. Pulmonary complications after hepatic artery chemoembolization or infusion via the inferior phrenic artery for primary liver cancer. *J Vasc Interv Radiol* 2002; **13**: 893-900 [PMID: 12354823 DOI: 10.1016/S1051-0443(07)61772-2]

26 **Kishi K**, Sonomura T, Satoh M, Nishida N, Terada M, Shioyama Y, Yamada R. Acute toxicity of lipiodol infusion into the hepatic arteries of dogs. *Invest Radiol* 1994; **29**: 882-889 [PMID: 7852039 DOI: 10.1097/00004424-199410000-00004]

P- Reviewer: Pompili M, Xiao E, Yao DF **S- Editor**: Wang XJ
L- Editor: A **E- Editor**: Yin SY

Published by **Baishideng Publishing Group Inc**
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.f6publishing.com/helpdesk
http://www.wjgnet.com

ISSN 1007-9327