

World Journal of *Clinical Cases*

World J Clin Cases 2018 September 26; 6(10): 308-405





REVIEW

- 308 Algorithm for the multidisciplinary management of hemorrhage in EUS-guided drainage for pancreatic fluid collections

Jiang TA, Xie LT

- 322 Mystery behind labial and oral melanotic macules: Clinical, dermoscopic and pathological aspects of Laugier-Hunziker syndrome

Duan N, Zhang YH, Wang WM, Wang X

MINIREVIEWS

- 335 Research progress on signaling pathways in cirrhotic portal hypertension

Xu W, Liu P, Mu YP

- 344 Gastrointestinal toxicity induced by microcystins

Wu JX, Huang H, Yang L, Zhang XF, Zhang SS, Liu HH, Wang YQ, Yuan L, Cheng XM, Zhuang DG, Zhang HZ

ORIGINAL ARTICLE

Basic Study

- 355 *PNPLA3* rs139051 is associated with phospholipid metabolite profile and hepatic inflammation in nonalcoholic fatty liver disease

Luo JJ, Cao HX, Yang RX, Zhang RN, Pan Q

Retrospective Study

- 365 Recurrent carpal tunnel syndrome: Evaluation and treatment of the possible causes

Eroğlu A, Sarı E, Topuz AK, Şimşek H, Pusat S

- 373 Adjuvant chemotherapy with S-1 plus oxaliplatin improves survival of patients with gastric cancer after D2 gastrectomy: A multicenter propensity score-matched study

Ren DF, Zheng FC, Zhao JH, Shen GS, Ahmad R, Zhang SS, Zhang Y, Kan J, Dong L, Wang ZY, Zhao FX, Zhao JD

CASE REPORT

- 384 Rectal perforation by inadvertent ingestion of a blister pack: A case report and review of literature

Fleres F, Ieni A, Saladino E, Speciale G, Aspromonte M, Cannà A, Macrì A



Contents

Semimonthly Volume 6 Number 10 September 26, 2018

- 393** Unusual complication in patient with Gardner's syndrome: Coexistence of triple gastrointestinal perforation and lower gastrointestinal bleeding: A case report and review of literature
Akbulut S, Koc C, Dirican A
- 398** Laparoscopic repair via the transabdominal preperitoneal procedure for bilateral lumbar hernia: Three cases report and review of literature
Huang DY, Pan L, Chen MY, Fang J

ABOUT COVER

Editorial Board Member of *World Journal of Clinical Cases*, Young-Seok Cho, MD, PhD, Professor, Division of Gastroenterology, Department of Internal Medicine, Seoul St. Mary's Hospital, the Catholic University of Korea, Seoul 06591, South Korea

AIM AND SCOPE

World Journal of Clinical Cases (*World J Clin Cases*, *WJCC*, online ISSN 2307-8960, DOI: 10.12998) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

The primary task of *WJCC* is to rapidly publish high-quality Autobiography, Case Report, Clinical Case Conference (Clinicopathological Conference), Clinical Management, Diagnostic Advances, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Clinical Practice, Meta-Analysis, Minireviews, Review, Therapeutics Advances, and Topic Highlight, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, geriatrics and gerontology, hematology, immunology, infectious diseases, internal medicine, obstetrics and gynecology, oncology, ophthalmology, orthopedics, otolaryngology, pathology, pediatrics, peripheral vascular disease, psychiatry, radiology, rehabilitation, respiratory medicine, rheumatology, surgery, toxicology, transplantation, and urology and nephrology.

INDEXING/ABSTRACTING

World Journal of Clinical Cases (*WJCC*) is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), and Journal Citation Reports/Science Edition. The 2018 Edition of Journal Citation Reports cites the 2017 impact factor for *WJCC* as 1.931 (5-year impact factor: N/A), ranking *WJCC* as 60 among 154 journals in Medicine, General and Internal (quartile in category Q2).

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Xiang Li*
Responsible Electronic Editor: *Yun-XiaoJian Wu*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

Responsible Science Editor: *Fang-Fang Ji*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Clinical Cases

ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

FREQUENCY
Semimonthly

EDITORS-IN-CHIEF
Sandro Vento, MD, Department of Internal Medicine, University of Botswana, Private Bag 00713, Gaborone, Botswana

EDITORIAL BOARD MEMBERS
All editorial board members resources online at <http://www.wjnet.com/2307-8960/editorialboard.htm>

EDITORIAL OFFICE
Jin-Lei Wang, Director

World Journal of Clinical Cases
Baishideng Publishing Group Inc
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjnet.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@wjnet.com
Help Desk: <http://www.f6publishing.com/helpdesk>
<http://www.wjnet.com>

PUBLICATION DATE
September 26, 2018

COPYRIGHT

© 2018 Baishideng Publishing Group Inc. Articles published by this Open Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT

All articles published in journals owned by the Baishideng Publishing Group (BPG) represent the views and opinions of their authors, and not the views, opinions or policies of the BPG, except where otherwise explicitly indicated.

INSTRUCTIONS TO AUTHORS

<http://www.wjnet.com/bpg/gerinfo/204>

ONLINE SUBMISSION

<http://www.f6publishing.com>

Algorithm for the multidisciplinary management of hemorrhage in EUS-guided drainage for pancreatic fluid collections

Tian-An Jiang, Li-Ting Xie

Tian-An Jiang, Li-Ting Xie, Department of Ultrasound, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, Zhejiang Province, China

ORCID number: Tian-An Jiang (0000-0002-7672-8394); Li-Ting Xie (0000-0002-0498-193X).

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

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Tian-An Jiang, MD, PhD, Professor, Surgeon, Department of Ultrasound, the First Affiliated Hospital, College of Medicine, Zhejiang University, Qingchun Road, No. 79, Hangzhou 310003, Zhejiang Province, China. tiananjiang@zju.edu.cn

Received: May 17, 2018

Peer-review started: May 21, 2018

First decision: June 20, 2018

Revised: July 5, 2018

Accepted: July 15, 2018

Article in press: July 16, 2018

Published online: September 26, 2018

Abstract

Pancreatic fluid collections (PFCs), common sequelae

of acute or chronic pancreatitis, are broadly classified as pancreatic pseudocysts or walled-off necrosis according to the revised Atlanta classification. Endoscopic ultrasound (EUS)-guided drainage is often considered a standard first-line therapy preferable to surgical or interventional radiology approaches for patients with symptomatic PFC. EUS-guided drainage is effective and successful; it has a technical success rate of 90%-100% and a clinical success rate of 85%-98%. Recent studies have shown a 5%-30% adverse events (AEs) rate for the procedure. The most common AEs include infection, hemorrhage, perforation and stent migration. Hemorrhage, a severe and sometimes deadly outcome, requires a well-organized and appropriate treatment strategy. However, few studies have reported the integrated management of hemorrhage during EUS-guided drainage of PFC. Establishing a practical therapeutic strategy is an essential and significant step in standardized management. The aim of this review is to describe the current situation of EUS-guided drainage of PFCs, including the etiology and treatment of procedure-related bleeding as well as current problems and future perspectives. We propose a novel and meaningful algorithm for systematically managing hemorrhage events. To our limited knowledge, a multidisciplinary algorithm for managing EUS-guided drainage for PFC-related bleeding has not been previously reported.

Key words: Pancreatic fluid collections; Hemorrhage; Endoscopic ultrasound-guided; Treatment algorithm; Adverse events

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Currently, endoscopic ultrasound (EUS)-guided drainage has been viewed as the optimal therapy for symptomatic pancreatic fluid collections (PFCs). Bleeding is one of the most dangerous adverse events.

However, few studies have addressed the treatment of hemorrhage in the EUS-guided drainage of PFCs. The aim of this review is to describe the etiology, therapy, and prevention of EUS-guided drainage procedure-related bleeding and to present current problems and future perspectives. Moreover, we propose a meaningful and multidisciplinary algorithm for the clinical management of hemorrhagic events in EUS-guided drainage for PFCs.

Jiang TA, Xie LT. Algorithm for the multidisciplinary management of hemorrhage in EUS-guided drainage for pancreatic fluid collections. *World J Clin Cases* 2018; 6(10): 308-321 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/308.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.308>

INTRODUCTION

Pancreatic fluid collections (PFCs) occur in approximately 50% of cases of acute pancreatitis (AP)^[1]. PFCs are defined by the revised Atlanta classification as “a collection” that develops over time (usually at or after 4 wk), including pancreatic pseudocyst (PPC) and walled-off necrosis (WON)^[2]. Recent studies have recognized endoscopic ultrasound (EUS)-guided drainage as the standard first-line therapy for symptomatic PFCs due to its minimal invasiveness, lower mortality and morbidity, shorter length of hospital stays, better physical recovery and lower cost compared to non-endoscopic treatments^[3-5].

EUS-guided drainage with stents is recognized as the gold standard for symptomatic PFCs because of its high technical success rate of 90%-100% and clinical success rate of 85%-98%, and low mortality rate of 1%-5%^[6,7]. Furthermore, multiple previous studies have indicated that EUS-guided drainage is effective, successful, and increasingly used. Despite the advantages of EUS-guided drainage for PFC compared to surgical and percutaneous methods, previous research has also found that it is associated with a significant number of adverse events (AEs), namely, bleeding, infection, perforation and stent migration^[8-10]. Hemorrhage in particular is fatal and difficult to control once it begins. Several published studies have revealed a 3%-20% bleeding rate^[11-13], and some cases have even ended in death^[14,15]. However, to date, there are minimal data regarding the optimal management of procedure-related hemorrhage. The establishment of an adequate and effective treatment strategy is an essential step for standardized management.

The purpose of this review is to describe the development of EUS-guided drainage of PFCs and the present situation and future perspective of procedural-related bleeding. Moreover, we propose a novel and meaningful algorithm for the treatment of hemorrhage. To our limited knowledge, this is the first report of an integrated and multidisciplinary method for the

management of EUS-guided drainage of PFCs with bleeding.

WHAT ARE PANCREATIC FLUID COLLECTIONS?

PFCs develop due to the sequelae of acute pancreatitis, exacerbations of chronic pancreatitis, trauma, malignancy and surgery^[16-18]. The majority of PFCs are asymptomatic and will resolve spontaneously; however, patients with obvious abdominal pain, gastric outlet obstruction, biliary obstruction, and organ failure warrant intervention^[19,20]. PFCs are being recognized at a growing frequency largely because of the increased use of abdominal cross-sectional imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI)^[10]. Recently, the role of EUS has evolved, and its increasing reproducibility allows it to further identify these collections^[21-25]. Furthermore, EUS is not only a diagnostic approach but a therapeutic one. PFCs are being identified at an increasing frequency, mainly due to the enhanced use of abdominal imaging approaches^[26].

EUS-GUIDED MANAGEMENT OF PANCREATIC FLUID COLLECTIONS

The management of symptomatic PFCs (PPC or WON) has evolved over time. Traditionally, the drainage of PFCs was connected to surgical or percutaneous methods. Currently, endoscopic drainage is now generally preferred over non-endoscopic drainage procedures and has become an optimal approach, preferable to surgical or percutaneous methods^[26,27]. EUS has developed as a backbone of PFC management, representing a rapid advance in clinical medicine that has markedly improved the treatment level of this complex disease. With the availability of EUS, the safety and efficacy of PFC drainage has improved further. Because the surgical approach has the disadvantages of invasiveness, high mortality, long hospital stays and high costs, endoscopic options have increasingly been used for initial management^[28]. Recent studies have also favored the endoscopic approach for the management of PFCs when endoscopic and surgical techniques were compared^[29,30]. Siddiqui *et al.*^[31] revealed that the percutaneous approach is associated with infection, a high recurrence rate, and fistula formation. A 14-year experience study by Keane *et al.*^[32] also confirmed that endoscopic drainage was superior to percutaneous drainage. Several studies in the literature have demonstrated that endoscopic drainage is superior to surgical and percutaneous drainage in terms of minimal invasiveness, lower morbidity, significantly better clinical success, lower re-intervention rates, shorter lengths of hospital stay, a better post-operative condition of the patient, and lower cost^[33,34]. EUS-guided drainage has the following advantages compared with other non-EUS-guided drainage

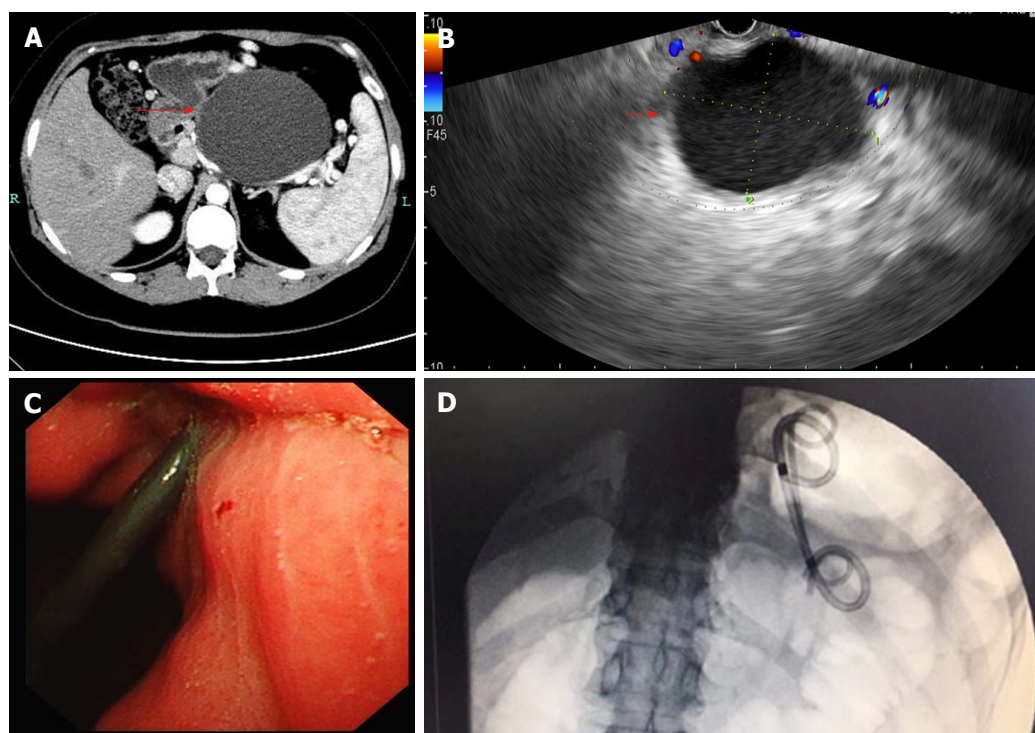


Figure 1 Imaging findings of pancreatic pseudocyst. A: A contrast-enhanced computed tomography (CECT) scan of the upper abdomen. A relatively homogenous cystic mass is identified in the region of the pancreatic tail (arrow). B: Endoscopic ultrasound (EUS) image showing a pancreatic pseudocyst (PPC) (arrow). C: Endoscopic image showing plastic stent deployment. D: Digital subtraction angiography (DSA) image revealing double pigtail plastic stent deployment.

options^[35-37]: (1) EUS can be used to determine lesion characteristics, such as whether the lesion is a simple cyst or whether significant necrotic debris is present. Thus, EUS can distinguish PPC from different true cysts, cystic tumors, WON, lymphoceles, and the gallbladder; (2) EUS-guided drainage is widespread to enhance the diagnosis of cystic pancreatic lesions and enable real-time image-guided control of cystic drainage; (3) EUS Doppler can identify the vessels and potentially reduce the risk of bleeding; and (4) EUS can adjust the angle of the transducer (up and down) in order to acquire better Doppler signal and ultrasound imaging (Figure 1).

OUTCOME MEASURES AND ADVERSE EVENTS OF EUS-GUIDED DRAINAGE

Over the past 30 years, diagnostic and therapeutic EUS has been applied to both benign and malignant diseases of the pancreas; its use to diagnose and treat patients with symptomatic PFC in particular has vastly expanded. Recently, Lang *et al*^[38], in their retrospective single-center study of 103 PFC patients treated by EUS-guided drainage, used both double-pigtail plastic stents (DDPS) and lumen-apposing covered self-expanding metal stents (LAMS). They concluded that the overall technical success rate was 99%, the overall clinical success rate as determined by complete resolution was 95% after 6 mo of follow-up; furthermore, AEs occurred in 19 out of 103 patients (18%) and mainly included hemorrhage (5%), perforation (1%), and unplanned

endoscopy (13%). In addition, a retrospective multicenter trial of a nationwide database involving all hospitals in Spain performed EUS-guided drainage for patients with PFC^[39]. This study included 211 patients (53% PPC and 47% WON) and reported 91% technical success, 93% clinical success, and 21% AEs. The AEs included infection (11%), bleeding (7%), and stent migration and/or perforation (3%). These findings are in accordance with a systematic review that demonstrated treatment success rates of 98% and 93% for technical success and clinical success, respectively^[40]. In another retrospective study, Bang *et al*^[6] reported 100% technical success and 93% treatment success for EUS-guided transmural drainage of symptomatic PPC with plastic stents. One of the largest prospective studies was reported by Shah *et al*^[41] and used LAMS to drain PFC. They reported a technical success rate of 91% and a PFC resolution rate of 93%. Similar results have been shown by Fabbri *et al*^[42] and Penn *et al*^[43]. These studies included 20 patients each and reported rates of 100%, 81%, and 15% for technical success, clinical success and AEs, respectively. A recently published retrospective study by Rinniella *et al*^[44] performed at 13 experienced institutions and including 93 PFCs drained with LAMS reported a 99% technical success rate, a 92% clinical success rate and a 5% AE rate. They indicated major procedure-related AEs, mainly including massive hemorrhage (1%), infection (1%), perforation (1%), stent migration (1%) and pneumoperitoneum (1%). In another prospective multicenter European

study, Walter *et al*^[45] reported success rates of 93% for pseudocysts and 81% for WON and a 9% rate of major AEs, which included infection (7%) and perforation (2%). In a retrospective two-center study of 49 PFC patients who underwent EUS-guided drainage, Ang *et al*^[20] reported the following rates for AEs: 2% infection, 4% bleeding, 2% perforation and 2% pneumoperitoneum. Therefore, the high success rate is the best indicator of the great development of EUS-guided drainage for PFC. The detailed outcome measures for EUS-guided drainage of PFCs are described in Table 1.

MANAGEMENT OF HEMORRHAGE:

WHERE DO WE STAND?

Although the EUS-guided drainage procedure is effective and has a high success rate, which diminishes the risk of death, AE rates of 5%-30% have been previously reported^[45-47]. As with any endoscopic procedure, EUS and its guided interventions may be accompanied by AEs, which mainly include bleeding, infection, perforation and stent migration^[48,49]. The adverse events associated with EUS-guided drainage of PFC are presented in detail in Table 2. In particular, bleeding is a dangerous, even deadly, adverse event. Further details regarding hemorrhage are shown in Table 3. An Italian study by Cavallini *et al*^[15] revealed that 11% of patients with massive hemorrhage died from a pseudoaneurysm of the gastroduodenal artery. Similarly, Mukai *et al*^[14] and Rinninella *et al*^[44] reported bleeding-related mortality rates of 3% and 1%, respectively. However, there are minimal data regarding the causes of and optimal treatments for hemorrhage. Recently, a researcher studied EUS-guided drainage of PFC with LAMS and DPPS^[38]. The hemorrhages that occurred in the DPPS group were due to plastic stent erosion into the opposite gastric wall. Esophagogastroduodenoscopy was conducted, and a visible vessel was cauterized for durable hemostasis. In the LAMS group, 4 bleeding episodes were observed. One was a collateral vessel bleed and was managed by conservative therapy, such as blood transfusion. The second was an intracavitary vessel bleed and was treated with balloon tamponade under endoscopic guidance. No further hemorrhage was noted. Another two patients developed bleeding caused by splenic artery pseudoaneurysm. One patient underwent successful hemostasis with left gastric and splenic artery embolization. Unfortunately, the other patient died before his embolization procedure. In a randomized controlled trial by Bang *et al*^[50] using LAMS for PFC drainage, three patients presented with serious gastrointestinal tract bleeding that required intensive care unit admission and blood transfusions. Furthermore, on EUS, interlacing vessels were visualized within the distal flange of the LAMS and CT

angiograms, confirming pseudoaneurysms. All three patients were successfully managed by interventional radiology-guided coil embolization.

Furthermore, Bapaye *et al*^[13] reported a 10-year retrospective study of 133 patients who underwent EUS-guided drainage for WON with multiple plastic stents (MPS) and fully covered bi-flanged metal stents (BFMS). Five patients in the MPS group developed hemorrhage due to tract dilatation and required blood transfusion. Bleeding was controlled by endoscopic clip application in all except one patient, who required surgery. Another two patients in the BFMS group developed hemorrhage due to the erosion of the splenic artery by the indwelling inner end of the stent; their bleeding was controlled with embolization. In another multicenter study, nine patients (4%) experienced hemorrhagic events^[47]. Two patients developed severe hemorrhage due to the inadvertent puncture of an artery and were successfully treated with interventional radiology (IR)-guided coil embolization. A prospective randomized trial reported by Varadarajulu *et al*^[33] showed that one patient suffered post-procedural bleeding. On endoscopy and arteriography, hemorrhage was distinct from the site of stent placement or a branch of the gastric artery, but it could not be controlled with epinephrine injection or coil placement. The patient had to undergo surgery. However, the patient then developed hemorrhage at the site of venous access, indicating the presence of acquired factor VIII inhibitors; it was corrected by administering anti-inhibitor coagulant complex. Finally, the patient was discharged on the sixth post-operative day. Interestingly, a retrospective study by Vazquez-Sequeiros *et al*^[39] revealed that in 64% of patients who developed delayed hemorrhage and portal hypertension, imaging studies showed that splenic vein thrombosis was the cause, and antithrombotic therapy with stent-induced ulceration may have contributed to this. This was a first clinically significant observation, and it will need to be supported with larger prospective randomized studies in the near future.

Cohen *et al*^[51] reported a case that used the tamponade of fully covered self-expandable metal stent (FCSEMS) to control bleeding and addressed dangerous hemorrhage. Similar events were also reported by Akbar *et al*^[52]. Talreja *et al*^[53] reported a prospective case series in which patients who experienced bleeding from the pseudocyst wall were treated with balloon tamponade and arterial embolization. Furthermore, Lakhtakia *et al*^[54], Siddiqui *et al*^[31] and Itoi *et al*^[12] described several cases of minor bleeding that were self-limiting and did not require treatment. However, some studies did not explain why the hemorrhage occurred^[20,27]; furthermore, several researchers did not describe how to address the hemorrhage^[14,55], and some studies did not demonstrate the methods used to diagnose the bleeding^[14,15,48,55]. Although these studies were limited to describing the etiology and treatment of these hemorrhagic episodes, future prospective studies

Table 1 Detailed outcome measures for endoscopic ultrasound-guided drainage of pancreatic fluid collections

Study	Year	Country	Design	Single/ multi-center	Stent style	No.	PFC style	Technical success	Clinical success	Adverse events	Mortality	Cause of death	Follow-up period
Lang <i>et al</i> ^[38]	2018	United States	RS	Single	LAMS	19	PPC/WON	99%	94%	53%	5%	Due to bleeding from splenic artery	≥ 6 mo
Aburajab <i>et al</i> ^[10]	2018	United States	RS	Single	DPPS LAMS with no DPPS	84 46	PPC/WON PPC	99% 96%	96% 91%	11% 25%	0% 0%	pseudoaneurysms None	2-4 mo
Siddiqui <i>et al</i> ^[46]	2017	United States	RS	Multi	LAMS with DPPS LAMS	23 86	PPC WON	100% 97.70%	100% 90%	0% 12%	0% 0%	None	≥ 6 mo
Bapaye <i>et al</i> ^[13]	2017	India	RS	Single	DPPS FCSEMS	106 121	WON WON	99% 100%	81% 95%	15% 2%	0% 0%	Died from sepsis; organ failure; pulmonary embolism	2 mo
Bang <i>et al</i> ^[30]	2017	United States	RCT	Single	BEMS MPS	72 61	WON WON	100% 100%	94% 74%	6% 36%	4% 7%	None	4-6 wk
Bang <i>et al</i> ^[6]	2017	United States	RS	Single	LAMS	12	WON	NR	NR	50%	0%	None	1-2 yr
Lakhtakia <i>et al</i> ^[54]	2016	India	RS	Single	DPPS	9	WON	NR	NR	0%	0%	Died of progressive sepsis	12 mo
Sharaiha <i>et al</i> ^[7]	2016	United States	RS	Multi	LAMS	20	PPC/WON	100%	95%	10%	5%	None	3 mo
Gornals <i>et al</i> ^[11]	2016	Spain	RS	Single	DPPS	40	PPC/WON	100%	93%	13%	0%	None	6 mo
Siddiqui <i>et al</i> ^[31]	2016	United States	RS	Multi	FCSEMS	205	WON	99.00%	75%	4%	0%	None	NR
Ang <i>et al</i> ^[20]	2016	Singapore	RS	Multi	LAMS	124	WON	100%	86%	19%	0%	None	NR
Vazquez-Sequeiros <i>et al</i> ^[39]	2016	Spanish	RS	Multi	LAMS	82	PPC/WON	100%	100%	33%	0%	None	≥ 6 mo
Shah <i>et al</i> ^[41]	2015	United States	PS	Single	LAMS	12	WON	100%	100%	10%	0%	None	NR
Walter <i>et al</i> ^[45]	2015	The Netherlands	PS	Single	LAMS	61	PPC/WON	98%	93% 81%WON	9%	1%	Died from an unrelated cause (myocardial infarction)	≥ 6 mo
Sharaiha <i>et al</i> ^[47]	2015	United States	RS	Multi	FCSEMS	112	PPC	98%	98%	16%	0%	None	10 mo
Rinninella <i>et al</i> ^[44]	2015	Italy	RS	Multi	DPPS FCSEMS	118 69	PPC PPC/WON	92% 99%	89% 93%	31% 5%	0% 4%	Died because of multi-organ failure and massive bleeding	1-2 yr
Mukai <i>et al</i> ^[14]	2015	Japan	RS	Single	FC BEMS	43	WON	100%	98%	7%	7%	None	6-7 mo
Lee <i>et al</i> ^[55]	2014	South Korea	PS	Single	DPPS FCSEMS	27 25	WON PFC	100% 100%	93% 87%	19% 0%	0% 0%	None	2 mo
Yamamoto <i>et al</i> ^[56]	2013	Japan	RS	Single	FCSEMS	25	PPC/WON	100%	78%	22%	10%	Because of multiple organ failure	1-2 yr
Bang <i>et al</i> ^[53]	2013	United States	RS	Multi	MTGT	76	WON	NR	70%	15%	15%	Due to portal hypertension	48 mo
Puri <i>et al</i> ^[48]	2012	India	PS	Single	DPPS	40	PPC	100%	98%	8%	0%	None	11 mo
Itoi <i>et al</i> ^[12]	2012	Japan	RS	Single	LAMS	15	PPC	100%	100%	0%	0%	None	1-2 yr
Varadarajulu <i>et al</i> ^[27]	2011	United States	RS	Single	DPPS	211	PPC/WON	NR	85%	8%	1%	Due to delayed bleeding	5 mo
Varadarajulu <i>et al</i> ^[64]	2011	United States	RS	Single	MTGT	12	WON	100%	92%	0%	0%	None	5 mo

PPC: Pancreatic pseudocyst; WON: Walled-off necrosis; PFC: Pancreatic fluid collections; PS: Prospective study; RS: Retrospective study; RCT: Randomized controlled trial; Single: Single-center; Multi: Multi-center; FCSEMS: Fully covered self-expandable metal stent; LAMS: Lumen-apposing metal stent; DPPS: Double-pigtail plastic stents; BEMS: Fully covered bi-flanged metal stents; MPS: Multiple plastic stents; MTGT: Multiple transluminal gateway technique; NR: Not reported.

Table 2 Details of the adverse events associated with endoscopic ultrasound-guided drainage of pancreatic fluid collections

Study	Year	Country	Design	Single/multi-center	Stent style	No.	PFC style	Infection	Bleeding	Perforation	Migration	Occlusion	Other adverse events
Lang <i>et al.</i> ^[8]	2018	United States	RS	Single	LAMS/DPPS	103	80 PP 23 WON	0%	5%	1%	0%	0%	13% Unplanned endoscopy
Aburajab <i>et al.</i> ^[10]	2018	United States	RS	Single	LAMS with DPPS/ with no DPPS	46	PP	9%	0%	2%	15%	0%	9% Re-intervention
Siddiqui <i>et al.</i> ^[6]	2017	United States	RS	Multi	LAMS/ DPPS/ FCSEMS	313	WON	3%	3%	2%	1%	8%	12% (No details)
Bapaye <i>et al.</i> ^[13]	2017	India	RS	Single	BFMS / MPS	133	WON	2%	5%	0%	2%	0%	14% Persistent sepsis
Bang <i>et al.</i> ^[30]	2017	United States	RCT	Single	LAMS/ DPPS	21	WON	0%	14%	0%	0%	0%	2% Buried stent
Bang <i>et al.</i> ^[6]	2017	United States	RS	Single	LAMS/ DPPS	60	21 PP 39 WON	12%	0%	0%	5%	0%	5% Biliary stricture
Lakhtakia <i>et al.</i> ^[54]	2016	India	RS	Single	FCSEMS	205	WON	0%	3%	1%	1%	10%	28% Re-intervention
Sharaiha <i>et al.</i> ^[7]	2016	United States	RS	Multi	LAMS	124	WON	6%	3%	0%	6%	6%	1% Buried stent
Gornals <i>et al.</i> ^[11]	2016	Spain	RS	Single	LAMS	12	WON	17%	17%	0%	0%	0%	19% Re-intervention
Siddiqui <i>et al.</i> ^[31]	2016	United States	RS	Multi	LAMS	82	14 PPC 68 WON	6%	7%	0%	0%	5%	None
Ang <i>et al.</i> ^[20]	2016	Singapore	RS	Multi	FCSEMS / DPPS	49	WON 14 PP 35	2%	4%	2%	0%	0%	2% Stent maldeployment; 1% pneumoperitoneum
Vazquez-Sequeiroset <i>et al.</i> ^[39]	2016	Spain	RS	Multi	FCSEMS	211	112 PP 99 WON	23%	7%	-	5%	0%	2% Pneumoperitoneum
Shah <i>et al.</i> ^[41]	2015	United States	PS	Multi	LAMS	33	PPC/ WON	6%	0%	0%	3%	0%	3% Perforation / pneumoperitoneum
Walker <i>et al.</i> ^[45]	2015	The Netherlands	PS	Multi	LAMS	61	PPC/ WON	7%	0%	2%	0%	0%	None
Sharaiha <i>et al.</i> ^[67]	2015	United States	RS	Multi	FCSEMS	112	PPC	14%	4%	3%	1%	7%	None
Rinninella <i>et al.</i> ^[44]	2015	Italy	RS	Multi	FCSEMS	93	WON 52 PPC18 PA 19 APFC 4	1%	1%	1%	1%	0%	1% Pneumoperitoneum
Mukai <i>et al.</i> ^[14]	2015	Japan	RS	Single	FCSEMS / DPPS	70	WON	0%	4%	1%	4%	0%	1% Mediastinal emphysema
Lee <i>et al.</i> ^[55]	2014	South Korea	PS	Single	FCSEMS/ DPPS	50	PFC	0%	2%	0%	4%	0%	None
Yamamoto <i>et al.</i> ^[56]	2013	Japan	RS	Multi	FCSEMS	9	5 PPC 4 WON	0%	11%	0%	11%	0%	None
Bang <i>et al.</i> ^[65]	2013	United States	RS	Multi	MTGT	76	WON	8%	1%	1%	4%	0%	None
Puri <i>et al.</i> ^[48]	2012	India	PS	Single	DPPS	40	40 PPC	3%	3%	0%	0%	0%	3% Pneumoperitoneum
Itoi <i>et al.</i> ^[12]	2012	Japan	RS	Single	LAMS	15	9 PPC	0%	20%	0%	7%	0%	None
Varadarajulu <i>et al.</i> ^[27] (Endo Trans Dra of pfc)	2011	United States	RS	Single	DPPS	211	6 WON 154 PPC 57 WON	3%	1%	1%	1%	0%	None

PPC: Pancreatic pseudocyst; WON: Walled-off necrosis; PFC: Pancreatic fluid collections; PS: Prospective study; RS: Retrospective study; RCT: Randomized controlled trial; Single: Single-center; Multi: Multi-center; FCSEMS: Fully covered self-expandable metal stent; LAMS: Lumen-apposing metal stents; DPPS: Double-pigtail plastic stents; BFMS: Fully covered bi-flanged metal stents; MPS: Multiple plastic stents; MTGT: Multiple transluminal gateway technique; NR: Not reported.

Table 3 Details of hemorrhage associated with endoscopic ultrasound-guided drainage of pancreatic fluid collections

Study	Year	Country	Design	Center	Stent style	No.	PFC style	Location of drainage	Age	Male	Bleeding rate	Cause and treatment of bleeding	Diagnosis of bleeding
Lang <i>et al</i> ^[38]	2018	United States	RS	Single	LAMS	19	PPC/WON	TG	51.6	NR	19%	1 Splenic artery pseudoaneurysms-left gastric and splenic artery embolization after cyst-gastrostomy; 2 Collateral vessel bleed-conservative transfused blood;	NR
Siddiqui <i>et al</i> ^[6]	2017	United States	RS	Multi	LAMS	86	WON	TG/TD/ Multiport	51.5	89%	7%	3 Intracavitary variceal bleed-endoscopically using balloon tamponade Stent erosion into the gastric wall-EGD was performed, and a visible vessel was treated with cautery for durable hemostasis Related to stent erosion into a vessel as the WON cavity wall collapses or related to pseudoaneurysm in the cavity wall-significant hemorrhage treated by coil embolization by interventional radiology	NR
												Due to inadvertent puncture of an artery; another was not reported- Successfully treated with coil embolization by interventional radiology	NR
												None	None
Bapaye <i>et al</i> ^[13]	2017	India	RS	Single	BFMS	72	WON	Mostly TG	43.8	86%	3%	Because of erosion of the splenic artery by the indwelling inner end of the stent-treated by blood transfusion; splenic artery arteriography embolization	Angiograms
												Because of tract dilatation-Blood transfusion; endoscopic clip application; surgery	Angiograms
Bang <i>et al</i> ^[20]	2017	United States	RCT	Single	LAMS	12	WON	NR	NR	NR	25%	With the resultant friction against regional vasculature surrounding the necrotic cavity precipitating bleeding-Blood transfusions interventional radiology-guided coil embolization	EUS and CT angiograms
Lakhtakia <i>et al</i> ^[54]	2016	India	RS	Single	FCSEMS	205	WON	NR	34.8	88%	3%	None No details of the cause of bleeding-Minor bleeding was self-limiting; major bleeding treated by selective coil embolization; another major bleeding treated by surgery	None Abdominal angiography
												No details of the cause of bleeding-Self-limited bleeding	NR
Siddiqui <i>et al</i> ^[51] Ang <i>et al</i> ^[20]	2016	United States Singapore	RS	Multi	LAMS	82	PPC/WON	TG/TD	53.1	67%	7%	No details of the cause of bleeding-Treated by blood transfusion	NR
												Portal hypertension due to splenic vein thrombosis and antithrombotic drug therapy in the presence of stent-induced ulceration may have been responsible-Interventional radiology embolization; surgical intervention; repeated endoscopic treatments (sclerotherapy); percutaneous radiology-guided drainage	NR
												No details of the cause of bleeding-Embolization by interventional radiology	NR
Vazquez-Sequeiros <i>et al</i> ^[59]	2016	Spanish	RS	Multi	FCSEMS	211	PPC/WON	TG/TD	58.1	69%	7%	Significant bleeding due to inadvertent puncture of an artery-Successfully treated with coil embolization by interventional radiology	NR
Sharaiha <i>et al</i> ^[71]	2016	United States	RS	Multi	LAMS	124	WON	NR	54.2	61%	2%	All fluid is evacuated and the cavity lumen is collapsed, or due to a vessel injury from the inner stent end-Arteriography plus endoscopic treatment	Abdominal angiography
Sharaiha <i>et al</i> ^[47]	2015	United States	RS	Multi	ECSEMS	112	PPC	TG/TD	53.2	55%	4%	Massive bleeding related to the concomitant use of a NCDC-Surgery	NR
Gornalls <i>et al</i> ^[11]	2015	Spain	RS	Single	DDPS	118	PPC	TG/TD	52.2	69%	NR		NR
Rinninella <i>et al</i> ^[44]	2015	Italy	RS	Multi	FCSEMS	93	PFC	TG/TD	60	76%	1%		NR

ious

Mukai <i>et al</i> ^[14]	2015	Japan	RS	Single	FC BFMS	43	WON	NR	54.4	86%	5%	The specific reason for bleeding is unclear-No details regarding the treatment of the bleeding events	NR
Lee <i>et al</i> ^[55]	2014	South Korea	PS	Single	Plastic FCSEMS	27 25	WON PFC	NR TG/TD	55.9 53.7	78% 88%	0% 0%	None None	None None
Yamamoto <i>et al</i> ^[56]	2013	Japan	RS	Single	DDPS FCSEMS	25 9	PFC PPC/WON	TG/TD TG	51.6 45	76% 67%	4% 11%	NR The bleeding was caused by vessel damage because of inflammation-Trans-arterial embolization	NR Angiography
Bang <i>et al</i> ^[63]	2013	United States	RS	Multi	MTGT	53	WON	TG/TD	53	69%	2%	No details of the cause of bleeding-Coil embolization under radiological guidance	NR
Puri <i>et al</i> ^[48]	2012	India	PS	Single	DDPS	40	PPC	TG/TD	39	78%	3%	Pseudoaneurysm of the blood vessel in the cystic wall ruptured-Surgery	NR
Itoi <i>et al</i> ^[12]	2012	Japan	RS	Single	LAMS	15	PPC	TG/TD	55.4	80%	20%	No details of the cause of bleeding-Minor, self-limited	None
Varadarajulu <i>et al</i> ^[27]	2011	United States	RS	Single	DDPS	211	PPC/WON	TG/TD/ TE/TJ	52	61%	1%	No details of the cause of bleeding-Required embolization with interventional radiology; conservatively	NR
Varadarajulu <i>et al</i> ^[64]	2011	United States	PS	Single	DDPS	110	PPC/WON	TG/TD	NR	NR	1%	Coagulant disorder (with underlying acquired factor VIII inhibitors) -Conservative treatment	Arteriography
Talreja <i>et al</i> ^[53]	2008	United States	PS	Single	FCSEMS	18	PFC	TG/TD	51	67%	13%	Bleeding from the pseudocyst wall-Treated with balloon tamponade and arterial embolization	NR

PPC: Pancreatic pseudocyst; WON: Walled-off necrosis; PFC: Pancreatic fluid collections; PS: Prospective study; RS: Retrospective study; RCT: Randomized controlled trial; Single: Single-center; Multi: Multi-center; FCSEMS: Fully covered self-expandable metal stent; LAMS: Lumen-apposing metal stents; DDPS: Double-pigtail plastic stents; BFMS: Double-covered bi-flanged metal stents; MPS: Multiple plastic stents; MGT: Multiple transluminal gateway technique; TG: Trans-gastric route; TD: Trans-duodenal route; TE: Trans-esophageal route; TJ: Trans-jejunal route; CT: Computed tomography; EUS: Endoscopic ultrasound; NCDC: Nasocystic drainage catheter; NR: Not reported.

findings.

MULTIDISCIPLINARY HEMOSTATIC ALGORITHM FOR EUS-GUIDED DRAINAGE

In light of previous experiences with hemostasis in the literature, we propose a simple and meaningful hemostasis algorithm for the management of bleeding during EUS-guided drainage of PFCs. According to the above analysis, the main causes of hemorrhage in cases of EUS-guided drainage of PFC are intra-procedural bleeding and post-procedural bleeding. Intra-procedural bleeding events are usually caused by pseudoaneurysms^[48], intracavitary vessel bleeds^[38], inadvertent puncture of vessels^[46], and the bursting of collateral vessels^[38]. Post-procedural bleeding events are associated with stent erosion into vessels^[11,13] and coagulation disorders^[33]. In addition, the main methods for checking hemorrhage are EUS angiograms and CT angiograms^[13,50]. There are four major hemostatic approaches: conventional treatment^[20], endoscopic treatment^[39,51], interventional radiology-guided embolization^[50,56], and surgery^[48,54]. Among these treatment approaches, the endoscopic methods used to control bleeding during or after EUS drainage include medicine injection (dilute epinephrine, hemostatic powder), endoscopic clip application, electrocautery, balloon tamponade, and the placement of large-diameter FCSEMS^[57]. Most importantly, once conventional treatment, endoscopic intervention, and radiological embolization have failed, surgery is performed immediately. Details are shown below.

Regarding intra-procedural hemorrhage

Pseudoaneurysm events are a common general AE, and IR-guided embolization is the most commonly used method for controlling hemorrhage. If an intracavitary vessel or cystic wall bleed occurs, it can be treated endoscopically using a balloon or FCSEMS tamponade. If required, a balloon dilator or FCSEMS may provide more sustained tamponade. Hemorrhage can also be caused by the inadvertent puncture of vessels or the bursting of a collateral vessel. The main principles of treatment are as follows: (1) For mild bleeding, first observe for self-limited bleeding; if the hemoglobin levels continue to decrease, then conservative treatment such as intensive care unit admission and blood transfusion may be needed; and (2) For moderate to severe hemorrhage, endoscopic therapy such as clip application, cautery, and balloon tamponade should

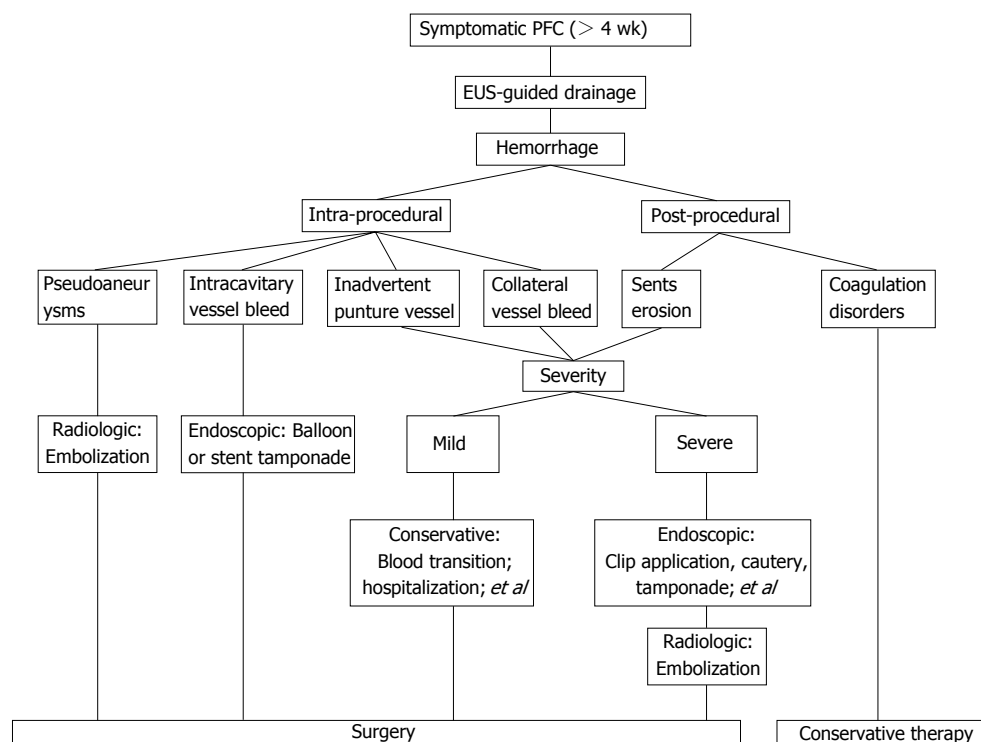


Figure 2 Imaging of a meaningful treatment algorithm. An integrated and multidisciplinary treatment algorithm is proposed for the endoscopic ultrasound (EUS)-guided drainage of pancreatic fluid collections. PFC: Pancreatic fluid collections.

be performed. Embolization by IR or surgery should be performed if endoscopic intervention fails.

Regarding post-procedural hemorrhage

Bleeding caused by stent erosion is common during post-procedural events, and the typical therapy is endoscopic or radiologic treatment. The principle of management is the same as in 1c above. Coagulation disorders are a dangerous cause of hemorrhage that requires conservative treatment.

As presented above, this study proposes an integrated, multidisciplinary and helpful hemostasis algorithm that offers appropriate therapeutic strategies to address the emergence of serious bleeding. The detailed multidisciplinary treatment algorithm is shown in Figure 2.

WHAT PROBLEMS SHOULD WE FOCUS ON?

There is evidence that EUS-guided drainage has become the first-line and most successful therapeutic method for PFC. However, there are still some problems to be addressed. Some common factors that may or may not affect the bleeding rate require further exploration. Herein, first of all, we present an in-depth exploration of whether the type of stent affects AEs (including hemorrhage), which remains controversial. Several studies have compared the use of plastic vs metal stents in the EUS-guided drainage of PFCs. A recent retrospective study of EUS-guided drainage for PFCs

using both LAMS and DPPS^[38] revealed hemorrhagic episodes in 21% of the LAMS group compared with 1% of the DPPS group, a difference that was statistically significant ($P = 0.0003$). Furthermore, the study confirmed that LAMS was associated with higher rates of AEs, specifically hemorrhage. A retrospective single-center analysis of EUS-guided drainage in 103 PFC patients reported by Bang *et al*^[50] demonstrated that there were significantly more bleeding episodes in the LAMS group (19%) than in the DPPS group (1%). Interestingly, both Lang *et al*^[38] and Bang *et al*^[50] speculated that perhaps the quick decompression of the cyst lumen by LAMS leads to irritating friction of the surrounding vessels and causes hemorrhage. In particular, Lang *et al*^[38] suggested that the wide cavity of the LAMS may allow more gastric acid to enter the cyst lumen, which leads to a low pH that may irritate the intracavitary vessels and stimulate hemorrhage. A multicenter retrospective study by Siddiqui *et al*^[46] compared the clinical outcomes and AEs of EUS-guided drainage in WON patients with three different stents: DPPS, FCSEMS and LAMS. There was no statistically significant difference in the technical success among the three groups (99.05% vs 100% vs 97.7% respectively, $P = 0.37$). At a 6-mo follow-up, the clinical success rates of WON drainage using FCSEMS and LAMS were higher than in drainage performed with DPPS. More importantly, the hemorrhage rates were 1%, 0% and 7% with DPPS, FCSEMS and LAMS, respectively. The hemorrhage rate associated with WON drainage in the LAMS group was lower than that in the DPPS group (1%

vs 7%).

A current systematic review and meta-analysis suggested that LAMS have superior efficacy and safety in the management of PFC^[40]. They may be preferred over plastic stents as they are associated with better clinical success and a lower AE rate. Similarly, a recent meta-analysis concluded that metal stents are superior to plastic stents for endoscopic drainage of PFCs because metal stents have a higher success rate and a lower AE rate^[58]. However, a Spanish multicenter study by Vazquez-Sequeiros^[39] and a Japanese retrospective trial by Yamamoto *et al.*^[56] suggest that the type of stent used may not influence whether bleeding occurs. A systematic review by Bang *et al.*^[59] did not find any difference in terms of AE rates and treatment success between metal and plastic stents for endoscopic drainage of PFCs. To date, whether the type of stent affects the hemorrhage rate is controversial. There is no consensus on these issues. We speculate that perhaps the different characteristics of different types of stents are related to the controversial problems. The use of metal stents for PFC has the advantages of quick and easy deployment, large calibers and a high technical success rate but also have the disadvantages of difficult extubation and possible gastrointestinal tract injury; in contrast, plastic stents have the advantages of low cost, easy extubation, small calibers and easy deployment but have the disadvantages of poor visibility under fluoroscopy and a long treatment period^[60].

Additionally, a large retrospective study by Sharaiha *et al.*^[47] included 230 patients with PPC treated with EUS-guided drainage. There was no statistically significant difference in the technical success rate between the DPPS and FCSEMS groups (92% vs 98%, $P = 0.06$), and the use of PS was associated with lower clinical success rates (89% vs 98%, $P = 0.01$) and higher rates of procedural AEs (31% vs 16%, $P = 0.006$). Multivariate analysis revealed that patients treated with PS were almost three times more likely to experience AE than those treated with FCSEMS. Regarding WON, there were no differences in the success and AE rates between the plastic and metal stent groups. Therefore, the study found that clinical outcomes are directly correlated with the type of fluid collections, and thus, accurate distinction is critical before any interventional therapy is conducted. Furthermore, in a study of 211 patients with symptomatic PFC, the treatment success rate for infective PPC was 93.5%, compared with only 63.2% for WON^[27]. The researchers concluded that clinical results are directly associated with the type of fluid collections, and thus, rigid classification is helpful for choosing the appropriate treatment for different types of collections. We presume that the type of pancreatic collections is related to the occurrence of bleeding, which is due to the different intracavitary components. Many randomized controlled trials are needed to prove this assumption.

In addition to the abovementioned factors that can impact the occurrence of hemorrhage, it is possible

that the size and location of PFC, the diameter and length of stents, the time and steps of the procedure, the timing of stent removal, the operator's experience, and the general health condition of the patient may be related to the hemorrhage rate. Moreover, it is necessary to explore whether cautery dilator affects the clinical outcomes and bleeding events or not. Further details are shown in Table 4. Despite recent advances in interventional endoscopy, many questions remain. Future prospective, randomized, comparative studies on the current topic are thus recommended.

HOW TO PREVENT THE OCCURRENCE OF HEMORRHAGE?

What can we draw from the literature to further improve the endoscopic management of patients with bleeding? There are five factors that are critical to improve the treatment of hemorrhage. First, the implementation of a standard procedure is the most significant step for safe and successful EUS-guided drainage of PFC. It is important to strictly comply with the indications and contraindications prior to the process. Before the initial EUS, patients must undergo cross-sectional imaging via CT or MRI^[10]. In addition, to prevent bleeding, coagulation function should be checked in high-risk patients or those with relevant history^[46]. Furthermore, correct categorization of the PFCs is a vital and appropriate step in therapeutic management. Patients with PPC can be drained by straightforward stent placement, while WON requires a multidisciplinary treatment approach^[19]. In addition, EUS, although a safe procedure in experienced hands, is not without AEs (including bleeding and perforation), and not all endoscopists have the technical expertise to perform such complicated procedures. In addition to the ability to master the EUS technique, mastery in the management of AE is compulsory^[61]. Thus, it is crucial for the operator to obtain sufficient knowledge of the techniques and potential risks and to undergo additional specific training before performing any procedure^[57]. Moreover, establishing a standard follow-up system management is essential; for example, what items need to be reviewed after an operation? When should the imaging check be reviewed? When should the stent be removed? A multi-center retrospective study by Vazquez-Sequeiros^[39] revealed a higher rate of delayed bleeding 3-6 mo after drainage, which suggests that it may be rational to remove the stent no later than 3 mo after placement. Therefore, follow-up CT and US are suggested for early assessment of the therapeutic effect to maximally reduce the risk of AE and improve the treatment effects, especially for patients with pseudoaneurysms^[62]. Finally, we recommend that EUS-guided procedures be performed under Doppler guidance, which can reduce the risk of hemorrhage and the incidence of stent erosion into vessels when the cavity wall collapses.

Table 4 Details of clinical outcomes between cautery and non-cautery dilator in endoscopic ultrasound-guided drainage for pancreatic fluid collections

Study	Year	Country	Design	Center	Stent style	No.	PFC style	Overall Technical success	Overall Clinical success	Overall bleeding rate	Dilated approaches (A. balloon/tapered dilator; B. needle knife; C. cystostome catheter; D. ERCP cannula)	Cautery dilator
Lang <i>et al</i> ^[38]	2018	United States	RS	Single	LAMS/DDPS	103	PPC/WON	99%	95%	5%	A + B	YES
Siddiqui <i>et al</i> ^[46]	2017	United States	RS	Multi	LAMS/DDPS/FCSEMS	313	WON	99%	90%	3%	A + B	YES
Bapaye <i>et al</i> ^[3]	2017	India	RS	Single	BFMS/MPS	133	WON	100%	82%	5%	A + C	YES
Lakhtakia <i>et al</i> ^[54]	2016	India	RS	Single	FCSEMS	205	WON	99%	75%	3%	A + C	YES
Siddiqui <i>et al</i> ^[51]	2016	United States	RS	Multi	LAMS	82	PPC/WON	86% PPC 100% WON	100% PPC 88% WON	7%	A + B	YES
Ang <i>et al</i> ^[20]	2016	Singapore	RS	Multi	FCSEMS/DDPS	49	PPC/WON	100%	96%	4%	A + B + C	YES
Vazquez-Sequeiros <i>et al</i> ^[39]	2016	Spanish	RS	Multi	FCSEMS	211	PPC/WON	97%	94%	7%	A + B + C	YES
Sharaiha <i>et al</i> ^[7]	2016	United States	RS	Multi	LAMS	124	WON	100%	86%	3%	A + B + C	YES
Sharaiha <i>et al</i> ^[47]	2015	United States	RS	Multi	ECSEMS/DDPS	230	PPC	96%	90%	4%	A + B	YES
Gornals <i>et al</i> ^[11]	2015	Spain	RS	Single	LAMS	12	WON	100%	100%	17%	A + C	YES
Mukai <i>et al</i> ^[44]	2015	Japan	RS	Single	FC BFMS/Plastic	70	WON	100%	96%	4%	A + C	YES
Lee <i>et al</i> ^[55]	2014	South Korea	PS	Single	FCSEMS/DDPS	50	PFC	100%	10%	2%	A + B	YES
Puri <i>et al</i> ^[48]	2012	India	PS	Single	DPS	40	PPC	100%	98%	3%	A + B	YES
Itoi <i>et al</i> ^[12]	2012	Japan	RS	Single	LAMS	15	PPC	100%	100%	20%	A + C	YES
Bang <i>et al</i> ^[50]	2017	United States	RCT	Single	LAMS/DDPS	21	WON	NR	NR	14%	A	NO
Rinninella <i>et al</i> ^[44]	2015	Italy	RS	Multi	FCSEMS	93	PFC	99%	93%	1%	A	NO
Yamamoto <i>et al</i> ^[56]	2013	Japan	RS	Single	FCSEMS	9	PPC/WON	100%	78%	11%	A	NO
Bang <i>et al</i> ^[61]	2013	United States	RS	Multi	MTGT	53	WON	NR	70%	1%	A	NO
Varadarajulu <i>et al</i> ^[27]	2011	United States	RS	Single	DDPS	211	PPC/WON	NR	85%	1%	A + D	NO
Varadarajulu <i>et al</i> ^[65]	2011	United States	PS	Single	DDPS	110	PPC/WON	100%	92%	1%	A + D	NO
Talreja <i>et al</i> ^[53]	2008	United States	PS	Single	FCSEMS	18	PFC	95%	78%	13%	A	NO

PPC: Pancreatic pseudocyst; WON: Walled-off necrosis; PFC: Pancreatic fluid collections; PS: Prospective study; RS: Retrospective study; RCT: Randomized controlled trial; Single: Single-center; Multi: Multi-center; FCSEMS: Fully covered self-expandable metal stent; LAMS: Lumen-apposing metal stent; DDPS: Double-pigtail plastic stents; BFMS: Fully covered bi-flanged metal stents; MPS: Multiple plastic stents; MTGT: Multiple transluminal gateway technique; ERCP: Endoscopic retrograde cholangiopancreatography; NR: Not reported.

In conclusion, this study demonstrates for the first time that an integrated and novel algorithm can be used to manage hemorrhage in EUS-guided drainage of PFCs and provides an initial and clinically meaningful treatment strategy for AEs. Hemorrhage presents a challenging problem for physicians, and the multidisciplinary and multicenter collaboration of accomplished endoscopists, radiologists, biomedical engineers, and surgeons is necessary to manage cases of severe bleeding and other AEs. Prospective, multi-center studies with larger sample sizes may be necessary to overcome the limitations and generalize these results. In the future, we aim to formulate optimal treatments and present additional works on bleeding treatment. The development of new implementation and equipment is the basis for alternative minimally invasive approaches to various pancreatic diseases and will make this technique safer and more effective.

REFERENCES

- Dhaka N**, Samanta J, Kochhar S, Kalra N, Appasani S, Manrai M, Kochhar R. Pancreatic fluid collections: What is the ideal imaging technique? *World J Gastroenterol* 2015; **21**: 13403-13410 [PMID: 26730150 DOI: 10.3748/wjg.v21.i48.13403]
- Banks PA**, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013; **62**: 102-111 [PMID: 23100216 DOI: 10.1136/gutjnl-2012-302779]
- Varadarajulu S**, Bang JY, Sutton BS, Trevino JM, Christein JD, Wilcox CM. Equal efficacy of endoscopic and surgical cystogastrostomy for pancreatic pseudocyst drainage in a randomized trial. *Gastroenterology* 2013; **145**: 583-90.e1 [PMID: 23732774 DOI: 10.1053/j.gastro.2013.05.046]
- Holt BA**, Varadarajulu S. The endoscopic management of pancreatic pseudocysts (with videos). *Gastrointest Endosc* 2015; **81**: 804-812 [PMID: 25805460 DOI: 10.1016/j.gie.2014.12.026]
- Venkatachalapathy SV**, Bekkali N, Pereira S, Johnson G, Oppong K, Nayar M, Leeds J, Paranandi B, Penman I, Carroll N, Godfrey E, James M, Aithal G, McKay C, Devlin J, Wong T, Makin A, Ryan B, Huggett M. Multicenter experience from the UK and Ireland of use of lumen-apposing metal stent for transluminal drainage of pancreatic fluid collections. *Endosc Int Open* 2018; **6**: E259-E265 [PMID: 29497684 DOI: 10.1055/s-0043-125362]
- Bang JY**, Hasan MK, Navaneethan U, Sutton B, Frandah W, Siddique S, Hawes RH, Varadarajulu S. Lumen-apposing metal stents for drainage of pancreatic fluid collections: When and for whom? *Dig Endosc* 2017; **29**: 83-90 [PMID: 27199157 DOI: 10.1111/den.12681]
- Sharaiha RZ**, Tyberg A, Khashab MA, Kumta NA, Karia K, Nieto J, Siddiqui UD, Waxman I, Joshi V, Benias PC, Darwin P, DiMaio CJ, Mulder CJ, Friedland S, Forcione DG, Sejpal DV, Gonda TA, Gress FG, Gaidhane M, Koons A, DeFilippis EM, Salgado S, Weaver KR, Poneros JM, Sethi A, Ho S, Kumbhari V, Singh VK, Tieu AH, Parra V, Likhitsup A, Womeldorph C, Casey B, Jonnalagadda SS, Desai AP, Carr-Locke DL, Kahaleh M, Siddiqui AA. Endoscopic Therapy With Lumen-apposing Metal Stents Is Safe and Effective for Patients With Pancreatic Walled-off Necrosis. *Clin Gastroenterol Hepatol* 2016; **14**: 1797-1803 [PMID: 27189914 DOI: 10.1016/j.cgh.2016.05.011]
- Romagnuolo J**, Cotton PB, Eisen G, Vargo J, Petersen BT. Identifying and reporting risk factors for adverse events in endoscopy. Part I: cardiopulmonary events. *Gastrointest Endosc* 2011; **73**: 579-585 [PMID: 21353857 DOI: 10.1016/j.gie.2010.11.022]
- Romagnuolo J**, Cotton PB, Eisen G, Vargo J, Petersen BT. Identifying and reporting risk factors for adverse events in endoscopy. Part II: noncardiopulmonary events. *Gastrointest Endosc* 2011; **73**: 586-597 [PMID: 21353858 DOI: 10.1016/j.gie.2010.11.023]
- Aburajab M**, Smith Z, Khan A, Dua K. Safety and efficacy of lumen-apposing metal stents with and without simultaneous double-pigtail plastic stents for draining pancreatic pseudocyst. *Gastrointest Endosc* 2018; **87**: 1248-1255 [PMID: 29233670 DOI: 10.1016/j.gie.2017.11.033]
- Gornals JB**, Consiglieri CF, Busquets J, Salord S, de-la-Hera M, Secanella L, Redondo S, Pelaez N, Fabregat J. Endoscopic necrosectomy of walled-off pancreatic necrosis using a lumen-apposing metal stent and irrigation technique. *Surg Endosc* 2016; **30**: 2592-2602 [PMID: 26335077 DOI: 10.1007/s00464-015-4505-2]
- Itoi T**, Binmoeller KF, Shah J, Sofuni A, Itokawa F, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Clinical evaluation of a novel lumen-apposing metal stent for endosonography-guided pancreatic pseudocyst and gallbladder drainage (with videos). *Gastrointest Endosc* 2012; **75**: 870-876 [PMID: 22301347 DOI: 10.1016/j.gie.2011.10.020]
- Bapaye A**, Dubale NA, Sheth KA, Bapaye J, Ramesh J, Gadhihar H, Mahajani S, Date S, Pujari R, Gaadhe R. Endoscopic ultrasonography-guided transmural drainage of walled-off pancreatic necrosis: Comparison between a specially designed fully covered bi-flanged metal stent and multiple plastic stents. *Dig Endosc* 2017; **29**: 104-110 [PMID: 27463528 DOI: 10.1111/den.12704]
- Mukai S**, Itoi T, Baron TH, Sofuni A, Itokawa F, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Tanaka R, Umeda J, Tonoizuka R, Honjo M, Gotoda T, Moriyasu F, Yasuda I. Endoscopic ultrasound-guided placement of plastic vs. biflanged metal stents for therapy of walled-off necrosis: a retrospective single-center series. *Endoscopy* 2015; **47**: 47-55 [PMID: 25264765 DOI: 10.1055/s-0034-1377966]
- Cavallini A**, Butturini G, Malleo G, Bertuzzo F, Angelini G, Abu Hilal M, Pederzoli P, Bassi C. Endoscopic transmural drainage of pseudocysts associated with pancreatic resections or pancreatitis: a comparative study. *Surg Endosc* 2011; **25**: 1518-1525 [PMID: 20976483 DOI: 10.1007/s00464-010-1428-9]
- Akshintala VS**, Saxena P, Zaheer A, Rana U, Hutfless SM, Lennon AM, Canto MI, Kallou AN, Khashab MA, Singh VK. A comparative evaluation of outcomes of endoscopic versus percutaneous drainage for symptomatic pancreatic pseudocysts. *Gastrointest Endosc* 2014; **79**: 921-928; quiz 983.e2, 983.e5 [PMID: 24315454 DOI: 10.1016/j.gie.2013.10.032]
- Singhal S**, Rotman SR, Gaidhane M, Kahaleh M. Pancreatic fluid collection drainage by endoscopic ultrasound: an update. *Clin Endosc* 2013; **46**: 506-514 [PMID: 24143313 DOI: 10.5946/ce.2013.46.5.506]
- ASGE Standards of Practice Committee**, Muthusamy VR, Chandrasekhara V, Acosta RD, Bruining DH, Chathadi KV, Eloubeidi MA, Faulx AL, Fonkalsrud L, Gurudu SR, Khashab MA, Kothari S, Lightdale JR, Pasha SF, Saltzman JR, Shaikat A, Wang A, Yang J, Cash BD, DeWitt JM. The role of endoscopy in the diagnosis and treatment of inflammatory pancreatic fluid collections. *Gastrointest Endosc* 2016; **83**: 481-488 [PMID: 26796695 DOI: 10.1016/j.gie.2015.11.027]
- Bang JY**, Varadarajulu S. Endoscopic ultrasound-guided management of pancreatic pseudocysts and walled-off necrosis. *Clin Endosc* 2014; **47**: 429-431 [PMID: 25325003 DOI: 10.5946/ce.2014.47.5.429]
- Ang TL**, Kongkam P, Kwek AB, Orkoonsawat P, Rerknimitr R, Fock KM. A two-center comparative study of plastic and lumen-apposing large diameter self-expandable metallic stents in endoscopic ultrasound-guided drainage of pancreatic fluid collections. *Endosc Ultrasound* 2016; **5**: 320-327 [PMID: 27803905 DOI: 10.4103/2303-9027.191659]
- Tenner S**, Baillie J, DeWitt J, Vege SS; American College of Gastroenterology. American College of Gastroenterology guideline: management of acute pancreatitis. *Am J Gastroenterol* 2013; **108**: 1400-1415; 1416 [PMID: 23896955 DOI: 10.1038/ajg.2013.218]
- Zerem E**, Hauser G, Loga-Zec S, Kunosić S, Jovanović P, Crnkic D. Minimally invasive treatment of pancreatic pseudocysts. *World J Gastroenterol* 2015; **21**: 6850-6860 [PMID: 26078561 DOI: 10.3748/wjg.v21.i22.6850]
- Aghdassi A**, Mayerle J, Kraft M, Sielenkämper AW, Heidecke CD, Lerch MM. Diagnosis and treatment of pancreatic pseudocysts in chronic pancreatitis. *Pancreas* 2008; **36**: 105-112 [PMID: 18376299 DOI: 10.1097/MPA.0b013e31815a8887]
- Lerch MM**, Stier A, Wahnschaffe U, Mayerle J. Pancreatic pseudocysts: observation, endoscopic drainage, or resection? *Dtsch Arztebl Int* 2009; **106**: 614-621 [PMID: 19890418 DOI: 10.3238/arztebl.2009.0614]
- Rana SS**, Bhasin DK, Reddy YR, Sharma V, Rao C, Sharma RK, Gupta R. Morphological features of fluid collections on endoscopic ultrasound in acute necrotizing pancreatitis: do they change over time? *Ann Gastroenterol* 2014; **27**: 258-261 [PMID: 24975052]
- Elmunzer BJ**. Endoscopic drainage of pancreatic fluid collections. *Clin Gastroenterol Hepatol* 2018; [Epub ahead of print] [PMID: 29601903 DOI: 10.1016/j.cgh.2018.03.021]
- Varadarajulu S**, Bang JY, Phadnis MA, Christein JD, Wilcox CM.

- Endoscopic transmural drainage of peripancreatic fluid collections: outcomes and predictors of treatment success in 211 consecutive patients. *J Gastrointest Surg* 2011; **15**: 2080-2088 [PMID: 21786063 DOI: 10.1007/s11605-011-1621-8]
- 28 **Bakker OJ**, van Santvoort HC, van Brunschot S, Geskus RB, Besselink MG, Bollen TL, van Eijck CH, Fockens P, Hazebroek EJ, Nijmeijer RM, Poley JW, van Ramshorst B, Vleggaar FP, Boermeester MA, Gooszen HG, Weusten BL, Timmer R; Dutch Pancreatitis Study Group. Endoscopic transgastric vs surgical necrosectomy for infected necrotizing pancreatitis: a randomized trial. *JAMA* 2012; **307**: 1053-1061 [PMID: 22416101 DOI: 10.1001/jama.2012.276]
 - 29 **Saul A**, Ramirez Luna MA, Chan C, Uscanga L, Valdovinos Andraca F, Hernandez Calleros J, Elizondo J, Tellez Avila F. EUS-guided drainage of pancreatic pseudocysts offers similar success and complications compared to surgical treatment but with a lower cost. *Surg Endosc* 2016; **30**: 1459-1465 [PMID: 26139498 DOI: 10.1007/s00464-015-4351-2]
 - 30 **Khan MA**, Hammad T, Khan Z, Lee W, Gaidhane M, Tyberg A, Kahaleh M. Endoscopic versus percutaneous management for symptomatic pancreatic fluid collections: a systematic review and meta-analysis. *Endosc Int Open* 2018; **6**: E474-E483 [PMID: 29607399 DOI: 10.1055/s-0044-102299]
 - 31 **Siddiqui AA**, Adler DG, Nieto J, Shah JN, Binmoeller KF, Kane S, Yan L, Laique SN, Kowalski T, Loren DE, Taylor LJ, Munigala S, Bhat YM. EUS-guided drainage of peripancreatic fluid collections and necrosis by using a novel lumen-apposing stent: a large retrospective, multicenter U.S. experience (with videos). *Gastrointest Endosc* 2016; **83**: 699-707 [PMID: 26515956 DOI: 10.1016/j.gie.2015.10.020]
 - 32 **Keane MG**, Sze SF, Cieplik N, Murray S, Johnson GJ, Webster GJ, Thorburn D, Pereira SP. Endoscopic versus percutaneous drainage of symptomatic pancreatic fluid collections: a 14-year experience from a tertiary hepatobiliary centre. *Surg Endosc* 2016; **30**: 3730-3740 [PMID: 26675934 DOI: 10.1007/s00464-015-4668-x]
 - 33 **Varadarajulu S**, Christein JD, Wilcox CM. Frequency of complications during EUS-guided drainage of pancreatic fluid collections in 148 consecutive patients. *J Gastroenterol Hepatol* 2011; **26**: 1504-1508 [PMID: 21575060 DOI: 10.1111/j.1440-1746.2011.06771.x]
 - 34 **Seewald S**, Ang TL, Richter H, Teng KY, Zhong Y, Groth S, Omar S, Soehendra N. Long-term results after endoscopic drainage and necrosectomy of symptomatic pancreatic fluid collections. *Dig Endosc* 2012; **24**: 36-41 [PMID: 22211410 DOI: 10.1111/j.1443-1661.2011.01162.x]
 - 35 **Park DH**, Lee SS, Moon SH, Choi SY, Jung SW, Seo DW, Lee SK, Kim MH. Endoscopic ultrasound-guided versus conventional transmural drainage for pancreatic pseudocysts: a prospective randomized trial. *Endoscopy* 2009; **41**: 842-848 [PMID: 19798610 DOI: 10.1055/s-0029-1215133]
 - 36 **Ikeuchi N**, Itoi T, Sofuni A, Itokawa F, Tsuchiya T, Kurihara T, Ishii K, Tsuji S, Tanaka R, Umeda J. Su1577 Endoscopic Ultrasound-Guided Pancreatic Pseudocyst With Metal Stent Appears to Be Effective and Safe Procedure. *Gastrointest Endosc* 2013; **77**: AB373-AB374 [DOI: 10.1016/j.gie.2013.03.1230]
 - 37 **van Brunschot S**, van Grinsven J, van Santvoort HC, Bakker OJ, Besselink MG, Boermeester MA, Bollen TL, Bosscha K, Bouwense SA, Bruno MJ, Cappendijk VC, Consten EC, Dejong CH, van Eijck CH, Erkelens WG, van Goor H, van Grevenstein WMU, Haveman JW, Hofker SH, Jansen JM, Laméris JS, van Lienden KP, Meijssen MA, Mulder CJ, Nieuwenhuijs VB, Poley JW, Quispel R, de Ridder RJ, Römkens TE, Scheepers JJ, Schepers NJ, Schwartz MP, Seerden T, Spanier BW, Straathof JWA, Strijker M, Timmer R, Venneman NG, Vleggaar FP, Voermans RP, Witteman BJ, Gooszen HG, Dijkgraaf MG, Fockens P; Dutch Pancreatitis Study Group. Endoscopic or surgical step-up approach for infected necrotizing pancreatitis: a multicentre randomised trial. *Lancet* 2018; **391**: 51-58 [PMID: 29108721 DOI: 10.1016/S0140-6736(17)32404-2]
 - 38 **Lang GD**, Fritz C, Bhat T, Das KK, Murad FM, Early DS, Edmundowicz SA, Kushnir VM, Mullady DK. EUS-guided drainage of peripancreatic fluid collections with lumen-apposing metal stents and plastic double-pigtail stents: comparison of efficacy and adverse event rates. *Gastrointest Endosc* 2018; **87**: 150-157 [PMID: 28713067 DOI: 10.1016/j.gie.2017.06.029]
 - 39 **Vazquez-Sequeiros E**, Baron TH, Pérez-Miranda M, Sánchez-Yagüe A, Gornals J, Gonzalez-Huix F, de la Serna C, Gonzalez Martin JA, Gimeno-Garcia AZ, Marra-Lopez C, Castellot A, Alberca F, Fernandez-Urrien I, Aparicio JR, Legaz ML, Sendino O, Loras C, Subtil JC, Nerin J, Perez-Carreras M, Diaz-Tasende J, Perez G, Repiso A, Vilella A, Dolz C, Alvarez A, Rodriguez S, Esteban JM, Juzgado D, Albillos A; Spanish Group for FCSEMS in Pancreas Collections. Evaluation of the short- and long-term effectiveness and safety of fully covered self-expandable metal stents for drainage of pancreatic fluid collections: results of a Spanish nationwide registry. *Gastrointest Endosc* 2016; **84**: 450-457.e2 [PMID: 26970012 DOI: 10.1016/j.gie.2016.02.044]
 - 40 **Hammad T**, Khan MA, Alastal Y, Lee W, Nawras A, Ismail MK, Kahaleh M. Efficacy and Safety of Lumen-Apposing Metal Stents in Management of Pancreatic Fluid Collections: Are They Better Than Plastic Stents? A Systematic Review and Meta-Analysis. *Dig Dis Sci* 2018; **63**: 289-301 [PMID: 29282638 DOI: 10.1007/s10620-017-4851-0]
 - 41 **Shah RJ**, Shah JN, Waxman I, Kowalski TE, Sanchez-Yague A, Nieto J, Brauer BC, Gaidhane M, Kahaleh M. Safety and efficacy of endoscopic ultrasound-guided drainage of pancreatic fluid collections with lumen-apposing covered self-expanding metal stents. *Clin Gastroenterol Hepatol* 2015; **13**: 747-752 [PMID: 25290534 DOI: 10.1016/j.cgh.2014.09.047]
 - 42 **Fabbri C**, Luigiano C, Cennamo V, Polifemo AM, Barresi L, Jovine E, Traina M, D'Imperio N, Tarantino I. Endoscopic ultrasound-guided transmural drainage of infected pancreatic fluid collections with placement of covered self-expanding metal stents: a case series. *Endoscopy* 2012; **44**: 429-433 [PMID: 22382852 DOI: 10.1055/s-0031-1291624]
 - 43 **Penn DE**, Draganov PV, Wagh MS, Forsmark CE, Gupte AR, Chauhan SS. Prospective evaluation of the use of fully covered self-expanding metal stents for EUS-guided transmural drainage of pancreatic pseudocysts. *Gastrointest Endosc* 2012; **76**: 679-684 [PMID: 22732874 DOI: 10.1016/j.gie.2012.04.457]
 - 44 **Rinninella E**, Kunda R, Dollhopf M, Sanchez-Yague A, Will U, Tarantino I, Gornals Soler J, Ullrich S, Meining A, Esteban JM, Enz T, Vanbiervliet G, Vleggaar F, Attili F, Larghi A. EUS-guided drainage of pancreatic fluid collections using a novel lumen-apposing metal stent on an electrocautery-enhanced delivery system: a large retrospective study (with video). *Gastrointest Endosc* 2015; **82**: 1039-1046 [PMID: 26014960 DOI: 10.1016/j.gie.2015.04.006]
 - 45 **Walter D**, Will U, Sanchez-Yague A, Brenke D, Hampe J, Wollny H, López-Jamar JM, Jechart G, Vilmann P, Gornals JB, Ullrich S, Fährndrich M, de Tejada AH, Junquera F, Gonzalez-Huix F, Siersema PD, Vleggaar FP. A novel lumen-apposing metal stent for endoscopic ultrasound-guided drainage of pancreatic fluid collections: a prospective cohort study. *Endoscopy* 2015; **47**: 63-67 [PMID: 25268308 DOI: 10.1055/s-0034-1378113]
 - 46 **Siddiqui AA**, Kowalski TE, Loren DE, Khalid A, Soomro A, Mazhar SM, Isby L, Kahaleh M, Karia K, Yoo J, Ofosu A, Ng B, Sharaiha RZ. Fully covered self-expanding metal stents versus lumen-apposing fully covered self-expanding metal stent versus plastic stents for endoscopic drainage of pancreatic walled-off necrosis: clinical outcomes and success. *Gastrointest Endosc* 2017; **85**: 758-765 [PMID: 27566053 DOI: 10.1016/j.gie.2016.08.014]
 - 47 **Sharaiha RZ**, DeFilippis EM, Kedia P, Gaidhane M, Boumitri C, Lim HW, Han E, Singh H, Ghumman SS, Kowalski T, Loren D, Kahaleh M, Siddiqui A. Metal versus plastic for pancreatic pseudocyst drainage: clinical outcomes and success. *Gastrointest Endosc* 2015; **82**: 822-827 [PMID: 25936453 DOI: 10.1016/j.gie.2015.02.035]
 - 48 **Puri R**, Mishra SR, Thandassery RB, Sud R, Eloubeidi MA. Outcome and complications of endoscopic ultrasound guided

- pancreatic pseudocyst drainage using combined endoprosthesis and naso-cystic drain. *J Gastroenterol Hepatol* 2012; **27**: 722-727 [PMID: 22313377 DOI: 10.1111/j.1440-1746.2012.07089.x]
- 49 **Siddiqui AA**, Dewitt JM, Strongin A, Singh H, Jordan S, Loren DE, Kowalski T, Eloubeidi MA. Outcomes of EUS-guided drainage of debris-containing pancreatic pseudocysts by using combined endoprosthesis and a nasocystic drain. *Gastrointest Endosc* 2013; **78**: 589-595 [PMID: 23660566 DOI: 10.1016/j.gie.2013.03.1337]
 - 50 **Bang JY**, Hasan M, Navaneethan U, Hawes R, Varadarajulu S. Lumen-apposing metal stents (LAMS) for pancreatic fluid collection (PFC) drainage: may not be business as usual. *Gut* 2017; **66**: 2054-2056 [PMID: 27582509 DOI: 10.1136/gutjnl-2016-312812]
 - 51 **Cohen M**, Kedia P, Sharaiha R, Kahaleh M. Tamponade of a bleeding pseudocyst with a fully covered metal stent. *Gastrointest Endosc* 2015; **81**: 229-230 [PMID: 24890424 DOI: 10.1016/j.gie.2014.04.019]
 - 52 **Akbar A**, Reddy DN, Baron TH. Placement of fully covered self-expandable metal stents to control entry-related bleeding during transmural drainage of pancreatic fluid collections (with video). *Gastrointest Endosc* 2012; **76**: 1060-1063 [PMID: 23078930 DOI: 10.1016/j.gie.2012.07.014]
 - 53 **Talreja JP**, Shami VM, Ku J, Morris TD, Ellen K, Kahaleh M. Transenteric drainage of pancreatic-fluid collections with fully covered self-expanding metallic stents (with video). *Gastrointest Endosc* 2008; **68**: 1199-1203 [PMID: 19028232 DOI: 10.1016/j.gie.2008.06.015]
 - 54 **Lakhtakia S**, Basha J, Talukdar R, Gupta R, Nabi Z, Ramchandani M, Kumar BVN, Pal P, Kalpala R, Reddy PM, Pradeep R, Singh JR, Rao GV, Reddy DN. Endoscopic "step-up approach" using a dedicated biflanged metal stent reduces the need for direct necrosectomy in walled-off necrosis (with videos). *Gastrointest Endosc* 2017; **85**: 1243-1252 [PMID: 27845053 DOI: 10.1016/j.gie.2016.10.037]
 - 55 **Lee BU**, Song TJ, Lee SS, Park DH, Seo DW, Lee SK, Kim MH. Newly designed, fully covered metal stents for endoscopic ultrasound (EUS)-guided transmural drainage of peripancreatic fluid collections: a prospective randomized study. *Endoscopy* 2014; **46**: 1078-1084 [PMID: 25412095 DOI: 10.1055/s-0034-1390871]
 - 56 **Yamamoto N**, Isayama H, Kawakami H, Sasahira N, Hamada T, Ito Y, Takahara N, Uchino R, Miyabayashi K, Mizuno S, Kogure H, Sasaki T, Nakai Y, Kuwatani M, Hirano K, Tada M, Koike K. Preliminary report on a new, fully covered, metal stent designed for the treatment of pancreatic fluid collections. *Gastrointest Endosc* 2013; **77**: 809-814 [PMID: 23453183 DOI: 10.1016/j.gie.2013.01.009]
 - 57 **Lakhtakia S**. Complications of diagnostic and therapeutic Endoscopic Ultrasound. *Best Pract Res Clin Gastroenterol* 2016; **30**: 807-823 [PMID: 27931638 DOI: 10.1016/j.bpg.2016.10.008]
 - 58 **Yoon SB**, Lee IS, Choi MG. Metal versus plastic stents for drainage of pancreatic fluid collection: A meta-analysis. *United European Gastroenterol J* 2018; **6**: 729-738 [PMID: 30083335 DOI: 10.1177/2050640618761702]
 - 59 **Bang JY**, Hawes R, Bartolucci A, Varadarajulu S. Efficacy of metal and plastic stents for transmural drainage of pancreatic fluid collections: a systematic review. *Dig Endosc* 2015; **27**: 486-498 [PMID: 25515976 DOI: 10.1111/den.12418]
 - 60 **Kawakami H**, Itoi T, Sakamoto N. Endoscopic ultrasound-guided transluminal drainage for peripancreatic fluid collections: where are we now? *Gut Liver* 2014; **8**: 341-355 [PMID: 25071899 DOI: 10.5009/gnl.2014.8.4.341]
 - 61 **Fabbri C**, Luigiano C, Maimone A, Polifemo AM, Tarantino I, Cennamo V. Endoscopic ultrasound-guided drainage of pancreatic fluid collections. *World J Gastrointest Endosc* 2012; **4**: 479-488 [PMID: 23189219 DOI: 10.4253/wjge.v4.i11.479]
 - 62 **Chen M**, Zhu H, Jin Z, Li Z, Du Y. Safety of lumen-apposing metal stents for pancreatic fluid drainage: waiting for a clear answer. *Gastrointest Endosc* 2018; **87**: 319-320 [PMID: 29241858 DOI: 10.1016/j.gie.2017.08.030]
 - 63 **Bang JY**, Wilcox CM, Trevino J, Ramesh J, Peter S, Hasan M, Hawes RH, Varadarajulu S. Factors impacting treatment outcomes in the endoscopic management of walled-off pancreatic necrosis. *J Gastroenterol Hepatol* 2013; **28**: 1725-1732 [PMID: 23829423 DOI: 10.1111/jgh.12328]
 - 64 **Varadarajulu S**, Phadnis MA, Christein JD, Wilcox CM. Multiple transluminal gateway technique for EUS-guided drainage of symptomatic walled-off pancreatic necrosis. *Gastrointest Endosc* 2011; **74**: 74-80 [PMID: 21612778 DOI: 10.1016/j.gie.2011.03.1122]
 - 65 **Varadarajulu S**, Phadnis MA, Christein JD, Wilcox CM. Multiple transluminal gateway technique for EUS-guided drainage of symptomatic walled-off pancreatic necrosis. *Gastrointestinal endoscopy* 2011; **74**(1): 74-80 [PMID: 21612778 DOI: 10.1016/j.gie.2011.03.1122]

P- Reviewer: Friedel D, Kitamura K, Velayos B **S- Editor:** Ma YJ
L- Editor: Filipodia **E- Editor:** Wu YXJ



Mystery behind labial and oral melanotic macules: Clinical, dermoscopic and pathological aspects of Laugier-Hunziker syndrome

Ning Duan, Yang-Heng Zhang, Wen-Mei Wang, Xiang Wang

Ning Duan, Wen-Mei Wang, Xiang Wang, Department of Oral Medicine, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing 210008, Jiangsu Province, China

Yang-Heng Zhang, Department of Periodontology, Nanjing Stomatological Hospital, Medical School of Nanjing University, Nanjing 210008, Jiangsu Province, China

ORCID number: Ning Duan (0000-0002-4776-0029); Yang-Heng Zhang (0000-0002-2151-1737); Wen-Mei Wang (0000-0003-4098-6495); Xiang Wang (0000-0002-2135-8576).

Author contributions: Duan N, Zhang YH and Wang WM contributed to this paper equally, and should be considered as co-first author; all the authors read and approved the final version of the manuscript before submission.

Supported by The National Natural Scientific Foundation of China, No. 81570978; the Nonprofit Industry Research Specific Fund of National Health and Family Planning Commission of China, No. 201502018; the Key Project of Science and Technology Department of Jiangsu Province, No. BL2014018; and the Project of Invigorating Health Care through Science, Technology and Education: the Project of Jiangsu Provincial Medical Youth Talent, No. QNRC2016118.

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

Open-Access: This article is an open-access article, which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Xiang Wang, MD, PhD, Associate Specialist, Department of Oral Medicine, Nanjing Stomatological

Hospital, Medical School of Nanjing University, 30 Zhongyang Road, Nanjing 210008, Jiangsu Province, China. yuwx999@sina.com
Telephone: +86-25-83620220
Fax: +86-25-83620202

Received: April 21, 2018
Peer-review started: April 21, 2018
First decision: June 15, 2018
Revised: June 28, 2018
Accepted: July 23, 2018
Article in press: July 24, 2018
Published online: September 26, 2018

Abstract

Labial and oral melanotic macules are commonly encountered in a broad range of conditions ranging from physiologic pigmentation to a sign of an underlying life-threatening disease. Although Laugier-Hunziker syndrome (LHS) shares some features of labial and oral pigmentation with a variety of conditions, it is a benign and acquired condition, frequently associated with longitudinal melanonychia. Herein, the demographic, clinical, dermoscopic, and pathological aspects of LHS were reviewed comprehensively. The important differential diagnoses of mucocutaneous and nail pigmentation are provided. An accurate diagnosis is crucial to design a reasonable medical strategy, including management options, malignant transformation surveillance, and psychological support. It is important that clinicians conduct long-term follow-up and surveillance due to the potential risks of malignant transformation and local severe complications in some conditions.

Key words: Laugier-Hunziker syndrome; Dermoscopy; Pathology; Pigmentation; Differential diagnosis; Peutz-Jeghers syndrome; Neurofibromatosis type 1; Carney complex; Melanonychia

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Although Laugier-Hunziker syndrome (LHS) is an uncommon disorder, labial or oral pigmentation is often encountered daily and clinically. By conducting a thorough review of the topic, the aims of the paper are to present the clinical, dermoscopic, and pathological features of LHS concisely and clearly. More to the point, the outlined typical features of various conditions associated with labial, oral, and nail pigmentation are conducive to facilitate differential diagnosis, promote early recognition of underlying diseases, and prevent unnecessary testing.

Duan N, Zhang YH, Wang WM, Wang X. Mystery behind labial and oral melanotic macules: Clinical, dermoscopic and pathological aspects of Laugier-Hunziker syndrome. *World J Clin Cases* 2018; 6(10): 322-334 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/322.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.322>

INTRODUCTION

In 1970, Laugier and Hunziker reported five cases of unusual acquired macular hyperpigmentation on the lips and oral mucosa, and two of these patients displayed longitudinal pigmented streaks on the nails^[1]. Based on the names of the original reporters, Laugier-Hunziker syndrome (LHS) was used to represent the clinical entity subsequently. Since the original description, approximately 200 cases of LHS have been reported in the literature.

To date, the exact etiology of LHS remains unknown. Regarding the syndrome, evidence of underlying disorders or systemic abnormalities and increased malignancy risk has not been established. LHS occurs predominantly among middle-aged adults and affects women more frequently^[2-6]. Clinically, the mucocutaneous pigmentary lesions in LHS characteristically present with lenticular melanotic macules on labial, oral, and acral areas. Longitudinal melanonychia is identified in approximately half of LHS patients. Dermoscopic findings of LHS mainly include regular reticular, granular, linear, curvilinear, arc streaks, fish scale-like, parallel furrow, or parallel ridge patterns in mucocutaneous pigmentary lesions^[7-10]. Pathologically, melanin accumulates in the basal layer of the epithelium or epidermis and the number of melanophages in the lamina propria or dermis increases. However, the number of melanocytes is unaffected^[4,11-13].

Labial and oral pigmentation is encountered in a broad range of conditions. Although the pigmentary lesions might appear trivial and insignificant, proper identification of characteristic pigmentation and subsequent establishment of differential diagnoses can promote essential examination and evaluation,

facilitate an early diagnosis of potential underlying diseases, guide a reasonable medical strategy, prevent unnecessary testing, and reduce mortality due to malignancies and severe complications.

DEMOGRAPHIC ASPECT

An extensive search of published case reports and review literatures on LHS since 1970 was performed based on the key words "Laugier-Hunziker syndrome," "Laugier-Hunziker-Baran syndrome," "essential lenticular melanotic pigmentation," "idiopathic lenticular mucocutaneous pigmentation," "Laugier and Hunziker pigmentation," "Laugier's disease," or "longitudinal melanonychia." Most of the studies that were assessed and studied were in English. A certain number of LHS cases from Europe have been reported in early articles in different languages. Therefore, these studies were also reviewed.

Through the literature review, demographic data were extracted and analyzed. In the intervening 48 years, only 206 cases of LHS were reported in 87 studies, and the majority was from Europe^[1,3-88]. Among them, 187 cases were reported with sufficient age data, and 197 cases were reported with complete gender information. According to the literature review results, the average age of the reported cases at diagnosis is 47.5 years old (range 12-87 years). Women are affected more frequently than men, with an overall female-to-male ratio of 1.8:1 (127 females, 70 males). LHS cases with various nationalities have been reported in the literature (Table 1).

CLINICAL CHARACTERISTICS

LHS is typically acquired in adulthood and cases tend to be sporadic in nature^[4,5,14,15]. A female preponderance with an overall female to male ratio of 2:1 has been proposed in the previous studies^[3,16]. To date, evidence supporting a malignant tendency associated with LHS is lacking. In addition, no systemic abnormality or familial factor is associated with the syndrome. To date, only one report described a woman and her daughters affected with the condition^[17]. Environmental risk factors have not been identified. Generally, pigmentary changes of individuals with LHS do not disappear naturally but slowly increase with age. To date, complete remission of pigmentation was reported in only one case^[18].

The most common lesion sites are the lips, especially the lower lip, and the oral cavity, particularly the buccal mucosa. Frequently, pigmentation is also found on the tongue, gingiva, and palatal mucosa. However, the mouth floor is an extremely rare site^[19]. Increased pigmentation occurs in the acral area and the genital region^[7,12,20-23]. The finger pulp or tip and the periungual area are frequently affected with pigmentary macules. The palmoplantar area is less frequently affected. The location distribution of pigmentary lesions in LHS is provided in Table 2.

Table 1 Geographical distribution of published cases affected with Laugier-Hunziker syndrome

	Country	No. of cases	Percentage (%)	Ref.
1	France	59	29	[1,14,20-22,24,26,27,30,31]
2	Italy	33	16	[11,12,18,25,28,29,33,34-37,40,63]
3	China	30	14.5	[5,9,10,19,23,58,72]
4	United States	14	7	[13,16,32,42,46,51,60,67-69,73,77,85,88]
5	United Kingdom	13	6	[3,39,45,49,52,54,76]
6	Germany	12	6	[40,61]
7	India	8	4	[47,56,57,70,71,81,86]
8	Turkey	6	3	[7,50,53,62,75,83]
9	Greece	5	2	[6,40,48]
10	Japan	5	2	[8,44,55,59]
11	Lebanon	3	1.5	[17]
12	Portugal	2	1	[23,43]
13	Brazil	2	1	[65,78]
14	Spain	2	1	[66]
15	Australia	2	1	[87]
16	Finland	1	0.5	[4]
17	Ireland	1	0.5	[15]
18	Russia	1	0.5	[38]
19	Switzerland	1	0.5	[41]
20	South Korea	1	0.5	[64]
21	Austria	1	0.5	[74]
22	Serbia	1	0.5	[79]
23	Chile	1	0.5	[80]
24	Romania	1	0.5	[82]
25	Singapore	1	0.5	[84]

Table 2 Distribution of involved locations of pigmentary lesions in Laugier-Hunziker syndrome

Involved locations	Frequency	Ref.
Lip	75% (154/206)	[1,3-16,18,19,21-25,27-29,31-38,40,41,43-52,54-68,70-73,75-78,80,83,85-88]
Oral cavity	68% (140/206)	[1,3-9,11-19,21-24,26,27,29,31-33,35-44,46,47,50-60,62-76,78,79,81,82,84-88]
Acral area	47% (96/206)	[1,3-10,12,14-17,19,22,23,25-27,31,32,34,39-41,43,44,47,49,50,52-54,56-59,61,63-66,68-72,77-84,86]
Periungual area	8% (17/206)	[8,14,16,25,31,34,43,47,50,54,64,65,71,73,77,79,81]
Finger	13% (26/206)	[5,8,9,11,14,19,21,41,43,49,55-57,59,60,66,70-72,74,76,84-86,88]
Palm	4% (8/206)	[7,43,44,46,59,60,62,81]
Toes	3% (6/206)	[19,56,59,70,84,86]
Sole	3% (6/206)	[7,21,46,59,62]
Genitalia	24% (17/70) ¹	[20-23,46]
Vulva or labia majora	10% (13/127) ¹	[7,12,15,17,21,30,58,74]
Anal mucosa and perianal area	1.5% (3/206)	[21,41]
Conjunctiva and sclera	4% (9/206)	[6,7,17,19,23,46,60,83]
Eyebrow and periorbital area	1.5% (3/206)	[41,53,71]
Pharynx	0.5% (1/206)	[4]
Esophagus	0.5% (1/206)	[44]
Neck, thorax, and abdomen	1% (2/206)	[25,81]
Back	0.5% (1/206)	[50]
Elbow	1% (2/206)	[43,76]
Pretibial area	0.5% (1/206)	[62]

¹Frequency of genitalia involvement of pigmentation was assessed according to the known sum of male or female cases.

Typically, the cutaneous or mucosal lesions manifest as gray, brown, blue-black, or black macules with a flat, smooth surface and relatively well-defined or indistinct margin. The lesions are generally 2 to 5 mm in diameter and lenticular, oval, or irregular in shape. Hyperpigmented macules are distributed in variable numbers. Single or multiple lesions are observed, and occasionally, the lesions are confluent^[11,12,19,21,41,42,44]. Symptoms are generally absent. Figure 1 presents typical lenticular melanotic macules on the lower lip of a patient affected with LHS.

Labial or oral hyperpigmentation may be accompanied by nail pigmentation. Longitudinal melanonychia is observed in approximately 44%-60% of LHS patients^[3,15,27,39,46,59]. The typical nail pigmentation in LHS manifests as single or double longitudinal brownish-black streaks with a homogeneous, smooth, and flat appearance. According to the classification by Baran^[27], the nail pigmentation in LHS is mainly categorized into three types: A single 1 to 2 mm wide longitudinal streak, a double 2 to 3 mm wide longitudinal streak on the lateral portion of the nail plate, and homogeneous



Figure 1 Lenticular melanotic macule on the lower lip of a Laugier-Hunziker syndrome patient.

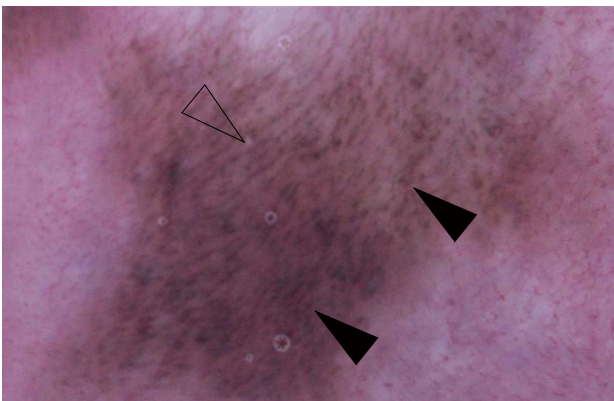


Figure 2 Dermoscopic findings of the Laugier-Hunziker syndrome patient. Granular (closed arrowhead) and linear (open arrowhead) patterns coexist in the labial pigmented lesion.

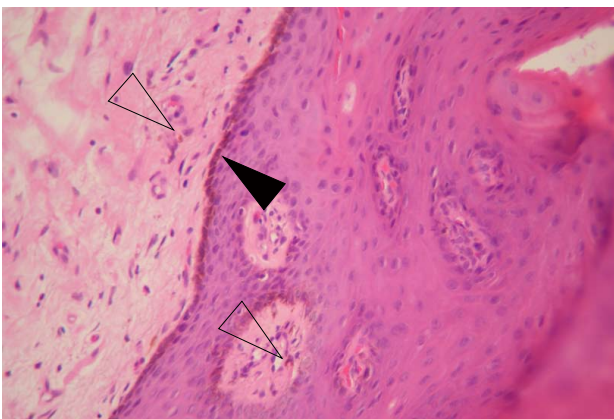


Figure 3 Pathological findings of the Laugier-Hunziker syndrome patient. Accumulated melanin in the basal layer (closed arrowhead) and slightly increased melanophages in the lamina propria (open arrowhead) (HE stain $\times 200$).

pigmentation of the radial or ulnar half of the nail plate. Veraldi *et al.*^[11] reported a fourth type, namely, complete pigmentation of the nail plate. All four types of nail involvement may simultaneously affect one or more fingernails and/or toenails. However, the degree of the pigmentation does not correspond to different stages of the syndrome. Fingernails are more frequently involved

compared to the toenails^[11]. Pseudo-Hutchinson's sign, which refers to nail matrix pigmentation that is visible through a translucent cuticle on periungual tissues and more frequently on the hyponychium, is commonly observed.

DERMOSCOPIC FEATURES

As a useful and noninvasive auxiliary diagnostic method, dermoscopy has been used for a more accurate diagnosis of pigmented lesions, including in LHS lesions. The dermoscopic findings of labial lesions in LHS consist of parallel furrow pattern with multiple brown dots^[7]; multiple brown or blue-gray granular patterns^[8]; a regular brownish reticular pattern including arc streaks, granules, and networks^[10]; a regular brown network pattern with a homogenous blue area^[61]; brown reticular lines, globules, parallel lines^[83]; and a fish scale-like pattern^[88]. These dermoscopic patterns are frequently noted along with linear and dotted vessels on whitish pink areas^[10,61]. Figure 2 presents the dermoscopic findings of the aforementioned LHS patient.

Similarly, the dermoscopic findings of mucosal pigmentary lesions in LHS are composed of a regular brownish reticulate pattern with linear or curvilinear vasculature on the buccal mucosa^[9], a parallel furrow pattern with linear and curvilinear streaks on the vulva^[7] and a light brown colored homogeneous pattern on the conjunctiva^[88]. Furthermore, the dermoscopic findings of cutaneous pigmentary lesions in LHS consist of a parallel furrow pattern in the palmoplantar region^[7]; a parallel ridge pattern on the finger pulp^[8,9,66], fingertip and palm^[66]; a brown to grayish homogeneous pattern^[88]; and a fibrillar pattern in the periungual area^[8].

Additionally, the dermoscopic findings of ungual pigmentary lesions in LHS mainly include homogenous, regular, brownish or grayish, bandlike^[7,9,66,82] and linear^[8,9,82,83] patterns with ill-defined margins on the nail plate. Sometimes, dermoscopic examination of the nail unit can reveal micro-Hutchinson sign, which is almost invisible to the naked eye^[82].

PATHOLOGICAL CHARACTERISTICS

Typically, the pathological findings of pigmented lesions in LHS involve an accumulation of melanin in the cells of the basal layer of the epithelium or epidermis. Melanocytes are normal in number, morphologic appearance, and distribution. An increased number of melanophages, *i.e.*, pigmentary incontinence, may be observed in the upper lamina propria or superficial connective tissues. Nevus cells are never observed. These features demonstrate that the condition is due to increased melanocytic activity rather than to an increased number of melanocytes^[12]. Figure 3 presents the pathological findings of the aforementioned LHS patient.

The accumulation of melanin in basal layer cells

Table 3 Various conditions associated with labial or oral pigmentation

	Focal	Diffuse
Exogenous origin	Amalgam tattoo	Tobacco-associated melanin pigmentation (smoker's melanosis)
	Topical medications	Drugs (e.g., antimalarials, tetracyclines, ketoconazole, zidovudine, phenothiazines, oral contraceptives, and chemotherapeutic agents)
	Graphite tattoo (e.g., carbon, lead pencils)	Heavy metals (including bismuth, mercury, silver, lead, gold, arsenic, tin, copper, brass, zinc, cadmium, chrome, and manganese)
Endogenous origin	Melanotic macule	Physiologic (racial) pigmentation
	Melanocytic nevus	Posttraumatic or postinflammatory pigmentation
	Melanoacanthoma	Lichen planus
	Melanoma	Discoid lupus erythematosus
	Hemangioma	LHS
	Lentigo maligna	Peutz-Jeghers syndrome
	Kaposi sarcoma	Addison's disease
		McCune-Albright syndrome
		Neurofibromatosis type 1 (von Recklinghausen's disease)
		Carney complex (NAME/LAMB syndrome)
		LEOPARD syndrome (lentiginosis profusa syndrome)
		Cronkhite-Canada syndrome
		Cushing syndrome
		Incontinentia pigmenti syndrome (Bloch-Sulzberger syndrome)

is confined to the tips of epithelial rete ridges, which has been reported and observed through a review of the histopathologic findings in some reported cases^[5,6,11,15,19,40,62,67,85]. Thus, this phenomenon is unclear and needs further investigation. In several cases, increased numbers of nonnested melanocytes were observed in the epithelial basal layer^[32,51,53,81]. An atypical report described slight cellular atypia of intraepidermal melanocytes in a sun-exposed skin lesion^[51]. Acanthosis^[6,13,17,22,42,44,70,81,86,88], hyperkeratosis^[60,70,81,86], hyperparakeratosis^[6,88], or spongiosis^[13,53] of the epithelium and elongated rete ridges^[6,32,81,88] have been noted in some reported cases.

DIFFERENTIAL DIAGNOSIS

Differential diagnosis of mucocutaneous pigmentation

A broad differential diagnosis should be considered when evaluating labial, oral, and cutaneous hyperpigmentation (Table 3). Generally, a diagnosis of exclusion should be taken into consideration. Labial and oral pigmentation is either focal or diffuse. Although focal lesions may be more worrying and require a biopsy for an accurate diagnosis, diffuse lesions usually have no specific histological features and may be the first sign of an underlying systemic disease^[4].

Amalgam tattoo: Accidental displacement of metal particles into oral soft tissues during restorative procedures using the dental material amalgam may result in amalgam pigmentation, the so-called "amalgam tattoo"^[89,90]. Amalgam tattoos are generally painless,

solitary or multiple macules that range from a few millimeters to greater than one centimeter in size. The most frequently involved sites include the gingiva and alveolar mucosa. Histologically, fine black fibrillar or granular material distributed in the connective tissue or in a perivascular location with minimal or no inflammatory response is noted^[89].

Smoker's melanosis: Smoker's melanosis is a specific entity characterized by black-brown hyperpigmentation of the oral cavity of heavy smokers^[91]. Smoker's melanosis is more common in women. The most common locations of smoker's melanosis are the labial attached gingiva and interdental papillae^[89-91].

Drug-induced mucocutaneous pigmentation:

Diffuse pigmentation may be associated with systemic intake of drugs. The most common drugs associated with pigmentation include tetracyclines (minocycline), antimalarials (chloroquine and hydroxychloroquine), antifungals (ketoconazole), antimycobacterial agents (clofazimine), antiretroviral agents (zidovudine), chemotherapeutics (cyclophosphamide, doxorubicin, and hydroxyurea), amiodarone, psychotropics (chlorpromazine), and oral contraceptives. Drug-induced oral pigmentation typically occurs following long-term medicine use and often resolves after cessation of the causative drug^[5,89].

Melanotic macule: Melanotic macule is also known as focal melanosis. Unlike ephelides, melanotic macules do not darken with sun exposure^[91]. Clinically, pigmented

macules are typically solitary, well circumscribed lesions that average 6 mm in size. Histologically, increased melanin is predominantly present in the basal cell layer without an increase in the number of melanocytes. Incontinence of pigmentation and melanophages may be noted in the superficial lamina propria^[89]. In contrast to simple lentigines, melanotic macules do not exhibit elongation of the rete ridges. In contrast to oral nevi or melanomas, melanotic macules are HMB-45 negative based on immunohistochemical staining^[91,92].

Melanocytic nevus: Intraoral melanocytic nevi appear frequently during the third and fourth decades of life^[91]. In general, the lesions are asymptomatic, pigmented macules or papules that are brown to black or blue in color. Histologically, approximately half of intraoral nevi cases classified as the intramucosal (intradental) type, and another one-third of cases are blue nevi. Importantly, intraoral nevi share clinical similarities and a predilection for the hard palate with oral melanoma^[91]. Therefore, a complete excision of all lesions is both practical and advisable.

Melanoacanthoma: Oral melanoacanthoma is considered a reactive process most frequently affecting black women in their third and fourth decades of life^[13,89]. The buccal mucosa is the most commonly involved site, and most lesions are solitary. The clinical appearance is characterized by a sharply demarcated, irregularly textured pigmented plaque^[91]. Histological examination reveals that large, dendritic melanocytes normally confined to the basal layer are instead dispersed throughout the epithelium^[89].

Melanoma: Oral melanoma is uncommon, with a higher incidence in Asians, Hispanics, and blacks^[93]. Oral melanoma is generally encountered in the fifth decade, with an increased incidence in men compared with women. A male-to-female ratio of 2.5-3.1 is reported among patients affected with oral melanoma^[78,94]. Within the oral cavity, the most common site is the palate, followed by the anterior labial gingiva^[94]. Clinically, oral melanoma is an asymptomatic, slow-growing, brown-to-black macule with irregular, asymmetric borders. Patients often present at an advanced stage with a rapidly enlarging mass with pain, ulceration, bleeding, mobile teeth, and bone involvement^[78]. Histopathologically, oral melanoma is indistinguishable from cutaneous melanoma and exhibits epithelioid or spindle nuclei^[91]. Pagetoid spread with large melanoma cells either singly or in nests can be noted in the superficial epithelium.

Any suspicious pigmentary lesion with irregular or asymmetric margins or those that are ulcerated, exophytic, or heterogeneous should be excised and assessed to exclude melanoma^[78,92]. Dermoscopic findings include some malignant features, such as "blue-white veil", irregular, asymmetrical, pinpoint, and hairpin patterns.

Physiological (racial) pigmentation: Physiological pigmentation or racial pigmentation is commonly identified in blacks, Asians, and other dark-skinned persons^[4,12,91]. The attached gingiva is the most common location, but other sites including the tips of the fungiform papillae on the dorsal tongue may also be affected^[13,89,91]. Physiological pigmentation of the oral cavity is typically diffuse, bilateral, and irregular in shape, commonly with poorly defined borders^[89,91].

Peutz-Jeghers syndrome: The most important differential diagnosis of LHS is Peutz-Jeghers syndrome (PJS) (Table 4). PJS is an autosomal dominant condition characterized by mucocutaneous pigmentation, gastrointestinal hamartomatous polyps, and cancer predisposition^[49]. PJS is caused by mutations in the serine/threonine kinase 11 (*STK11*) gene. PJS affects approximately one in 8300 individuals. Hyperpigmented macules on the lips, oral, perianal, genital mucosa, and acral skin are common^[95]. PJS must be excluded because patients with PJS exhibit an increased incidence of gastrointestinal carcinoma as well as genital and mammary tumors. The lifetime risk of cancer in this condition is estimated to be 76%-85%^[96]. Patients with PJS are recommended to undergo lifelong cancer surveillance, including colonoscopy, gastroduodenoscopy, and capsule endoscopy, as well breast imaging and gynecological assessment in women and testicular examination in men^[97]. *STK11* gene testing facilitates the differential diagnosis, ongoing management, and cancer surveillance^[87].

Addison's disease: Addison's disease is an endocrine disorder due to adrenocortical insufficiency. This disease affects approximately one in 100000 people. In developed countries, the disease is usually related to autoimmune disorders, whereas it is commonly associated with infection, especially tuberculosis, in developing nations^[98].

Addison's disease is characterized by hyperpigmentation of pressure exposed areas and associated with scanty body hair, hypotension, hyponatremia, hyperkalemia, hypoglycemia, and elevated blood urea nitrogen^[98]. Diffuse pigmentation may be noted on the skin, lip, oral cavity, conjunctiva, and genitalia. Labial and oral hyperpigmentation may be the first sign of the disease^[12].

McCune-Albright syndrome: McCune-Albright syndrome is a sporadic disorder characterized by café-au-lait pigmentation on the skin, polyostotic fibrous dysplasia, and hyperfunctioning endocrinopathies including precocious puberty in women, Leydig and Sertoli cell hyperplasia in men, hyperthyroidism, excess growth hormone, and Cushing syndrome. The disorder is caused by a somatic mutation of the guanine nucleotide-binding protein, α -stimulating (*GNAS*) complex locus gene, leading to constitutive activation of

Table 4 Differential diagnosis between Peutz-Jeghers syndrome and Laugier-Hunziker syndrome

	PJS	LHS
Inheritance	Autosomal dominant (<i>STK11</i> gene)	Sporadic and acquired
Age of onset	Birth to infancy	Adult onset
Shape of mucocutaneous pigmented macules	Freckle-like	Lenticular
Labial pigmentation	Very common	Very common
Oral pigmentation	Common	Very common
Perioral, perirhinal, or periorbital pigmentation	Common	Uncommon
Nail pigmentation	Uncommon	Very common
Acral skin pigmentation	Common	Common
Systemic involvement	Gastrointestinal polyposis	None
Risk of malignancy	Colon, gastric, small intestinal, pancreatic, breast, ovarian, thyroid, lung, and Sertoli cell (in men) cancers	None

PJS: Peutz-Jeghers syndrome; LHS: Laugier-Hunziker syndrome.

the α subunit of the stimulatory G protein (G_{α})^[99].

The most common locations of café-au-lait pigmentation include the posterior neck, sacrum, and head. The pigmentary lesions are light to dark brown macules and patches that may be segmental and tend to be unilateral. The size of café-au-lait pigmentation, which typically ranges from 0.5 to 20 cm, does not correlate with the extent of bone disease^[100]. The pigmentation often covers a large geographic area with an irregular border that is frequently described as a "coast of Maine" border. A small increase in risk of malignancies in affected thyroid, bone, or pancreas tissues is noted^[99].

Neurofibromatosis type 1: Neurofibromatosis type 1 is one of the most common human genetic diseases, occurring in approximately one of every 3000 births. No sex or race predilection is noted. Neurofibromatosis type 1 is an autosomal dominant inherited disease due to a mutation in the *NF1* gene on the long arm of chromosome 17^[101].

Neurofibromatosis type 1 is characterized primarily by café-au-lait pigmentation, multiple neurofibromas, optic glioma, iris Lisch nodules (pigmented hamartomas of the iris), central nervous system tumors, and bone malformations. The café-au-lait spots are yellow to dark brown macules that vary in diameter from a few millimeters to several centimeters^[101]. Café-au-lait pigmentation in neurofibromatosis type 1 has characteristic smooth borders analogous to the "coast of California," in contrast to the irregular borders of the café-au-lait pigmentation in McCune-Albright syndrome. Axillary or inguinal freckling (Crowe's sign) is a highly suggestive sign. Although neurofibromas are benign tumors, plexiform neurofibromas may progress to malignant peripheral nerve sheath tumors. Other neoplasias frequently observed in neurofibromatosis type 1 patients include pilocytic astrocytomas, gastrointestinal stromal tumors, pheochromocytomas, and juvenile myelomonocytic leukemia^[102].

Carney complex: The Carney complex is an autosomal-dominant inherited disorder associated with an inactivating mutation in the protein kinase, cAMP-

dependent regulatory, type 1, α (*PRKAR1 α*) gene located on chromosome 17 and a second genetic locus on chromosome 2p16 that produces a milder phenotype^[103].

The Carney complex is characterized by lentigines, myxomas, endocrine tumors or overactivity, and schwannomas. Pale brown to black lentigines are the most common feature and are present in 70% to 80% of affected individuals. The frequently affected sites include the face, lips, conjunctiva, inner and outer canthi, and genital mucosa^[99].

The most serious component of the Carney complex is life threatening cardiac myxoma, which causes death or serious disability in a significant percentage of patients. Spotty facial pigmentation is labeled as a marker of the Carney complex^[91]. Therefore, early recognition of characteristic pigmentation leading to prompt assessment of the presence of cardiac myxoma can reduce mortality in affected individuals.

There is a strong association between the Carney complex and endocrine disease, including primary pigmented nodular adrenocortical disease (PPNAD)-associated Cushing syndrome, acromegaly, elevated prolactin, thyroid abnormalities, testicular large-cell calcifying Sertoli cell tumors, ovarian cystadenomas, and pancreatic lesions^[99].

LEOPARD syndrome: LEOPARD syndrome is an autosomal dominant multisystemic disorder^[104]. Mutations in the ubiquitous protein tyrosine phosphatase, nonreceptor type 11 gene (*PTPN11*), Raf-1 proto-oncogene (*RAF1*), B-Raf proto-oncogene (*BRAF*) or mitogen-activated protein kinase kinase 1 gene (*MAP2K1*) are related to the syndrome^[105].

The acronym "LEOPARD" refers to the presence of distinctive clinical features: Lentigines (L), electrocardiographic conduction abnormalities (E), ocular hypertelorism (O), pulmonary stenosis (P), genital abnormalities (A), retardation of growth (R), and sensorineural deafness (D).

Lentigines appear in early childhood and increase in number until puberty. Multiple well-defined brownish lentigines appear mostly on the neck, upper extremities,

Table 5 Differential diagnosis of nail pigmentation

Causation of nail pigmentation		Condition
Nonmelanocytic origin	Exogenous	Dirt
		Tobacco
		Tar
		Potassium permanganate
		Silver nitrate
	Infectious	Bacterial infection (<i>e.g.</i> , <i>Pseudomonas aeruginosa</i>)
		Onychomycosis (fungal infection)
		Subungual hemorrhage
	Traumatic	Subungual hematoma
		Hemangioma
Melanocytic origin	Neoplastic	Ethnic (racial) melanonychia
	Physiological	Vitamin B12 deficiency
	Nutritional	Onychotillomania (nail biting)
		Frictional melanonychia
	Traumatic	Systemic drug-induced melanonychia (<i>e.g.</i> , zidovudine, hydroxyurea, and minocycline)
		Radiotherapy-induced melanonychia
		Lichen planus
	Iatrogenic	Psoriasis
		Addison's disease
	Inflammatory	Pregnancy
	Endocrinic	LHS
		AIDS
	Syndromic	Benign melanocytic hyperplasia
		Lentigo simplex
	Activated melanocytic	Nevus
		Onychopapilloma
		Onychomatricoma
		Bowen's disease
		Melanoma

LHS: Laugier-Hunziker syndrome; AIDS: Acquired immunodeficiency syndrome.

trunk, and below the knees. The face, scalp, palms, soles, and genitals may also be involved, but the mucosa is invariably unaffected. Typical lentigines are flat, small, dark brown macules that are histologically characterized by increased numbers of melanocytes and copious melanin production^[105].

Cronkhite-Canada syndrome: Cronkhite-Canada syndrome is a nonhereditary condition characterized by cutaneous hyperpigmentation, alopecia, onychodystrophy, and diffuse gastrointestinal polyposis associated with diarrhea, abdominal pain, and protein-losing enteropathy and malnutrition^[106]. The condition occurs most frequently in middle-aged or older adults, with a slight male predominance, and a male-to-female ratio of 3:2^[107]. Homogeneous, diffuse acral pigmentation is often noted in Cronkhite-Canada syndrome, but no pigmentation is noted within the oral cavity^[107,108]. In contrast to other polyposis syndromes, the polyps in Cronkhite-Canada syndrome patients can develop throughout the gastrointestinal tract (except the esophagus)^[107]. To facilitate differential diagnosis, the relationships between several syndromes or systemic disorders characterized by diffuse mucocutaneous pigmentation are graphically illustrated in Figure 4.

Differential diagnosis of melanonychia

Melanonychia can be found in a wide variety of conditions ranging from physiological pigmentation to life-threatening tumors (Table 5). Nail pigmentation in LHS should be distinguished from melanonychia caused by other disorders. The first and most important task is to determine whether the pigment is of melanocytic or nonmelanocytic origin. In most cases this task can be accomplished by clinical inspection and examination with a dermoscope^[109].

Exogenous pigmentation: Most exogenous pigmentation is due to dirt, tobacco, chemical agents, cosmetics, and topical therapeutic agents such as silver nitrate. Generally, exogenous pigmentation does not manifest as a longitudinal streak. Most of the pigmentation can be easily removed^[109,110].

Onychomycosis: Onychomycosis may commonly present as nail pigmentation and abnormalities of the nail plate surface. White, yellow, green, or black changes in nails are due to fungal pigment that invades the nail plate. The most commonly responsible fungi include *Scytalidium dimidiatum* and *Trichophyton rubrum* var. *nigricans*^[111].

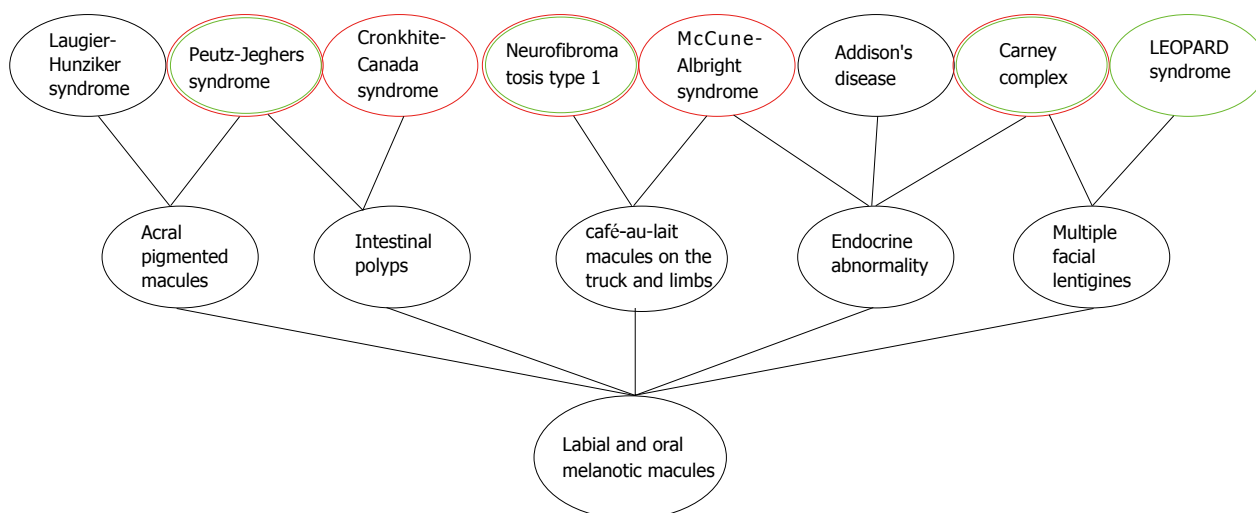


Figure 4 The differentiation and relationship between several main syndromes or systemic disorders characterized by diffuse mucocutaneous pigmentation. Red circle: Malignancy risk; Green circle: Inheritance tendency.

Bacterial infection: Bacterial nail pigmentation, which is most commonly due to *Pseudomonas aeruginosa*, *Klebsiella* and *Proteus spp.*, exhibits a greenish or grayish hue and the discoloration is often confined to the lateral edge of the nail^[110]. The pigmentation appears black to the naked eye, but it is clearly deep green when observed with a dermoscope.

Subungual hemorrhage and subungual hematoma: Typically, subungual hemorrhage or hematoma is due to a history of a single acute and heavy trauma. Dermoscopic findings typically involve a small reddish-black globular pattern and the proximal and lateral margins of the pigment^[109]. Generally, subungual hematoma does not pose a diagnostic difficulty. However, it must be kept in mind that a tumor with neovascularization may bleed. Therefore, the presence of subungual blood should not be a sign used to exclude the diagnosis of melanoma^[109,110].

Ethnic nail pigmentation: Ethnic or racial nail pigmentation is physiological longitudinal melanonychia observed in dark-skinned individuals^[109]. Longitudinal melanonychia, which is distinctly uncommon in whites, has been reported to occur in 77%-96% of blacks and 11% of Asians^[50]. Ethnic longitudinal melanonychia can present as single or multiple pigmented bands involving one or more digits.

Traumatic longitudinal melanonychia: The two common types of traumatic longitudinal melanonychia are nail pigmentation associated with onychotillomania (nail biting) and frictional melanonychia, which typically involves the fourth and/or fifth toenails due to friction with shoes^[109].

Drug-induced melanonychia: Drug-induced longitudinal melanonychia appears as horizontal or longitudinal bands or as diffuse nail darkening^[109].

The three most frequently responsible drugs include zidovudine, hydroxyurea, and minocycline^[112].

Longitudinal melanonychia associated with inflammatory nail disorders: Multiple longitudinal melanonychia may occur in patients with lichen planus, which is typically associated with nail plate thinning, onychotrophy, onychorrhexis, longitudinal splitting, longitudinal grooves or ridges of the nail plate, nail scarring, and abnormalities^[19].

Nevus: Nail matrix nevi may be congenital or acquired and are typically observed in children and young adults. Generally, nail matrix nevi occur more often on fingernails compared with toenails. Typically, the width of the pigmented band of nail matrix nevi is 3 mm or less. Dermoscopic features of nail matrix nevi involve longitudinal parallel lines with a homogeneous brown color and regular spacing and thickness^[113].

Bowen's disease: Nonmelanocytic nail tumors may activate melanocytes resulting in longitudinal melanonychia. This entity is most commonly observed in association with Bowen's disease^[109]. Bowen's disease or squamous cell carcinoma *in situ* is a slowly progressive malignancy.

Melanoma: Nail matrix melanoma is the most frequently observed melanoma subtype in blacks and Asians. It usually affects middle-aged or elderly individuals^[109]. Nail matrix melanoma most frequently affects the index finger, the thumb, the large toe, or all of them^[114].

Longitudinal melanonychia in malignancies is typically characterized by lesions wider than 5 mm, and gray to black streaks with variable color and shape within one lesion may occur in nail matrix melanoma. Moreover, nail dystrophy, nail erosion, and a bleeding mass strongly suggest a malignancy^[110]. Brown or black

pigmentation spreading from the nail bed, matrix and nail plate to the adjacent cuticle and proximal or lateral nail folds (Hutchinson's sign) is a crucial clinical clue to diagnose or exclude melanoma^[78].

Typically, the dermoscopic features of nail matrix melanoma include the absence of both homogeneity of both color and thickness of each longitudinal line, Hutchinson and micro-Hutchinson signs, and granular pigmentation^[112,115].

TREATMENT

In general, no treatment is required for LHS given the lack of associated systemic complications or malignant transformation. Patients may seek treatment for removal of labial hyperpigmented macules based on aesthetic consideration. Cryosurgery, Q-switched Nd:YAG lasers and Q-switched alexandrite lasers represent therapeutic options. Recurrence may occur after treatment. Sun protection is crucial to prevent reoccurrence^[70].

CONCLUSION

LHS shares some features of labial, oral, and nail pigmentation with a variety of conditions. These conditions span a wide gamut from normal variation to a sign of an underlying life-threatening abnormality. Clinicians should be more familiar with typical features of these conditions and aware of important differential diagnosis, which contributes to obtaining an early final diagnosis. Accurate diagnosis is crucial to developing a reasonable medical strategy, including management options, malignant transformation surveillance, and psychological support. The evaluation involves a detailed history and physical examination, including visual inspection of all the affected sites. Dermoscopy and biopsy of any suspicious lesion should be performed for dermoscopic and histopathological evaluation. Other necessary tests or examinations may be considered in case of a difficult diagnosis. It is crucial that clinicians conduct long-term follow-up and surveillance due to the potential risks for malignant transformation and local severe complications in some conditions.

REFERENCES

- 1 **Laugier P**, Hunziker N. [Essential lenticular melanic pigmentation of the lip and cheek mucosa]. *Arch Belg Dermatol Syphiligr* 1970; **26**: 391-399 [PMID: 5515564]
- 2 **Nayak RS**, Kotrashetti VS, Hosmani JV. Laugier-Hunziker syndrome. *J Oral Maxillofac Pathol* 2012; **16**: 245-250 [PMID: 22923898 DOI: 10.4103/0973-029X.99079]
- 3 **Kemmett D**, Ellis J, Spencer MJ, Hunter JA. The Laugier-Hunziker syndrome—a clinical review of six cases. *Clin Exp Dermatol* 1990; **15**: 111-114 [PMID: 2347100 DOI: 10.1111/j.1365-2230.1990.tb02044.x]
- 4 **Siponen M**, Salo T. Idiopathic lenticular mucocutaneous pigmentation (Laugier-Hunziker syndrome): a report of a case. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; **96**: 288-292 [PMID: 12973285 DOI: 10.1016/S1079-2104(03)00295-6]
- 5 **Zuo YG**, Ma DL, Jin HZ, Liu YH, Wang HW, Sun QN. Treatment of Laugier-Hunziker syndrome with the Q-switched alexandrite laser in 22 Chinese patients. *Arch Dermatol Res* 2010; **302**: 125-130 [PMID: 20012075 DOI: 10.1007/s00403-009-0930-1]
- 6 **Nikitakis NG**, Koumaki D. Laugier-Hunziker syndrome: case report and review of the literature. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013; **116**: e52-e58 [PMID: 23562360 DOI: 10.1016/j.oooo.2012.12.012]
- 7 **Gencoglan G**, Gerceker-Turk B, Kilinc-Karaarslan I, Akalin T, Ozdemir F. Dermoscopic findings in Laugier-Hunziker syndrome. *Arch Dermatol* 2007; **143**: 631-633 [PMID: 17515514 DOI: 10.1001/archderm.143.5.631]
- 8 **Tamiya H**, Kamo R, Sowa J, Haruta Y, Tanaka M, Ishii M, Kobayashi H. Dermoscopic features of pigmentation in Laugier-Hunziker-Baran syndrome. *Dermatol Surg* 2010; **36**: 152-154 [PMID: 19889156 DOI: 10.1111/j.1524-4725.2009.01371.x]
- 9 **Ko JH**, Shih YC, Chiu CS, Chuang YH. Dermoscopic features in Laugier-Hunziker syndrome. *J Dermatol* 2011; **38**: 87-90 [PMID: 21175762 DOI: 10.1111/j.1346-8138.2010.01077.x]
- 10 **Wei Z**, Li GY, Ruan HH, Zhang L, Wang WM, Wang X. Laugier-Hunziker syndrome: A case report. *J Stomatol Oral Maxillofac Surg* 2018; **119**: 158-160 [PMID: 29246753 DOI: 10.1016/j.jormas.2017.12.003]
- 11 **Veraldi S**, Cavicchini S, Benelli C, Gasparini G. Laugier-Hunziker syndrome: a clinical, histopathologic, and ultrastructural study of four cases and review of the literature. *J Am Acad Dermatol* 1991; **25**: 632-636 [PMID: 1791220 DOI: 10.1016/0190-9622(91)70244-V]
- 12 **Mignogna MD**, Lo Muzio L, Ruoppo E, Errico M, Amato M, Satriano RA. Oral manifestations of idiopathic lenticular mucocutaneous pigmentation (Laugier-Hunziker syndrome): a clinical, histopathological and ultrastructural review of 12 cases. *Oral Dis* 1999; **5**: 80-86 [PMID: 10218046 DOI: 10.1111/j.1601-0825.1999.tb00068.x]
- 13 **Zaki H**, Sabharwal A, Kramer J, Aguirre A. Laugier-Hunziker Syndrome Presenting with Metachronous Melanoacanthomas. *Head Neck Pathol* 2018 [PMID: 29450847 DOI: 10.1007/s12105-018-0897-3]
- 14 **Porneuf M**, Dandurand M. Pseudo-melanoma revealing Laugier-Hunziker syndrome. *Int J Dermatol* 1997; **36**: 138-141 [PMID: 9109016 DOI: 10.1111/j.1365-4362.1997.tb03076.x]
- 15 **Lenane P**, Sullivan DO, Keane CO, Loughlin SO. The Laugier-Hunziker syndrome. *J Eur Acad Dermatol Venereol* 2001; **15**: 574-577 [PMID: 11843221 DOI: 10.1046/j.1468-3083.2001.00352.x]
- 16 **Sterling GB**, Libow LF, Grossman ME. Pigmented nail streaks may indicate Laugier-Hunziker syndrome. *Cutis* 1988; **42**: 325-326 [PMID: 3234031]
- 17 **Makhoul EN**, Ayoub NM, Helou JF, Abadjian GA. Familial Laugier-Hunziker syndrome. *J Am Acad Dermatol* 2003; **49**: S143-S145 [PMID: 12894104 DOI: 10.1067/mjd.2003.300]
- 18 **Bisighini G**, Davalli R. Malattia di Laugier: tre casi clinici (in Italian). *Chron Dermatol* 1985; **16**: 821-825
- 19 **Wang WM**, Wang X, Duan N, Jiang HL, Huang XF. Laugier-Hunziker syndrome: a report of three cases and literature review. *Int J Oral Sci* 2012; **4**: 226-230 [PMID: 23174847 DOI: 10.1038/ijos.2012.60]
- 20 **Revuz J**, Clerici T. Penile melanosis. *J Am Acad Dermatol* 1989; **20**: 567-570 [PMID: 2715403 DOI: 10.1016/S0190-9622(89)70064-5]
- 21 **Dupré A**, Viraben R. Laugier's disease. *Dermatologica* 1990; **181**: 183-186 [PMID: 2269375 DOI: 10.1159/000247920]
- 22 **Seoane Lestón JM**, Vázquez García J, Cazenave Jiménez AM, de la Cruz Mera A, Aguado Santos A. [Laugier-Hunziker syndrome. A clinical and anatomopathologic study. Presentation of 13 cases]. *Rev Stomatol Chir Maxillofac* 1998; **99**: 44-48 [PMID: 9615354]
- 23 **Ayoub N**, Barete S, Bouaziz JD, Le Pelletier F, Frances C. Additional conjunctival and penile pigmentation in Laugier-Hunziker syndrome: a report of two cases. *Int J Dermatol* 2004; **43**: 571-574 [PMID: 15304179 DOI: 10.1111/j.1365-4632.2004.02173.x]
- 24 **Pellerat J**, Hermier C, Thivolet J, Vittori F. [Laugier's labiojugal essential melanic pigmentation]. *Bull Soc Fr Dermatol Syphiligr*

- 1971; **78**: 563-564 [PMID: 5152910]
- 25 **Sartoris S**, Pippone M, De Paoli MA, Cervetti O. Pigmentazione melanica idiopatica (in Italian). *Giorn e Min Derm* 1975; **110**: 382-385
- 26 **Laugier P**, Hunziker N, Olmos L. [Essential melanic pigmentation of the mouth and the lips]. *Ann Dermatol Venereol* 1977; **104**: 181-184 [PMID: 869459]
- 27 **Baran R**. Longitudinal melanotic streaks as a clue to Laugier-Hunziker syndrome. *Arch Dermatol* 1979; **115**: 1448-1449 [PMID: 533292 DOI: 10.1001/archderm.1979.04010120044018]
- 28 **Bundino S**, Zima AM. Pigmentazione melanica lentiginosa essenziale della mucosa orale (Laugier). Studio di un caso al microscopio elettronico (in Italian). *Giorn Ital Dermatol* 1979; **114**: 145-148
- 29 **Bertazzoni MG**, Botticelli A, Cimitan A. Su di un caso di pigmentazione melanica essenziale labio-buccale (M. di Laugier) (in Italian). *Chron Dermatol* 1984; **15**: 565-570
- 30 **Beurey J**, Weber M, Jeandel C. Pigmentation melanique essentielle de la muqueuse genitale: maladie de Laugier vulvaire (in French)? *Med Hyg* 1986; **44**: 786-790
- 31 **Baran R**, Barrière H. Longitudinal melanonychia with spreading pigmentation in Laugier-Hunziker syndrome: a report of two cases. *Br J Dermatol* 1986; **115**: 707-710 [PMID: 3801308 DOI: 10.1111/j.1365-2133.1986.tb06651.x]
- 32 **Koch SE**, LeBoit PE, Odom RB. Laugier-Hunziker syndrome. *J Am Acad Dermatol* 1987; **16**: 431-434 [PMID: 3819089 DOI: 10.1016/S0190-9622(87)70055-3]
- 33 **Offidani A**, Morroni M, Lazzaretti G, Mambelli V. [Histological and ultrastructural aspects of Laugier's disease. Presentation of a case]. *G Ital Dermatol Venereol* 1987; **122**: 535-538 [PMID: 3443467]
- 34 **Borrello P**, Toni F, Misciali C, Valeri F, Barone M. [A case of Laugier-Hunziker syndrome]. *G Ital Dermatol Venereol* 1988; **123**: 537-538 [PMID: 3254321]
- 35 **Dal Tio R**, Di Vito F, Grasso F. [Hyperpigmentation of the Laugier-Hunziker-syndrome type appearing during antineoplastic polychemotherapy]. *G Ital Dermatol Venereol* 1989; **124**: 77-83 [PMID: 2807389]
- 36 **Patrone P**, Cafini G, Schianchi S, Passarini B. [Laugier-Hunziker syndrome. Description of 3 cases]. *G Ital Dermatol Venereol* 1989; **124**: 95-96 [PMID: 2807391]
- 37 **Satriano RA**. Pigmentazione melanica essenziale della mucosa orale e delle labbra (Malattia di Laugier-Hunziker) (in Italian). *Chron Derm* 1989; **21**: 743-745
- 38 **Golovinov ED**. [A case of Laugier's disease]. *Vestn Dermatol Venerol* 1990; **(10)**: 60-61 [PMID: 2278200]
- 39 **Lamey PJ**, Nolan A, Thomson E, Lewis MA, Rademaker M. Oral presentation of the Laugier-Hunziker syndrome. *Br Dent J* 1991; **171**: 59-60 [PMID: 1873096 DOI: 10.1038/sj.bdj.4807604]
- 40 **Haneke E**. [Laugier-Hunziker-Baran syndrome]. *Hautarzt* 1991; **42**: 512-515 [PMID: 1917472]
- 41 **Gerbig AW**, Hunziker T. Idiopathic lentiginous mucocutaneous pigmentation or Laugier-Hunziker syndrome with atypical features. *Arch Dermatol* 1996; **132**: 844-845 [PMID: 8678590 DOI: 10.1001/archderm.132.7.844]
- 42 **Mowad CM**, Shrager J, Elenitsas R. Oral pigmentation representing Laugier-Hunziker syndrome. *Cutis* 1997; **60**: 37-39 [PMID: 9252732]
- 43 **Ferreira MJ**, Ferreira AM, Soares AP, Rodrigues JC. Laugier-Hunziker syndrome: case report and treatment with the Q-switched Nd-Yag laser. *J Eur Acad Dermatol Venereol* 1999; **12**: 171-173 [PMID: 10343950 DOI: 10.1111/j.1468-3083.1999.tb01011.x]
- 44 **Yamamoto O**, Yoshinaga K, Asahi M, Murata I. A Laugier-Hunziker syndrome associated with esophageal melanocytosis. *Dermatology* 1999; **199**: 162-164 [PMID: 10559586 DOI: 10.1159/000018227]
- 45 **Sheridan AT**, Dawber RP. Laugier-Hunziker syndrome: treatment with cryosurgery. *J Eur Acad Dermatol Venereol* 1999; **13**: 146-148 [PMID: 10568501 DOI: 10.1111/j.1468-3083.1999.tb00874.x]
- 46 **Began D**, Mirowski G. Perioral and acral lentiginous in an African American man. *Arch Dermatol* 2000; **136**: 419, 422 [PMID: 10724212]
- 47 **Kanwar AJ**, Kaur S, Kaur C, Thami GP. Laugier-Hunziker syndrome. *J Dermatol* 2001; **28**: 54-57 [PMID: 11280468 DOI: 10.1111/j.1346-8138.2001.tb00088.x]
- 48 **Papadavid E**, Walker NP. Q-switched Alexandrite laser in the treatment of pigmented macules in Laugier-Hunziker syndrome. *J Eur Acad Dermatol Venereol* 2001; **15**: 468-469 [PMID: 11763394 DOI: 10.1046/j.1468-3083.2001.00322.x]
- 49 **Lampe AK**, Hampton PJ, Woodford-Richens K, Tomlinson I, Lawrence CM, Douglas FS. Laugier-Hunziker syndrome: an important differential diagnosis for Peutz-Jeghers syndrome. *J Med Genet* 2003; **40**: e77 [PMID: 12807976 DOI: 10.1136/jmg.40.6.e77]
- 50 **Aytek S**, Alp S. Laugier-Hunziker syndrome associated with actinic lichen planus. *J Eur Acad Dermatol Venereol* 2004; **18**: 221-223 [PMID: 15009312 DOI: 10.1111/j.1468-3083.2004.00876.x]
- 51 **Moore RT**, Chae KA, Rhodes AR. Laugier and Hunziker pigmentation: a lentiginous proliferation of melanocytes. *J Am Acad Dermatol* 2004; **50**: S70-S74 [PMID: 15097932 DOI: 10.1016/j.jaad.2003.09.016]
- 52 **Fisher D**, Field EA, Welsh S. Laugier-Hunziker Syndrome. *Clin Exp Dermatol* 2004; **29**: 312-313 [PMID: 15115520 DOI: 10.1111/j.1365-2230.2004.01507.x]
- 53 **Akcali C**, Serarslan G, Atik E. The Laugier-Hunziker syndrome. *East Afr Med J* 2004; **81**: 544-545 [PMID: 15715135 DOI: 10.4314/eamj.v81i10.9240]
- 54 **Sabesan T**, Ramchandani PL, Peters WJ. Laugier-Hunziker syndrome: a rare cause of mucocutaneous pigmentation. *Br J Oral Maxillofac Surg* 2006; **44**: 320-321 [PMID: 15964106 DOI: 10.1016/j.bjoms.2005.04.008]
- 55 **Ozawa T**, Fujiwara M, Harada T, Muraoka M, Ishii M. Q-switched alexandrite laser therapy for pigmentation of the lips owing to Laugier-Hunziker syndrome. *Dermatol Surg* 2005; **31**: 709-712 [PMID: 15996427 DOI: 10.1111/j.1524-4725.2005.31618]
- 56 **Ajith C**, Handa S. Laugier-Hunziker pigmentation. *Indian J Dermatol Venereol Leprol* 2005; **71**: 354-356 [PMID: 16394464 DOI: 10.4103/0378-6323.16790]
- 57 **Sardana K**, Mishra D, Garg V. Laugier Hunziker syndrome. *Indian Pediatr* 2006; **43**: 998-1000 [PMID: 17151406]
- 58 **Tan J**, Greaves MW, Lee LH. Laugier-Hunziker syndrome and hypocellular marrow: a fortuitous association? *Clin Exp Dermatol* 2007; **32**: 584-585 [PMID: 17692057 DOI: 10.1111/j.1365-2230.2007.02484.x]
- 59 **Yago K**, Tanaka Y, Asanami S. Laugier-Hunziker-Baran syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; **106**: e20-e25 [PMID: 18468464 DOI: 10.1016/j.tripleo.2008.03.037]
- 60 **Blossom J**, Altmayer S, Jones DM, Slominski A, Carlson JA. Volar melanotic macules in a gardener: a case report and review of the literature. *Am J Dermatopathol* 2008; **30**: 612-619 [PMID: 19033941 DOI: 10.1097/DAD.0b013e318184bc57]
- 61 **Simionescu O**, Dumitrescu D, Costache M, Blum A. Dermatoscopy of an invasive melanoma on the upper lip shows possible association with Laugier-Hunziker syndrome. *J Am Acad Dermatol* 2008; **59**: S105-S108 [PMID: 19119112 DOI: 10.1016/j.jaad.2008.07.023]
- 62 **Aliagaoglu C**, Yanik ME, Albayrak H, Güvenç SC, Yildirim U. Laugier-Hunziker syndrome: diffuse large hyperpigmentation on atypical localization. *J Dermatol* 2008; **35**: 806-807 [PMID: 19239567 DOI: 10.1111/j.1346-8138.2008.00577.x]
- 63 **Montebugnoli L**, Gelli I, Cervellati F, Misciali C, Raone B. Laugier-hunziker syndrome: an uncommon cause of oral pigmentation and a review of the literature. *Int J Dent* 2010; **2010**: 525404 [PMID: 20671949 DOI: 10.1155/2010/525404]
- 64 **Kim EJ**, Cho SH, Lee JD. A Case of Laugier-Hunziker Syndrome. *Ann Dermatol* 2008; **20**: 126-129 [PMID: 27303175 DOI: 10.5021/ad.2008.20.3.126]
- 65 **Pereira PM**, Rodrigues CA, Lima LL, Reyes SA, Mariano AV. Do you know this syndrome? *An Bras Dermatol* 2010; **85**: 751-753 [PMID: 21152810 DOI: 10.1590/S0365-05962010000500029]
- 66 **Sendagorta E**, Feito M, Ramirez P, Gonzalez-Beato M, Saida T, Pizarro A. Dermoscopic findings and histological correlation of

- the acral volar pigmented maculae in Laugier-Hunziker syndrome. *J Dermatol* 2010; **37**: 980-984 [PMID: 21039787 DOI: 10.1111/j.1346-8138.2010.00924.x]
- 67 **Lema B**, Najarian DJ, Lee M, Miller C. JAAD Grand Rounds quiz. Numerous hyperpigmented macules of the oral mucosa. *J Am Acad Dermatol* 2010; **62**: 171-173 [PMID: 20082906 DOI: 10.1016/j.jaad.2008.11.009]
 - 68 **Rangwala S**, Doherty CB, Katta R. Laugier-Hunziker syndrome: A case report and review of the literature. *Dermatol Online J* 2010; **16**: 9 [PMID: 21199635]
 - 69 **Jabbari A**, Gonzalez ME, Franks AG Jr, Sanchez M. Laugier Hunziker syndrome. *Dermatol Online J* 2010; **16**: 23 [PMID: 21163174]
 - 70 **Sachdeva S**, Sachdeva S, Kapoor P. Laugier-hunziker syndrome: a rare cause of oral and acral pigmentation. *J Cutan Aesthet Surg* 2011; **4**: 58-60 [PMID: 21572687 DOI: 10.4103/0974-2077.79199]
 - 71 **Asati DP**, Tiwari S. Laugier-Hunziker syndrome. *Indian J Dermatol Venereol Leprol* 2011; **77**: 536 [PMID: 21727718 DOI: 10.4103/0378-6323.82422]
 - 72 **Ma DL**, Vano-Galvan S. Hyperpigmentation in Laugier-Hunziker syndrome. *CMAJ* 2011; **183**: 1402 [PMID: 21825050 DOI: 10.1503/cmaj.110211]
 - 73 **Kosari P**, Kelly KM. Asymptomatic lower lip hyperpigmentation from Laugier-Hunziker syndrome. *Cutis* 2011; **88**: 235-236 [PMID: 22272486]
 - 74 **Wondratsch H**, Feldmann R, Steiner A, Breier F. Laugier-hunziker syndrome in a patient with pancreatic cancer. *Case Rep Dermatol* 2012; **4**: 174-176 [PMID: 22949900 DOI: 10.1159/000342070]
 - 75 **Ergun S**, Saruhanoglu A, Migliari DA, Maden I, Tanyeri H. Refractory Pigmentation Associated with Laugier-Hunziker Syndrome following Er:YAG Laser Treatment. *Case Rep Dent* 2013; **2013**: 561040 [PMID: 24367727 DOI: 10.1155/2013/561040]
 - 76 **Bhojru B**, Paulus J. Macular pigmentation complicating irritant contact dermatitis and viral warts in Laugier-Hunziker syndrome. *Clin Exp Dermatol* 2016; **41**: 294-296 [PMID: 26508289 DOI: 10.1111/ced.12764]
 - 77 **Mahmood T**, Menter A. The Laugier-Hunziker syndrome. *Proc (Bayl Univ Med Cent)* 2015; **28**: 41-42 [PMID: 25552795 DOI: 10.1080/08998280.2015.11929182]
 - 78 **Fernandes D**, Ferrisse TM, Navarro CM, Massucato EM, Onofre MA, Bufalino A. Pigmented lesions on the mucosa: a wide range of diagnoses. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2015; **119**: 374-378 [PMID: 25687194 DOI: 10.1016/j.oooo.2014.11.015]
 - 79 **Lalosevic J**, Zivanovic D, Skiljevic D, Medenica L. Laugier-Hunziker syndrome--Case report. *An Bras Dermatol* 2015; **90**: 223-225 [PMID: 26312723 DOI: 10.1590/abd1806-4841.20153840]
 - 80 **Fajre X**, Aspillaga M, McNab M, Navarrete J, Sanhueza V, Benedetto J. [Laugier-Hunziker syndrome in a patient with Sjögren's syndrome: Report of one case]. *Rev Med Chil* 2016; **144**: 671-674 [PMID: 27552020 DOI: 10.4067/S0034-98872016000500017]
 - 81 **Barman PD**, Das A, Mondal AK, Kumar P. Laugier-Hunziker Syndrome Revisited. *Indian J Dermatol* 2016; **61**: 338-339 [PMID: 27293265 DOI: 10.4103/0019-5154.182429]
 - 82 **Voicu C**, Cărbunaru A, Berevoescu M, Mihalcea O, Clătici VG. A rare association between Laugier-Hunziker, Sjogren syndromes and other autoimmune disorders-case report and literature review. *Rom J Clin Exp Dermatol* 2016; **3**: 136-142
 - 83 **Kaçar N**, Yildiz CC, Demirkan N. Dermoscopic features of conjunctival, mucosal, and nail pigmentations in a case of Laugier-Hunziker syndrome. *Dermatol Pract Concept* 2016; **6**: 23-24 [PMID: 26937304 DOI: 10.5826/dpc.0601a07]
 - 84 **Abid MB**, Mughal P, Abid MA. Anaemia with Laugier-Hunziker Syndrome: a diagnostic dilemma. *Singapore Med J* 2017; **58**: 281 [PMID: 28536726 DOI: 10.11622/smedj.2017040]
 - 85 **Paul J**, Harvey VM, Sbicca JA, O'Neal B. Laugier-Hunziker syndrome. *Cutis* 2017; **100**: E17-E19 [PMID: 29121134]
 - 86 **Verma B**, Behra A, Ajmal AK, Sen S. Laugier-Hunziker Syndrome in a Young Female. *Indian Dermatol Online J* 2017; **8**: 148-150 [PMID: 28405564 DOI: 10.4103/2229-5178.202282]
 - 87 **Duong BT**, Winship I. The role of STK 11 gene testing in individuals with oral pigmentation. *Australas J Dermatol* 2017; **58**: 135-138 [PMID: 26768676 DOI: 10.1111/ajd.12443]
 - 88 **Cusick EH**, Marghoob AA, Braun RP. Laugier-Hunziker syndrome: a case of asymptomatic mucosal and acral hyperpigmentation. *Dermatol Pract Concept* 2017; **7**: 27-30 [PMID: 28515989 DOI: 10.5826/dpc.0702a05]
 - 89 **Müller S**. Melanin-associated pigmented lesions of the oral mucosa: presentation, differential diagnosis, and treatment. *Dermatol Ther* 2010; **23**: 220-229 [PMID: 20597941 DOI: 10.1111/j.1529-8019.2010.01319.x]
 - 90 **Meleti M**, Vescovi P, Mooi WJ, van der Waal I. Pigmented lesions of the oral mucosa and perioral tissues: a flow-chart for the diagnosis and some recommendations for the management. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; **105**: 606-616 [PMID: 18206403 DOI: 10.1016/j.tripleo.2007.07.047]
 - 91 **Eisen D**. Disorders of pigmentation in the oral cavity. *Clin Dermatol* 2000; **18**: 579-587 [PMID: 11134853 DOI: 10.1016/S0738-081X(00)00148-6]
 - 92 **Greenberg SA**, Schlosser BJ, Mirowski GW. Diseases of the lips. *Clin Dermatol* 2017; **35**: e1-e14 [PMID: 29289276 DOI: 10.1016/j.clindermatol.2017.11.003]
 - 93 **McLaughlin CC**, Wu XC, Jemal A, Martin HJ, Roche LM, Chen VW. Incidence of noncutaneous melanomas in the U.S. *Cancer* 2005; **103**: 1000-1007 [PMID: 15651058 DOI: 10.1002/cncr.20866]
 - 94 **Hicks MJ**, Flaitz CM. Oral mucosal melanoma: epidemiology and pathobiology. *Oral Oncol* 2000; **36**: 152-169 [PMID: 10745167 DOI: 10.1016/S1368-8375(99)00085-8]
 - 95 **Tomlinson IP**, Houlston RS. Peutz-Jeghers syndrome. *J Med Genet* 1997; **34**: 1007-1011 [PMID: 9429144 DOI: 10.1136/jmg.34.12.1007]
 - 96 **Hearle N**, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, Keller JJ, Westerman AM, Scott RJ, Lim W, Trimboth JD, Giardiello FM, Gruber SB, Offerhaus GJ, de Rooij FW, Wilson JH, Hansmann A, Möslin G, Royer-Pokora B, Vogel T, Phillips RK, Spigelman AD, Houlston RS. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clin Cancer Res* 2006; **12**: 3209-3215 [PMID: 16707622 DOI: 10.1158/1078-0432.CCR-06-0083]
 - 97 **Beggs AD**, Latchford AR, Vasen HF, Moslein G, Alonso A, Aretz S, Bertario L, Blanco I, Bülow S, Burn J, Capella G, Colas C, Friedl W, Möller P, Hes FJ, Järvinen H, Mecklin JP, Nagengast FM, Parc Y, Phillips RK, Hyer W, Ponz de Leon M, Renkonen-Sinisalo L, Sampson JR, Stormorken A, Tejpar S, Thomas HJ, Wijnen JT, Clark SK, Hodgson SV. Peutz-Jeghers syndrome: a systematic review and recommendations for management. *Gut* 2010; **59**: 975-986 [PMID: 20581245 DOI: 10.1136/gut.2009.198499]
 - 98 **Sarkar SB**, Sarkar S, Ghosh S, Bandyopadhyay S. Addison's disease. *Contemp Clin Dent* 2012; **3**: 484-486 [PMID: 23633816 DOI: 10.4103/0976-237X.107450]
 - 99 **Pichard DC**, Boyce AM, Collins MT, Cowen EW. Oral pigmentation in McCune-Albright syndrome. *JAMA Dermatol* 2014; **150**: 760-763 [PMID: 24671640 DOI: 10.1001/jamadermatol.2014.184]
 - 100 **Collins MT**, Singer FR, Eugster E. McCune-Albright syndrome and the extraskeletal manifestations of fibrous dysplasia. *Orphanet J Rare Dis* 2012; **7** Suppl 1: S4 [PMID: 22640971 DOI: 10.1186/1750-1172-7-S1-S4]
 - 101 **Cunha KS**, Barboza EP, Dias EP, Oliveira FM. Neurofibromatosis type I with periodontal manifestation. A case report and literature review. *Br Dent J* 2004; **196**: 457-460 [PMID: 15105854 DOI: 10.1038/sj.bdj.4811175]
 - 102 **Rosenbaum T**, Wimmer K. Neurofibromatosis type 1 (NF1) and associated tumors. *Klin Padiatr* 2014; **226**: 309-315 [PMID: 25062113 DOI: 10.1055/s-0034-1382021]
 - 103 **Salpea P**, Stratakis CA. Carney complex and McCune Albright syndrome: an overview of clinical manifestations and human molecular genetics. *Mol Cell Endocrinol* 2014; **386**: 85-91 [PMID: 24012779 DOI: 10.1016/j.mce.2013.08.022]
 - 104 **Kalev I**, Muru K, Teek R, Zordania R, Reimand T, Köbas K, Ounap K. LEOPARD syndrome with recurrent PTPN11 mutation

- Y279C and different cutaneous manifestations: two case reports and a review of the literature. *Eur J Pediatr* 2010; **169**: 469-473 [PMID: 19768645 DOI: 10.1007/s00431-009-1058-1]
- 105 **Zhang J**, Cheng R, Liang J, Ni C, Li M, Yao Z. Lentiginous phenotypes caused by diverse pathogenic genes (SASH1 and PTPN11): clinical and molecular discrimination. *Clin Genet* 2016; **90**: 372-377 [PMID: 27659786 DOI: 10.1111/cge.12728]
 - 106 **Suzuki R**, Irisawa A, Hikichi T, Takahashi Y, Kobayashi H, Kumakawa H, Ohira H. Cronkhite-Canada syndrome associated with myelodysplastic syndrome. *World J Gastroenterol* 2009; **15**: 5871-5874 [PMID: 19998513 DOI: 10.3748/wjg.15.5871]
 - 107 **Fan RY**, Wang XW, Xue LJ, An R, Sheng JQ. Cronkhite-Canada syndrome polyps infiltrated with IgG4-positive plasma cells. *World J Clin Cases* 2016; **4**: 248-252 [PMID: 27574615 DOI: 10.12998/wjcc.v4.i8.248]
 - 108 **Wen XH**, Wang L, Wang YX, Qian JM. Cronkhite-Canada syndrome: report of six cases and review of literature. *World J Gastroenterol* 2014; **20**: 7518-7522 [PMID: 24966624 DOI: 10.3748/wjg.v20.i23.7518]
 - 109 **Braun RP**, Baran R, Le Gal FA, Dalle S, Ronger S, Pandolfi R, Gaide O, French LE, Laugier P, Saurat JH, Marghoob AA, Thomas L. Diagnosis and management of nail pigmentations. *J Am Acad Dermatol* 2007; **56**: 835-847 [PMID: 17320240 DOI: 10.1016/j.jaad.2006.12.021]
 - 110 **Haneke E**, Baran R. Longitudinal melanonychia. *Dermatol Surg* 2001; **27**: 580-584 [PMID: 11442597 DOI: 10.1111/j.1524-4725.2001.01916.x]
 - 111 **Piraccini BM**, Dika E, Fanti PA. Tips for diagnosis and treatment of nail pigmentation with practical algorithm. *Dermatol Clin* 2015; **33**: 185-195 [PMID: 25828711 DOI: 10.1016/j.det.2014.12.002]
 - 112 **Ronger S**, Touzet S, Ligeron C, Balme B, Viallard AM, Barrut D, Colin C, Thomas L. Dermoscopic examination of nail pigmentation. *Arch Dermatol* 2002; **138**: 1327-1333 [PMID: 12374538 DOI: 10.1001/archderm.138.10.1327]
 - 113 **Haenssle HA**, Blum A, Hofmann-Wellenhof R, Kreusch J, Stolz W, Argenziano G, Zalaudek I, Brehmer F. When all you have is a dermatoscope- start looking at the nails. *Dermatol Pract Concept* 2014; **4**: 11-20 [PMID: 25396079 DOI: 10.5826/dpc.0404a02]
 - 114 **Möhrle M**, Häfner HM. Is subungual melanoma related to trauma? *Dermatology* 2002; **204**: 259-261 [PMID: 12077517 DOI: 10.1159/000063354]
 - 115 **Benati E**, Ribero S, Longo C, Piana S, Puig S, Carrera C, Cicero F, Kittler H, Deinlein T, Zalaudek I, Stolz W, Scope A, Pellacani G, Moscarella E, Piraccini BM, Starace M, Argenziano G. Clinical and dermoscopic clues to differentiate pigmented nail bands: an International Dermoscopy Society study. *J Eur Acad Dermatol Venereol* 2017; **31**: 732-736 [PMID: 27696528 DOI: 10.1111/jdv.13991]

P- Reviewer: Aksoy B, Fiorentini G, Jakhar D, Ziogas DE
S- Editor: Ji FF **L- Editor:** Filipodia **E- Editor:** Wu YXJ



Research progress on signaling pathways in cirrhotic portal hypertension

Wen Xu, Ping Liu, Yong-Ping Mu

Wen Xu, Ping Liu, Yong-Ping Mu, Institute of Liver Diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (TCM), Shanghai 201203, China

Wen Xu, Ping Liu, Yong-Ping Mu, Key Laboratory of Liver and Kidney Disease of the Ministry of Education, Shanghai University of TCM, Shanghai 201203, China

Wen Xu, Ping Liu, Yong-Ping Mu, Clinical key laboratory of TCM of Shanghai, Shanghai 201203, China

ORCID number: Wen Xu (0000-0003-2132-9537); Ping Liu (0000-0002-6152-4508); Yong-Ping Mu (0000-0002-4533-5563).

Author contributions: Xu W wrote the paper and performed the research; Liu P and Mu YP designed the study.

Supported by the National Natural Science Foundation of China, No. 81573948.

Conflict-of-interest statement: We declare that we have no conflicts of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Yong-Ping Mu, PhD, Attending Doctor, Department of Gastroenterology and Hepatology, Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine (TCM), 528 Zhangheng Road, Pudong District, Shanghai 201203, China. yymu8888@126.com
Telephone: +86-21-20256526
Fax: +86-21-20256521

Received: May 28, 2018

Peer-review started: May 28, 2018

First decision: July 3, 2018

Revised: July 23, 2018

Accepted: August 3, 2018

Article in press: August 4, 2018

Published online: September 26, 2018

Abstract

Portal hypertension (PHT) is an important consequence of liver cirrhosis, which can lead to complications that adversely affect a patient's quality of life and survival, such as upper gastrointestinal bleeding, ascites, and portosystemic encephalopathy. In recent years, advances in molecular biology have led to major discoveries in the pathological processes of PHT, including the signaling pathways that may be involved: PI3K-AKT-mTOR, RhoA/Rho-kinase, JAK2/STAT3, and farnesoid X receptor. However, the pathogenesis of PHT is complex and there are numerous pathways involved. Therefore, the targeting of signaling pathways for medical management is lagging. This article summarizes the progress that has been made in understanding the signaling pathways in PHT, and provides ideas for treatment of the disorder.

Key words: PI3K-AKT-mTOR; Portal hypertension; Rho-associated kinases; Liver cirrhosis; Signaling pathways; Farnesoid X-activated receptors; JAK2/STAT3

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Portal hypertension (PHT) is a syndrome of portal venous system hemodynamics in liver cirrhosis. Current therapeutic options are often insufficient to prevent progression of the disease. We therefore may find more effective clinical treatments by understanding the signal pathways involved in the disease. This paper is an up-to-date and thorough review of the signaling

pathways that may be involved in the pathogenesis of PHT in liver cirrhosis.

Xu W, Liu P, Mu YP. Research progress on signaling pathways in cirrhotic portal hypertension. *World J Clin Cases* 2018; 6(10): 335-343 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/335.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.335>

INTRODUCTION

Portal hypertension (PHT), the main consequence of cirrhosis, can lead to complications, such as variceal hemorrhage, ascites, and portosystemic encephalopathy. These complications may cause both diminished quality of life and mortality, and also may necessitate liver transplantation^[1-3]. PHT is characterized by abnormally elevated intrahepatic venous pressure, which is due to various etiological factors. Increased intrahepatic vascular resistance (IHVR) and increased portal venous blood flow are the major pathological processes in the development of PHT^[4]. IHVR is mainly determined by liver fibrosis, intrahepatic vasoconstriction, intrahepatic angiogenesis, and abnormal blood flow. Narrowing of intrahepatic microvessels, caused by fibrosis, can increase resistance and be responsible, in part, for increased responsiveness of these venules to vasoconstrictive substances^[5]. Angiogenesis, the formation of new vessels from preexisting vasculature, is an important pathophysiological feature of PHT that enhances IHVR^[4,6]. Another feature of PHT is the development of hyper-dynamic splanchnic circulation, with an increased blood flow in splanchnic organs that drain into the portal vein and a consequent increase in portal venous inflow^[7]. The increased splanchnic and portal blood flow further elevates portal pressure. Although the pathophysiology of PHT has been studied extensively, its precise mechanisms are undefined.

An improved understanding of the molecular mechanisms of PHT is crucial to developing effective treatment strategies. Despite the incomplete knowledge of PHT pathophysiology, certain molecular pathways have been identified. The current review reveals that PI3K-AKT-mTOR, RhoA/Rho-kinase, JAK2/STAT3, farnesoid X receptor (FXR) and other signaling pathways might be targetable in the treatment of PHT. We believe that this summary of the roles of the signaling pathways in PHT may help investigators find relevant targets for PHT treatment.

PI3K-AKT-MTOR SIGNALING

Overview of mTOR

The kinase known as targets of rapamycin (TOR), belonging to the phosphatidylinositol kinase-related kinase, is an evolutionarily conserved protein kinase that

was first found in yeast. The homologous substance of TOR in mammals is referred to collectively as mTOR^[8]. mTOR is a ubiquitously expressed serine kinase that regulates cell growth and proliferation, and mTOR signaling plays a role in immunological processes, angiogenesis and fibrogenesis^[9,10]. The mTOR signaling pathway is highly active in the evolution of PHT^[11]. Mejias *et al*^[9] found that rapamycin could reduce portal pressure by blocking mTOR and thus alleviate hyperdynamic visceral circulation, an effect that may be due to rapamycin's inhibition of lymphocyte proliferation, neovascularization, and fibrosis.

mTOR regulates fibrosis

Mammals have two mTOR complexes: mTORC1 and mTORC2. mTORC1 is an important regulator of ribosome production and translation and has two downstream effectors, including the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and ribosomal protein S6 kinase. mTORC1 integrates signaling from growth factor receptors, then activates the p70 ribosomal protein S6 kinase (p70S6K) by phosphorylation and inhibits the eukaryotic initiation factor 4E-BP1. Thus, mTORC1 forms two different signaling pathways to regulate mRNA translation and to control protein synthesis^[12,13]. mTORC2 phosphorylates the serine/threonine protein kinase Akt/protein kinase B at serine residue Ser473^[14] and participates in the regulation of phosphorylation and activation of cytoskeletal actin, protein kinase B (Akt/protein kinase B), protein kinase C, and glucocorticoid-induced protein kinase 1 in serum^[8]. *In vivo*, growth factors, mitogen and other hormones lead to the activation of p70S6K by phosphorylation of mTOR through phosphatidylinositol 3-kinase-related kinase (PI3K)-Akt pathways, which upregulate the expression of cyclin D1, D3 and E to control cell-cycle progression^[10]. One effect of this activity is increased proliferation of hepatic stellate cells (HSCs). Activation of HSCs increases the contractility of intrahepatic vessels, thereby increasing resistance to liver blood flow. p70S6K phosphorylation stimulates the production of the synthesis of collagen and other extracellular matrix components, predominately type I collagen^[15]. It is generally accepted that the activation of HSCs leads to fibrosis, which is one of the important steps in the development of PHT^[16]. It has been proposed that activation of mTOR promotes both HSC proliferation as well as the synthesis of extracellular matrix, which accelerates liver fibrosis and the development of PHT^[15,16].

PI3K-Akt regulates mTOR signaling involved in angiogenesis

Akt, also called protein kinase B, is a threonine protein kinase akin to the PI3K protein family. One of the functions of Akt is direct phosphorylation of mTOR. Another is maintaining Rheb's GTP-binding state by inactivating tuberous sclerosis complex 2 to enhance

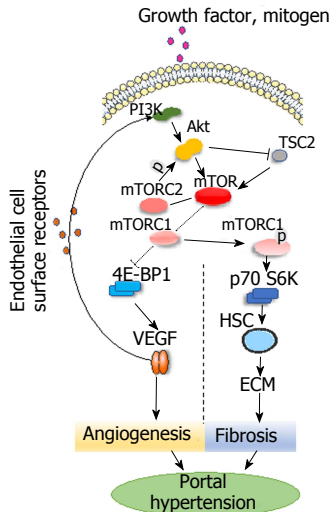


Figure 1 PI3K-AKT-mTOR signaling pathways in the development of hepatic portal hypertension. PI3K-AKT-mTOR: *In vivo*, growth factors, mitogens and hormones interact with PI3K, which activates Akt that then activates mTOR. Mammals have two mTOR complexes: mTORC1 and mTORC2. Akt can phosphorylate mTORC1 and then activate p70S6K via phosphorylation to inhibit 4E-BP1. Activated p70S6K promotes the proliferation of HSCs and stimulates both the production of ribosomes and the synthesis of collagen and other extracellular matrix constituents. mTORC1 can inhibit 4E-BP1, promote the splitting of VEGF, and regulate angiogenesis. Akt can inactivate tuberous sclerosis complex 2 and enhance mTOR activity. mTORC2 regulates Akt. Akt: Protein kinase B; ECM: Extracellular matrix; HSC: Hepatic stellate cell; mTOR: Mammalian targets of rapamycin; mTORC: Mammalian targets of rapamycin complex; p70S6K: p70 ribosomal protein S6 Kinase; TSC2: Tuberous sclerosis complex 2; VEGF: Vascular endothelial growth factor; 4E-BP1: 4E-binding protein-1.

mTOR activity^[17,18]. Akt is an important upstream mediator of mTOR and is regulated by mTORC2^[14]. In previous studies^[15,19], the Akt/mTOR signaling pathway was activated in bile duct ligation-induced cirrhotic rats and was implicated in the activation of HSCs. The Akt/mTOR signaling pathway is the major downstream effector of PI3K and regulates cell growth, proliferation, motility and apoptosis^[14,17]. While AKT directly affects mTOR, mTORC1 reciprocally regulates the growth factor responsiveness of PI3K and Akt through feedback inhibition^[20,21]. The direct phosphorylation of 4E-BP1 by mTORC1 reportedly initiates translation of hypoxia inducible factor-1 α (HIF-1 α), which promotes the expression of vascular endothelial growth factor (VEGF), thereby regulating angiogenesis in physiological and pathological conditions^[22,23]. Under certain conditions, VEGF and endothelial cell surface receptors bind to activate the PI3K-Akt signaling pathway and further activate mTOR kinase, thereby enhancing portal pressure (Figure 1). It may be effective for inhibiting the development of PHT by inhibiting Akt or mTOR directly. However, the specific effect needs further investigation.

RHOA/RHO-KINASE SIGNALING

Activation of the RhoA/Rho-kinase signaling pathway,

by participating in vasoconstriction and vasodilation responses^[24-26], is one of the key mechanisms of PHT development.

Overview of RhoA

The Ras homolog gene family member A (RhoA) is a member of the small molecular weight G proteins in the Ras superfamily. RhoA signaling participates in many cellular responses, including cell contraction, adhesion, proliferation and migration^[27]. RhoA, a member of the GTP-binding protein Rho GTPase family, circulates between activated GTP-RhoA and stationary GDP-RhoA. RhoA-GDP and RhoA-GTP are interconverted by a dephosphorylation/phosphorylation process and then trigger or terminate a cellular cascade activation/reaction, acting as a “molecular switch”. Only RhoA activates Rho-kinase in the membrane-bound activated state and has downstream effects^[28,29].

RhoA/Rho-kinase signaling and portal pressure

Geranylgeranyl pyrophosphate (GGPP), as a key substance in the transfer of RhoA to cell membranes, plays a role in the activation of the RhoA/Rho kinase signaling pathway. RhoA is linked to GGPP, a byproduct of cholesterol synthesis, to “lipidize” GGPP so it can be inserted into the cell membrane to form membrane-bound RhoA (a small GTPase protein on the cell surface) and activate the RhoA/Rho kinase pathway by binding to angiotensin^[26,30,31]. Trebicka *et al.*^[32] found that statins, 3-hydroxy-3-methyl-glutaryl CoA reductase inhibitors, inhibited the expression of 3-hydroxy-3-methyl-glutaryl CoA reductase, which downregulated the expression of GGPP and then blocked the RhoA/Rho-kinase signaling pathway, leading to reduced activation of HSC. Liu *et al.*^[33] found that sodium ferulate can affect the activation of RhoA and the contraction of activated HSC by reducing the synthesis of GGPP in HSCs in liver cirrhosis. These actions reduce the intrahepatic resistance in cirrhotic rats. Zhang *et al.*^[34] found that a selective agonist of estrogen receptor β could reduce IHVR by reducing RhoA expression, thus inhibiting the myosin light chain activity and increasing the levels of endothelial nitric oxide synthase (eNOS), which leads to decreased portal vein pressure in cirrhotic ovariectomized rats.

Wei *et al.*^[35] demonstrated that sodium ferulate inhibits the hepatic RhoA/Rho-kinase signaling pathway and increases eNOS synthesis, eventually leading to reduced hepatic portal pressure in cirrhotic rats. This reaction indicates that the RhoA/Rho-kinase signaling pathway is involved in the formation of PHT. The activation of Rho kinase increases portal pressure in two ways: first, by inhibition of myosin light chain phosphatase, which produces a downstream effect that increases smooth muscle contractions^[29,36]. Myosin phosphatase, myosin light chain, adducin, mono serine protein kinase, and protein kinase C-protein inhibitor protein (CPI-17) are substrates of Rho kinase. The

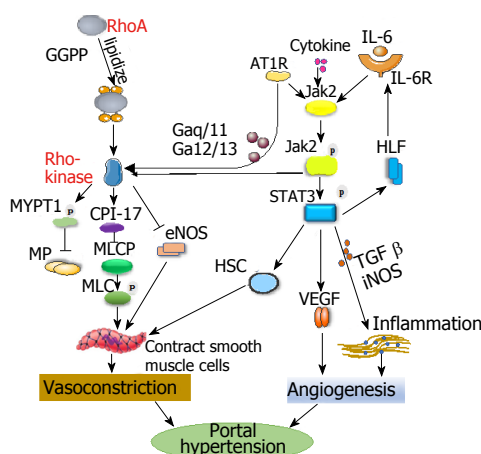


Figure 2 Proposed signaling pathways in the development of hepatic portal hypertension. RhoA/Rho-kinase: GGPP “lipidizes” RhoA to form membrane-bound RhoA and to activate Rho-kinase. AT1R stimulates JAK2 to phosphorylate and then activate Rho-kinase. Activated Rho-kinase phosphorylates the myosin targeting subunit 1, which inactivates myosin phosphatase. The inactivation of myosin phosphatase increases the phosphorylation of myosin light chain and promotes vasoconstriction. CPI-17 combines with myosin light chain phosphatase to inhibit the activation of the enzymes and promote the contraction of smooth muscle cells. The activation of Rho-kinase could downregulate the expression of eNOS and reduce its activity. The coupling of AT1R to Gαq/11 and Gα12/13 promotes Rho-kinase activation, which is involved in extracellular matrix production. JAK2/STAT3: Cytokines activate the JAK2/STAT3 pathway. Enhanced JAK2/STAT3 signaling upregulates TGF-β and iNOS expression and accelerates intestinal inflammation, which aggravates endothelial dysfunction and angiogenesis. IL-6 binds to its receptor, activates JAK2, phosphorylates it, and then causes the phosphorylation of STAT3 in cells, thereby activating HSC and promoting hypoxia inducible factor expression. These events can upregulate the activation of IL-6 and reactivate the STAT3 pathway, which forms a loop, constantly activating HSC, causing vessel contraction, and ultimately increasing portal pressure. AT1R: Angiotensin-type II receptor 1; CPI-17: C-protein inhibitor protein; eNOS: Endothelial nitric oxide synthase; GGPP: Geranylgeranyl pyrophosphate; HIF: Hypoxia inducible factor; HSC: Hepatic stellate cell; IL-6: Interleukin-6; IL-6R: Interleukin-6 receptor; iNOS: Inducible nitric oxide synthase; JAK2: Janus kinase 2; MLCP: Myosin light chain phosphatase; MP: Myosin phosphatase; MYPT1: Myosin phosphatase target subunit 1; RhoA: Ras homolog gene family member A; STAT3: Signal transducers and activators of transcription; TGF-β: Transforming growth factor β; VEGF: Vascular endothelial growth factor.

most studied Rho kinase substrates involved in PHT formation are myosin phosphatase, myosin light chain and CPI-17. Activation of Rho kinase leads to the phosphorylation of myosin targeting subunit 1, which inactivates myosin phosphatase. Inactivation of myosin phosphatase did not lead to dephosphorylation of myosin light chain. This, in turn, leads to an increase in cytoplasmic myosin light chain phosphorylation and increased crosslinking of myosin, thereby promoting vasoconstriction. CPI-17 was combined with myosin light chain phosphatase to inhibit the activation of myosin light chain phosphatase and promote the contraction of smooth muscle cells^[37]. The second way of increasing portal pressure is the downregulation of eNOS expression and reduction of its activity^[38]. The activity of eNOS, which is another downstream target of the RhoA/Rho-kinase pathway^[39] that is involved

in regulating portal pressure, may be negatively-regulated by RhoA/Rho-eNOS activity; this effect causes the relaxation of vascular smooth muscle and plays a key role in maintaining the steady state of the vascular wall^[40]. Rosado *et al.*^[41] found that terutroban, a thromboxane-A2/prostaglandin-endoperoxide receptor antagonist, reduced portal pressure by inhibiting Rho-kinase activity and enhancing eNOS-dependent vasodilatation in cirrhotic rats.

In addition, coupling of the angiotensin-type II receptor 1 (AT1R) to heterotrimeric G proteins (Gαq/11 and Gα12/13) allows stimulation and activation of the RhoA/Rho kinase pathway, which is involved in extracellular matrix production; these reactions are crucial in the development of fibrosis and PHT^[42,43] (Figure 2).

Thus, the RhoA/Rho kinase signaling pathway is important in regulating IHVR and increasing portal pressure. It may be effective for inhibiting vasoconstriction by inhibiting the key mechanisms in the RhoA/Rho-kinase signaling pathway, such as phosphorylation of myosin light chain directly. However, the specific effect needs further investigation.

JAK2/STAT3 SIGNALING

Overview of JAK2/STAT3

The janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathway participates in numerous pathophysiological processes. Various cytokines produce corresponding tissue and cell-specific effects through combinations of four members of the JAK family and seven members of the STAT family. JAK2, the most conserved isoform of the JAK family, acts directly on downstream STAT3 in JAK/STAT signaling^[44]. The JAK2/STAT3 pathway interacts with numerous cytokines that can be activated by angiotensin II, interferon-γ, transforming growth factor β (TGF-β), etc. Upon activation, STAT3 is phosphorylated to become p-STAT3, which can form homodimers or heterodimers, translocate to the nucleus, and bind to specific regulatory sequences on DNA^[45]; these products then regulate the expression of VEGF, TGF-β, eNOS, and inducible nitric oxide synthase (iNOS), a process that is important in cell proliferation, fibrosis and angiogenesis^[46-48]. It has been found that JAK2/STAT3 signaling is overactive in PHT and involved in its development^[44,49].

JAK2/STAT3 regulate angiogenesis and vasoconstriction

Angiogenesis is considered one of the factors in the development of PHT. Pathological angiogenesis may lead to increased intrahepatic circulatory resistance, and subsequently cause PHT and its severe complications such as variceal bleeding. VEGF is one of the cytokines involved in the development of angiogenesis^[50,51]. Wang *et al.*^[52] found that AG490, a specific antagonist of JAK2, decreased the formation

of new blood vessels in the liver by inhibiting the expression of JAK2/STAT3 signaling, which suppressed the activation of HSCs and reduced the expression of VEGF. JAK2/STAT3 signaling may stimulate vascular hyperplasia and decrease vascular tone by increasing the expression of VEGF, thus promoting the development of PHT. Interleukin-17 activates HSCs by STAT3 signaling, and activation of HSCs plays a key role in the formation of PHT^[53]. IL-6 also activates the STAT3 pathway. IL-6 binds to its receptor, activates JAK2, phosphorylates it, and then causes the phosphorylation of STAT3 in cells; these reactions result in the activation of HSCs and promotion of HLF expression, which can upregulate the activation of IL-6 and reactivate the STAT3 pathway. Thus, a loop is formed that constantly activates HSCs, ultimately causing hepatic vessel contractions that lead to increased portal pressure^[54].

Endogenous angiogenesis and increased eNOS-derived nitric oxide levels in PHT have been considered important in the maintenance of PHT, and JAK2/STAT3 has been reported to promote eNOS protein expression^[52,55].

Visceral inflammation is usually present in patients with PHT, especially in those with advanced PHT, and the inflammation can accentuate endothelial dysfunction and angiogenesis^[56]. Relevant studies^[52] have shown that enhanced JAK2/STAT3 signaling accelerates intestinal inflammation in PHT rats by upregulating TGF- β and iNOS expression. These findings suggest that JAK2/STAT3 participates in the pathogenesis of PHT by regulating factors such as VEGF, eNOS, TGF- β and iNOS.

Recent studies^[49,57] have found a relationship between the JAK2/STAT3 signaling pathway and RhoA/Rho-kinase signaling. JAK2 was shown to establish a link between AT1R and the RhoA/Rho-kinase pathway in smooth muscle cells. AT1R stimulates JAK2 to phosphorylate and then induce Arhgef1, the nucleotide exchange factor responsible for activating RhoA, which activates Rho-kinase, eventually leading to vasoconstriction (Figure 2).

FXR PATHWAY

Activation of the above three signaling pathways mainly promotes the formation of PHT, while activation of the FXR pathway can reduce PHT.

Overview of FXR

FXR is a bile acid-reactive transcription factor and member of the nuclear receptor superfamily (NR1H4)^[58] that is highly expressed in the liver and small intestine. Like other nuclear receptor members, FXR has an N-terminal activation domain (AF1) that interacts with a cofactor, a conserved DNA-binding domain, and a unique ligand-binding domain, allowing receptor dimerization and the C-terminal activation domain (AF2) to regulate the interaction^[59,60]. In recent years,

it has been recognized that the FXR plays a key role in the metabolism of bile acids and intestinal flora, as bile acids and FXR closely interact^[61,62]. In many liver diseases, FXR is involved in fibrosis, and in the gastrointestinal tract it has immunological activity and vascular function^[63]. FXR is a major transcriptional regulator of genes involved in bile acid homeostasis and is a regulator of lipid and carbohydrate metabolism in the normal liver^[64].

Studies have documented deficiency of the FXR system in rat cirrhosis models, and FXR agonists can improve PHT through various pathways by activating FXR, which is related to vascular regulation and PHT^[64]. Small heterodimer partner, the direct target gene of FXR, is a downstream orphan nuclear receptor for FXR that inhibits many other nuclear receptors, including cholesterol 7 α -hydroxylase^[65]; it is increased after stimulation of FXR agonist INT-747 in a cirrhosis model^[64]. The beneficial effects of this process involve hemodynamic changes of intrahepatic endothelial dysfunction and the molecular repair of intrahepatic eNOS activity^[64].

FXR regulates vasodilation

In cirrhotic PHT, a decrease in intrahepatic eNOS activity is key to the pathogenesis of increased IHVR^[6], which is mainly caused by impaired hepatic vascular dilatation through the combination of reduced eNOS activity and nitric oxide bioavailability^[66,67]. FXR affects blood vessel nitric oxide signaling by increasing eNOS concentrations^[68]. In animal models of cirrhosis, obeticholic acid, a steroid FXR agonist and chenodeoxycholic acid derivative, restored intrahepatic eNOS levels and enhanced the expression of dimethylarginine dimethylamidohydrolase-1 (DDAH-1). Increases in DDAH-1 reduce the level of systemic asymmetric dimethylarginine (ADMA), thus upregulating the expression of eNOS, and then modulating nitric oxide production, which eventually results in decreased portal vein pressure^[64,69]. ADMA is a competitive inhibitor of the eNOS substrate L-arginine and decreases eNOS phosphorylation of vascular endothelial cells *in vitro* and *in vivo*^[70]. DDAH-1 is a key enzyme that metabolizes liver ADMA^[69]. In addition, studies have found that alanine-glyoxylate aminotransferase-2 (AGXT2), which is present in mitochondria, is involved in the metabolism of ADMA. Rodionov *et al.*^[71] found that ADMA levels were significantly reduced in the liver and plasma of AGXT2-overexpressing mice. Caplin *et al.*^[72] found that the ADMA levels were significantly increased in the plasma of AGXT2 knockout mice. The FXR agonist PX20606 upregulates GTP cyclohydrolase-1, a key enzyme in the synthesis of cofactor tetrahydrobiopterin (BH4), resulting in increased amounts of BH4; sufficient concentrations of BH4 are essential for eNOS to catalyze nitric oxide. The enhancement of eNOS activity and BH4 has improved nitric oxide-mediated sinus endothelial function^[68]. FXR agonism also decreases inflammatory

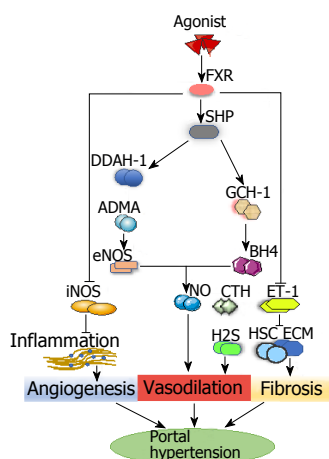


Figure 3 FXR-mediated pathways in angiogenesis, vasodilation and fibrosis during portal hypertension. FXR pathway: FXR agonist enhances the expression of FXR, which enhances the expression of DDAH-1 and GTP cyclohydrolase-1. DDAH-1 can reduce the levels of ADMA and upregulate the expression of eNOS. GTP cyclohydrolase-1 can increase the expression of BH4. This synergistic enhancement of eNOS and BH4 activity has improved nitric oxide-mediated sinus endothelial function. In addition, FXR agonism reduces the inflammatory response by reducing the expression of iNOS and cyclooxygenase 2 to improve PHT. FXR agonism decreases the expression of endothelin-1 and then inhibits HSC proliferation and extracellular matrix synthesis, which can ameliorate fibrosis. Repressed endothelin-1 can increase the production of cystathionase-mediated hydrogen sulfide, which can cause vasodilation. ADMA: Asymmetric dimethylarginine; BH4: Tetrahydrobiopterin; DDAH-1: Dimethylarginine dimethylamidohydrolase-1; ECM: Extracellular matrix; eNOS: Endothelial nitric oxide synthase; FXR: Farnesoid X receptor; GCH-1: GTP cyclohydrolase-1; H₂S: Hydrogen sulfide; HSC: Hepatic stellate cell; iNOS: Inducible nitric oxide synthase; NO: Nitric Oxide; SHR: Small heterodimer partner.

responses, and the associated development of PHT, by reducing the expression of iNOS and cyclooxygenase 2^[73].

FXR regulates endothelial dysfunction

The increase of internal vascular resistance caused by endothelial dysfunction is one of the factors in PHT formation. In some studies^[68,74], endothelial dysfunction was mainly due to increased activity of vasoconstrictive factor (endothelin-1) and impaired nitric oxide signaling in sinusoidal endothelial cells. Endothelin-1 is a powerful vasoconstrictor in hepatic sinuses^[75]. In liver damage, enhanced synthesis of endothelin-1 has activated HSCs, which promoted sine refactoring^[6,68] and increased the amount of phosphorylated moesin, a marker of HSC contraction^[75]. Endothelin-1 not only induces HSC proliferation and contraction, with consequent sinusoidal vasoconstriction, but also increases extracellular matrix synthesis^[68]. FXR agonism ameliorated intrahepatic resistance^[75] by decreasing the expression of endothelin-1^[76], which inhibited endothelin-1-mediated contraction of hepatic stellate cell and increased the production of liver cystathionase-mediated hydrogen sulfide^[68]. Cystathionase is a key enzyme for the local production of hydrogen sulfide, a potent nitric oxide-independent vasodilator^[77] (Figure 3).

The above four signaling pathways have been extensively studied, however some novel signaling pathways need further study. Recent studies have shown that the increase in reactive oxygen leads to increased expression of Nuclear Factor-E2-related factor 2/Heme Oxygenase 1 (Nrf2/HO-1) in portal hypertensive rats. HO-1 is regulated by Nrf2 and can be used to induce hypovascular reactivity or as a vasodilator, which also results in increased expression of VEGF in the mesenteric artery of patients with PHT, which then forms the collateral portal vessels^[78]. Therefore, reducing the portal pressure by inhibiting Nrf2/HO-1 signaling is effective. Zeng *et al.*^[79] found that Kruppel-like factor 2 inhibits the proliferation of sinusoidal endothelial cells and vascular formation by downregulating extracellular signal-regulated kinases 1/2 signaling, which inhibits the process of angiogenesis, and then ameliorates elevated portal pressure. Gao *et al.*^[80] found that combining celecoxib and octreotide not only significantly inhibited the expression of phospho-extracellular regulated kinase (p-ERK), HIF-1 α , and VEGF, but also prevented HIF-1 α from binding to VEGF by blocking the MAPK-ERK signaling pathway, which synergistically improves hepatic fibrosis and portal hypertonia in thioacetamide-induced cirrhotic rats by inhibiting both intrahepatic and extrahepatic angiogenesis. The mechanism responsible may be inactivation of the p-ERK-HIF-1 α -VEGF signaling pathway.

CONCLUSION

In recent years, progress has been made in understanding how PHT develops and in the development of potential nonsurgical therapeutic approaches to PHT. The limitations of current PHT treatments are directed towards the outcomes of PHT, such as bleeding varices, and not towards the underlying causes. Several signaling pathways are involved in the pathogenesis of PHT, including PI3K-AKT-mTOR, RhoA/Rho kinase, JAK2/STAT3 and FXR. These pathways affect the development of PHT by regulating IHVR and portal vein blood flow. In addition, some newly discovered signaling pathways may be novel therapeutic targets, such as p-ERK-HIF-1 α -VEGF signaling. Efforts directed toward modifying the pathways should be explored for the effective prevention and treatment of PHT, however the pathways are incompletely understood and deserve further investigation.

REFERENCES

- 1 Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. *Gastroenterology* 2008; **134**: 1715-1728 [PMID: 18471549 DOI: 10.4274/Turk]
- 2 Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet* 2008; **371**: 838-851 [PMID: 18328931 DOI: 10.1016/S0140-6736(08)60383-9]
- 3 Garcia-Tsao G. Portal hypertension. *Curr Opin Gastroenterol* 2001; **17**: 281-290 [PMID: 17031170]
- 4 Bosch J, Abraldes JG, Fernández M, García-Pagán JC. Hepatic

- endothelial dysfunction and abnormal angiogenesis: new targets in the treatment of portal hypertension. *J Hepatol* 2010; **53**: 558-567 [PMID: 20561700 DOI: 10.1016/j.jhep.2010.03.021]
- 5 **Zhou Q**, Hennenberg M, Trebicka J, Jochem K, Leifeld L, Biecker E, Sauerbruch T, Heller J. Intrahepatic upregulation of RhoA and Rho-kinase signalling contributes to increased hepatic vascular resistance in rats with secondary biliary cirrhosis. *Gut* 2006; **55**: 1296-1305 [PMID: 16492715 DOI: 10.1136/gut.2005.081059]
- 6 **Fernandez M**. Molecular pathophysiology of portal hypertension. *Hepatology* 2015; **61**: 1406-1415 [PMID: 25092403 DOI: 10.1002/hep.27343]
- 7 **Fernandez M**, Mejias M, Garcia-Pras E, Mendez R, Garcia-Pagan JC, Bosch J. Reversal of portal hypertension and hyperdynamic splanchnic circulation by combined vascular endothelial growth factor and platelet-derived growth factor blockade in rats. *Hepatology* 2007; **46**: 1208-1217 [PMID: 17654489 DOI: 10.1002/hep.21785]
- 8 **Jung CH**, Ro SH, Cao J, Otto NM, Kim DH. mTOR regulation of autophagy. *FEBS Lett* 2010; **584**: 1287-1295 [PMID: 20083114 DOI: 10.1016/j.febslet.2010.01.017]
- 9 **Mejias M**, Garcia-Pras E, Gallego J, Mendez R, Bosch J, Fernandez M. Relevance of the mTOR signaling pathway in the pathophysiology of splenomegaly in rats with chronic portal hypertension. *J Hepatol* 2010; **52**: 529-539 [PMID: 20206401 DOI: 10.1016/j.jhep.2010.01.004]
- 10 **Neef M**, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. *J Hepatol* 2006; **45**: 786-796 [PMID: 17050028 DOI: 10.1016/j.jhep.2006.07.030]
- 11 **Patsenker E**, Schneider V, Ledermann M, Saegesser H, Dorn C, Hellerbrand C, Stickel F. Potent antifibrotic activity of mTOR inhibitors sirolimus and everolimus but not of cyclosporine A and tacrolimus in experimental liver fibrosis. *J Hepatol* 2011; **55**: 388-398 [PMID: 21168455 DOI: 10.1016/j.jhep.2010.10.044]
- 12 **Sarbassov DD**, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 2005; **17**: 596-603 [PMID: 16226444 DOI: 10.1016/j.ceb.2005.09.009]
- 13 **Choo AY**, Yoon SO, Kim SG, Roux PP, Blenis J. Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proc Natl Acad Sci USA* 2008; **105**: 17414-17419 [PMID: 18955708 DOI: 10.1073/pnas.0809136105]
- 14 **Sarbassov DD**, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**: 1098-1101 [PMID: 15718470 DOI: 10.1126/science.1106148]
- 15 **Gäbele E**, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schrum LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. *J Biol Chem* 2005; **280**: 13374-13382 [PMID: 15677443 DOI: 10.1074/jbc.M409444200]
- 16 **Thoen LF**, Guimarães EL, Dollé L, Mannaerts I, Najimi M, Sokal E, van Grunsven LA. A role for autophagy during hepatic stellate cell activation. *J Hepatol* 2011; **55**: 1353-1360 [PMID: 21803012 DOI: 10.1016/j.jhep.2011.07.010]
- 17 **Yang Q**, Guan KL. Expanding mTOR signaling. *Cell Res* 2007; **17**: 666-681 [PMID: 17680028 DOI: 10.1038/cr.2007.64]
- 18 **Chen Y**, Klionsky DJ. The regulation of autophagy - unanswered questions. *J Cell Sci* 2011; **124**: 161-170 [PMID: 21187343 DOI: 10.1242/jcs.064576]
- 19 **Wang W**, Yan J, Wang H, Shi M, Zhang M, Yang W, Peng C, Li H. Rapamycin ameliorates inflammation and fibrosis in the early phase of cirrhotic portal hypertension in rats through inhibition of mTORC1 but not mTORC2. *PLoS One* 2014; **9**: e83908 [PMID: 24404143 DOI: 10.1371/journal.pone.0083908]
- 20 **O'Reilly KE**, Rojo F, She QB, Solit D, Mills GB, Smith D, Lane H, Hofmann F, Hicklin DJ, Ludwig DL, Baselga J, Rosen N. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. *Cancer Res* 2006; **66**: 1500-1508 [PMID: 16452206 DOI: 10.1158/0008-5472.CAN-05-2925]
- 21 **Zhang HH**, Lipovsky AI, Dibble CC, Sahin M, Manning BD. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. *Mol Cell* 2006; **24**: 185-197 [PMID: 17052453 DOI: 10.1016/j.molcel.2006.09.019]
- 22 **Geerts AM**, Vanheule E, Van Vlierberghe H, Leybaert L, Van Steenkiste C, De Vos M, Colle I. Rapamycin prevents mesenteric neo-angiogenesis and reduces splanchnic blood flow in portal hypertensive mice. *Hepatol Res* 2008; **38**: 1130-1139 [PMID: 18564143 DOI: 10.1111/j.1872-034X.2008.00369.x]
- 23 **Karar J**, Maity A. PI3K/AKT/mTOR Pathway in Angiogenesis. *Front Mol Neurosci* 2011; **4**: 51 [PMID: 22144946 DOI: 10.3389/fnmol.2011.00051]
- 24 **Lauriol J**, Keith K, Jaffré F, Couvillon A, Saci A, Goonasekera SA, McCarthy JR, Kessinger CW, Wang J, Ke Q, Kang PM, Molkentin JD, Carpenter C, Kontaridis MI. RhoA signaling in cardiomyocytes protects against stress-induced heart failure but facilitates cardiac fibrosis. *Sci Signal* 2014; **7**: ra100 [PMID: 25336613 DOI: 10.1126/scisignal.2005262]
- 25 **Sai X**, Yonemura S, Ladher RK. Junctionally restricted RhoA activity is necessary for apical constriction during phase 2 inner ear placode invagination. *Dev Biol* 2014; **394**: 206-216 [PMID: 25173873 DOI: 10.1016/j.ydbio.2014.08.022]
- 26 **Lee MH**, Cho YS, Han YM. Simvastatin suppresses self-renewal of mouse embryonic stem cells by inhibiting RhoA geranylgeranylation. *Stem Cells* 2007; **25**: 1654-1663 [PMID: 17464088 DOI: 10.1634/stemcells.2006-0753]
- 27 **Johnson LA**, Rodansky ES, Haak AJ, Larsen SD, Neubig RR, Higgins PD. Novel Rho/MRTF/SRF inhibitors block matrix-stiffness and TGF- β -induced fibrogenesis in human colonic myofibroblasts. *Inflamm Bowel Dis* 2014; **20**: 154-165 [PMID: 24280883 DOI: 10.1097/01.MIB.0000437615.98881.31]
- 28 **Trebicka J**, Hennenberg M, Laleman W, Shelest N, Biecker E, Schepke M, Nevens F, Sauerbruch T, Heller J. Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. *Hepatology* 2007; **46**: 242-253 [PMID: 17596891 DOI: 10.1002/hep.21673]
- 29 **Bishop AL**, Hall A. Rho GTPases and their effector proteins. *Biochem J* 2000; **348 Pt 2**: 241-255 [PMID: 10816416 DOI: 10.1042/0264-6021:3480241]
- 30 **Charlton-Menys V**, Durrington PN. Human cholesterol metabolism and therapeutic molecules. *Exp Physiol* 2008; **93**: 27-42 [PMID: 18165431 DOI: 10.1113/expphysiol.2007.035147]
- 31 **Schmidmaier R**, Baumann P, Simsek M, Dayyani F, Emmerich B, Meinhardt G. The HMG-CoA reductase inhibitor simvastatin overcomes cell adhesion-mediated drug resistance in multiple myeloma by geranylgeranylation of Rho protein and activation of Rho kinase. *Blood* 2004; **104**: 1825-1832 [PMID: 15161667 DOI: 10.1182/blood-2003-12-4218]
- 32 **Trebicka J**, Schierwagen R. Statins, Rho GTPases and KLF2: new mechanistic insight into liver fibrosis and portal hypertension. *Gut* 2015; **64**: 1349-1350 [PMID: 25596180 DOI: 10.1136/gutjnl-2014-308800]
- 33 **Liu J**, Peng L, Yang J, Wang M, Xu S, Liu J, Han P, He J, Tian D, Zhou Q. Sodium Ferulate Reduces Portal Pressure Through Inhibition of RhoA/Rho-Kinase and Activation of Endothelial Nitric Oxide Synthase in Cirrhotic Rats. *Dig Dis Sci* 2015; **60**: 2019-2029 [PMID: 25724163 DOI: 10.1007/s10620-015-3544-9]
- 34 **Zhang CG**, Zhang B, Deng WS, Duan M, Chen W, Wu ZY. Role of estrogen receptor β selective agonist in ameliorating portal hypertension in rats with CCl₄-induced liver cirrhosis. *World J Gastroenterol* 2016; **22**: 4484-4500 [PMID: 27182159 DOI: 10.3748/wjg.v22.i18.4484]
- 35 **Wei L**, Yang J, Wang M, Xu SN, Liang HM, Zhou Q. Sodium ferulate lowers portal pressure in rats with secondary biliary cirrhosis through the RhoA/Rho-kinase signaling pathway: a preliminary study. *Int J Mol Med* 2014; **34**: 1257-1267 [PMID: 25174394 DOI: 10.3892/ijmm.2014.1905]
- 36 **Wang Y**, Zheng XR, Riddick N, Bryden M, Baur W, Zhang X, Surks HK. ROCK isoform regulation of myosin phosphatase

- and contractility in vascular smooth muscle cells. *Circ Res* 2009; **104**: 531-540 [PMID: 19131646 DOI: 10.1161/CIRCRESAHA.108.188524]
- 37 **Antoniou SA**. Targeting RhoA/ROCK pathway in pulmonary arterial hypertension. *Expert Opin Ther Targets* 2012; **16**: 355-363 [PMID: 22449260 DOI: 10.1517/14728222.2012.671811]
- 38 **Ming XF**, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. *Mol Cell Biol* 2002; **22**: 8467-8477 [PMID: 12446767 DOI: 10.1128/MCB.22.24.8467-8477.2002]
- 39 **Anegawa G**, Kawanaka H, Yoshida D, Konishi K, Yamaguchi S, Kinjo N, Taketomi A, Hashizume M, Shimokawa H, Maehara Y. Defective endothelial nitric oxide synthase signaling is mediated by rho-kinase activation in rats with secondary biliary cirrhosis. *Hepatology* 2008; **47**: 966-977 [PMID: 18167063 DOI: 10.1002/hep.22089]
- 40 **Dudzinski DM**, Michel T. Life history of eNOS: partners and pathways. *Cardiovasc Res* 2007; **75**: 247-260 [PMID: 17466957 DOI: 10.1016/j.cardiores.2007.03.023]
- 41 **Rosado E**, Rodríguez-Villarrupla A, Gracia-Sancho J, Tripathi D, García-Calderó H, Bosch J, García-Pagán JC. Terutroban, a TP-receptor antagonist, reduces portal pressure in cirrhotic rats. *Hepatology* 2013; **58**: 1424-1435 [PMID: 23703868 DOI: 10.1002/hep.26520]
- 42 **Mehta PK**, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007; **292**: C82-C97 [PMID: 16870827 DOI: 10.1152/ajpcell.00287.2006]
- 43 **Klein S**, Van Beuge MM, Granzow M, Beljaars L, Schierwagen R, Kilic S, Heidari I, Huss S, Sauerbruch T, Poelstra K, Trebicka J. HSC-specific inhibition of Rho-kinase reduces portal pressure in cirrhotic rats without major systemic effects. *J Hepatol* 2012; **57**: 1220-1227 [PMID: 22878469 DOI: 10.1016/j.jhep.2012.07.033]
- 44 **Wang D**, Yin J, Dong R, Zhao J, Wang Q, Wang N, Wang S, Du X, Lu J. Inhibition of Janus kinase-2 signalling pathway ameliorates portal hypertensive syndrome in partial portal hypertensive and liver cirrhosis rats. *Dig Liver Dis* 2015; **47**: 315-323 [PMID: 25637451 DOI: 10.1016/j.dld.2014.12.017]
- 45 **Vera J**, Rateitschak K, Lange F, Kossow C, Wolkenhauer O, Jaster R. Systems biology of JAK-STAT signalling in human malignancies. *Prog Biophys Mol Biol* 2011; **106**: 426-434 [PMID: 21762720 DOI: 10.1016/j.pbiomolbio.2011.06.013]
- 46 **Liu RY**, Zeng Y, Lei Z, Wang L, Yang H, Liu Z, Zhao J, Zhang HT. JAK/STAT3 signaling is required for TGF- β -induced epithelial-mesenchymal transition in lung cancer cells. *Int J Oncol* 2014; **44**: 1643-1651 [PMID: 24573038 DOI: 10.3892/ijo.2014.2310]
- 47 **Chong HC**, Chan JS, Goh CQ, Gounko NV, Luo B, Wang X, Foo S, Wong MT, Choong C, Kersten S, Tan NS. Angiopoietin-like 4 stimulates STAT3-mediated iNOS expression and enhances angiogenesis to accelerate wound healing in diabetic mice. *Mol Ther* 2014; **22**: 1593-1604 [PMID: 24903577 DOI: 10.1038/mt.2014.102]
- 48 **Kim D**, Lee IH, Kim S, Choi M, Kim H, Ahn S, Saw PE, Jeon H, Lee Y, Jon S. A specific STAT3-binding peptide exerts antiproliferative effects and antitumor activity by inhibiting STAT3 phosphorylation and signaling. *Cancer Res* 2014; **74**: 2144-2151 [PMID: 24576829 DOI: 10.1158/0008-5472.CAN-13-2187]
- 49 **Granzow M**, Schierwagen R, Klein S, Kowallick B, Huss S, Linhart M, Mazar IG, Görtzen J, Vogt A, Schildberg FA, Gonzalez-Carmona MA, Wojtalla A, Krämer B, Nattermann J, Siegmund SV, Werner N, Fürst DO, Laleman W, Knolle P, Shah VH, Sauerbruch T, Trebicka J. Angiotensin-II type 1 receptor-mediated Janus kinase 2 activation induces liver fibrosis. *Hepatology* 2014; **60**: 334-348 [PMID: 24619965 DOI: 10.1002/hep.27117]
- 50 **Iwakiri Y**, Shah V, Rockey DC. Vascular pathobiology in chronic liver disease and cirrhosis - current status and future directions. *J Hepatol* 2014; **61**: 912-924 [PMID: 24911462 DOI: 10.1016/j.jhep.2014.05.047]
- 51 **Hsu SJ**, Lee FY, Wang SS, Hsin IF, Lin TY, Huang HC, Chang CC, Chuang CL, Ho HL, Lin HC, Lee SD. Caffeine ameliorates hemodynamic derangements and portosystemic collaterals in cirrhotic rats. *Hepatology* 2015; **61**: 1672-1684 [PMID: 25557829 DOI: 10.1002/hep.27679]
- 52 **Wang D**, Wang Q, Yin J, Dong R, Wang Q, Du X, Lu J. Combined administration of propranolol+AG490 offers better effects on portal hypertensive rats with cirrhosis. *J Gastroenterol Hepatol* 2016; **31**: 1037-1044 [PMID: 26487394 DOI: 10.1111/jgh.13207]
- 53 **Meng F**, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Österreich CH, Stickel F, Ley K, Brenner DA, Kisseleva T. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. *Gastroenterology* 2012; **143**: 765-776.e3 [PMID: 22687286 DOI: 10.1053/j.gastro.2012.05.049]
- 54 **Xiang DM**, Sun W, Ning BF, Zhou TF, Li XF, Zhong W, Cheng Z, Xia MY, Wang X, Deng X, Wang W, Li HY, Cui XL, Li SC, Wu B, Xie WF, Wang HY, Ding J. The HLF/IL-6/STAT3 feedforward circuit drives hepatic stellate cell activation to promote liver fibrosis. *Gut* 2017; pii: gutjnl-2016-313392 [PMID: 28754776 DOI: 10.1136/gutjnl-2016-313392]
- 55 **Schwabl P**, Payer BA, Grahovac J, Klein S, Horvatits T, Mitterhauser M, Stift J, Boucher Y, Trebicka J, Trauner M, Angermayr B, Fuhrmann V, Reiberger T, Peck-Radosavljevic M. Pioglitazone decreases portosystemic shunting by modulating inflammation and angiogenesis in cirrhotic and non-cirrhotic portal hypertensive rats. *J Hepatol* 2014; **60**: 1135-1142 [PMID: 24530596 DOI: 10.1016/j.jhep.2014.01.025]
- 56 **Reiberger T**, Angermayr B, Schwabl P, Rohr-Udilova N, Mitterhauser M, Gangl A, Peck-Radosavljevic M. Sorafenib attenuates the portal hypertensive syndrome in partial portal vein ligated rats. *J Hepatol* 2009; **51**: 865-873 [PMID: 19726100 DOI: 10.1016/j.jhep.2009.06.024]
- 57 **Klein S**, Rick J, Lehmann J, Schierwagen R, Schierwagen IG, Verbeke L, Hittatiya K, Uschner FE, Manekeller S, Strassburg CP, Wagner KU, Sayeski PP, Wolf D, Laleman W, Sauerbruch T, Trebicka J. Janus-kinase-2 relates directly to portal hypertension and to complications in rodent and human cirrhosis. *Gut* 2017; **66**: 145-155 [PMID: 26385087 DOI: 10.1136/gutjnl-2015-309600]
- 58 **Lee FY**, Lee H, Hubbert ML, Edwards PA, Zhang Y. FXR, a multipurpose nuclear receptor. *Trends Biochem Sci* 2006; **31**: 572-580 [PMID: 16908160 DOI: 10.1016/j.tibs.2006.08.002]
- 59 **Thomas C**, Pellicciari R, Pruzanski M, Auwerx J, Schoonjans K. Targeting bile-acid signalling for metabolic diseases. *Nat Rev Drug Discov* 2008; **7**: 678-693 [PMID: 18670431 DOI: 10.1038/nrd2619]
- 60 **Li Y**, Jadhav K, Zhang Y. Bile acid receptors in non-alcoholic fatty liver disease. *Biochem Pharmacol* 2013; **86**: 1517-1524 [PMID: 23988487 DOI: 10.1016/j.bcp.2013.08.015]
- 61 **DeGirolamo C**, Rainaldi S, Bovenga F, Murzilli S, Moschetta A. Microbiota modification with probiotics induces hepatic bile acid synthesis via downregulation of the Fxr-Fgf15 axis in mice. *Cell Rep* 2014; **7**: 12-18 [PMID: 24656817 DOI: 10.1016/j.celrep.2014.02.032]
- 62 **Sayin SI**, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, Angelin B, Hyötyläinen T, Orešič M, Bäckhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab* 2013; **17**: 225-235 [PMID: 23395169 DOI: 10.1016/j.cmet.2013.01.003]
- 63 **Halilbasic E**, Claudel T, Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *J Hepatol* 2013; **58**: 155-168 [PMID: 22885388 DOI: 10.1016/j.jhep.2012.08.002]
- 64 **Verbeke L**, Farre R, Trebicka J, Komuta M, Roskams T, Klein S, Elst IV, Windmolders P, Vanuytsel T, Nevens F, Laleman W. Obeticholic acid, a farnesoid X receptor agonist, improves portal hypertension by two distinct pathways in cirrhotic rats. *Hepatology* 2014; **59**: 2286-2298 [PMID: 24259407 DOI: 10.1002/hep.26939]
- 65 **Lu TT**, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, Mangelsdorf DJ. Molecular basis for feedback regulation of bile

- acid synthesis by nuclear receptors. *Mol Cell* 2000; **6**: 507-515 [PMID: 11030331 DOI: 10.1016/S1097-2765(00)00050-2]
- 66 **Van de Castele M**, Omasta A, Janssens S, Roskams T, Desmet V, Nevens F, Fevery J. In vivo gene transfer of endothelial nitric oxide synthase decreases portal pressure in anaesthetised carbon tetrachloride cirrhotic rats. *Gut* 2002; **51**: 440-445 [PMID: 12171971 DOI: 10.1136/gut.51.3.440]
 - 67 **Laviña B**, Gracia-Sancho J, Rodríguez-Vilarrupla A, Chu Y, Heistad DD, Bosch J, García-Pagán JC. Superoxide dismutase gene transfer reduces portal pressure in CCl₄ cirrhotic rats with portal hypertension. *Gut* 2009; **58**: 118-125 [PMID: 18829979 DOI: 10.1136/gut.2008.149880]
 - 68 **Schwabl P**, Hambruch E, Seeland BA, Hayden H, Wagner M, Garnys L, Strobel B, Schubert TL, Riedl F, Mitteregger D, Burnet M, Starlinger P, Oberhuber G, Deuschle U, Rohr-Udilova N, Podesser BK, Peck-Radosavljevic M, Reiberger T, Kremoser C, Trauner M. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. *J Hepatol* 2017; **66**: 724-733 [PMID: 27993716 DOI: 10.1016/j.jhep.2016.12.005]
 - 69 **Mookerjee RP**, Mehta G, Balasubramanian V, Mohamed Fel Z, Davies N, Sharma V, Iwakiri Y, Jalan R. Hepatic dimethylarginine-dimethylaminohydrolase 1 is reduced in cirrhosis and is a target for therapy in portal hypertension. *J Hepatol* 2015; **62**: 325-331 [PMID: 25152204 DOI: 10.1016/j.jhep.2014.08.024]
 - 70 **Kajimoto H**, Kai H, Aoki H, Yasuoka S, Anegawa T, Aoki Y, Ueda S, Okuda S, Imaizumi T. Inhibition of eNOS phosphorylation mediates endothelial dysfunction in renal failure: new effect of asymmetric dimethylarginine. *Kidney Int* 2012; **81**: 762-768 [PMID: 22297680 DOI: 10.1038/ki.2011.476]
 - 71 **Rodionov RN**, Murry DJ, Vaulman SF, Stevens JW, Lentz SR. Human alanine-glyoxylate aminotransferase 2 lowers asymmetric dimethylarginine and protects from inhibition of nitric oxide production. *J Biol Chem* 2010; **285**: 5385-5391 [PMID: 20018850 DOI: 10.1074/jbc.M109.091280]
 - 72 **Caplin B**, Wang Z, Slaviero A, Tomlinson J, Dowsett L, Delahaye M, Salama A; International Consortium for Blood Pressure Genome-Wide Association Studies, Wheeler DC, Leiper J. Alanine-glyoxylate aminotransferase-2 metabolizes endogenous methylarginines, regulates NO, and controls blood pressure. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2892-2900 [PMID: 23023372 DOI: 10.1161/ATVBAHA.112.254078]
 - 73 **Li YT**, Swales KE, Thomas GJ, Warner TD, Bishop-Bailey D. Farnesoid x receptor ligands inhibit vascular smooth muscle cell inflammation and migration. *Arterioscler Thromb Vasc Biol* 2007; **27**: 2606-2611 [PMID: 18029909 DOI: 10.1161/ATVBAHA.107.152694]
 - 74 **Gupta TK**, Toruner M, Chung MK, Groszmann RJ. Endothelial dysfunction and decreased production of nitric oxide in the intrahepatic microcirculation of cirrhotic rats. *Hepatology* 1998; **28**: 926-931 [PMID: 9755227 DOI: 10.1002/hep.510280405]
 - 75 **Li J**, Kuruba R, Wilson A, Gao X, Zhang Y, Li S. Inhibition of endothelin-1-mediated contraction of hepatic stellate cells by FXR ligand. *PLoS One* 2010; **5**: e13955 [PMID: 21085652 DOI: 10.1371/journal.pone.0013955]
 - 76 **He F**, Li J, Mu Y, Kuruba R, Ma Z, Wilson A, Alber S, Jiang Y, Stevens T, Watkins S, Pitt B, Xie W, Li S. Downregulation of endothelin-1 by farnesoid X receptor in vascular endothelial cells. *Circ Res* 2006; **98**: 192-199 [PMID: 16357303 DOI: 10.1161/01.RES.0000200400.55539.85]
 - 77 **Zhao W**, Zhang J, Lu Y, Wang R. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *EMBO J* 2001; **20**: 6008-6016 [PMID: 11689441 DOI: 10.1093/emboj/20.21.6008]
 - 78 **Qin J**, He Y, Duan M, Luo M. Effects of Nuclear Factor-E2-related factor 2/Heme Oxygenase 1 on splanchnic hemodynamics in experimental cirrhosis with portal hypertension. *Microvasc Res* 2017; **111**: 12-19 [PMID: 28025064 DOI: 10.1016/j.mvr.2016.12.009]
 - 79 **Zeng XQ**, Li N, Pan DY, Miao Q, Ma GF, Liu YM, Tseng YJ, Li F, Xu LL, Chen SY. Kruppel-like factor 2 inhibit the angiogenesis of cultured human liver sinusoidal endothelial cells through the ERK1/2 signaling pathway. *Biochem Biophys Res Commun* 2015; **464**: 1241-1247 [PMID: 26212440 DOI: 10.1016/j.bbrc.2015.07.113]
 - 80 **Gao JH**, Wen SL, Feng S, Yang WJ, Lu YY, Tong H, Liu R, Tang SH, Huang ZY, Tang YM, Yang JH, Xie HQ, Tang CW. Celecoxib and octreotide synergistically ameliorate portal hypertension via inhibition of angiogenesis in cirrhotic rats. *Angiogenesis* 2016; **19**: 501-511 [PMID: 27380212 DOI: 10.1007/s10456-016-9522-9]

P- Reviewer: Gorrell MD, Gonzalez-Reimers E, Manenti A
S- Editor: Dou Y **L- Editor:** Filipodia **E- Editor:** Wu YXJ



Gastrointestinal toxicity induced by microcystins

Jin-Xia Wu, Hui Huang, Lei Yang, Xiao-Feng Zhang, Shen-Shen Zhang, Hao-Hao Liu, Yue-Qin Wang, Le Yuan, Xue-Min Cheng, Dong-Gang Zhuang, Hui-Zhen Zhang

Jin-Xia Wu, Hui Huang, Hao-Hao Liu, Yue-Qin Wang, Le Yuan, Xue-Min Cheng, Dong-Gang Zhuang, Hui-Zhen Zhang, Department of Environmental Hygiene, College of Public Health, Zhengzhou University, Zhengzhou 450001, Henan Province, China

Lei Yang, Xiao-Feng Zhang, Shen-Shen Zhang, Department of Nutriology, College of Public Health, Zhengzhou University, Zhengzhou 450001, Henan Province, China

ORCID number: Jin-Xia Wu (0000-0003-1440-8034); Hui Huang (0000-0003-4083-0247); Lei Yang (0000-0002-0655-8123); Xiao-Feng Zhang (0000-0002-5577-7891); Shen-Shen Zhang (0000-0002-7849-371X); Hao-Hao Liu (0000-0002-4951-6230); Yue-Qin Wang (0000-0003-4789-936X); Le Yuan (0000-0002-5119-0444); Xue-Min Cheng (0000-0001-5402-767X); Dong-Gang Zhuang (0000-0001-9472-5683); Hui-Zhen Zhang (0000-0003-0520-1955).

Supported by Henan Natural Science Foundation, No. 162300410267 and the National Nature Science Foundation of China, Nos. 81773384 and 81472948.

Author contributions: Wu JX contributed to the design, analysis of data, and drafting and writing the article; Huang H, Yang L, Zhang XF and Zhang SS critically revised the manuscript for intellectual content; Liu HH, Wang YQ, Yuan L, Cheng XM and Zhuang DG contributed to literature collection; Zhang HZ contributed to critical revision and final approval.

Conflict-of-interest statement: The authors declare that no conflicts of interest exist in this study.

Open-Access: This article is an open-access article, which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Hui-Zhen Zhang, PhD, Professor,

Department of Environmental Hygiene, College of Public Health, Zhengzhou University, No. 100 Science Avenue, Zhengzhou 450001, Henan Province, China. huizhenzhang@zzu.edu.cn
Telephone: +86-371-67781461

Received: April 23, 2018

Peer-review started: April 23, 2018

First decision: May 23, 2018

Revised: June 8, 2018

Accepted: June 27, 2018

Article in press: June 28, 2018

Published online: September 26, 2018

Abstract

Microcystins (MCs) are produced by certain bloom-forming cyanobacteria that can induce toxicity in various organs, including renal toxicity, reproductive toxicity, cardiotoxicity, and immunosuppressive effects. It has been a significant global environmental issue due to its harm to the aquatic environment and human health. Numerous investigators have demonstrated that MC exposure can induce a widespread epidemic of enterogastritis with symptoms similar to food poisoning in areas close to lakes. Both *in vivo* and *in vitro* studies have provided evidence of positive associations between MC exposure and gastrointestinal toxicity. The toxicity of MCs on the gastrointestinal tract is multidimensional. MCs can affect gastrointestinal barrier function and shift the structure of gut microbiota in different gut regions. Furthermore, MCs can inhibit the secretion of gastrointestinal digestive enzymes and the release of inflammatory cytokines, which affects the expression of immune-related genes in the intestine. The damage of the intestine is closely correlated to MC exposure because the intestine is the main site for the digestion and absorption of nutrients. The damage to the gastrointestinal tract due to MCs was summarized from different aspects, which can be used as a foundation for further exploration of molecular damage mechanisms.

Key words: Immunotoxicity; Gastrointestinal toxicity;

Intestine; Depuration; Oxidative stress; Microcystins

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: First, the gastrointestinal toxicity of microcystins (MCs) on a population was described. Second, the concentration or localization of MCs in the small intestine after exposure to various concentrations and different time points, as well as after the depuration of MCs in the intestine, was summarized. Third, the change in morphologic pathology and other effects such as oxidative stress, immunotoxicity, digestive enzymes, and gut microbiota in the intestine with exposure to MCs were discussed. Further challenges that need to be addressed were also summarized.

Wu JX, Huang H, Yang L, Zhang XF, Zhang SS, Liu HH, Wang YQ, Yuan L, Cheng XM, Zhuang DG, Zhang HZ. Gastrointestinal toxicity induced by microcystins. *World J Clin Cases* 2018; 6(10): 344-354 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/344.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.344>

INTRODUCTION

The development of industrialization accompanied by the increase of effluent discharge leads to the occurrence of cyanobacterial bloom and red tide due to nitrogen and phosphorous overload. Previous studies have demonstrated that almost 80% of algal blooms could produce secondary metabolites. This includes microcystins (MCs), which have a general structure of cyclo (-D-Ala1-L-R12-D-erythro-β-methylAsp3-L-R24-Adda5-D-Glu6-N-methyldehydro-Ala7), in which R1 and R2 are the variable L amino acids responsible for most of the congeners (Figure 1)^[1]. Furthermore, more than 100 structural variants have been identified, among which microcystin-LR (MC-LR) was the most widespread and virulent type, followed by microcystin-RR (MC-RR), and microcystin-YR (MC-YR)^[2,3].

MCs pose a threat to human health in many ways, such as oral, dermal and inhalation pathways, and exposure may occur during recreation depending on the types of activities undertaken in the water^[4,5]. Freshwater aquaculture ponds are important artificially regulated aquatic ecosystems that provide a large number of freshwater fish products in China^[6]. Moreover, China's freshwater aquaculture area and production stands first in the world^[7]. Studies have shown that MCs can accumulate in a variety of aquatic organisms, such as zooplankton, bivalves, crustaceans, fish, and aquatic vertebrates^[6]. It may exert a potential toxic hazard to humans after the consumption of MC-contaminated aquatic products. Recently, studies have shown that MCs can accumulate in edible crops and soils irrigated with MC-contaminated water^[3]. It has a potential risk to be transferred to the human body through the ingestion of

these vegetables^[3]. Moreover, if MC-contaminated water is applied during medical treatment, this may lead to acute intoxication in patients and even death. Although such incidents seldom happen, this should arouse our attention for its serious consequences.

Studies have indicated that MCs accumulate mainly in the liver, and can be transported to the kidneys, muscle, brain, and intestines through blood circulation^[6]. Furthermore, MCs have been confirmed to be transported into cells *via* organic anion transporting polypeptides (OATPs), which exist in almost every organ^[8], while some OATPs are preferentially or even selectively expressed in specific tissues^[9]. Thus, the susceptibility among tissues toward MC exposure may be explained by OATP subtypes and the OATP subtype-selective transport of specific MC congeners^[8,10]. Therefore, OATPs are the prerequisite for the toxicity that MCs can exert. Previous reports have shown that MCs can cross the intestinal barrier *via* OATP3A1 and OATP4A1, which are located in the small intestine epithelium^[11]. This follows that MCs may pose a potential threat to human health. Therefore, the present study aimed to perform a compilation of increasing information that involves intestinal toxicity with regard to fish, mammals, cells, and group surveys, in order to summarize the present research gaps that should be addressed through further studies.

GASTROINTESTINAL TOXICITY OF MCs ON THE POPULATION

The detriments caused by MCs produced by blue-green algae have attracted worldwide concern. Epidemiological investigation and toxicology research have been used to illuminate the perniciousness of MCs. Epidemiological investigations can reflect the direct association between the health and MC exposure of the population, which is especially important to identify the hazards of MCs on mankind.

Study on enterogastritis with exposure to MC-LR

Widespread epidemics of enterogastritis with symptoms similar to those during food poisoning were found in a series of towns alongside Elk River in Charleston, West Virginia. A survey revealed that the acute gastroenteritis outbreak was on a large scale, and was not caused by bacterial infection but by toxins. After all measures were ineffective in removing toxins, it was speculated that the toxin was most likely MC^[12]. Pilotto *et al.*^[13] discovered that there was a positive association between the incidence of gastroenteritis and the exposure time and density of cyanobacteria through a prospective investigation. Furthermore, there was a significant trend of the increasing occurrence of enterogastritis when exposed to more than 5000 cyanobacterial cells/mL for more than one hour^[13]. Those results show that MCs can lead to acute gastroenteritis in humans.

Effect on gastroenteric carcinomas

Zhou *et al.*^[14] investigated the association of MCs in

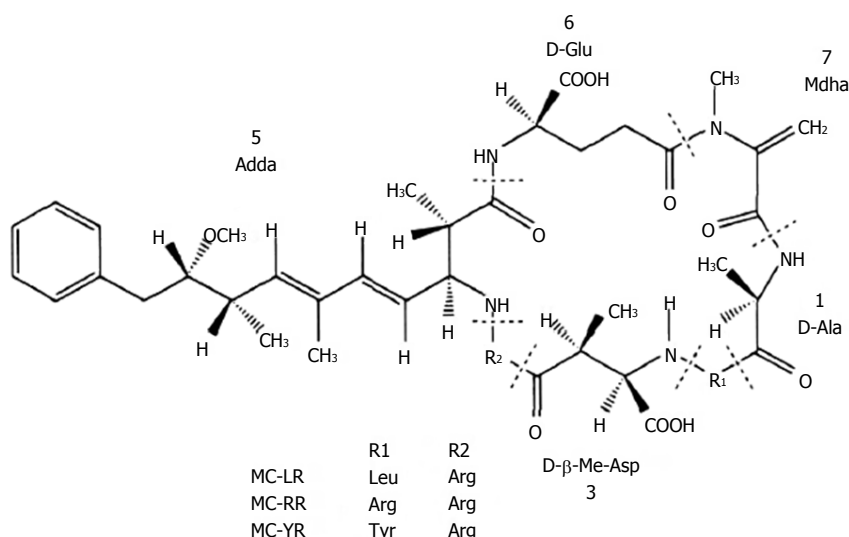


Figure 1 Chemical structure of microcystins.

drinking water with the incidence of colorectal cancer through a retrospective cohort. In that study, eight towns in Haining City of Zhejiang Province, China were randomly selected as study sites, and 408 cases of colon and rectum carcinomas were selected for study. The results revealed that the incidence of colorectal cancer was significantly higher in the population that drank river and pond water compared to the population that drank well and tap water, suggesting that MCs may play a causative role in the carcinogenesis of the colon and rectum^[14]. Other studies also manifested the positive association between MCs and the incidence of colon and rectum carcinomas. Lin *et al.*^[15] discovered that male colorectal cancer mortality increases as the MC content increases in water. Furthermore, a consistent trend between the positive detection rate of MCs and the mortality of colorectal cancer was found. Moreover, Chen *et al.*^[16] discovered the positive association between the concentration of MCs and the morbidity of colorectal cancer. In addition, Falconer *et al.*^[17] demonstrated that MC exposure is a contributing factor to the increased mortality of gastric cancer.

CONCENTRATION OR LOCALIZATION OF MCs IN THE SMALL INTESTINE

The toxicity of MCs mainly depends on the accumulation dose^[2], which can be determined through the intake and depuration of MCs in the body. Furthermore, the location of MCs in cells changes along with the accumulation of MCs thereby causing different degrees of damage.

Localization of MCs in the small intestine

Previous studies have demonstrated that MCs can be detected in the small intestine because a specific OATP membrane transport system can carry it into the enterocytes^[11]. After exposure of 0-50 $\mu\text{mol/L}$ of MC-LR concentrations for 24 h in IEC-6 cells, the intracellular

localization of MC-LR was in the cytoplasm and around the nucleus. Furthermore, western blot results revealed that there was a dose-dependent content following exposure to MC-LR^[18].

Caco-2 cells have microvilli and cellulose associated with brush border epithelium with a similarity in structure and function in small intestine epithelial cells^[19]. Therefore, it can be used to simulate intestinal transshipment. Caco-2 cells were treated with concentrations of MC-LR or MC-RR ranging from 1-50 $\mu\text{mol/L}$ for four hours. There was no difference in the subcellular localization of MC-LR or MC-RR among concentrations (Figure 2A). After incubation of Caco-2 cells in a fixed concentration of 20 $\mu\text{mol/L}$ of MC-LR or MC-RR, the intracellular location of MC-LR or MC-RR was observed over a period of time. These results show that staining at the cell membrane could be observed after thirty minutes of treatment, which subsequently progressed to the cytoplasm after two hours and around the nucleus after six hours (Figure 2B). These results confirm that MC-LR and MC-RR could reach the nucleus^[11], suggesting that MC-LR may induce DNA damage. In addition, it has been demonstrated that DNA damage can be induced in intestinal tissues after exposure to 50 $\mu\text{g/kg}$ of body weight of MC-LR in mice for 24 h^[20].

In another *in vivo* study, medaka fish were administered an oral gavage of MC-LR extracts at a dose of 100 mg/L for two hours. The results revealed that the labeled areas were detectable in the intestinal submucosa, and can be seen in the cytoplasm of the submucosal macrophages in adult medaka intestine^[21].

Concentration of MCs in the small intestine

OATPs are present in almost every organ or tissue^[8]. MCs can be transported to organs such as the kidney, intestine, heart, spleen, and pancreas, and is mediated by blood circulation and OATPs. Various studies have revealed that MC-LR concentrations are higher in the intestines than in other organs^[22-26], but MC-RR

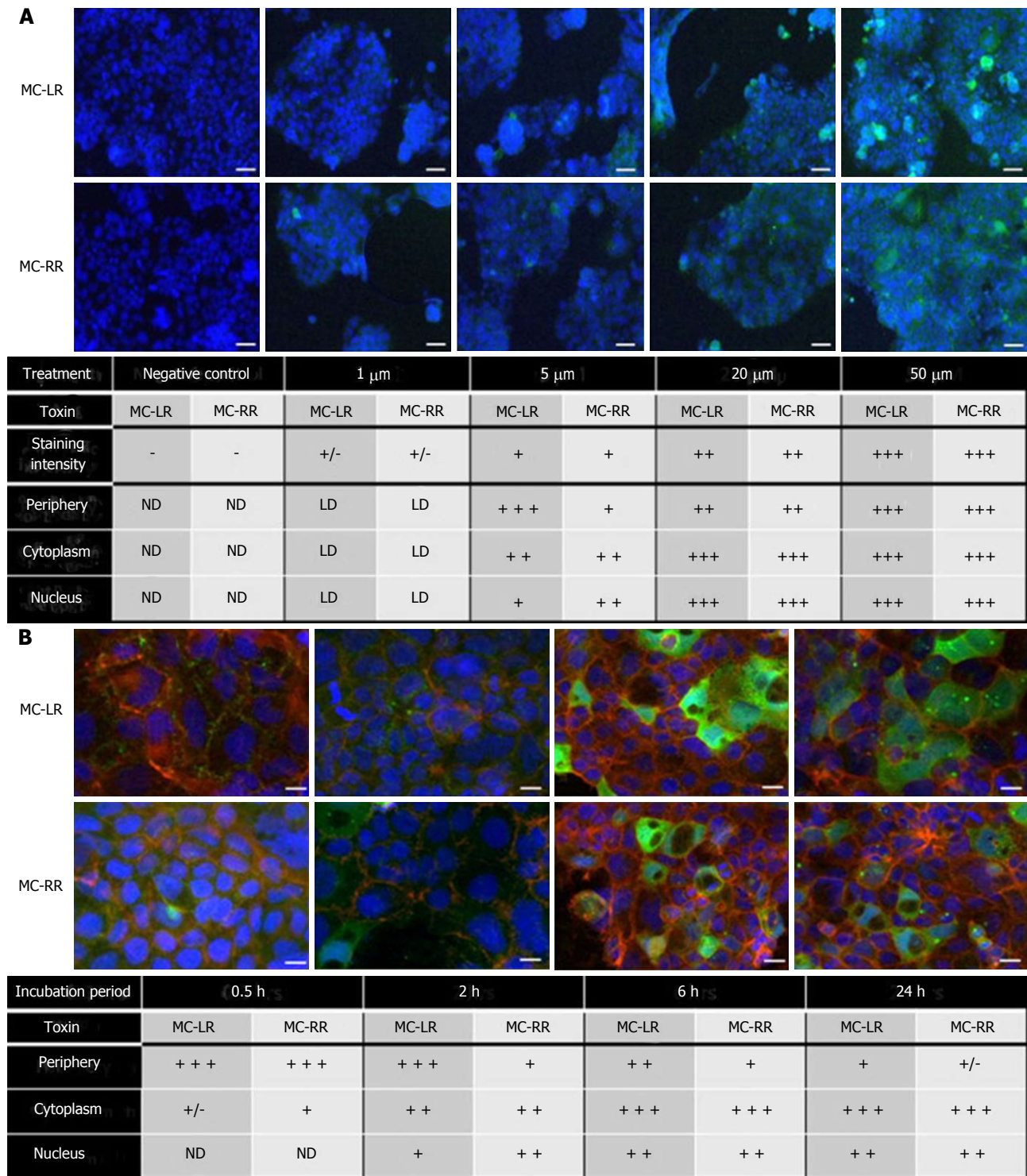


Figure 2 Subcellular localization of MC-LR and MC-RR in Caco-2 cells, which was adapted from a reference^[11]. A: Caco-2 cells were treated for four hours with concentrations of MC-LR or MC-RR ranging from 1-50 $\mu\text{mol/L}$; B: Caco-2 cells were treated for several time points with 20 $\mu\text{mol/L}$ of MC-LR or MC-RR. The cellular localization of these toxins was detected using an anti-ADDA antibody and an Alexa fluor 488 secondary antibody. Nuclei were counterstained with DAPI. The symbols (-), (+), (++) and (+++) represent the relative importance of the staining localization into the cell. Scale bars: A: 20 μm ; B: 10 μm . ND: Alexa fluor 488 staining not detected; LD: Low signal of Alexa fluor 488 staining; (+/-) low signal intensity; MC: Microcystin.

concentrations are higher in the hepatopancreas than in the intestines under the same condition^[7,23,24,27]. The difference in the susceptibility of tissues towards MC-LR and MC-RR exposure may be explained by the OATP subtype-selective transport of specific MC congeners. Moreover, studies have shown that the intestine had the

highest MC concentration (MC-LR + MC-RR)^[28]. Other studies have also shown that MC content was higher in the hepatopancreas, followed by the intestine and other organs^[29,30]. Furthermore, studies manifested that the intestine had relatively low MC content^[27,31]. The difference in MC content in the intestine and other organs

Table 1 Summary of concentrations or locations of microcystins in experimental models

Model	Toxins	Exposure	Dose	Time	Experiments	Con. or loc.	Ref.
Caco-2 cell	MC-LRMC-RR	Incubation	1, 5, 20, 50 µmol/L	0.5, 2, 6, 24 h	Immunofluorescence	Periphery, cytoplasm, nucleus	[11]
IEC-6 cell	MC-LR	Incubation	6.25, 12.5, 25, 50 µmol/L	6, 12, 24 h	Immunofluorescence WB	Cytoplasm, around the nucleus	[18]
Medaka fish	MC-LR	Gavage	100 mg/L	2 h	Immunohistochemistry	In the cytoplasm of the submucosal macrophages	[21]
Cyprinus carpio, anguilla anguilla	MC-LR MC-RR	Immersion	Lake Oubeira	12 mo	HPLC	Intestine > hepatopancreas liver > intestine	[22]
Aristichthys nobilis	MC-LR MC-RR	<i>i.p.</i>	50, 200 µg/kg body weight	1, 3, 12, 24, 48, 72 h	LC-ESI-MS	Higher in intestine than in other organs liver > intestine	[23]
Silver carp	MC-LR/MC-RR	Immersion	40 mm plankton net	40, 80 d	HPLC	49.2 and 115.3 (average 78.8) µg/g DW	[24]
Crayfish	MC-LR	Immersion	0.1, 1, 10, 100 µg/L	8 h, 1, 3, 4, 7 d	HPLC	Higher in intestine than in other organs	[25]
Bellamyia aeruginosa	MC-LR MC-RR MC-YR	Immersion	Lake Taihu	12 mo	LC-MS	Intestine > hepatopancreas	[26]
Jenynsia multidentata, corydoras paleatus	MC-RR	Immersion	50 µg/L	24 h	HPLC LC-ESI-TOF-MS	Liver > intestine	[27]
Aristichthys nobilis	MC-LR	Immersion	Lake Taihu	12 mo	LC-MS/HPLC-UV	85.67 mg/g DW	[28]
Freshwater mussels	MC-LR MC-RR MC-YR	Immersion	Lake Taihu	12 mo	LC-MS HPLC	20.65 µg/g DW	[30]
Carassius auratus	MC-LRMC-RR	<i>i.p.</i>	200 µg/kg body weight	1, 3, 12, 24, 48 h	LC-MS	Less than 0.1% of the injected MCs	[31]
Bivalves	MC-LR MC-RR MC-YR	Immersion	Lake Taihu	6 mo	LC-MS	Hepatopancreas > intestine	[32]
Wistar rat	MC-LR	<i>i.v.</i>	80 µg/kg body weight	1, 2, 4, 6, 12, 24 h	LC-MS	Less than 0.2% of injected MCs	[33]
Carassius carassius	MC-LR MC-RR	<i>i.p.</i>	50 µg/kg body weight	1, 3, 12, 24, 48, 168 h	LC-MS	Less than 0.05% of injected MCs	[34]
Freshwater fish at different trophic levels	MC-LR/MC-RR	Immersion	Lake Chaohu	-	HPLC/LC/ESI-MS	Higher in intestine than in other organs	[35]

"Con.", "loc." and "-" represent the concentration, location and no data, respectively. MC: Microcystin; LC-MS: Liquid chromatography-mass spectrometry; HPLC: High-performance liquid chromatography.

among species and the different categories of the same species may be explained by the different mechanisms of uptake and depuration among other factors^[32].

In studies *in vivo*, MC concentrations in different parts of the gut were detected by liquid chromatography-mass spectrometry (LC-MS), in which the MC concentration was much higher in the mid-gut walls than in the hind- and fore-gut walls. This clarifies the importance of the mid-gut wall as a major site for MC absorption^[32]. The concentration or localization of MCs in the small intestine is summarized in Table 1.

Depuration of MCs in the intestine

MCs can accumulate and metabolize in various tissues through the blood and exert toxic effects. Studies have shown that no MC-LR was detectable despite the abundant presence of MC-RR in the intestine^[24], while Wang and Yuan *et al.*^[25] found that MC-LR content was much higher in the intestine than in other organs, which may be the result of the selective absorption of MCs in small intestine epithelial cells, or the difference between the elimination and inhibition in the intestine among species^[23,25]. Chen *et al.*^[29] demonstrated that the highest concentration of MCs was found in the kidneys, and

two peaks were observed, indicating that MCs can be directly excreted *via* the kidney in rats. The kidneys function as an important excretory organ and play a significant role in filtering to form urine and discharge metabolic wastes, which regulate the balance between electrolytes and acid-base. Moreover, Lei *et al.*^[31] found that a significant negative correlation was present between MC-RR concentrations in blood and in the kidneys, confirming that the blood plays an important role in the transportation of MC-RR to the kidneys for excretion. From the above, it can be found that MCs may accumulate in the intestines. Studies have revealed that MCs exist mainly in two forms in animal tissues: Covalently bound MCs and methanol-extractable forms^[25]. MCs accumulate rapidly, but are slowly eliminated in the intestine^[25,31], which is probably due to the presence of MCs in the intestine with a covalently bound form^[25].

PATHOLOGICAL EFFECT OF MCs IN THE INTESTINES

MCs can not only induce damage to enterocytes *in vitro*, but also induce pathological injury *in vivo*, leading

to the production of oxides, the parasecretion of immunocytokines and digestive enzymes, and disorders in intestinal secretion of water, electrolytes, and intestinal flora.

Effect of MCs on Enterocytes

Previous studies indicated that MCs can be transported across enterocyte membranes *via* OATPs^[2]. Moreover, studies have manifested that cell viability significantly decreased, while the ratio of apoptotic cells increased, after intestinal epithelial cells (IEC-6) and human intestinal Caco-2 cells were exposed to MCs^[18]. MCs can affect the integrity of the intestinal barrier by decreasing its transepithelial electrical resistance (TEER) values and inducing the cytoskeletal protein expression of occludin and ZO-1 in IECs in a dose-dependent decrease^[18]. Tight junctions (TJs) are composed of occludin and ZO-1. The dysfunction of the intestinal TJ barrier is an important event in the pathogenesis of enteropathies. MCs may influence the barrier function of the intestine by affecting the TJs of IEC-6. Furthermore, MCs can increase intracellular ROS production and promote proinflammatory cytokine secretion, including IL-6 and IL-8^[36], which contributes to the damage to the small intestinal epithelial cells. Humpage *et al.*^[37] confirmed that MCs could induce the transformation of normal crypt cells (NCC). This follows that MCs can induce damage to enterocytes.

Effect of MCs on morphology

The intestines hollow body comprises of four concentric layers: mucosa, submucosa, muscle and serosa^[38]. MCs may have different effects on these layers. MCs can induce the pathological lesions on the small intestinal mucosa in a dose-dependent manner. A large quantity of macrophages has been found below the epithelium^[38], illustrating the loss of adhesion between loose connective tissues and the tract. Large amounts of macrophages were noted in the submucosa with prolonged exposure time and concentration^[21,38]. Erythrocyte cells, such as goblet cells, play a significant role in the protection and digestion function of the intestine. Several zones of lysis and fewer goblet cells were detected in the intestinal epithelium in MC-LR-treated fish^[39]. Nevertheless, the most prominent characteristic was the loss of microvilli and exfoliation of epithelial cells^[38-41]. Intestinal pathological changes were characterized by hyperplasia, which thickened at several points in the lining of the intestinal mucosa, resulting in an undulating surface^[42,43]. Fish treated with 25 µg and 50 µg of MC-LR exhibited a catarrhal enteritis process and necrotic enteritis, respectively^[40]. An ultrastructural study revealed that in severe damage zones of the intestine, the vacuolization of enterocytes and hemorrhaging vessels in the submucosa were observed, as well as loss of normal architecture in smooth muscle fibers and the necrosis of fibers^[38,40]. The pathological damage on the intestine is

presented in Figure 3.

Oxidative stress related to MC exposure

The toxicity of MCs is primarily due to the irreversible inhibition of serine/threonine protein phosphatase 1 and 2A^[44,45], which leads to disruptions in the cytoskeleton, necrosis, cytoplasmic vacuolization, and consequent apoptosis. Furthermore, recent reports have shown that oxidative stress induced by MCs is the initial factor that causes other various injuries^[4]. MCs can induce the oxidative stress of mitochondria, leading to the increase of mitochondrial permeability transition and the release of cytochrome c. This would consequently increase the protein expression of Bax and caspases-3, -8 and -9 ($P < 0.01$), and inhibits the protein expression of Bcl-2^[46,47]. The toxicity of MC-LR is involved in alterations in oxidation and the antioxidant system^[40,48,49]. Studies have shown that oxidative stress biomarkers (ROS, TBARS, and MDA) have a dose-dependent increase with exposure to MCs, while antioxidase (GSH, SOD, GPx, GR, and GST) activities in general increase at lower concentrations, and decreased at higher concentrations^[48-50]. The results above suggest that the production of excessive oxidative substances and the dysfunction of the anti-oxidative system induced by MC-LR can lead to small intestinal lesions.

Immunotoxicity of MCs on the intestines

The mucosa plays important pleiotropic roles, including absorption, secretion and barrier functions. Furthermore, the mucosa has a lot of lymphocytes and macrophages, and a decrease in intraepithelial lymphocytes in the mucous of the intestine was detected with exposure to 50 µg/kg and 100 µg/kg of MC-LR for 48 h^[49]. Furthermore, the mRNA levels of IFN-1, IL-1b, IL-8, TGF-b, and TNF-a dramatically increased in the intestine in all MC-LR treated groups^[51]. Moreover, the destruction of the intestinal mucosal structure induced by MC-LR may be responsible for the dysfunction of mucosal immunity.

Effect of MCs on digestive enzymes

Digestive enzymes exist in the brush border of the intestinal mucosa and play an important role in the final digestion phase. Its activity is closely correlated to the integrity of the intestinal mucosa. Liu *et al.*^[52] observed that the activity of disaccharides, alkaline phosphatase and gamma-glutamyltransferase (γ -glutamyltransferase) declined after the intraperitoneal injection of MC-LR for 28 d. Yao *et al.*^[53] discovered that the activity of diastase and protease significantly declined in the intestines of silver carp in water contaminated by MCs. However, Moreno *et al.*^[50] were able to catch sight that the activity of diastase decreased, while other intestinal apical membrane enzymes (lactase, maltase, and alkaline phosphatase) were not modified after intravenous injection of MC-LR for eight hours. The results reported by Liu and Yao are not completely consistent, which was possibly due to the discrepancy in exposure dose and

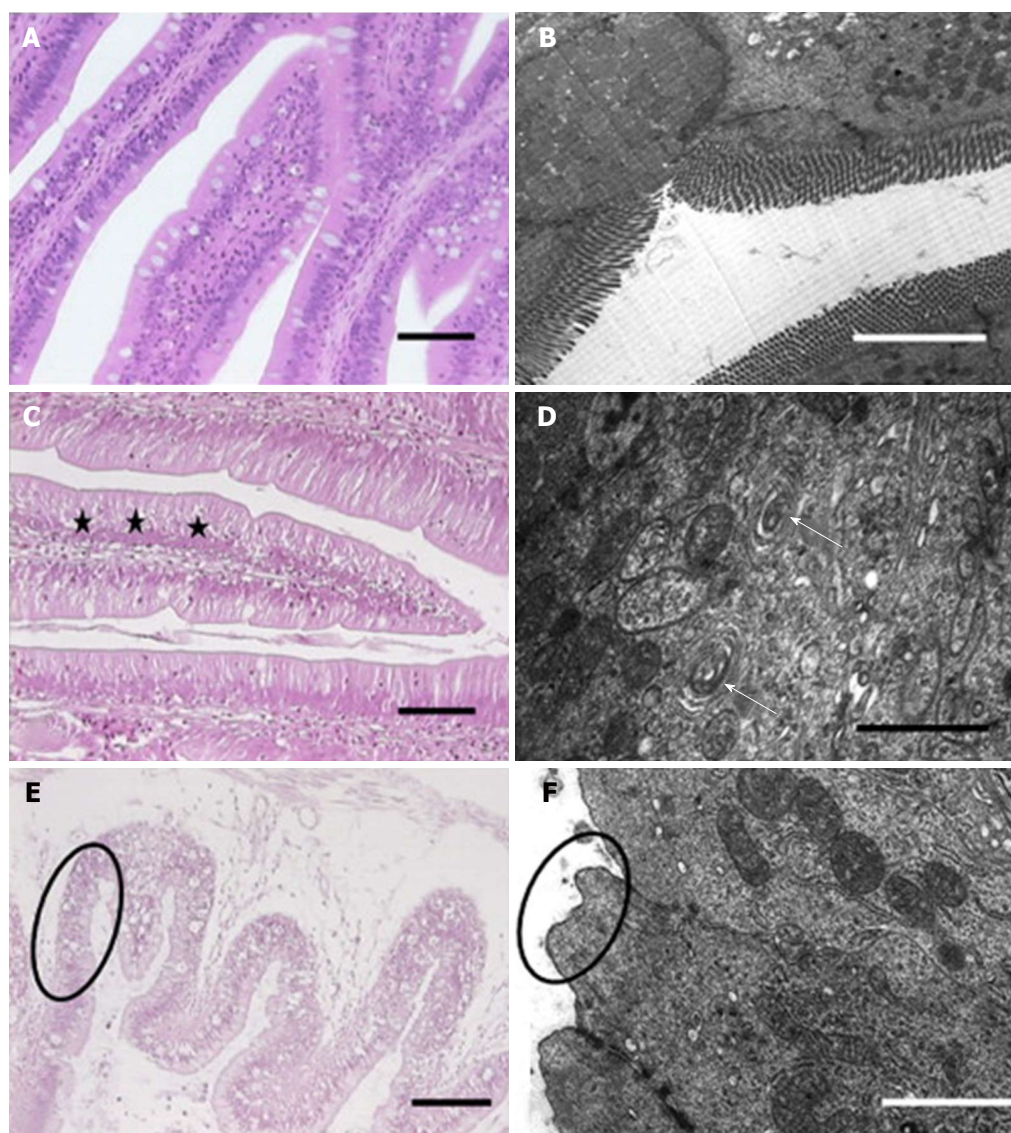


Figure 3 Histopathological and ultrastructural observations in the gastrointestinal tract of *Tenca* fish exposed to MCs. Adapted from a ref.[40]. H and E staining (A) and ultrastructural observations (B). A and B: Control groups; C and D: 25 mg of MC-LR/fish group; E and F: 55 mg of MC-LR/fish groups. Vacuolated enterocytes (star). Vacuolization of the endoplasmic reticulum (arrow). Pycnotic nuclei, vacuolated cytoplasm (E, circle). Total microvilli loss (F, circle).

methods. Above all, MCs can affect the digestive function through altering the integrity of the intestinal mucosa.

Effect of MCs on the intestinal secretion of water and electrolytes

MCs can inhibit protein phosphatase and stimulate macrophagocytes to secrete the corresponding cytokines. Rocha *et al*^[54] observed that MC-LR can induce the release of interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) through peritoneal macrophages *in vitro* and the secretion of electrolytes in rabbit ileal mucosa. Nobre *et al*^[55] detected that MC-LR promoted the secretion of water and electrolytes (potassium, chloride, and sodium). The phosphorylation of residues in the N-terminus of the Na-K-Cl cotransporter is the mechanism that regulates the activation of intestinal fluid secretion in diarrheal toxicity^[55]. A previous study has implicated protein phosphatase 1 (PP1) in the dephosphorylation of the Na-

K-Cl cotransporter^[55]. It has been recognized that MC-LR is a potent inhibitor of PP1 and protein phosphatase 2A (PP2A), which demonstrates that MC-LR may indirectly inhibit the dephosphorylation of the Na-K-Cl cotransporter by inhibiting the activity of PP1^[55].

Effects of MCs on gut microbiota and other aspects

The small intestine consists of the duodenum, jejunum and ileum. The intestinum crassum can be divided into the cecum, colon and rectum. The toxicity of MCs on the gut significantly depends on the absorption capacity, which is determined by the structure of the gut. MCs have a higher affinity with OATPs and can be absorbed rapidly and efficiently eliminated with bile acids. Dahlem *et al*^[56] revealed that MCs are absorbed mainly in the ileum but are relatively low in the jejunum because the ileum is an active site for the transport of bile salt. The gut microbiome is a diverse and carefully balanced ecosystem

of great importance in keeping the intestine healthy. If this balance is broken, many diseases can occur. Studies found that MC-LR can increase the microbial species richness as well as the microbial diversity in the cecum and colon with no effect in the jejunum and ileum. The increase of *Barnesiella* was most remarkable. Therefore, the toxicological effects of MC-LR varied between the jejunum and ileum and the other two gut regions^[2]. However, there are few studies on the effects of MC-LR on the gut microbiome. The gut microbiome has a significant impact on human health as the first defense line in the intestine. Therefore, the effect of MC-LR on the intestinal flora needs to be further studied. Furthermore, studies have also shown that MC-LR could change the glycosylation pattern of the intestinal wall, and suppress the efflux activity of multidrug resistance proteins^[57].

FURTHER CHALLENGES

The concentration of MCs in the intestines vary among species

The present study comprehensively summarized the increasing information on the influence of MCs on the intestines. A positive association between intestinal toxicity and MC exposure were observed. The concentration or localization of MCs in the small intestine is summarized in Table 1. MCs can be absorbed into the bloodstream and transported to the intestine through blood circulation, and with the mediation of OATPs, MCs can be transferred from blood to enterocytes. No differences were observed in the localization of MC-LR or MC-RR among concentrations. Nevertheless, MCs can cross from the cell membrane to reach the cytoplasm and subsequently the nucleus with prolonged MC exposure. Hence, DNA damage may be induced by MCs and the apoptotic process would be triggered when DNA damage exceeds its repair capacity. This is perhaps one of the mechanisms for MCs to induce enterocyte apoptosis. However, further studies are needed to confirm this. There was a discrepancy in MC content in various organs within species. This may be the result of the different doses and methods used. Furthermore, the distribution of MCs in the intestines differs among tissues across separate species. The difference in MC content in the intestine across separate species may be explained by the differential expression of OATP subtypes. The difference in uptake capacity of the intestine between MC-LR and MC-RR may be the result of the OATP subtype-selective transport of specific MC congeners. The uptake ability of MCs varies in different sites of the intestine. There may be some substances (*e.g.*, bile acids) that competed with MCs in the transport by OATPs. All of these explanations are speculation and further research is needed to confirm these hypotheses.

The majority of reports have mainly focused on the uptake and transport of MCs in the jejunum and ileum and rarely focused on other parts of the gut. Moreover, it remains uncertain whether the uptake mechanism of MCs in other parts of the intestine is the same as

that in the jejunum, ileum, and colon. Simultaneously, there are few reports with regard to the depuration of MCs in the intestine. Studies have shown that MCs can be excreted directly *via* the kidneys, since a negative correlation was present between MC-RR concentration in the blood and in the kidneys^[29,31]. It has long been recognized that glutathione (GSH) and cysteine (Cys) conjugation play an important role in the detoxification of MCs in animal organs^[23]. However, further validation and exploration are needed to reduce the physical toxic effects of MCs. Otherwise, whether the intestine is an accumulation organ or intestinal microflora has a role in the degradation of MCs remains to be further studied.

Other mechanisms that could damage the intestines may exist

MCs can induce pathological damage of the small intestine, including the loss of microvilli, cytoplasmic vacuolization, the exfoliation of epithelial cells, the hyperplasia of the intestinal mucosa, the hemorrhage of vessels in the submucosa, fibrosis of smooth muscle, disruption of cytoskeleton, and necrosis. These molecular mechanisms may be involved in the disruption of cytoskeleton-associated proteins, the production of reactive oxygen species (ROS) and suppression of anti-oxidation resistance, the activation of pro-apoptotic proteins and inhibition of anti-apoptotic proteins, the release of IL-1 β and TNF- α by peritoneal macrophages, and the inhibition of PP1 activity, which would eventually lead to changes in morphology, alteration in digestive enzyme activity, shifts in gut microbial patterns, the prohibition of multidrug resistance protein efflux activity, the decline in the immunity of the small intestine mucosa, disorder in water and electrolytes, and even DNA damage and carcinogenesis. Studies have mainly focused on the pathological injury induced by MCs in the intestine but rarely on its molecular mechanisms. The most important molecular toxic mechanism of MCs in eukaryotes is it can strongly and specifically inhibit the activity of serine and threonine PP1 and PP2A, which are involved in many important intracellular processes such as cell growth, differentiation, protein synthesis, cell signaling, *etc.* Studies have confirmed that there is an irreversible covalent bond between MCs and PP1/PP2A^[58]. The toxic mechanisms reported in the small intestine are related to oxidative stress and the expression of apoptosis-related proteins after exposure to MC-LR. Therefore, further studies are needed to explore the specific cell signaling or receptors in MC-LR-induced intestinal damage. Furthermore, other mechanisms such as the endoplasmic reticulum stress pathway, caspase-dependent pathway, and mitochondrial-dependent pathway still require further investigation.

Long-term exposure experiments and cohort studies are necessary

As it is known, the confirmation of human carcinogen requirement is as follows: (1) a rigorous design, reliable

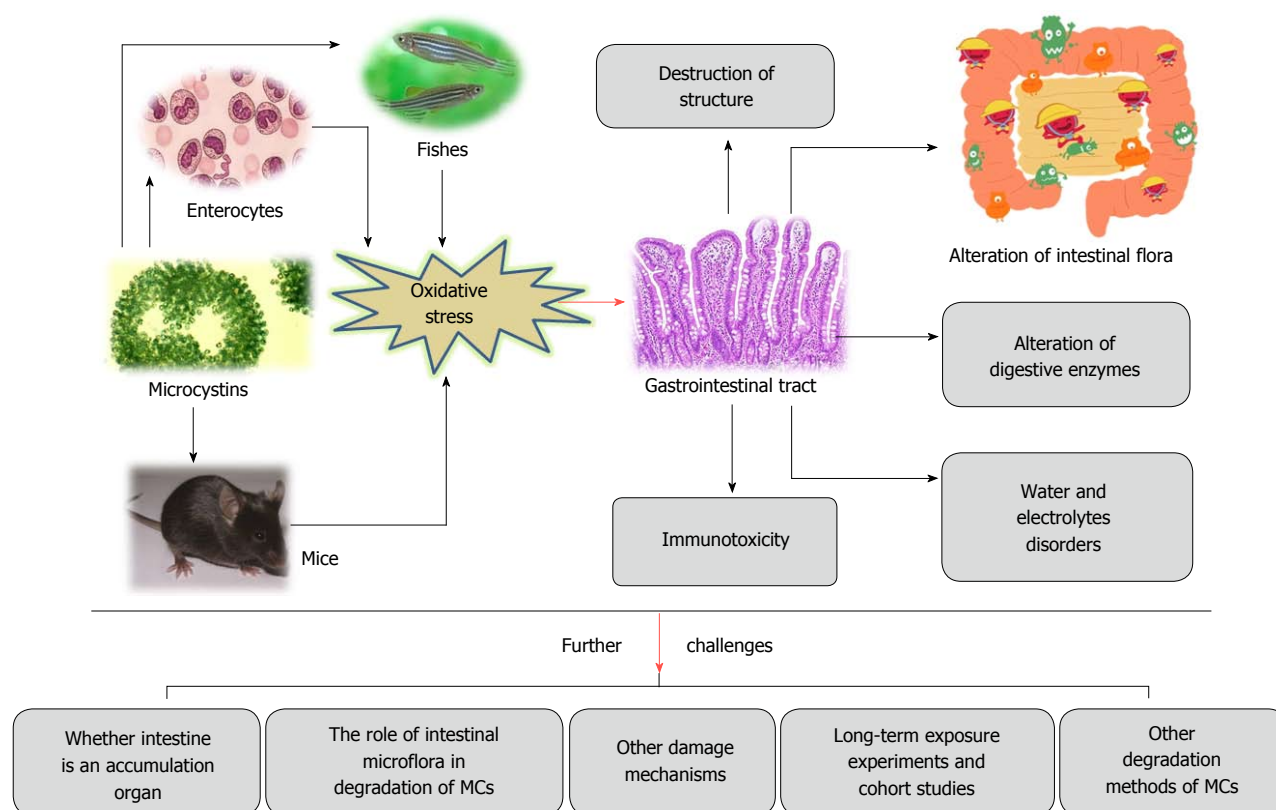


Figure 4 Summarization of the damages of microcystins to the gastrointestinal tract and further challenges that need to be addressed. MCs: Microcystins.

method to exclude the confounding epidemiological survey; (2) dose-response relationship; and (3) other survey data validation or animal experiments support. There is a certain correlation between the pollution of MCs and the occurrence of gastrointestinal cancer according to existing population research data. However, the majority of present population studies are retrospective investigations with more confounding factors and biases. Therefore, the causal relationship between the exposure of MCs and the occurrence of cancer remains unclear. More appropriate methods (cohort study) should be taken to determine its causal relationship. Present animal experiments are mostly acute or subacute experiments, and tumorigenesis is usually triggered by long term exposure to low doses of carcinogens. Therefore, long-term animal exposure experiments with low-dose MCs are needed.

The intestine is the main site for digestion and absorption, and directly determines the physical nutritional status. In previous studies conducted by the investigators, weight decreased significantly after intraperitoneal injection of 25 µg/kg of body weight of MC-LR for 14 days (data not shown). The damage induced by MCs to the gastrointestinal tract and further challenges that need to be addressed are summarized in Figure 4.

Routine clinical practice

MCs can enter the body through the mouth, inhalation, skin contact, medical treatment, uptake of aquatic

products contaminated by MCs, etc. Epidemiological surveys showed that MCs in drinking water sources are one of the major causes of the high incidence of primary liver cancer in some areas of southern China. The gastroenteritis of children occurs every year in an area of a specific reservoir water supply in Harare, Zimbabwe. However, children who used other water supplies in the city did not develop gastroenteritis^[59,60]. MCs were detected in many drinking water sources especially in the Taihu River of Wuxi, China in 2007^[26]. MCs pose a serious threat to human health. Thus, we advise in daily life to not drink river water directly, to not participate in water activities in bodies of water contaminated by water blooms, to not eat aquatic products contaminated with MCs, and to not use water contaminated with MCs as medical water in medical treatment. These recommendations will play a vital role in preventing the damage of MCs.

CONCLUSION

From the above discussion, it could be concluded that MCs induced damage to the gastrointestinal tract and caused various kinds of pathogenesis in the gastrointestinal tract, all of which poses a threat to human health. However, the mechanisms involved are unknown, and a broad number of issues raised in the present indicate the need to explore toxic mechanisms and seek detoxification methods. All of those proposed further challenges needs to be addressed.

REFERENCES

- 1 **Carmichael WW**, Beasley V, Bunner DL, Eloff JN, Falconer I, Gorham P, Harada K, Krishnamurthy T, Yu MJ, Moore RE. Naming of cyclic heptapeptide toxins of cyanobacteria (blue-green algae). *Toxicon* 1988; **26**: 971-973 [PMID: 3149803 DOI: 10.1016/0041-0101(88)90195-X]
- 2 **Chen L**, Giesy JP, Xie P. The dose makes the poison. *Sci Total Environ* 2018; **621**: 649-653 [PMID: 29197283 DOI: 10.1016/j.scitotenv.2017.11.218]
- 3 **Lee S**, Jiang X, Manubolu M, Riedl K, Ludsins SA, Martin JF, Lee J. Fresh produce and their soils accumulate cyanotoxins from irrigation water: Implications for public health and food security. *Food Res Int* 2017; **102**: 234-245 [PMID: 29195944 DOI: 10.1016/j.foodres.2017.09.079]
- 4 **Ma J**, Li Y, Duan H, Sivakumar R, Li X. Chronic exposure of nanomolar MC-LR caused oxidative stress and inflammatory responses in HepG2 cells. *Chemosphere* 2018; **192**: 305-317 [PMID: 29117589 DOI: 10.1016/j.chemosphere.2017.10.158]
- 5 **Vidal F**, Sedan D, D'Agostino D, Cavalieri ML, Mullen E, Parot Varela MM, Flores C, Caixach J, Andrinolo D. Recreational Exposure during Algal Bloom in Carrasco Beach, Uruguay: A Liver Failure Case Report. *Toxins (Basel)* 2017; **9**: pii: E267 [PMID: 28858213 DOI: 10.3390/toxins9090267]
- 6 **Hu X**, Zhang R, Ye J, Wu X, Zhang Y, Wu C. Monitoring and research of microcystins and environmental factors in a typical artificial freshwater aquaculture pond. *Environ Sci Pollut Res Int* 2018; **25**: 5921-5933 [PMID: 29235032 DOI: 10.1007/s11356-017-0956-4]
- 7 **Zhang Y**, Bleeker A, Liu J. Nutrient discharge from china's aquaculture industry and associated environmental impacts. *Environ Res Lett* 2015; **10**: 045002 [DOI: 10.1088/1748-9326/10/4/045002]
- 8 **Steiner K**, Zimmermann L, Hagenbuch B, Dietrich D. Zebrafish Oatp-mediated transport of microcystin congeners. *Arch Toxicol* 2016; **90**: 1129-1139 [PMID: 26055554 DOI: 10.1007/s00204-015-1544-3]
- 9 **Ding J**, Wang J, Xiang Z, Diao W, Su M, Shi W, Wan T, Han X. The organic anion transporting polypeptide 1a5 is a pivotal transporter for the uptake of microcystin-LR by gonadotropin-releasing hormone neurons. *Aquat Toxicol* 2017; **182**: 1-10 [PMID: 27842270 DOI: 10.1016/j.aquatox.2016.11.005]
- 10 **Feurstein D**, Kleinteich J, Heussner AH, Stemmer K, Dietrich DR. Investigation of microcystin congener-dependent uptake into primary murine neurons. *Environ Health Perspect* 2010; **118**: 1370-1375 [PMID: 20472527 DOI: 10.1289/ehp.0901289]
- 11 **Zeller P**, Clément M, Fessard V. Similar uptake profiles of microcystin-LR and -RR in an in vitro human intestinal model. *Toxicology* 2011; **290**: 7-13 [PMID: 21872638 DOI: 10.1016/j.tox.2011.08.005]
- 12 **Miller AP**, Tisdale ES. Public health engineering: epidemic of intestinal disorders in charleston, W. VA., occurring simultaneously with unprecedented water supply conditions. *Am J Public Health Nations Health* 1931; **21**: 198-200 [PMID: 18013204 DOI: 10.2105/AJPH.21.1.74]
- 13 **Pilotto LS**, Douglas RM, Burch MD, Cameron S, Beers M, Rouch GJ, Robinson P, Kirk M, Cowie CT, Hardiman S, Moore C, Attewell RG. Health effects of exposure to cyanobacteria (blue-green algae) during recreational water-related activities. *Aust N Z J Public Health* 1997; **21**: 562-566 [PMID: 9470258 DOI: 10.1111/j.1467-842X.1977.tb01114.x]
- 14 **Zhou L**, Yu H, Chen K. Relationship between microcystin in drinking water and colorectal cancer. *Biomed Environ Sci* 2002; **15**: 166-171 [PMID: 12244757]
- 15 **Lin YD**, Zhang YS, Xu M, Yang JB, Chen Y, Hu L, Shen WA. Study on the relationship between Wuxi Taihu waters pollution by algae toxin and health of the population (In Chinese). *Sh J Prev Med* 2003; **15**: 435-437
- 16 **Chen K**, Jiao DA, Lu L. Study on the relationship between the type of drinking water and the incidence of colorectal cancer (In Chinese). *Chinese Journal of Public Health*; 1991; **10**: 324-326
- 17 **Falconer IR**. Toxic cyanobacterial bloom problems in Australian waters: risks and impacts on human health. *Phycologia* 2001; **40**: 228-233 [DOI: 10.2216/i0031-8884-40-3-228.1]
- 18 **Zhou Y**, Xu X, Yu B, Yu G. Characterization of in vitro effects of microcystin-LR on intestinal epithelial cells. *Environ Toxicol* 2017; **32**: 1539-1547 [PMID: 27758031 DOI: 10.1002/tox.22375]
- 19 **Markowska M**, Oberle R, Juzwin S, Hsu CP, Gryszkiewicz M, Streeter AJ. Optimizing Caco-2 cell monolayers to increase throughput in drug intestinal absorption analysis. *J Pharmacol Toxicol Methods* 2001; **46**: 51-55 [PMID: 12164260 DOI: 10.1016/S1056-8719(01)00161-7]
- 20 **Gaudin J**, Huet S, Jarry G, Fessard V. In vivo DNA damage induced by the cyanotoxin microcystin-LR: comparison of intra-peritoneal and oral administrations by use of the comet assay. *Mutat Res* 2008; **652**: 65-71 [PMID: 18282792 DOI: 10.1016/j.mrgentox.2007.10.024]
- 21 **Djediat C**, Malécot M, de Luze A, Bernard C, Puiseux-Dao S, Edery M. Localization of microcystin-LR in medaka fish tissues after cyanotoxin gavage. *Toxicon* 2010; **55**: 531-535 [PMID: 19837107 DOI: 10.1016/j.toxicon.2009.10.005]
- 22 **Amrani A**, Nasri H, Azzouz A, Kadi Y, Bouaicha N. Variation in cyanobacterial hepatotoxin (microcystin) content of water samples and two species of fishes collected from a shallow lake in Algeria. *Arch Environ Contam Toxicol* 2014; **66**: 379-389 [PMID: 24445842 DOI: 10.1007/s00244-013-9993-2]
- 23 **He J**, Chen J, Xie P, Zhang D, Li G, Wu L, Zhang W, Guo X, Li S. Quantitatively evaluating detoxification of the hepatotoxic microcystins through the glutathione and cysteine pathway in the cyanobacteria-eating bighead carp. *Aquat Toxicol* 2012; **116-117**: 61-68 [PMID: 22466356 DOI: 10.1016/j.aquatox.2012.03.004]
- 24 **Xie L**, Xie P, Ozawa K, Honma T, Yokoyama A, Park HD. Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. *Environ Pollut* 2004; **127**: 431-439 [PMID: 14638304 DOI: 10.1016/j.envpol.2003.08.011]
- 25 **Yuan J**, Gu Z, Zheng Y, Zhang Y, Gao J, Chen S, Wang Z. Accumulation and detoxification dynamics of microcystin-LR and antioxidant responses in male red swamp crayfish *Procambarus clarkii*. *Aquat Toxicol* 2016; **177**: 8-18 [PMID: 27218425 DOI: 10.1016/j.aquatox.2016.05.004]
- 26 **Zhang D**, Xie P, Liu Y, Chen J, Liang G. Bioaccumulation of the hepatotoxic microcystins in various organs of a freshwater snail from a subtropical Chinese lake, Taihu Lake, with dense toxic *Microcystis* blooms. *Environ Toxicol Chem* 2007; **26**: 171-176 [PMID: 17269475 DOI: 10.1897/06-222R.1]
- 27 **Cazenave J**, Wunderlin DA, de Los Angeles Bistoni M, Amé MV, Krause E, Pflugmacher S, Wiegand C. Uptake, tissue distribution and accumulation of microcystin-RR in *Corydoras paleatus*, *Jenynsia multidentata* and *Odontesthes bonariensis*. A field and laboratory study. *Aquat Toxicol* 2005; **75**: 178-190 [PMID: 16157397 DOI: 10.1016/j.aquatox.2005.08.002]
- 28 **Chen J**, Xie P, Zhang D, Lei H. In situ studies on the distribution patterns and dynamics of microcystins in a biomanipulation fish-bighead carp (*Aristichthys nobilis*). *Environ Pollut* 2007; **147**: 150-157 [PMID: 17029683 DOI: 10.1016/j.envpol.2006.08.015]
- 29 **Chen J**, Xie P, Guo L, Zheng L, Ni L. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large shallow, eutrophic lake of the subtropical China. *Environ Pollut* 2005; **134**: 423-430 [PMID: 15620587 DOI: 10.1016/j.envpol.2004.09.014]
- 30 **Chen J**, Xie P. Seasonal dynamics of the hepatotoxic microcystins in various organs of four freshwater bivalves from the large eutrophic lake Taihu of subtropical China and the risk to human consumption. *Environ Toxicol* 2005; **20**: 572-584 [PMID: 16302170 DOI: 10.1002/tox.20146]
- 31 **Lei H**, Xie P, Chen J, Liang G, Dai M, Zhang X. Distribution of toxins in various tissues of crucian carp intraperitoneally injected with hepatotoxic microcystins. *Environ Toxicol Chem* 2008; **27**: 1167-1174 [PMID: 18419201 DOI: 10.1897/07-522.1]
- 32 **Chen J**, Xie P. Microcystin accumulation in freshwater bivalves from Lake Taihu, China, and the potential risk to human consumption. *Environ Toxicol Chem* 2007; **26**: 1066-1073 [PMID: 17521156 DOI: 10.1897/06-423R1.1]
- 33 **Wang Q**, Xie P, Chen J, Liang G. Distribution of microcystins in various organs (heart, liver, intestine, gonad, brain, kidney and lung) of Wistar rat via intravenous injection. *Toxicon* 2008; **52**: 721-727 [PMID: 18775740 DOI: 10.1016/j.toxicon.2008.08.004]
- 34 **Lei H**, Xie P, Chen J, Liang G, Yu T, Jiang Y. Tissue distribution and depuration of the extracted hepatotoxic cyanotoxin microcystins

- in crucian carp (*Carassius carassius*) intraperitoneally injected at a sublethal dose. *ScientificWorldJournal* 2008; **8**: 713-719 [PMID: 18677427 DOI: 10.1100/tsw.2008.101]
- 35 **Xie L**, Xie P, Guo L, Li L, Miyabara Y, Park HD. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Environ Toxicol* 2005; **20**: 293-300 [PMID: 15892067 DOI: 10.1002/tox.20120]
 - 36 **Huguet A**, Henri J, Petitpas M, Hogeveen K, Fessard V. Comparative cytotoxicity, oxidative stress, and cytokine secretion induced by two cyanotoxin variants, microcystin LR and RR, in human intestinal Caco-2 cells. *J Biochem Mol Toxicol* 2013; **27**: 253-258 [PMID: 23554253 DOI: 10.1002/jbt.21482]
 - 37 **Humpage AR**, Hardy SJ, Moore EJ, Froschio SM, Falconer IR. Microcystins (cyanobacterial toxins) in drinking water enhance the growth of aberrant crypt foci in the mouse colon. *J Toxicol Environ Health A* 2000; **61**: 155-165 [PMID: 11036504 DOI: 10.1080/00984100050131305]
 - 38 **Ferreira MF**, Oliveira VM, Oliveira R, da Cunha PV, Grisolia CK, Pires OR Jr. Histopathological effects of [D-Leu(1)]Microcystin-LR variants on liver, skeletal muscle and intestinal tract of *Hypophthalmichthys molitrix* (Valenciennes, 1844). *Toxicon* 2010; **55**: 1255-1262 [PMID: 20144637 DOI: 10.1016/j.toxicon.2010.01.016]
 - 39 **Trinchet I**, Djediat C, Huet H, Dao SP, Edery M. Pathological modifications following sub-chronic exposure of medaka fish (*Oryzias latipes*) to microcystin-LR. *Reprod Toxicol* 2011; **32**: 329-340 [PMID: 21839164 DOI: 10.1016/j.reprotox.2011.07.006]
 - 40 **Atencio L**, Moreno I, Jos A, Pichardo S, Moyano R, Blanco A, Cameán AM. Dose-dependent antioxidant responses and pathological changes in tenca (*Tinca tinca*) after acute oral exposure to Microcystis under laboratory conditions. *Toxicon* 2008; **52**: 1-12 [PMID: 18588906 DOI: 10.1016/j.toxicon.2008.05.009]
 - 41 **Djediat C**, Moyenga D, Malécot M, Comte K, Yéprémian C, Bernard C, Puisieux-Dao S, Edery M. Oral toxicity of extracts of the microcystin-containing cyanobacterium *Planktothrix agardhii* to the medaka fish (*Oryzias latipes*). *Toxicon* 2011; **58**: 112-122 [PMID: 21635913 DOI: 10.1016/j.toxicon.2011.05.011]
 - 42 **Huynh-Delerme C**, Edery M, Huet H, Puisieux-Dao S, Bernard C, Fontaine JJ, Crespeau F, de Luze A. Microcystin-LR and embryonal development of medaka fish, *Oryzias latipes*. I. Effects on the digestive tract and associated systems. *Toxicon* 2005; **46**: 16-23 [PMID: 15922383 DOI: 10.1016/j.toxicon.2005.03.009]
 - 43 **Molina R**, Moreno I, Pichardo S, Jos A, Moyano R, Monterde JG, Cameán A. Acid and alkaline phosphatase activities and pathological changes induced in *Tilapia* fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. *Toxicon* 2005; **46**: 725-735 [PMID: 16185737 DOI: 10.1016/j.toxicon.2005.07.012]
 - 44 **Lin W**, Hou J, Guo H, Li L, Wang L, Zhang D, Li D, Tang R. The synergistic effects of waterborne microcystin-LR and nitrite on hepatic pathological damage, lipid peroxidation and antioxidant responses of male zebrafish. *Environ Pollut* 2018; **235**: 197-206 [PMID: 29289830 DOI: 10.1016/j.envpol.2017.12.059]
 - 45 **Martins ND**, Yunes JS, Monteiro DA, Rantin FT, Kalinin AL. Microcystin-LR leads to oxidative damage and alterations in antioxidant defense system in liver and gills of *Brycon amazonicus* (SPIX & AGASSIZ, 1829). *Toxicon* 2017; **139**: 109-116 [PMID: 29024772 DOI: 10.1016/j.toxicon.2017.10.006]
 - 46 **La-Salette R**, Oliveira MM, Palmeira CA, Almeida J, Peixoto FP. Mitochondria a key role in microcystin-LR kidney intoxication. *J Appl Toxicol* 2008; **28**: 55-62 [PMID: 17461434 DOI: 10.1002/jat.1251]
 - 47 **Zhang H**, Cai C, Wu Y, Shao D, Ye B, Zhang Y, Liu J, Wang J, Jia X. Mitochondrial and endoplasmic reticulum pathways involved in microcystin-LR-induced apoptosis of the testes of male frog (*Rana nigromaculata*) in vivo. *J Hazard Mater* 2013; **252-253**: 382-389 [PMID: 23548922 DOI: 10.1016/j.jhazmat.2013.03.017]
 - 48 **Pavagadhi S**, Gong Z, Hande MP, Dionysiou DD, de la Cruz AA, Balasubramanian R. Biochemical response of diverse organs in adult *Danio rerio* (zebrafish) exposed to sub-lethal concentrations of microcystin-LR and microcystin-RR: a balneation study. *Aquat Toxicol* 2012; **109**: 1-10 [PMID: 22207040 DOI: 10.1016/j.aquatox.2011.11.009]
 - 49 **Sedan D**, Laguens M, Copparoni G, Aranda JO, Giannuzzi L, Marra CA, Andrinolo D. Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of Microcystin-LR. *Toxicon* 2015; **104**: 26-33 [PMID: 26210502 DOI: 10.1016/j.toxicon.2015.07.011]
 - 50 **Moreno IM**, Mate A, Repetto G, Vázquez CM, Cameán AM. Influence of microcystin-LR on the activity of membrane enzymes in rat intestinal mucosa. *J Physiol Biochem* 2003; **59**: 293-299 [PMID: 15164949 DOI: 10.1007/BF03179887]
 - 51 **Chen C**, Liu W, Wang L, Li J, Chen Y, Jin J, Kawan A, Zhang X. Pathological damage and immunomodulatory effects of zebrafish exposed to microcystin-LR. *Toxicon* 2016; **118**: 13-20 [PMID: 27085306 DOI: 10.1016/j.toxicon.2016.04.030]
 - 52 **Liu LY**, Xie P. Effect of microcystin - LR on intestinal digestive enzymes in mice (In Chinese). *Acta Hydrobiologia Sinica* 2014; **38**: 533-539
 - 53 **Yao YH**, Yu LN, He WH, Li G, Luo XS. Effects of toxic microcystin on the growth, activities of digestive enzymes and accumulation of microcystin in *Carassius auratus*. *Zhongguo Shuichan Kexue* 2007; **14**: 969-973
 - 54 **Rocha MF**, Sidrim JJ, Soares AM, Jimenez GC, Guerrant RL, Ribeiro RA, Lima AA. Supernatants from macrophages stimulated with microcystin-LR induce electrogenic intestinal response in rabbit ileum. *Pharmacol Toxicol* 2000; **87**: 46-51 [PMID: 10987215 DOI: 10.1111/j.0901-9928.2000.870108.x]
 - 55 **Nobre AC**, Nunes-Monteiro SM, Monteiro MC, Martins AM, Havt A, Barbosa PS, Lima AA, Monteiro HS. Microcystin-LR promote intestinal secretion of water and electrolytes in rats. *Toxicon* 2004; **44**: 555-559 [PMID: 15450931 DOI: 10.1016/j.toxicon.2004.07.014]
 - 56 **Dahlem AM**, Hassan AS, Swanson SP, Carmichael WW, Beasley VR. A model system for studying the bioavailability of intestinally administered microcystin-LR, a hepatotoxic peptide from the cyanobacterium *Microcystis aeruginosa*. *Pharmacol Toxicol* 1989; **64**: 177-181 [PMID: 2502775 DOI: 10.1111/j.1600-0773.1989.tb00625.x]
 - 57 **Bieczynski F**, Torres WD, Paineñil JC, Castro JM, Bianchi VA, Frontera JL, Paz DA, González C, Martín A, Villanueva SS, Luquet CM. Alterations in the intestine of Patagonian silverside (*Odontesthes hatcheri*) exposed to microcystin-LR: Changes in the glycosylation pattern of the intestinal wall and inhibition of multidrug resistance proteins efflux activity. *Aquat Toxicol* 2016; **178**: 106-117 [PMID: 27474942 DOI: 10.1016/j.aquatox.2016.07.016]
 - 58 **Svircev Z**, Baltić V, Gantar M, Juković M, Stojanović D, Baltić M. Molecular aspects of microcystin-induced hepatotoxicity and hepatocarcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 2010; **28**: 39-59 [PMID: 20390967 DOI: 10.1080/10590500903585382]
 - 59 **Zilberg B**. Gastroenteritis in Salisbury. European children--a five-year study. *Cent Afr J Med* 1966; **12**: 164-168 [PMID: 5919885]
 - 60 **Pouria S**, de Andrade A, Barbosa J, Cavalcanti RL, Barreto VT, Ward CJ, Preiser W, Poon GK, Neild GH, Codd GA. Fatal microcystin intoxication in haemodialysis unit in Caruaru, Brazil. *Lancet* 1998; **352**: 21-26 [PMID: 9800741 DOI: 10.1016/S0140-6736(97)12285-1]

P- Reviewer: Amorniyotin S, Chow WK, Slomiany BL, Teramoto-Matsubara OT **S- Editor:** Ji FF
L- Editor: Filipodia **E- Editor:** Song H



Basic Study

***PNPLA3* rs139051 is associated with phospholipid metabolite profile and hepatic inflammation in nonalcoholic fatty liver disease**

Ji-Jun Luo, Hai-Xia Cao, Rui-Xu Yang, Rui-Nan Zhang, Qin Pan

Ji-Jun Luo, Hai-Xia Cao, Rui-Xu Yang, Rui-Nan Zhang, Qin Pan, Department of Gastroenterology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200092, China

ORCID number: Ji-Jun Luo (0000-0002-3007-935X); Hai-Xia Cao (0000-0002-8265-9460); Rui-Xu Yang (0000-0001-9384-6408); Rui-Nan Zhang (0000-0001-9049-3010); Qin Pan (0000-0001-5855-4952).

Author contributions: Pan Q conceived and designed the experiments; Luo JJ, Yang RX and Zhang RN performed the experiments; Luo JJ and Cao HX analyzed the data; Pan Q wrote the paper; Luo JJ and Cao HX contributed equally to this work.

Supported by National Key Research and Development Plan "Precision Medicine Research", No. 2017YFC0908903; National Natural Science Foundation of China, No. 81070346, No. 81270492, No. 81470859, No. 81270491 and No. 81470840; State Key Development Program for Basic Research of China, No. 2012CB517501; 100 Talents Program, No. XBR2011007h; and Program of the Committee of Science and Technology, No. 09140903500.

Institutional review board statement: The study was reviewed and approved by the Xinhua Hospital Ethics Committee Affiliated to Shanghai Jiaotong University School of Medicine.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Qin Pan, MD, PhD, Professor, Department of Gastroenterology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Kongjiang Road NO. 1665, Yangpu District, Shanghai 200092, China. panqin@xinhumed.com.cn
Telephone: +86-21-63846590
Fax: +86-21-25077340

Received: June 19, 2018

Peer-review started: June 19, 2018

First decision: July 11, 2018

Revised: July 16, 2018

Accepted: August 3, 2018

Article in press: August 4, 2018

Published online: September 26, 2018

Abstract**AIM**

To investigate the effect of *PNPLA3* polymorphisms on serum lipidomics and pathological characteristics in nonalcoholic fatty liver disease (NAFLD).

METHODS

Thirty-four biopsy-proven NAFLD patients from Northern, Central, and Southern China were subjected to stratification by genotyping their single nucleotide polymorphisms (SNPs) in *PNPLA3*. Ultra performance liquid chromatography-tandem mass spectrometry was then employed to characterize the effects of *PNPLA3* SNPs on serum lipidomics. In succession, correlation analysis revealed the association of *PNPLA3*-related lipid profile and hepatic pathological characteristics on a basis of steatosis, activity, and fibrosis assessment. The variant-based scoring of hepatocyte steatosis, ballooning, lobular inflammation, and liver fibrosis was finally performed so as to uncover the actions of lipidomics-affecting *PNPLA3*

SNPs in NAFLD-specific pathological alterations.

RESULTS

PNPLA3 SNPs (rs139051, rs738408, rs738409, rs2072906, rs2294918, rs2294919, and rs4823173) demonstrated extensive association with the serum lipidomics, especially phospholipid metabolites [lysophosphatidylcholine (LPC), lysophosphatidylcholine plasmalogen (LPCO), lysophosphatidylethanolamine (LPE), phosphatidylcholine (PC), choline plasmalogen (PCO), phosphatidylethanolamine (PE), ethanolamine plasmalogen (PEO)], of NAFLD patients. *PNPLA3* rs139051 (A/A genotype) and rs2294918 (G/G genotype) dominated the up-regulatory effect on phospholipids of LPCs (LPC 17:0, LPC 18:0, LPC 20:0, LPC 20:1, LPC 20:2) and LPCOs (LPC O-16:1, LPC O-18:1). Moreover, subjects with high-level LPCs/LPCOs were predisposed to low-grade lobular inflammation of NAFLD (ρ : -0.407 to -0.585, $P < 0.05$ -0.001). The significant correlation of *PNPLA3* rs139051 and inflammation grading [A/A vs A/G + G/G: 0.50 (0.00, 1.75) vs 1.50 (1.00, 2.00), $P < 0.05$] further demonstrated its pathological role based on the modulation of phospholipid metabolite profile.

CONCLUSION

The A/A genotype at *PNPLA3* rs139051 exerts an up-regulatory effect on serum phospholipids of LPCs and LPCOs, which are associated with low-grade lobular inflammation of NAFLD.

Key words: Nonalcoholic fatty liver disease; Patatin-like phospholipase domain containing 3; Single nucleotide polymorphism; Phospholipid; Inflammation

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: *PNPLA3* single nucleotide polymorphisms reflect an important genetic basis of serum lipidomics, especially phospholipid metabolites, in nonalcoholic fatty liver disease (NAFLD) patients. *PNPLA3* rs139051 (A/A genotype) exerts an up-regulatory effect on phospholipids of lysophosphatidylcholines (LPCs) and lysophosphatidylcholine plasmalogens (LPCOs). Moreover, both the A/A genotype at *PNPLA3* rs139051 and high-level LPCs/LPCOs share an association with the low-grade lobular inflammation of NAFLD. Therefore, *PNPLA3* rs139051 may underlie the inflammatory progress of NAFLD with its modulation of phospholipid metabolite profiles.

Luo JJ, Cao HX, Yang RX, Zhang RN, Pan Q. *PNPLA3* rs139051 is associated with phospholipid metabolite profile and hepatic inflammation in nonalcoholic fatty liver disease. *World J Clin Cases* 2018; 6(10): 355-364 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/355.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.355>

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by excess triglyceride accumulation and lobular inflammatory infiltration and is a leading cause of most chronic liver diseases in European, Asian-Pacific, and American patients^[1]. According to the diagnosis and management guidelines, hyperlipidemia (e.g., hypertriglyceridemia or hypercholesterolemia) has been identified as one of the most important risk factors for NAFLD^[2]. Approximately 50% of patients with hyperlipidemia have ultrasonographic evidence of fatty infiltration in the liver^[3]. Patients with a spectrum of NAFLD, ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), show prevalence of dyslipidemia, including increased serum triglycerides and low-density lipoprotein (LDL), and low levels of high-density lipoprotein cholesterol (HDL-C)^[4]. Thus, the lipidemic properties are closely associated with the initiation and progression of NAFLD.

Recently, genome-wide association analysis and clinical investigations have found that single nucleotide polymorphisms (SNPs) of patatin-like phospholipase domain containing 3 (*PNPLA3*) (e.g., rs738409, rs1010023, rs2281135, rs139051 and rs2294918) underlie the genetic susceptibility of NAFLD, independent of gender, age and ethnic background^[5-11]. Functional studies have highlighted that adiponutrin, the encoded product of *PNPLA3*, is a crucial regulator of lipid metabolism in the liver via its activities on triacylglycerol lipase and acyl glycerol O-acyltransferase^[12]. Given the central role of the liver in systemic lipid homeostasis, these loss-of-function SNPs in *PNPLA3* are suggested to predispose individuals to NAFLD, probably by dysregulation of hepatic and serum lipid profiles^[4]. This lipid-metabolism-regulating role of *PNPLA3* SNPs has been confirmed in the liver of NAFLD patients^[13]. However, understanding of the SNP-specific impact on serum lipids and their correlation with pathological characteristics is still limited and controversial^[14-17].

We therefore stratified Chinese Han patients with biopsy-proven NAFLD by genotyping their *PNPLA3* SNPs. Ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was used to characterize the effects of *PNPLA3* SNPs on serum lipidomics of NAFLD patients. Correlation analysis uncovered the association of *PNPLA3*-related lipid profile and hepatic pathological characteristics by assessment of steatosis, activity and fibrosis (SAF). Finally, variant-based scoring of steatosis, ballooning, inflammation, and liver fibrosis was performed to reveal the actions of lipidomics-affecting *PNPLA3* SNPs on NAFLD-specific pathological alterations.

MATERIALS AND METHODS

Study population

Thirty-four Chinese Han patients with biopsy-proven NAFLD were enrolled from Shanghai Xinhua Hospital (n

= 17), Zhengxing Hospital ($n = 8$) and Tianjin Hospital of Infectious Diseases ($n = 9$) between January 2012 and June 2013. The exclusion criteria were as follows: historic or current high alcohol consumption equivalent to > 20 g/d for men and > 10 g/d for women^[18,19], viral hepatitis, drug-induced liver disease, Wilson's disease, autoimmune liver diseases and other diseases that lead to steatosis. The study was approved by the Xinhua Hospital Research Ethics Committee, and informed consent was obtained from all patients.

Anthropometric and biochemical assessments

The baseline data of age (41.03 ± 14.81 years), gender, height (167.44 ± 7.82 cm), weight (75.34 ± 9.49 kg), and body mass index (BMI) (26.90 ± 3.13) were characterized for the study population. Fasting blood samples were collected from the NAFLD patients, and a multichannel automatic analyzer (Advia 1650; Bayer, Moss, Norway) was used to test biochemical indexes of alanine aminotransferase (58.33 ± 33.89 U/L), aspartate aminotransferase (24.05 ± 28.63 U/L), alkaline phosphatase (101.90 ± 110.49 U/L), and γ -glutamyltransferase (115.73 ± 278.59 U/L). Fasting blood glucose (42.70 ± 28.07 μ U/mL), total cholesterol (4.70 ± 0.61 mg/dL), and triglyceride (1.70 ± 0.68 mg/dL) were subjected to assessment using Wako Bioproducts (Wako Pure Chemical Industries, Richmond, VA, United States).

Hepatic histopathological assessment

Liver samples from each NAFLD patient were obtained by needle biopsy after obtaining informed consent. Liver tissues were fixed in formalin, paraffin embedded, and 5- μ m sections were cut. Both hematoxylin and eosin and Masson's trichrome staining were used for pathological characterization, including hepatocyte steatosis, lobular inflammation, ballooning, and liver fibrosis according to the SAF scoring method^[20-22], by three pathologists who were not aware of the study.

Genotyping of *PNPLA3* SNPs

Blood samples from NAFLD patients were treated as follows: (1) centrifugation at 1500 rpm for 10 min; (2) separation of buffy-coat layer; and (3) extraction of genomic DNA from the buffy coat lymphocytes by QiAamp DNA Mini Kit (Qiagen, Venlo, Netherlands). A custom Ion AmpliSeq panel (Life Technologies Thermo Fisher Scientific, Waltham, MA, United States) of human *PNPLA3* was designed for the emulsion polymerase chain reaction of template DNA using a Ion OneTouch 2 System (Life Technologies). *PNPLA3* SNPs were genotyped by the Ion 318 Chip (Life Technologies) according to the Ion PGM 200 Sequencing kit protocol^[23].

UPLC-MS/MS

After 12-h fasting, serum lipidomics of the enrolled NAFLD patients were analyzed with a combination of

UPLC (Waters, Milford, MA, United States) and Triple TOF 5600 mass spectrometer (AB SCIEX, Framingham, MA, United States)^[24]. The column temperature was set to 55°C. The ratios of acetonitrile/water (mobile phase A) and propanol/acetonitrile (mobile phase B) were 3/2 and 9/1, respectively, with 10 mmol/L ammonium acetate added to all mobile phases. The elution gradient program was carried out as follows: 0-1.5 min: 32% B; 1.5-14 min: 85% B; 15.5-15.6 min: 97% B; 15.6-18 min: 97% B; 18-20 min: 32% B (flow rate: 0.26 mL/min). MS was applied by electrospray ionization operating in the positive and negative ion modes. The temperature of the interface heater was 600°C (–) and 500°C (+), with 4500 V (–) and 5500 V (+) ion spray voltage. The declustering potential was 100 V (–) and 100 V (+), and collision energy was 10 V (–) and 10 V (+). Thirteen quality control (QC) samples, being randomly inserted into the sequence, were analyzed for data precision. The raw data obtained from UPLC-MS/MS by Analyst TF 1.6 software (AB SCIEX) were subjected to identification of lipid composition using LipidView/PeakView and quantification of lipid concentrations using MultiQuant 2.0. The relative standard deviation (RSD) of 239 serum lipids in QC samples was evaluated against the internal standards.

Statistical analysis

The clinical data were expressed as mean \pm SD (continuous, normally distributed variables) or medians (interquartile range) (discontinuous, non-normally distributed variables). Differences in serum lipidomics among the groups of *PNPLA3* SNPs were investigated by unpaired Student's independent *t* tests. Correlation analysis of phospholipid metabolite profile and hepatic pathological characteristics was assessed by Spearman's correlation. The Mann-Whitney *U* test was performed to evaluate the differences in pathological grading among groups of *PNPLA3* SNPs. SPSS version 19.0 (SPSS Inc., Chicago, IL, United States) was used for statistical analysis with a two-side significant criterion at $P < 0.05$.

RESULTS

***PNPLA3* SNPs associated with serum lipidomics in NAFLD patients**

With the stable distribution and limited RSD of QC samples (Figure 1 and Table 1), an obvious association of *PNPLA3* SNPs (rs139051, rs738408, rs738409, rs2072906, rs2294918, rs2294919 and rs4823173) and serum lipidomic characteristics was observed in Chinese Han NAFLD patients by combination of *PNPLA3* genotyping and UPLC-MS/MS (Table 2). In detail, these *PNPLA3* SNPs significantly correlated to the serum level of various members of cholesteryl ester (CE), free fatty acid (FFA), lyso-phosphatidylcholine (LPC), lysophosphatidylcholine plasmalogen (LPCO), lysophosphatidylethanolamine

Table 1 Relative standard deviation distribution in quality control samples

Percentage	Relative SD				
	< 10%	10%-15%	15%-20%	20%-30%	30%-50%
Peak number in total	10	47	32	10	1
Sum of peak number in total	10	57	89	99	100

Table 2 *PNPLA3* single nucleotide polymorphisms associated with serum lipidomics of nonalcoholic fatty liver disease patients

SNPs	Number of SNP-associated serum lipids									
	CE	FFA	LPC	LPCO	LPE	PC	PCO	PE	PEO	TAG
rs139051 (A/A: A/G + G/G)	1	1	5	2	1	0	0	1	1	0
rs738408 (T/T: C/T + C/C)	1	0	0	0	0	0	0	0	0	0
rs738409 (G/G: C/G + C/C)	1	0	0	0	0	0	0	0	0	0
rs2072906 (G/G: A/G + A/A)	1	0	0	0	0	0	1	0	0	0
rs2294918 (G/G: A/A + A/G)	0	0	3	3	1	1	0	9	0	5
rs2294919 (C/C: C/T + T/T)	0	1	0	0	0	0	0	0	0	0
rs4823173 (A/A: A/G + G/G)	1	0	0	0	0	0	0	0	0	0

CE: Cholesteryl ester; FFA: Free fatty acid; LPC: Lysophosphatidylcholine; LPCO: Lysophosphatidylcholine plasmalogen; LPE: Lysophosphatidylethanolamine; NAFLD: Nonalcoholic fatty liver disease; PC: Phosphatidylcholine; PCO: Choline plasmalogen; PE: Phosphatidylethanolamine; PEO: Ethanolamine plasmalogen; *PNPLA3*: Patatin-like phospholipase domain containing 3; SNP: Single nucleotide polymorphisms; TAG: Triacylglycerol.

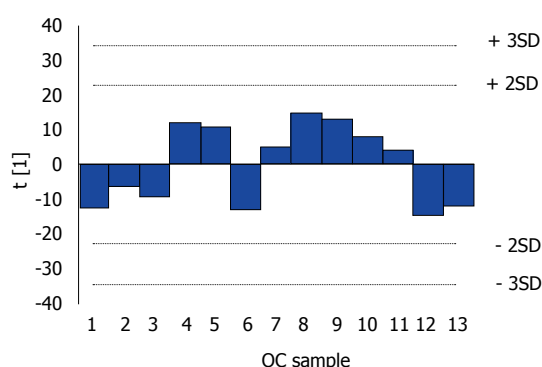


Figure 1 Dynamic distribution of quality control samples throughout the Ultra-high performance liquid chromatography-tandem mass spectrometry analysis. Colored bars indicate the quality control samples that are projected onto the first principal component with a range from -2 SD to +2 SD. QC: Quality control; SD: Standard deviation.

(LPE), phosphatidylcholine (PC), choline plasmalogen (PCO), phosphatidylethanolamine (PE), ethanolamine plasmalogen (PEO), and triacylglycerol (TAG) (Table 2).

***PNPLA3* rs139051 and rs2294918 exerted upregulatory effects on LPCs and LPCOs**

PNPLA3 rs139051 and rs2294918, with their effects on 12 and 22 different serum lipids, respectively, dominated the *PNPLA3* SNP-lipidomics association (Table 2). Compared to those with the A/G or G/G genotype, NAFLD patients carrying the A/A genotype at *PNPLA3* rs139051 demonstrated significantly higher serum

levels of LPC 17:0, LPC 18:0, LPC 20:0, LPC 20:1, LPC 20:2, LPC O-16:1, LPC O-18:1, and significantly lower levels of LPE 20:4, PE 34:0 and PE O-36:5 (Table 3). Quantitative analysis showed significantly increasing levels of LPC 17:0, LPC 20:0, LPC 20:1, LPC O-16:0, LPC O-16:1, and LPC O-18:1 in the NAFLD patients with the G/G phenotype compared to the A/A or A/G phenotype at *PNPLA3* rs2294918 (Table 3). Nevertheless, there was lower serum levels of LPE 22:6, PC 32:1, PE 34:0, PE 34:2, PE 36:2, PE 36:4, PE 38:4, PE 38:5, PE 38:6, PE 40:5 and PE 40:6 in these patients (Table 3).

High levels of LPCs and LPCOs demonstrated a correlation with low-grade hepatic inflammation

To shed light on the influence of *PNPLA3* SNP-related lipidomic characteristics, correlations of phospholipid metabolites and NAFLD-specific pathological disorders (hepatocyte steatosis, lobular inflammation, ballooning, and liver fibrosis) were analyzed. Various LPCs (LPC 17:0, LPC 18:0, LPC 20:0, LPC 20:1 and LPC 20:2) and LPCOs (LPC O-16:1 and LPC O-18:1) showed negative correlations with the grade of lobular inflammation. The serum levels of these phospholipid metabolites were high in NAFLD patients with the A/A genotype at *PNPLA3* rs139051 and/or the G/G genotype at rs2294918. Most of them shared similar Spearman's rank correlation coefficients (Table 4). In contrast, pathological indexes other than lobular inflammation correlated to neither high-level phospholipid metabolites

Table 3 Effects of *PNPLA3* rs139051 and rs2294918 on serum profile of phospholipid metabolites in nonalcoholic fatty liver disease patients

Phospholipid metabolites	SNPs					
	rs139051			rs2294918		
	A/A	A/G + G/G	P value	G/G	A/A + A/G	P value
LPC 17:0	0.148 ± 0.119	0.089 ± 0.032	0.045 ^a	0.143 ± 0.113	0.084 ± 0.023	0.023 ^a
LPC 18:0	5.501 ± 4.064	3.482 ± 1.032	0.045 ^a	5.199 ± 3.888	3.564 ± 0.941	0.068
LPC 20:0	0.019 ± 0.012	0.011 ± 0.005	0.013 ^a	0.018 ± 0.011	0.010 ± 0.005	0.008 ^a
LPC 20:1	0.035 ± 0.028	0.020 ± 0.072	0.031 ^a	0.033 ± 0.026	0.018 ± 0.007	0.017 ^a
LPC 20:2	0.046 ± 0.040	0.025 ± 0.090	0.037 ^a	0.042 ± 0.038	0.027 ± 0.010	0.091
LPC O-16:0	0.128 ± 0.109	0.078 ± 0.016	0.058	0.123 ± 0.102	0.075 ± 0.016	0.038 ^a
LPC O-16:1	0.111 ± 0.088	0.068 ± 0.016	0.046 ^a	0.106 ± 0.083	0.067 ± 0.014	0.041 ^a
LPC O-18:1	0.076 ± 0.071	0.041 ± 0.011	0.039 ^a	0.072 ± 0.067	0.040 ± 0.011	0.036 ^a
LPE 20:4	0.087 ± 0.037	0.116 ± 0.035	0.029 ^a	0.092 ± 0.040	0.113 ± 0.034	0.143
LPE 22:6	0.091 ± 0.044	0.113 ± 0.037	0.138	0.090 ± 0.036	0.122 ± 0.046	0.035 ^a
PC 32:1	1.574 ± 0.941	1.912 ± 1.167	0.358	1.450 ± 0.949	2.263 ± 1.036	0.030 ^a
PE 34:0	0.312 ± 0.018	0.325 ± 0.016	0.041 ^a	0.310 ± 0.015	0.332 ± 0.015	0.001 ^a
PE 34:2	0.335 ± 0.187	0.309 ± 0.159	0.673	0.280 ± 0.131	0.418 ± 0.219	0.028 ^a
PE 36:2	0.758 ± 0.349	0.702 ± 0.324	0.634	0.650 ± 0.271	0.913 ± 0.398	0.030 ^a
PE 36:4	0.282 ± 0.142	0.324 ± 0.169	0.435	0.246 ± 0.105	0.410 ± 0.181	0.015 ^a
PE 38:4	0.746 ± 0.349	0.830 ± 0.348	0.493	0.661 ± 0.228	1.031 ± 0.423	0.018 ^a
PE 38:5	0.079 ± 0.036	0.079 ± 0.034	0.960	0.070 ± 0.026	0.098 ± 0.043	0.025 ^a
PE 38:6	0.621 ± 0.431	0.613 ± 0.332	0.953	0.493 ± 0.232	0.878 ± 0.517	0.036 ^a
PE 40:5	0.053 ± 0.026	0.064 ± 0.033	0.305	0.047 ± 0.022	0.080 ± 0.030	0.001 ^a
PE 40:6	0.445 ± 0.316	0.451 ± 0.220	0.952	0.354 ± 0.174	0.644 ± 0.351	0.003 ^a
PE O-36:5	0.529 ± 0.192	0.699 ± 0.276	0.041 ^a	0.547 ± 0.171	0.707 ± 0.331	0.071

LPC: Lysophosphatidylcholine; LPCO: Lysophosphatidylcholine plasmalogen; LPE: Lysophosphatidylethanolamine; NAFLD: Nonalcoholic fatty liver disease; PC: Phosphatidylcholine; PCO: Choline plasmalogen; PE: Phosphatidylethanolamine; PEO: Ethanolamine plasmalogen; *PNPLA3*: Patatin-like phospholipase domain containing 3. Values are expressed as mean ± SD. ^a*P* < 0.05.

Table 4 Phospholipid metabolites correlated with pathological characteristics of nonalcoholic fatty liver disease

Phospholipid metabolites	Steatosis		Lobular inflammation		Ballooning		Fibrosis	
	rho	P value	rho	P value	rho	P value	rho	P value
LPC 17:0	0.149	0.399	-0.525	0.001 ^a	0.093	0.599	0.095	0.592
LPC 18:0	0.024	0.892	-0.478	0.004 ^a	0.136	0.442	0.089	0.618
LPC 20:0	-0.071	0.692	-0.585	0.000 ^a	0.010	0.956	0.082	0.645
LPC 20:1	-0.061	0.734	-0.489	0.003 ^a	-0.165	0.352	-0.072	0.686
LPC 20:2	0.092	0.604	-0.453	0.007 ^a	0.145	0.415	0.042	0.812
LPC O-16:0	0.297	0.088	-0.296	0.089	0.025	0.886	0.146	0.410
LPC O-16:1	0.070	0.695	-0.425	0.012 ^a	0.028	0.874	0.027	0.881
LPC O-18:1	-0.114	0.521	-0.407	0.017 ^a	-0.018	0.921	0.107	0.546
LPE 20:4	-0.079	0.658	0.107	0.547	0.071	0.690	-0.071	0.692
LPE 22:6	-0.274	0.117	0.054	0.764	0.125	0.481	0.173	0.327
PC 32:1	0.020	0.913	0.086	0.630	0.107	0.547	-0.069	0.699
PE 34:0	-0.012	0.946	0.193	0.275	-0.173	0.327	-0.146	0.410
PE 34:2	-0.054	0.762	-0.025	0.888	-0.043	0.809	-0.153	0.388
PE 36:2	-0.050	0.777	-0.079	0.659	-0.118	0.506	-0.257	0.143
PE 36:4	-0.091	0.608	0.150	0.398	0.121	0.495	-0.052	0.770
PE 38:4	-0.112	0.529	0.214	0.224	0.110	0.537	-0.065	0.716
PE 38:5	-0.234	0.182	0.178	0.313	-0.026	0.885	-0.096	0.587
PE 38:6	-0.151	0.393	0.096	0.588	0.176	0.320	0.105	0.554
PE 40:5	-0.013	0.944	0.132	0.457	0.049	0.783	-0.194	0.273
PE 40:6	-0.192	0.277	0.132	0.457	0.150	0.397	0.067	0.705
PE O-36:5	0.021	0.905	0.178	0.313	-0.205	0.245	-0.131	0.461

LPC: Lysophosphatidylcholine; LPCO: Lysophosphatidylcholine plasmalogen; LPE: Lysophosphatidylethanolamine; NAFLD: Nonalcoholic fatty liver disease; PC: Phosphatidylcholine; PCO: Choline plasmalogen; PE: Phosphatidylethanolamine; PEO: Ethanolamine plasmalogen; rho: Spearman's rank correlation coefficient. ^a*P* < 0.05.

(LPCs and LPCOs) nor low-level ones (LPEs, PC 32:1, PEs and PEO 36:5) in these NAFLD patients (Table 4). Given their impact on the serum profile of phospholipid metabolites, both *PNPLA3* rs139051 and rs2294918 are proposed to act in hepatic inflammation of NAFLD by

targeting, at least to a large extent, LPCs and LPCOs.

Low-grade hepatic inflammation occurred in NAFLD patients with the A/A genotype at *PNPLA3* rs139051

After the stratification of NAFLD patients by *PNPLA3*

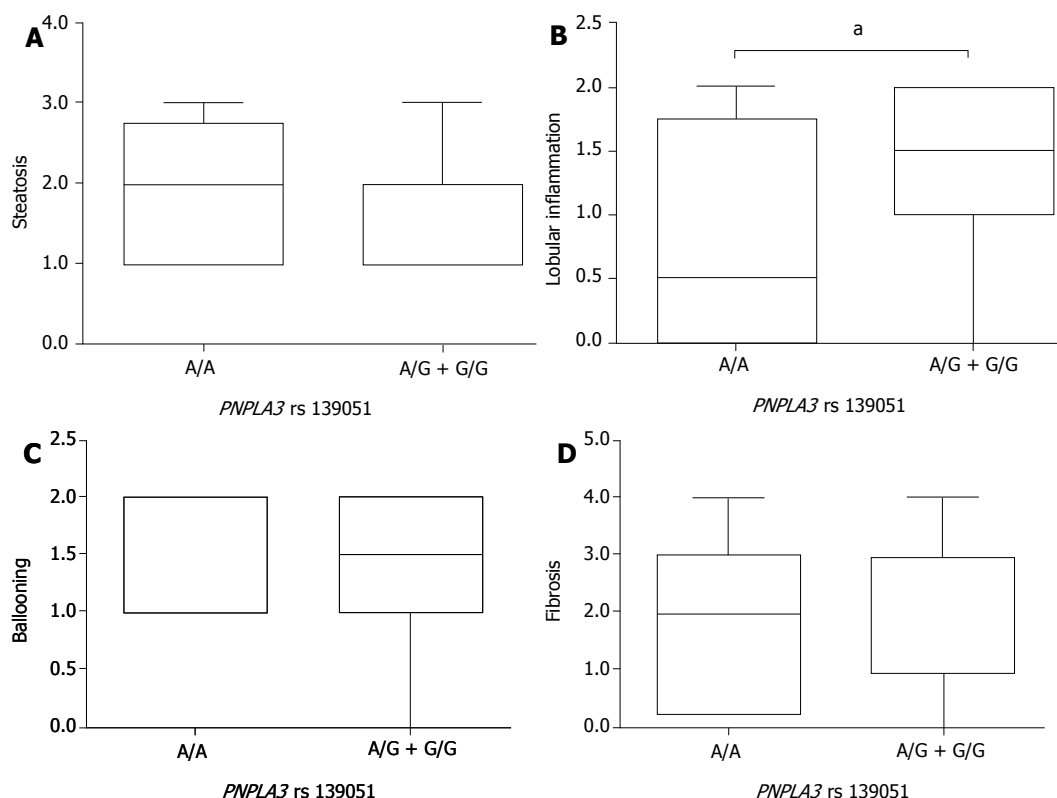


Figure 2 Nonalcoholic fatty liver disease patients carrying the A/A genotype at *PNPLA3* rs139051 demonstrated low-grade lobular inflammation. Box plots indicate the difference in pathologic characteristics of steatosis (A); lobular inflammation (B); ballooning (C); and fibrosis (D) between nonalcoholic fatty liver disease patients with the A/A or A/G + G/G genotype at *PNPLA3* rs139051. Results are presented as medians and Interquartile Range. ^a*P* < 0.05.

genotypes, comparison of steatosis, lobular inflammation, ballooning, and liver fibrosis was carried out to assess the pathological role of *PNPLA3* rs139051 and rs2294918. By the SAF-based scoring, the A/A genotype at *PNPLA3* rs139051 conferred significantly lower lobular inflammation than the A/G + G/G genotypes (Figure 2). However, *PNPLA3* rs139051 did not seem to exert any significant impact on NAFLD-specific steatosis, ballooning, and fibrosis (Figure 2). NAFLD patients with *PNPLA3* rs2294918, another SNP related to the inflammation-associated phospholipid metabolites, showed a comparable grade in each of these pathological indexes regardless of genotype (Figure 3).

DISCUSSION

PNPLA3 is the liver-enriched member of the PNPLA family, which is located on the membrane of lipid droplets and endoplasmic reticulum in hepatocytes^[25,26]. Its conserved patatin-like domain demonstrates hydrolase activity against TAG, diacylglycerol and monoacylglycerol^[15]. Activities of thioesterase and acylglycerol transacylase reflect the other aspects of the lipometabolic action of *PNPLA3*^[15]. *PNPLA3* was recently identified to promote the transfer of very-long-chain polyunsaturated fatty acids from TAG to phospholipids^[27]. These effects shed light on an important regulatory role of *PNPLA3* in lipid homeostasis^[13], which is supposed to be affected by

PNPLA3-related genetic variants.

In the present study, genotype-based, widespread effects of *PNPLA3* SNPs in lipid profiles were found in the sera of NAFLD patients from Northern (Tianjin), Central (Shanghai) and Southern China (Zhangzhou, Fujian). Being similar to previous reports^[13,28,29], members of TAG, CE and FFA were identified in the differential serum lipids associated with *PNPLA3* SNPs (e.g., rs738408, rs738409, rs2072906, rs2294919 and rs4823173). In contrast, *PNPLA3* rs139051 and rs2294918 primarily exerted their lipidomic impact on phospholipid metabolites. LPCs, LPCOs and PEs were confirmed to dominate the differential serum lipids because of their high abundance. Therefore, dysregulation of phospholipid metabolite profile reflects the major role of *PNPLA3* SNPs in serum lipidomics.

To gain further insight into the *PNPLA3*-related phospholipid characteristics, differences in serum levels of LPC, LPCO and PE were analyzed between various genotypes of *PNPLA3* rs139051 and rs2294918. NAFLD patients with the A/A instead of A/G + G/G genotype at *PNPLA3* rs139051 exhibited significantly higher levels of LPCs and LPCOs. The G/G, but not the A/A + A/G, genotype of *PNPLA3* rs2294918 also predisposed NAFLD patients to statistical elevation of serum LPCs and LPCOs. Various types of LPCs (e.g., LPC 17:0 and LPC 18:0) have been shown to play a critical role in the spectrum of NAFLD^[30,31]. Their reduction in blood samples was detected in patients with ¹H-MRS- or

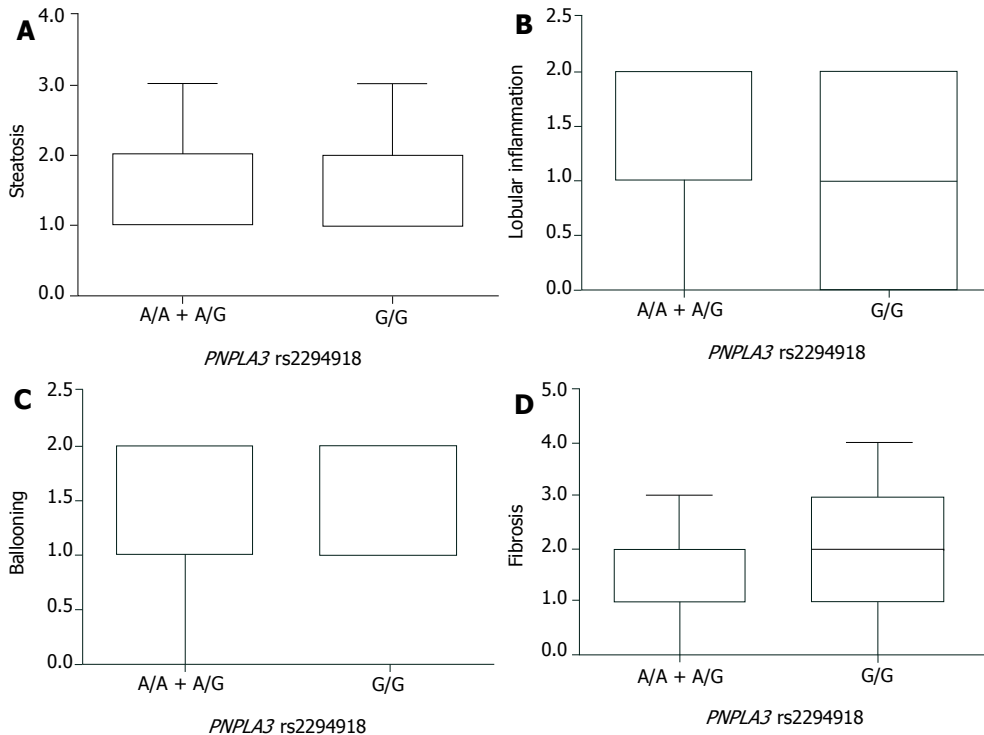


Figure 3 *PNPLA3* rs2294918 showed no association with pathologic characteristics of nonalcoholic fatty liver disease patients. Box plots indicate the grade of steatosis (A); lobular inflammation (B); ballooning (C); and fibrosis (D) in nonalcoholic fatty liver disease patients with the G/G or A/A + A/G genotype at *PNPLA3* rs2294918. Results are presented as medians and Interquartile Range.

biopsy-proven hepatic steatosis^[30]. Similarly, significant decreases in serum palmitoyl-, stearoyl- and oleoyl-LPC were obtained in an experimental rodent model of NASH^[32]. In contrast, uptake of LPC metabolite (PC) ameliorates the hepatic injury of NAFLD with a reduction of serum aspartate aminotransferase and alanine aminotransferase^[33]. Given the similarity in LPC- and LPCO-based lipid metabolism^[32,34-37], *PNPLA3* rs139051 and rs2294918 are suggested to protect the liver from NAFLD-related impairment by their upregulatory effect on both LPCs and LPCOs.

In contrast to its correlation with LPCs and LPCOs upregulation, the G/G genotype at *PNPLA3* rs2294918 conferred significantly lower levels of PEs in the NAFLD patients compared to those with the A/A + A/G genotype. A similar decrease in serum PE 34:0 was found in these NAFLD patients in association with the A/A genotype at *PNPLA3* rs139051. Recent studies have highlighted a growing increase in PE concentration during progression of NAFLD (healthy control < simple steatosis < NASH)^[38]. Nevertheless, individuals carrying the Val175Met variant allele of PE N-methyltransferase (PEMT), which inhibits PE to PC conversion, show high susceptibility to NASH^[39]. PEMT^{-/-} mice also suffer from NASH after being fed a choline-deficient or high-fat diet^[40,41]. Thus, *PNPLA3* rs139051 and rs2294918 represent a novel layer of genetic regulation that down-regulates the harmful components of lipidomics.

Metabolically, LPCs are catabolized into PCs by the lysophosphatidylcholine acyltransferase 1/2/4^[32].

Glycerophosphocholine, the lysoplasmalogenase-dependent catalysate of LPCOs, serves as another precursor of PCs^[36,37]. As a result, the upregulation of LPCs and LPCOs, together with the downregulation of PEs, may lead to an increased ratio of PC to PE in NAFLD patients with the A/A genotype at *PNPLA3* rs139051 and/or G/G at *PNPLA3* rs2294918. Acting as the major source of serum phospholipids, hepatic PCs and PEs play an essential part in the integrity of cellular and organelle membranes^[41,42]. An abnormally low molar ratio of PC/PE has been linked to steatohepatitis due to its induction of membrane leakage^[43]. On the contrary, administration of polyene PC prevents patients from hepatic injury, with an improvement in PC/PE ratio^[44]. Because of the antioxidant propensity of plasmalogen, LPCO deficiency is responsible for additional effects of NASH that sensitize patients to reactive-oxygen-species-dependent peroxidation, and successive lobular inflammation^[45,46]. An inflammation-attenuating effect of *PNPLA3* rs139051 and rs2294918 is then proposed on the basis of LPCs and LPCOs augmentation.

Indeed, our experimental observations relating to NAFLD patients revealed that an increase in LPCs and LPCOs significantly correlated with an attenuation of hepatic inflammation. Both high-level LPCs/LPCOs and low-grade lobular inflammation characterized patients with the A/A genotype at *PNPLA3* rs139051 and/or the G/G genotype at rs2294918. However, pathological characteristics other than hepatic inflammation, including hepatocyte steatosis, ballooning, and liver

fibrosis, were not associated with either of these phospholipid metabolites. *PNPLA3* genotyping and SAF-based pathological grading confirmed the phospholipid-mediated effect of *PNPLA3* SNPs on NAFLD. There was a lower grade of lobular inflammation in patients with the A/A vs A/G + G/G genotype at *PNPLA3* rs139051. Similar, yet mild, amelioration of lobular inflammation occurred in NAFLD patients carrying the G/G rather than other genotypes at *PNPLA3* rs2294918.

In conclusion, *PNPLA3* SNPs reflect an important genetic basis of lipidomic characteristics in NAFLD patients, with the focus on phospholipid metabolite profile. The A/A genotype at *PNPLA3* rs139051 exerts an upregulatory effect on serum LPCs and LPCOs. Both the genotype of *PNPLA3* rs139051 and the increased levels of LPCs/LPCOs share an association with the low-grade lobular inflammation of NAFLD. *PNPLA3* rs139051, therefore, may underlie the inflammatory progress of NAFLD by its modulation of the phospholipid metabolite profile.

ARTICLE HIGHLIGHTS

Research background

Genome-wide association analysis and clinical investigations have found that single nucleotide polymorphisms (SNPs) of patatin-like phospholipase domain containing 3 (*PNPLA3*) underlie the genetic susceptibility of nonalcoholic fatty liver disease (NAFLD), independent of gender, age and ethnic background.

Research motivation

The understanding of the SNP-specific impact on serum lipids and their correlation with pathological characteristics is still limited and controversial. In this study, the authors stratified Chinese Han patients with biopsy-proven NAFLD by genotyping their *PNPLA3* SNPs.

Research objectives

In this study, the authors investigated the effect of *PNPLA3* polymorphisms on serum lipidomics and pathological characteristics of NAFLD.

Research methods

Thirty-four biopsy-proven NAFLD patients from China were subjected to stratification by genotyping their SNPs in *PNPLA3*. Ultra-performance liquid chromatography-tandem mass spectrometry was employed to characterize the effects of *PNPLA3* SNPs on serum lipidomics. The variant-based scoring of hepatocyte steatosis, ballooning, lobular inflammation, and liver fibrosis was performed to uncover the actions of lipidomics-affecting *PNPLA3* SNPs in NAFLD-specific pathological alternations.

Research results

PNPLA3 SNPs demonstrated extensive association with the serum lipidomics, especially phospholipid metabolites of NAFLD patients. The significant correlation of *PNPLA3* rs139051 and inflammation grading further convinced its pathological role that was based on the modulation of phospholipid metabolite profile.

Research conclusions

The authors found that the A/A genotype at *PNPLA3* rs139051 exerts an up-regulatory effect on serum phospholipids of lysophosphatidylcholine (LPC) and lysophosphatidylcholine plasmalogen (LPCO), which are associated with low-grade lobular inflammation of NAFLD.

Research perspectives

These experimental observations relating to NAFLD patients revealed that

an increase in LPCs and LPCOs significantly correlated with an attenuation of hepatic inflammation. However, the pathological characteristics other than hepatic inflammation displayed no association with either of these phospholipid metabolites.

REFERENCES

- 1 **Younossi ZM**, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* 2016; **64**: 73-84 [PMID: 26707365 DOI: 10.1002/hep.28431]
- 2 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Gastroenterological Association; American Association for the Study of Liver Diseases; American College of Gastroenterology. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]
- 3 **Assy N**, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci* 2000; **45**: 1929-1934 [PMID: 11117562 DOI: 10.1023/A:1005661516165]
- 4 **Cohen DE**, Fisher EA. Lipoprotein metabolism, dyslipidemia, and nonalcoholic fatty liver disease. *Semin Liver Dis* 2013; **33**: 380-388 [PMID: 24222095 DOI: 10.1055/s-0033-1358519]
- 5 **Romeo S**, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, Boerwinkle E, Cohen JC, Hobbs HH. Genetic variation in *PNPLA3* confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008; **40**: 1461-1465 [PMID: 18820647 DOI: 10.1038/ng.257]
- 6 **Kottronen A**, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, Bergholm R, Arkkila P, Arola J, Kiviluoto T, Fisher RM, Ehrenborg E, Orho-Melander M, Ridderstråle M, Groop L, Yki-Järvinen H. A common variant in *PNPLA3*, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia* 2009; **52**: 1056-1060 [PMID: 19224197 DOI: 10.1007/s00125-009-1285-z]
- 7 **Soikoian S**, Castañón GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009; **50**: 2111-2116 [PMID: 19738004 DOI: 10.1194/jlr.P900013-JLR200]
- 8 **Pan Q**, Chen MM, Zhang RN, Wang YQ, Zheng RD, Mi YQ, Liu WB, Shen F, Su Q, Fan JG. *PNPLA3* rs1010023 Predisposes Chronic Hepatitis B to Hepatic Steatosis but Improves Insulin Resistance and Glucose Metabolism. *J Diabetes Res* 2017; **2017**: 4740124 [PMID: 28695131 DOI: 10.1155/2017/4740124]
- 9 **Li Q**, Qu HQ, Rentfro AR, Grove ML, Mirza S, Lu Y, Hanis CL, Fallon MB, Boerwinkle E, Fisher-Hoch SP, McCormick JB. *PNPLA3* polymorphisms and liver aminotransferase levels in a Mexican American population. *Clin Invest Med* 2012; **35**: E237-E245 [PMID: 22863562 DOI: 10.25011/cim.v35i4.17153]
- 10 **Peng XE**, Wu YL, Lin SW, Lu QQ, Hu ZJ, Lin X. Genetic variants in *PNPLA3* and risk of non-alcoholic fatty liver disease in a Han Chinese population. *PLoS One* 2012; **7**: e50256 [PMID: 23226254 DOI: 10.1371/journal.pone.0050256]
- 11 **Donati B**, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, Petta S, Xing C, Dongiovanni P, del Menico B, Rametta R, Mancina RM, Badiali S, Fracanzani AL, Craxi A, Fargion S, Nobili V, Romeo S, Valenti L. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. *Hepatology* 2016; **63**: 787-798 [PMID: 26605757 DOI: 10.1002/hep.28370]
- 12 **Jenkins CM**, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem* 2004; **279**: 48968-48975 [PMID: 15364929 DOI: 10.1074/jbc.M407841200]

- 13 **Peter A**, Kovarova M, Nadalin S, Cermak T, Königsrainer A, Machicao F, Stefan N, Häring HU, Schleicher E. PNPLA3 variant I148M is associated with altered hepatic lipid composition in humans. *Diabetologia* 2014; **57**: 2103-2107 [PMID: 24972532 DOI: 10.1007/s00125-014-3310-0]
- 14 **Basantani MK**, Sitnick MT, Cai L, Brenner DS, Gardner NP, Li JZ, Schoiswohl G, Yang K, Kumari M, Gross RW, Zechner R, Kershaw EE. Pnpla3/Adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. *J Lipid Res* 2011; **52**: 318-329 [PMID: 21068004 DOI: 10.1194/jlr.M011205]
- 15 **Huang Y**, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. *J Biol Chem* 2011; **286**: 37085-37093 [PMID: 21878620 DOI: 10.1074/jbc.M111.290114]
- 16 **Hyysalo J**, Gopalacharyulu P, Bian H, Hyötyläinen T, Leivonen M, Jaser N, Juuti A, Honka MJ, Nuutila P, Olkkonen VM, Oresic M, Yki-Järvinen H. Circulating triacylglycerol signatures in nonalcoholic fatty liver disease associated with the I148M variant in PNPLA3 and with obesity. *Diabetes* 2014; **63**: 312-322 [PMID: 24009255 DOI: 10.2337/db13-0774]
- 17 **Sharma M**, Mitnala S, Vishnubhotla RK, Mukherjee R, Reddy DN, Rao PN. The Riddle of Nonalcoholic Fatty Liver Disease: Progression From Nonalcoholic Fatty Liver to Nonalcoholic Steatohepatitis. *J Clin Exp Hepatol* 2015; **5**: 147-158 [PMID: 26155043 DOI: 10.1016/j.jceh.2015.02.002]
- 18 **Reinert DF**, Allen JP. The Alcohol Use Disorders Identification Test (AUDIT): a review of recent research. *Alcohol Clin Exp Res* 2002; **26**: 272-279 [PMID: 11964568 DOI: 10.1111/j.1530-0277.2002.tb02534.x]
- 19 **Pérula de Torres LA**, Fernández-García JA, Arias-Vega R, Muriel-Palmino M, Márquez-Rebollo E, Ruiz-Moral R. [Validation of the AUDIT test for identifying risk consumption and alcohol use disorders in women]. *Aten Primaria* 2005; **36**: 499-506 [PMID: 16324508 DOI: 10.1016/S0212-6567(05)70552-7]
- 20 **Bedossa P**, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. *Hepatology* 2014; **60**: 565-575 [PMID: 24753132 DOI: 10.1002/hep.27173]
- 21 **Munteanu M**, Tiniakos D, Anstee Q, Charlotte F, Marchesini G, Bugianesi E, Trauner M, Romero Gomez M, Oliveira C, Day C, Dufour JF, Bellentani S, Ngo Y, Traussnig S, Perazzo H, Deckmyn O, Bedossa P, Ratzliff V, Poynard T; FLIP Consortium and the FibroFrance Group. Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference. *Aliment Pharmacol Ther* 2016; **44**: 877-889 [PMID: 27549244 DOI: 10.1111/apt.13770]
- 22 **Goodman ZD**. Grading and staging systems for inflammation and fibrosis in chronic liver diseases. *J Hepatol* 2007; **47**: 598-607 [PMID: 17692984 DOI: 10.1016/j.jhep.2007.07.006]
- 23 **Pan Q**, Zhang RN, Wang YQ, Zheng RD, Mi YQ, Liu WB, Shen F, Chen GY, Lu JF, Zhu CY, Zhang SY, Chen YM, Sun WL, Fan JG. Linked PNPLA3 polymorphisms confer susceptibility to nonalcoholic steatohepatitis and decreased viral load in chronic hepatitis B. *World J Gastroenterol* 2015; **21**: 8605-8614 [PMID: 26229402 DOI: 10.3748/wjg.v21.i28.8605]
- 24 **Yang RX**, Hu CX, Sun WL, Pan Q, Shen F, Yang Z, Su Q, Xu GW, Fan JG. Serum Monounsaturated Triacylglycerol Predicts Steatohepatitis in Patients with Non-alcoholic Fatty Liver Disease and Chronic Hepatitis B. *Sci Rep* 2017; **7**: 10517 [PMID: 28874844 DOI: 10.1038/s41598-017-11278-x]
- 25 **Baulande S**, Lasnier F, Lucas M, Pairault J. Adiponutrin, a transmembrane protein corresponding to a novel dietary- and obesity-linked mRNA specifically expressed in the adipose lineage. *J Biol Chem* 2001; **276**: 33336-33344 [PMID: 11431482 DOI: 10.1074/jbc.M105193200]
- 26 **Huang Y**, He S, Li JZ, Seo YK, Osborne TF, Cohen JC, Hobbs HH. A feed-forward loop amplifies nutritional regulation of PNPLA3. *Proc Natl Acad Sci U S A* 2010; **107**: 7892-7897 [PMID: 20385813 DOI: 10.1073/pnas.1003585107]
- 27 **Mitsche MA**, Hobbs HH, Cohen JC. Patatin-like phospholipase domain-containing protein 3 promotes transfer of essential fatty acids from triglycerides to phospholipids in hepatic lipid droplets. *J Biol Chem* 2018; **293**: 6958-6968 [PMID: 29555681 DOI: 10.1074/jbc.RA118.002333]
- 28 **Chamoun Z**, Vacca F, Parton RG, Gruenberg J. PNPLA3/adiponutrin functions in lipid droplet formation. *Biol Cell* 2013; **105**: 219-233 [PMID: 23398201 DOI: 10.1111/boc.201200036]
- 29 **Perttilä J**, Huaman-Samanez C, Caron S, Tanhuanpää K, Staels B, Yki-Järvinen H, Olkkonen VM. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am J Physiol Endocrinol Metab* 2012; **302**: E1063-E1069 [PMID: 22338072 DOI: 10.1152/ajpendo.00125.2011]
- 30 **Orešič M**, Hyötyläinen T, Kotronen A, Gopalacharyulu P, Nygren H, Arola J, Castillo S, Mattila I, Hakkarainen A, Borra RJ, Honka MJ, Verrijken A, Francque S, Iozzo P, Leivonen M, Jaser N, Juuti A, Sørensen TI, Nuutila P, Van Gaal L, Yki-Järvinen H. Prediction of non-alcoholic fatty-liver disease and liver fat content by serum molecular lipids. *Diabetologia* 2013; **56**: 2266-2274 [PMID: 23824212 DOI: 10.1007/s00125-013-2981-2]
- 31 **Feldman A**, Eder SK, Felder TK, Kedenko L, Paulweber B, Stadlmayr A, Huber-Schönauer U, Niederseer D, Stickel F, Auer S, Haschke-Becher E, Patsch W, Datz C, Aigner E. Clinical and Metabolic Characterization of Lean Caucasian Subjects With Non-alcoholic Fatty Liver. *Am J Gastroenterol* 2017; **112**: 102-110 [PMID: 27527746 DOI: 10.1038/ajg.2016.318]
- 32 **Tanaka N**, Matsubara T, Krausz KW, Patterson AD, Gonzalez FJ. Disruption of phospholipid and bile acid homeostasis in mice with nonalcoholic steatohepatitis. *Hepatology* 2012; **56**: 118-129 [PMID: 22290395 DOI: 10.1002/hep.25630]
- 33 **Lee HS**, Nam Y, Chung YH, Kim HR, Park ES, Chung SJ, Kim JH, Sohn UD, Kim HC, Oh KW, Jeong JH. Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. *Life Sci* 2014; **118**: 7-14 [PMID: 25445436 DOI: 10.1016/j.lfs.2014.09.027]
- 34 **Taniyama Y**, Shibata S, Kita S, Horikoshi K, Fuse H, Shirafuji H, Sumino Y, Fujino M. Cloning and expression of a novel lysophospholipase which structurally resembles lecithin cholesterol acyltransferase. *Biochem Biophys Res Commun* 1999; **257**: 50-56 [PMID: 10092508 DOI: 10.1006/bbrc.1999.0411]
- 35 **Subramanian VS**, Goyal J, Miwa M, Sugatami J, Akiyama M, Liu M, Subbaiah PV. Role of lecithin-cholesterol acyltransferase in the metabolism of oxidized phospholipids in plasma: studies with platelet-activating factor-acetyl hydrolase-deficient plasma. *Biochim Biophys Acta* 1999; **1439**: 95-109 [PMID: 10395969 DOI: 10.1016/S1388-1981(99)00072-4]
- 36 **Wu LC**, Pfeiffer DR, Calhoon EA, Madias F, Marcucci G, Liu S, Jurkowitz MS. Purification, identification, and cloning of lysoplasmalogenase, the enzyme that catalyzes hydrolysis of the vinyl ether bond of lysoplasmalogen. *J Biol Chem* 2011; **286**: 24916-24930 [PMID: 21515882 DOI: 10.1074/jbc.M111.247163]
- 37 **Veldhuizen RA**, Mok A, McMurray WC, Possmayer F. Examination of the potential role of the glycerophosphorylcholine (GPC) pathway in the biosynthesis of phosphatidylcholine by liver and lung. *Biochim Biophys Acta* 1989; **1005**: 157-161 [PMID: 2775769 DOI: 10.1016/0005-2760(89)90181-1]
- 38 **Ma DW**, Arendt BM, Hillyer LM, Fung SK, McGilvray I, Guindi M, Allard JP. Plasma phospholipids and fatty acid composition differ between liver biopsy-proven nonalcoholic fatty liver disease and healthy subjects. *Nutr Diabetes* 2016; **6**: e220 [PMID: 27428872 DOI: 10.1038/nutd.2016.27]
- 39 **Dong H**, Wang J, Li C, Hirose A, Nozaki Y, Takahashi M, Ono M, Akisawa N, Iwasaki S, Saibara T, Onishi S. The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. *J Hepatol* 2007; **46**: 915-920 [PMID: 17391797 DOI: 10.1016/j.jhep.2006.12.012]
- 40 **Vance DE**. Physiological roles of phosphatidylethanolamine N-methyltransferase. *Biochim Biophys Acta* 2013; **1831**: 626-632

- [PMID: 22877991 DOI: 10.1016/j.bbalip.2012.07.017]
- 41 **van der Veen JN**, Kennelly JP, Wan S, Vance JE, Vance DE, Jacobs RL. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim Biophys Acta* 2017; **1859**: 1558-1572 [PMID: 28411170 DOI: 10.1016/j.bbamem.2017.04.006]
 - 42 **Kent C**, Carman GM. Interactions among pathways for phosphatidylcholine metabolism, CTP synthesis and secretion through the Golgi apparatus. *Trends Biochem Sci* 1999; **24**: 146-150 [PMID: 10322420 DOI: 10.1016/S0968-0004(99)01365-1]
 - 43 **Arendt BM**, Ma DW, Simons B, Noureldin SA, Therapondos G, Guindi M, Sherman M, Allard JP. Nonalcoholic fatty liver disease is associated with lower hepatic and erythrocyte ratios of phosphatidylcholine to phosphatidylethanolamine. *Appl Physiol Nutr Metab* 2013; **38**: 334-340 [PMID: 23537027 DOI: 10.1139/apnm-2012-0261]
 - 44 **Gundermann KJ**, Kuenker A, Kuntz E, Drożdżik M. Activity of essential phospholipids (EPL) from soybean in liver diseases. *Pharmacol Rep* 2011; **63**: 643-659 [PMID: 21857075 DOI: 10.1016/S1734-1140(11)70576-X]
 - 45 **Zoeller RA**, Lake AC, Nagan N, Gaposchkin DP, Legner MA, Lieberthal W. Plasmalogens as endogenous antioxidants: somatic cell mutants reveal the importance of the vinyl ether. *Biochem J* 1999; **338 (Pt 3)**: 769-776 [PMID: 10051451 DOI: 10.1042/0264-6021:3380769]
 - 46 **Nagan N**, Zoeller RA. Plasmalogens: biosynthesis and functions. *Prog Lipid Res* 2001; **40**: 199-229 [PMID: 11275267 DOI: 10.1016/S0163-7827(01)00003-0]

P- Reviewer: Schwarz SM **S- Editor:** Dou Y

L- Editor: Filipodia **E- Editor:** Wu YXJ



Retrospective Study

Recurrent carpal tunnel syndrome: Evaluation and treatment of the possible causes

Ahmet Eroğlu, Enes Sarı, Ali Kıvanç Topuz, Hakan Şimşek, Serhat Pusat

Ahmet Eroğlu, Hakan Şimşek, Serhat Pusat, Department of Neurosurgery, Haydarpaşa Sultan Abdülhamid Education and Research Hospital, Istanbul 34000, Turkey

Enes Sarı, Department of Orthopaedics and Traumatology, Near East University Hospital, Lefkoşa 99010, Cyprus

Ali Kıvanç Topuz, Department of Neurosurgery, Baypark Hospital, Istanbul 34000, Turkey

ORCID number: Ahmet Eroğlu (0000-0001-7848-1551); Enes Sarı (0000-0003-2385-1732); Ali Kıvanç Topuz (0000-0001-7544-1087); Hakan Şimşek (0000-0002-2621-9372); Serhat Pusat (0000-0003-2412-2320).

Author contributions: Eroğlu A contributed to the idea for research or article/hypothesis generation, supervision and responsibility for the organisation and course of the project and the manuscript preparation; Sarı E planed the methods to generate hypothesis and takes responsibility for creation of the entire or a substantial part of the manuscript; Topuz AK took responsibility for conducting literature search; Şimşek H reworked the final, before submission version of the manuscript for intellectual content, not just spelling and grammar check; Pusat S took responsibility for creation of the entire or a substantial part of the manuscript.

Institutional review board statement: The manuscript has been approved by Ministry of Health Haydarpaşa Sultan Abdülhamid Education and Research Hospital, Review Board of Neurosurgery.

Informed consent statement: It has been declared that all relevant persons involved (subjects or legally authorized representative) gave their informed consent (written or verbal, as appropriate).

Conflict-of-interest statement: All authors have no conflicts of interest to report.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this

work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Ahmet Eroğlu, MD, Surgeon, Department of Neurosurgery, Haydarpaşa Sultan Abdülhamid Education and Research Hospital, Selimiye Neighborhood, Tibbiye Street, Istanbul 34000, Turkey. drahmetoglu@gmail.com
Telephone: +90-506-2036231
Fax: +90-216-5422815

Received: April 8, 2018

Peer-review started: April 8, 2018

First decision: May 16, 2018

Revised: July 25, 2018

Accepted: August 11, 2018

Article in press: August 11, 2018

Published online: September 26, 2018

Abstract

AIM

To investigate the causes of the recurrent carpal tunnel syndrome (CTS) and implemented surgical interventions.

METHODS

Four hundred and eighty-seven patients, who were diagnosed with CTS and underwent surgical intervention between October 2016 and September 2007, were evaluated in this retrospective study. The age, gender, physical evaluation findings, electrophysiological examination reports and implemented surgical treatment methods were analyzed.

RESULTS

Thirty-nine of the cases were operated due to recur-

rent CTS. Further examination of the patients with recurrent CTS revealed that ten cases had diabetic polyneuropathy, three cases had hypothyroidism, two cases had rheumatoid arthritis and one case had systemic amyloidosis. Postoperative electromyography confirmed the neuropathy was due to systemic diseases. The remaining 23 patients with recurrent CTS did not have any systemic disease and all of them had applied previously to another health center.

CONCLUSION

We concluded that the recurrence rates in CTS might be decreased with exploration and incision of the entire transverse ligament. Damage to the motor and sensory branches of the median nerve could be avoided with an incision on the ulnar side.

Key words: Carpal tunnel; Electromyography; Median nerve; Retrospective study; Entrapment neuropathies

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: In this study, 23 cases of recurrent carpal tunnel syndrome did not have any systemic disease and all of them had undergone a surgical intervention in another center. The incision was made starting distal to the volar wrinkle, passed between the thenar and hypothenar region, 2-3 mm medially to the thenar wrinkle and extended 2-3 cm to the lateral side of the third finger. In recurrent cases, an appropriate differential diagnosis, re-operation without delay to avoid the development of the interfascial fibrosis, and implementation of a precise and careful surgical technique play important roles in improving the surgical outcome.

Eroğlu A, Sarı E, Topuz AK, Şimşek H, Pusat S. Recurrent carpal tunnel syndrome: Evaluation and treatment of the possible causes. *World J Clin Cases* 2018; 6(10): 365-372 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/365.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.365>

INTRODUCTION

Entrapment neuropathies are disorders of peripheral nerves characterized by pain, numbness or loss of function. The symptoms depend on the compression caused by the adjacent anatomical structures. Carpal tunnel syndrome (CTS) is the most common peripheral nerve entrapment neuropathy and CTS surgery is the most commonly performed operation in the hand region^[1-4]. Surgical decompression was first performed by Amadio^[5] in 1995 and by Learmonth^[6] in 1933. A variety of surgical decompression techniques have been described over the years^[7-9] (Table 1). The prevalence of CTS is 0.6%-3.4% in the general population^[10,11]. It has a higher prevalence in certain occupational groups^[12,13].

CTS is five times more common in women than men between the ages of 30-60 years and the involvement is usually bilateral^[12]. An increase in pressure within the carpal tunnel is the major factor known in the etiology. The increased pressure impairs the blood supply of the median nerve and causes nerve damage^[14].

A specific etiological factor may not be detected in the majority of patients with CTS. CTS is idiopathic in approximately 50% of the patients. Most patients are occupied in work requiring repetitive wrist motions^[12,15,16]. Patients with congenital narrow carpal tunnel are more prone to CTS. Secondary causes include anatomical causes such as abnormalities in bone structure, traumatic structural disorders such as occupational recurrent microtrauma, and systemic diseases such as amyloidosis, diabetes, hypothyroidism, rheumatic diseases, and cancer^[11,17]. The sense of prickling in the hand, radiating numbness in three fingers, and pain in the hand, wrist and medial side of the arm may emerge in early stages of the disease. Weakness and atrophy in the thenar muscles, loss of hand skills, and impairment in daily life activities are the major symptoms in advanced and chronic cases.

MATERIALS AND METHODS

Study objective

Four hundred and eighty-seven patients, who had undergone surgical intervention due to the diagnosis of CTS between September 2007 and October 2016, were evaluated retrospectively. The age, gender, physical evaluation findings, electrophysiological examination reports of the patients, and the implemented surgical treatment methods were recorded.

Inclusion and exclusion criteria

Of all cases, 448 (91.9%) had primary CTS and the remaining 39 (8.1%) cases had recurrent CTS. Twenty-three of the patients included in this study had recurrent CTS, complaints for at least 3 mo, no additional neural pathology, and persistent conduction disorders in sensory and motor fibers observed by electromyography (EMG). Sixteen patients with recurrent CTS who had systemic diseases such as diabetes mellitus and thyroid disorders were excluded from the study.

Operative procedures

Hypoesthesia in the median nerve sensation area, loss of strength in the radial three fingers, thenar muscle atrophy, and Tinel and Phalen signs were evaluated during the clinical examination. Preoperative wrist x-ray images were evaluated, and preoperative and postoperative (1st and 6th months) EMG images were examined for each patient. All patients were operated on by the same surgeon. Regarding the prophylaxis of the infection, a single dose of a parenteral antibiotic was administered before the intervention and continued with an oral antibiotic for the next 3 d. All patients

Table 1 Milestones of carpal tunnel syndrome decompression surgery

References	Year	Accomplishment
Marie <i>et al</i> ^[38]	1913	Defined median nerve compression
Amadio ^[5]	1924	Median nerve decompression by transecting the transverse carpal ligament
Learmonth ^[6]	1933	Median nerve decompression by transecting the transverse carpal ligament
Cannon <i>et al</i> ^[39]	1946	Reported good results with the release of transverse carpal ligament with median nerve compression
Phalen <i>et al</i> ^[8]	1950	Started using standard open approach
Chow ^[26]	1989	Described dual portal endoscopic decompression technique
Agee <i>et al</i> ^[27]	1992	Single proximal portal endoscopic decompression technique
Biyani <i>et al</i> ^[40]	1993	Described mini-open double-incision technique
Bromley ^[41]	1994	Single distal mini-open technique

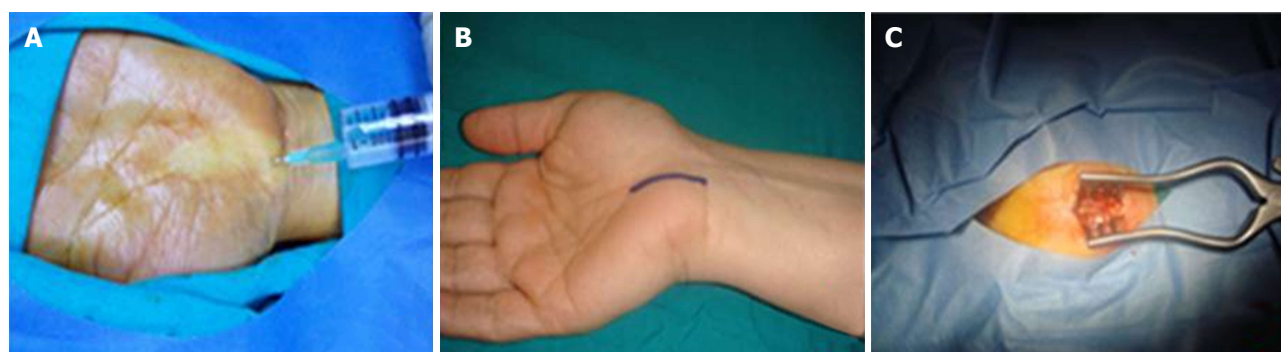


Figure 1 Mini open incision method. A: Local anesthetic application to the incision line; B: The standard incision starts from the distal volar wrinkle, passes between the thenar and hypothenar region 2-3 mm medially to thenar wrinkle and extends 2-3 cm distally to the lateral side of the third finger; C: Placement of the skin retractor after sharp dissection.

were discharged on the same day. An elastic bandage was used for the first 24 h and the arm was positioned in a 90° flexion. Postoperative wrist splinting was not used. The next day after the operation, dressings were changed and finger exercises were started. Stitches were removed on the 10th day and exercises with a softball and hot water bath were initiated. The mean follow-up time was 8.6 mo (range: 7.2-13 mo).

Surgical technique

Open surgery with a standard incision, open surgery with a mini-incision, or closed surgery such as endoscopic surgery and retinaculotomy may be used in CTS. We preferred a 2-3 cm mini-incision so that the entire transverse ligament could be visualized (Figure 1). Open surgery with a mini-incision was performed under local anesthesia without a tourniquet (Figure 1A). The patients were positioned supine on the operating table. The arm was placed on the surgical table slightly elevated and a small silicone pad was placed under the wrist, while the arm was in 90° abduction. Our standard incision starts distal to the volar wrinkle, passes between the thenar and hypothenar region, 2-3 mm medial to the thenar wrinkle and extends 2-3 cm distally to the lateral side of the third finger (Figure 1B). Regarding the patients with recurrent CTS, we preferred an open surgery with a 1-1.5 cm mini-incision over the previous long incision scar in patients who had previously undergone open surgery. A similar

incision was done just at the distal side of the previous incision scar in patients who had a transverse incision over the volar wrinkle, and a similar incision was done again between the previous incision lines in patients who had previously undergone endoscopic surgery (Figure 1C). Following the local anesthetic infiltration into the incision line, a vertical skin and subcutaneous incision was carried out. The skin and subcutaneous tissues were sharply incised with a No 15 blade and a skin retractor was inserted. The sharp dissection was deepened. After passing through the subcutaneous fat and the granulation tissue, the palmar aponeurosis and transverse ligament were exposed. The skin retractor was re-positioned and the transverse ligament was fully visualized. The ligament was completely and cautiously incised on the ulnar side of the median nerve with a No. 15 scalpel. Subsequently, a dissector was used to check whether the decompression was sufficient or not on the proximal and distal sides (Figure 2). Hemostasis was achieved by compressing the palm for a few minutes. The skin was sutured with 4/0 vicryl and the wound was closed with a sterile dressing. The strength of abductor pollicis brevis and other flexor muscles and sensation in the thenar region was controlled at the end-stage of the operation.

Statistical analysis

Due to small sub-group numbers and no subjects for comparison, statistical analysis was not carried out.

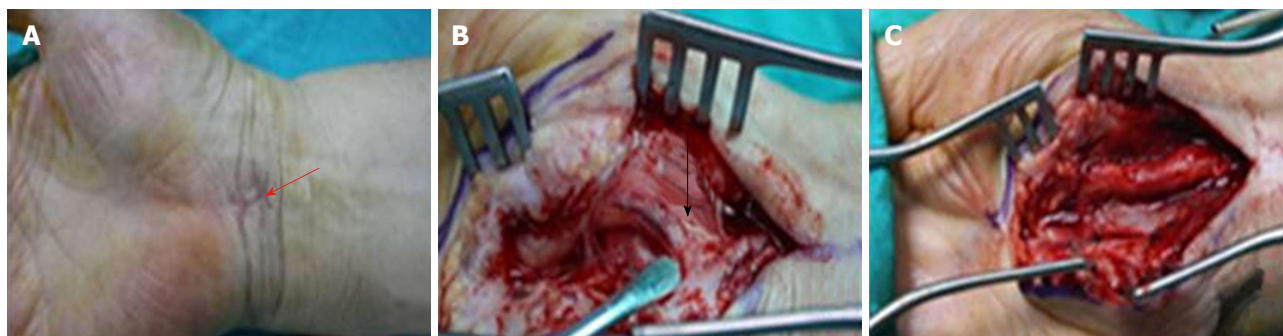


Figure 2 Extended mini-open incision technique in a patient previously operated on using the uniprotal endoscopic method. A: Endoscopic portal scar over the distal wrist wrinkle (red arrow); B: Incomplete incision of the transverse carpal ligament and compression on the median nerve (black arrow); C: The incision is completed and the median nerve is fully decompressed.

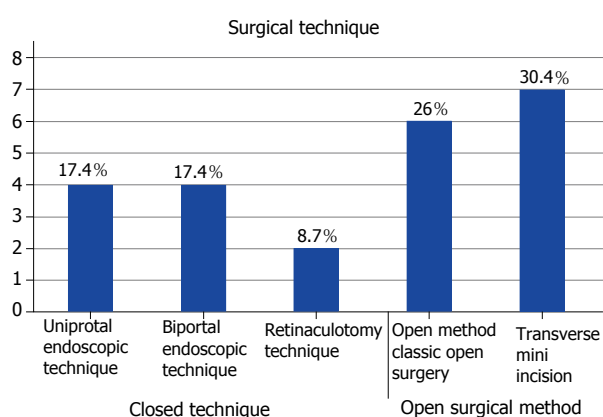


Figure 3 Ten (43.4%) recurrent cases were previously operated with closed technique (uniprotal endoscopic technique in four, biportal endoscopic technique in four and retinaculotomy technique in two cases). Six (26%) recurrent cases were previously operated with open surgical method and seven (30.4%) recurrent cases were previously operated with transverse mini incision.

RESULTS

Patient demographics and characteristics

Regarding the patients with recurrent CTS cases ($n = 23$), 15 were females (65.2%) and eight were males (34.8%). The mean age was 46.5 years (range: 21-69 years). In 12 (52.1%) of these cases the left hand was affected, and in 11 (47.9%) cases the right hand was affected. The closed technique was previously performed in ten (43.4%) of the patients with recurrent CTS (uniprotal endoscopic technique ($n = 4$), biportal endoscopic ($n = 4$) and retinaculotomy technique ($n = 2$) were used). Regarding the previous interventions, open surgery was used in six (26.1%) cases with recurrent CTS, and transverse mini-incision was used in seven (30.4%) cases with recurrent CTS (Figure 3).

Preoperative examination findings

Following the first operation, all patients continued to have one or more complaints, which included nocturnal pain, sensory loss, and pain increasing with activity over the median nerve distribution area. The patients with

recurrent symptoms stated that they still had the same complaints they had in the preoperative period for an average of 3.2 mo (1-7 mo) after the previous surgery. The clinical findings of the physical examination were the following: sensory impairment in 16 cases (69.5%), nocturnal pain (awoken from sleep) in 18 cases (78.2%), and loss of hand strength in 13 cases (56.5%). Tinel sign and Phalen test sign were positive in 16 (69.5%) and in 13 (56.5%) cases, respectively. The thenar atrophy was detected in 14 (60.8%) cases (Figure 4).

Preoperative EMG findings

Preoperative EMG examinations of patients with recurrent CTS revealed low amplitudes of action potentials (severe) ($n = 4$), conduction disorders (moderate) both in sensory and motor fibers ($n = 13$), and conduction disorders affecting only the sensory fibers ($n = 6$). Two cases of severe EMG changes had denervation potentials in the thenar muscles and severe damage to the median nerve.

Surgical results

Patients were operated on using the open mini-incision technique. The mean duration of the operation was 12 min (range: 10-15 min). None of the cases had any additional complications concerning the motor and sensory branch of the median nerve. Wound infection emerged in two cases and a hematoma in one case. The one patient who developed a hematoma was immediately re-operated on and the hematoma was excised. Two patients with wound site infection were treated with oral antibiotics. The mean duration of return to daily living was 21 d (range: 16-27 d).

Postoperative EMG findings

The EMG examination performed in the 6th month after the operation showed irreversible axonal damage to the median nerve in two patients (these patients had findings of denervation in the thenar muscles in the preoperative EMG examination). Improved latency in motor and sensory fibers of the median nerve was reported in all other cases.

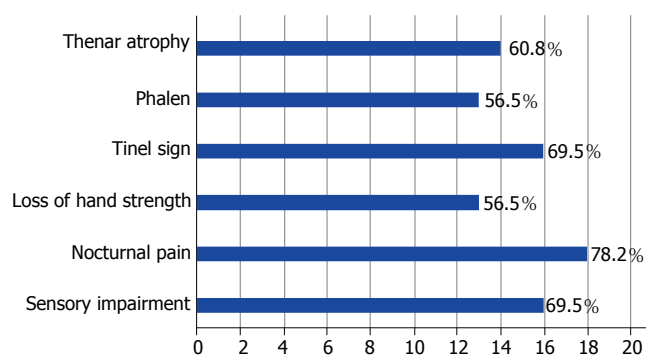


Figure 4 Thenar atrophy in 14 (60.8%) cases. Phalen test was positive in 13 (56.5%) cases. Tinel sign was found in 16 (69.5%) cases. Loss of hand strength in 13 (56.5%) cases, nocturnal pain in 18 (78.2%) cases and sensory impairment was detected in 16 (69.5%) cases.

DISCUSSION

Conservative methods should be primarily considered in the treatment of CTS, but surgical treatment should be preferred in cases where patients are not responding to conservative treatments^[16,18,19]. The goal of the surgical treatment is to release transverse carpal ligament (TCL) completely and to decompress the canal. Decompression of the median nerve with complete dissection of the TCL leads to a clinical improvement in the vast majority of patients^[16,19-21]. Several surgical methods have been described in CTS surgery, including open and closed techniques. Although there is no significant difference between these surgical methods in respect to clinical and electrophysiological outcome, recurrence may be encountered due to the incomplete or insufficient release of the transverse ligament^[19,21,22].

Carpal tunnel decompression surgery using open technique, which was first performed by Amadio^[5] in 1995 and later described by Learmont^[6] in 1933, is still preferred^[23-25]. Although it has been reported that this technique provides satisfying results, there are also certain disadvantages such as pain at the incision site, sensitivity to the scar tissue, and delayed return to daily activities and work^[16]. Thus, alternative methods have been developed to avoid postoperative morbidity after open surgery^[1]. Chow^[26] and Agee *et al.*^[27] reported that with the widespread use of endoscopic methods, new developments in endoscopic instruments and more experienced surgeons, the postoperative morbidity is decreased, the time until return to work after the surgery is shortened, and the scars are more cosmetic and painless. However, endoscopic techniques may also lead to high complication rates when performed without adequate knowledge of endoscopic anatomy and experience^[15,18,28].

An evaluation of the entire carpal tunnel may not be possible during endoscopic surgery and cutting the transverse ligament without adequate visualization may increase the risk of median nerve injury^[28]. Lee reported that the median nerve was injured in two cases in their series^[29]. It is also possible that space-occupying lesions may be overlooked during endoscopic

surgery. Endoscopic surgery also requires a high level of surgical experience and special instrumentation, and cannot be implemented in cases of neurolysis or tenosynovectomy^[30].

With the open mini-incision technique, the entire transverse ligament can be visualized and the median nerve and canal may be fully investigated. All 23 patients with recurrent CTS who were included in our study had been previously operated with different surgical techniques in different centers. We observed in all of these patients that the release of the transverse ligament was incomplete and the median nerve was still under compression. We resolved the inadequate decompression with a complete incision of the ligament (Figure 2). All cases stated that their nocturnal pain, which was awakening them from sleep, was relieved on the first day after the operation. The carpal tunnel decompression with open surgery is considered as the gold standard in the treatment of CTS^[1,18,21]. Although successful results have generally been reported with this method, certain disadvantages may also be encountered, such as weakness in the hand, sensitivity to the scar tissue, and delayed return to daily activities and work^[1,31]. Various complications including injury to the palmar cutaneous branch (PCB) of the median nerve, hypertrophic incision scarring, reflex sympathetic dystrophy, and increased tension in flexor tendons have also been reported after open surgery^[1]. In our cases, we did not encounter these aforementioned complications, with the exception of wound infection and hematoma.

PCB arises from the median nerve before the TCL. This branch provides the sensitive innervation of the thenar region of the hand and plays a major role in the planning of surgical incisions for carpal tunnel surgery. According to some authors, this sensory branch extends to the ulnar side^[10]. For this reason, Franzini *et al.*^[32] preferred a 1 cm longitudinal incision proximal to the wrist flexor line. Abdullah *et al.*^[22] reported that PCB was arising from the radial side of the median nerve and is always located lateral to the palmaris longus (PL) tendon, so they were using a transverse incision at the medial side of the PL tendon. In our cases, we used a

mini-incision starting from the distal side of the wrist flexor line and extending 2-3 mm to the medial side of the thenar line. We exposed the entire transverse ligament, identified PCB, and incised the transverse ligament on the medial side of the median nerve.

Regarding recurrence after CTS surgery, the most common reason is the incomplete release of the distal part of the TCL^[21]. A 2-3 cm open mini-incision enables the visualization of the distal part of the ligament. However, in patients with recurrent CTS in this study, we extended the mini-incision about 1-1.5 cm due to fibrotic scar tissue formation. The median nerve is divided into two main trunks (lateral and medial) at the distal end of the TCL. The branch, which provides motor innervation, originates from the lateral trunk. Several anatomic variations of this motor branch should be taken into consideration during the planning of the surgical incision^[10]. According to the Lanz classification, variations of the motor branch include extra-ligamentous, subligamentous, and less commonly transligamentous localizations^[33]. This branch rarely originates from the ulnar side of the median nerve and rarely gives recurrent motor branches^[33]. We believe that the implementation of the closed techniques (e.g., endoscopic methods) and the dissection of the transverse ligament without fully visualizing the median nerve may cause iatrogenic neural injuries, depending on the anatomic variations of the median nerve. In addition, recurrence may also be encountered in the closed techniques due to the incomplete dissection of TCL and the inadequate decompression of the median nerve.

We used an open mini-incision technique in our cases and carried out a small incision to release the median nerve and cut the TCL, volar carpal ligament and deep palmar fascia. Shapiro^[34] reported good results in 96% of patients with a technique named "carpal tunnel release with microsurgery", which is performed by a mini-incision using special instruments (microscope and Easyloupe). Decompression of the transligamentous motor branch with this incision is also possible. However, the most common disadvantage of this incision is a large scar and loss of hand function^[22]. One of the most frequently discussed issues is "should the incision be longitudinal or transverse?"^[35]. The authors, who prefer a longitudinal incision, suggest that PCB injuries may be avoided with this incision. However, according to the experience gained from the anatomic studies, PCB rarely extends to the medial side of the PL tendon^[10]. Therefore, PCB can be preserved by an incision that does not extend to the lateral side of the PL tendon^[35]. In our cases, we incised the transverse ligament from the ulnar side of the median nerve.

The average time to return to daily activities was longer after the open surgical method compared to the endoscopic and open mini-incision surgical methods^[30]. This duration was 14-17 d after the closed technique and 28 d after the open surgery technique^[24]. In our

cases, the average time to return to the daily activities was 17 d (14-21 d). The comparison of the outcome in both groups does not show any significant difference between open and closed surgical methods. However, 10-15% of patients who had undergone endoscopic surgery encountered an inadequate relief in symptoms or an early onset of recurrence^[25]. Median, ulnar and digital nerve injuries have been reported in the literature for both open and closed technique^[36]. In our cases, no additional neural damage was observed. In addition to the relatively simpler technique and it being easier to learn, the lower cost of the surgical instruments used in open mini-incision surgery is another advantage in comparison to the endoscopic and retinaculotomy techniques^[37]. Surgical experience, special instruments and appropriate assistance are required for the endoscopic surgery and retinaculotomy methods. One of the major disadvantages of the closed technique is the increased injury risk of the ulnar-radial artery arch^[26]. Other advantages of the open mini-incision surgical incision technique, which we used in our study, include the easy access to the proximal and the distal end of the TCL, prevention of the damage to the superficial palmar arch, and preservation of the motor branch, which innervates the *m. abductor pollicis brevis*.

Our patients stated that nocturnal paresthesia was immediately relieved the day after CTS surgery. If the pain is not immediately relieved after the surgery, an incomplete incision of TCL should be considered^[10]. We believe that the preference for an open surgical technique with complete incision of the carpal transverse ligament will enable a complete decompression of the median nerve and, consequently, a significant reduction in the recurrence and neuronal injury rates. In patients with recurrent CTS, an appropriate differential diagnosis, re-operation without delay to avoid the development of interfacial fibrosis, implementation of a precise and careful surgical technique, and initiation of an appropriate exercise program in the postoperative period are the factors contributing to the improvement of surgical outcome.

ARTICLE HIGHLIGHTS

Research background

The reasons for recurring carpal tunnel have been researched since the 1990s. Studies have investigated fibrosis and surgical techniques. This study, however, demonstrates that the median nerve should be relieved by full incision of the transverse ligament.

Research motivation

In carpal tunnel surgery, the recurrence rate was increased following the widespread use of the endoscopic and minimally-invasive techniques. A satisfying surgical outcome cannot be achieved if the compression caused by the transverse ligament cannot be completely relieved. The development of the endoscopic and minimally-invasive techniques and the proper training of relevant surgeons will decrease recurrence rates. The critical step in carpal tunnel syndrome surgery is the complete incision of the transverse ligament on the median nerve and the relief of the compression. Independent of the selected surgical technique, the complete incision of the transverse ligament

should be ensured.

Research objectives

The main aim of the study is to perform carpal tunnel surgery with the appropriate surgical method without the need for a second operation. Re-operation on patients with recurrence prolongs the hospitalization time with consequential economic loss. Careful and appropriate surgery will prevent this. Appropriate surgical methods will also prevent surgeons from encountering medicolegal problems. Complete incision of the transverse ligament will reduce recurrence rates following carpal tunnel surgery.

Research methods

Four hundred and eighty-seven patients were evaluated retrospectively. The age, gender, physical evaluation findings, electrophysiological examination reports of the patients, and the implemented surgical treatment methods were recorded in this research.

Research results

Fibrosis and surgical methods have been criticized in the literature. However, this manuscript emphasizes the importance of removing ligament integrity completely. If the complete incision of the transverse ligament is not ensured with endoscopic and minimally-invasive methods, an open surgery technique must be implemented.

Research conclusions

Relief of the median nerve in carpal tunnel surgery occurs when the transverse ligament is completely incised. Recurrence rates therein decrease. Regardless of the surgical procedure, it should be ensured that the transverse ligament is completely incised. If minimally-invasive methods are insufficient in nerve decompression, open surgery should be performed.

Research perspectives

Complete incision of the transverse ligament will reduce recurrence rates following carpal tunnel surgery. This study demonstrates that the median nerve should be relieved by full incision of the transverse ligament. This manuscript emphasizes the importance of completely removing ligament integrity. A satisfying surgical outcome cannot be achieved if the compression caused by the transverse ligament cannot be completely relieved. The relief of the median nerve in carpal tunnel surgery occurs when the transverse ligament is completely incised. Recurrence rates therein decrease. If minimally-invasive methods are insufficient in nerve decompression, open surgery should be performed.

REFERENCES

- 1 **Jimenez DF**, Gibbs SR, Clapper AT. Endoscopic treatment of carpal tunnel syndrome: a critical review. *J Neurosurg* 1998; **88**: 817-826 [PMID: 9576248 DOI: 10.3171/jns.1998.88.5.0817]
- 2 **Paget J**. Lectures on Surgical Pathology. Lindsay and Blakiston, 3rd ed. Philadelphia 1854: 173-179
- 3 **Tulipan JE**, Kim N, Abboudi J, Jones C, Liss F, Kirkpatrick W, Rivlin M, Wang ML, Matzon J, Ilyas AM. Open Carpal Tunnel Release Outcomes: Performed Wide Awake versus with Sedation. *J Hand Microsurg* 2017; **9**: 74-79 [PMID: 28867906 DOI: 10.1055/s-0037-1603200]
- 4 **Nazari G**, Shah N, MacDermid JC, Woodhouse L. The Impact of Sensory, Motor and Pain Impairments on Patient- Reported and Performance Based Function in Carpal Tunnel Syndrome. *Open Orthop J* 2017; **11**: 1258-1267 [PMID: 29290864 DOI: 10.2174/1874325001711011258]
- 5 **Amadio PC**. The first carpal tunnel release? *J Hand Surg Br* 1995; **20**: 40-41 [PMID: 7759932 DOI: 10.1016/S0266-7681(05)80013-0]
- 6 **Learmonth J**. The principle of decompression in treatment of certain diseases of peripheral nerves. *Surg Clin North Am* 1933; **13**: 905-913
- 7 **Love JG**. Median neuritis or carpal tunnel syndrome; diagnosis and treatment. *N C Med J* 1955; **16**: 463-469 [PMID: 13266163]
- 8 **Phalen GS**, Gardner WJ, La Londe AA. Neuropathy of the median nerve due to compression beneath the transverse carpal ligament. *J Bone Joint Surg Am* 1950; **32A**: 109-112 [PMID: 15401727 DOI: 10.2106/00004623-195032010-00011]
- 9 **Mirza MA**, King ET Jr. Newer techniques of carpal tunnel release. *Orthop Clin North Am* 1996; **27**: 355-371 [PMID: 8614584]
- 10 **Serarslan Y**, Melek İ, Duman T. Karpal Tünel Sendromu. *Pamukkale Tıp Dergisi* 2008; **1**: 45-49
- 11 **Kürkçü M**, Türkkan S, Tüzün HY. Karpal tünel Sendromu ve Median Sinirin Diğer Tuzak Nöropatileri. *TOTBİD Dergisi* 2015; **14**: 566-571 [DOI: 10.14292/totbid.dergisi.2015.78]
- 12 **Papanicolaou GD**, McCabe SJ, Firrell J. The prevalence and characteristics of nerve compression symptoms in the general population. *J Hand Surg Am* 2001; **26**: 460-466 [PMID: 11418908 DOI: 10.1053/jhsu.2001.24972]
- 13 **Stevens JC**, Beard CM, O'Fallon WM, Kurland LT. Conditions associated with carpal tunnel syndrome. *Mayo Clin Proc* 1992; **67**: 541-548 [PMID: 1434881 DOI: 10.1016/S0025-6196(12)60461-3]
- 14 **Bland JD**. Carpal tunnel syndrome. *Curr Opin Neurol* 2005; **18**: 581-585 [PMID: 16155444 DOI: 10.1097/01.wco.0000173142.58068.5a]
- 15 **Urbaniak JR**, Desai SS. Complications of nonoperative and operative treatment of carpal tunnel syndrome. *Hand Clin* 1996; **12**: 325-335 [PMID: 8724584]
- 16 **Şavk O**, Turgut M, Çullu E, Akyol A, Alparslan B. Karpal Tünel Sendromunun cerrahi dekompresyonunda standart ve mini insizyon tekniklerinin karşılaştırılması. *ADÜ Tıp Fakültesi Dergisi* 2002; **3**: 9-13
- 17 **Kıbıcı K**, Köksal V. Mini Açık Teknikle Yapılan Karpal Tünel Cerrahisi ve Fonksiyonel Sonuçları. *Türk Nöroşirürji Dergisi* 2010; **20**: 7-14
- 18 **Zamborsky R**, Kokavec M, Simko L, Bohac M. Carpal Tunnel Syndrome: Symptoms, Causes and Treatment Options. Literature Review. *Ortop Traumatol Rehabil* 2017; **19**: 1-8 [PMID: 28436376 DOI: 10.5604/15093492.1232629]
- 19 **Vázquez-Alonso MF**, Abdala-Dergal C. [Principal causes for recurrent carpal tunnel syndrome]. *Acta Ortop Mex* 2016; **30**: 17-20 [PMID: 27627773]
- 20 **Szabo RM**, Steinberg DR. Nerve Entrapment Syndromes in the Wrist. *J Am Acad Orthop Surg* 1994; **2**: 115-123 [PMID: 10708999 DOI: 10.5435/00124635-199403000-00005]
- 21 **Dahlin LB**, Salö M, Thomsen N, Stütz N. Carpal tunnel syndrome and treatment of recurrent symptoms. *Scand J Plast Reconstr Surg Hand Surg* 2010; **44**: 4-11 [PMID: 20136467 DOI: 10.3109/02844310903528697]
- 22 **Abdullah AF**, Wolber PH, Ditto EW 3rd. Sequelae of carpal tunnel surgery: rationale for the design of a surgical approach. *Neurosurgery* 1995; **37**: 931-935; discussion 935-936 [PMID: 8559342 DOI: 10.1227/00006123-199511000-00012]
- 23 **Cellocchio P**, Rossi C, El Boustany S, Di Tanna GL, Costanzo G. Minimally invasive carpal tunnel release. *Orthop Clin North Am* 2009; **40**: 441-448, vii [PMID: 19773048 DOI: 10.1016/j.joc.2009.06.002]
- 24 **Atroshi I**, Johnsson R, Ornstein E. Endoscopic carpal tunnel release: prospective assessment of 255 consecutive cases. *J Hand Surg Br* 1997; **22**: 42-47 [PMID: 9061522 DOI: 10.1016/S0266-7681(97)80013-7]
- 25 **Hulsizer DL**, Staebler MP, Weiss AP, Akelman E. The results of revision carpal tunnel release following previous open versus endoscopic surgery. *J Hand Surg Am* 1998; **23**: 865-869 [PMID: 9763263 DOI: 10.1016/S0363-5023(98)80164-0]
- 26 **Chow JC**. Endoscopic release of the carpal ligament: a new technique for carpal tunnel syndrome. *Arthroscopy* 1989; **5**: 19-24 [PMID: 2706047 DOI: 10.1016/0749-8063(89)90085-6]
- 27 **Agee JM**, McCarroll HR Jr, Tortosa RD, Berry DA, Szabo RM, Peimer CA. Endoscopic release of the carpal tunnel: a randomized prospective multicenter study. *J Hand Surg Am* 1992; **17**: 987-995 [PMID: 1430964 DOI: 10.1016/S0363-5023(09)91044-9]
- 28 **Chen AC**, Wu MH, Cheng CY, Chan YS. Outcomes and Satisfaction with Endoscopic Carpal Tunnel Releases and the Predictors - A Retrospective Cohort Study. *Open Orthop J* 2016; **10**:

- 757-764 [PMID: 28217200 DOI: 10.2174/1874325001610010757]
- 29 **Lee WP**, Strickland JW. Safe carpal tunnel release via a limited palmar incision. *Plast Reconstr Surg* 1998; **101**: 418-424; discussion 425-426 [PMID: 9462775 DOI: 10.1097/00006534-199802000-00025]
- 30 **Atroshi I**, Johnsson R, Ornstein E. Patient satisfaction and return to work after endoscopic carpal tunnel surgery. *J Hand Surg Am* 1998; **23**: 58-65 [PMID: 9523956 DOI: 10.1016/S0363-5023(98)80090-7]
- 31 **Bradley MP**, Hayes EP, Weiss AP, Akelman E. A prospective study of outcome following mini-open carpal tunnel release. *Hand Surg* 2003; **8**: 59-63 [PMID: 12923936 DOI: 10.1142/S021881040300187X]
- 32 **Franzini A**, Broggi G, Servello D, Dones I, Pluchino MG. Transillumination in minimally invasive surgery for carpal tunnel release. Technical note. *J Neurosurg* 1996; **85**: 1184-1186 [PMID: 8929518 DOI: 10.3171/jns.1996.85.6.1184]
- 33 **Lanz U**. Anatomical variations of the median nerve in the carpal tunnel. *J Hand Surg Am* 1977; **2**: 44-53 [PMID: 839054 DOI: 10.1016/S0363-5023(77)80009-9]
- 34 **Shapiro S**. Microsurgical carpal tunnel release. *Neurosurgery* 1995; **37**: 66-70 [PMID: 8587693 DOI: 10.1097/00006123-199507000-00010]
- 35 **Kulick RG**. Carpal tunnel syndrome. *Orthop Clin North Am* 1996; **27**: 345-354 [PMID: 8614583]
- 36 **Bozentka DJ**, Osterman AL. Complications of endoscopic carpal tunnel release. *Hand Clin* 1995; **11**: 91-95 [PMID: 7751336]
- 37 **Lee H**, Jackson TA. Carpal tunnel release through a limited skin incision under direct visualization using a new instrument, the carposcope. *Plast Reconstr Surg* 1996; **98**: 313-319; discussion 320 [PMID: 8764720 DOI: 10.1097/00006534-199608000-00016]
- 38 **Marie P**, Foix C. Atrophie isolée de l'éminence thenar d'origine névritique: rôle du ligament annulaire antérieur du carpe dans la pathogénie de la lésion. *Rev Neurol* 1913; **26**: 647-649
- 39 **Cannon BW**, Love JG. Tardy median palsy; median neuritis; median thenar neuritis amenable to surgery. *Surgery* 1946; **20**: 210-216 [PMID: 20994804]
- 40 **Biyani A**, Downes EM. An open twin incision technique of carpal tunnel decompression with reduced incidence of scar tenderness. *J Hand Surg Br* 1993; **18**: 331-334 [PMID: 8345260 DOI: 10.1016/0266-7681(93)90055-K]
- 41 **Bromley GS**. Minimal-incision open carpal tunnel decompression. *J Hand Surg Am* 1994; **19**: 119-120 [PMID: 8169355 DOI: 10.1016/0363-5023(94)90234-8]

P- Reviewer: Sergi CM, Vento S **S- Editor:** Ma YJ

L- Editor: Filipodia **E- Editor:** Wu YXJ



Retrospective Study

Adjuvant chemotherapy with S-1 plus oxaliplatin improves survival of patients with gastric cancer after D2 gastrectomy: A multicenter propensity score-matched study

Deng-Feng Ren, Fang-Chao Zheng, Jun-Hui Zhao, Guo-Shuang Shen, Raees Ahmad, Shui-Sheng Zhang, Yu Zhang, Jie Kan, Li Dong, Zi-Yi Wang, Fu-Xing Zhao, Jiu-Da Zhao

Deng-Feng Ren, Jun-Hui Zhao, Raees Ahmad, Zi-Yi Wang, Fu-Xing Zhao, Jiu-Da Zhao, Department of Medical Oncology, Affiliated Hospital of Qinghai University, Affiliated Cancer Hospital of Qinghai University, Xining 810000, Qinghai Province, China

Fang-Chao Zheng, Department of Medical Oncology, Shouguang Hospital of Traditional Chinese Medicine, Weifang 262700, Shandong Province, China

Guo-Shuang Shen, Department of Surgical Oncology, Affiliated Hospital of Qinghai University, Affiliated Cancer Hospital of Qinghai University, Xining 810000, Qinghai Province, China

Shui-Sheng Zhang, Department of Pancreatic and Gastric Surgery, National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Yu Zhang, Department of Medical Oncology, Qinghai Red Cross Hospital, Xining 810000, Qinghai Province, China

Jie Kan, Department of Medical Oncology, People's Hospital of Qinghai Province, Xining 810000, Qinghai Province, China

Li Dong, Institutes of Biomedical Sciences, Shanxi University, Taiyuan 030006, Shanxi Province, China

ORCID number: Deng-Feng Ren (0000-0002-4544-9528); Fang-Chao Zheng (0000-0002-2640-544X); Jun-Hui Zhao (0000-0002-5443-853X); Guo-Shuang Shen (0000-0002-9381-8658); Raees Ahmad (0000-0002-9975-5213); Shui-Sheng Zhang (0000-0001-6660-7495); Yu Zhang (0000-0002-8383-8364); Jie Kan (0000-0001-7259-0474); Li Dong (0000-0002-3292-7834); Zi-Yi Wang (0000-0002-3261-7457); Fu-Xing Zhao (0000-0003-0976-5184); Jiu-Da Zhao (0000-0002-1266-8943).

Author contributions: Ren DF and Zheng FC contributed equally to this work; Zhao JD and Ren DF conceived, designed and wrote the manuscript; Zheng FC, Zhao JD, Zhang SS, Zhang Y, Kan J and Wang ZY collected the clinical data and followed

up the patients; Ren DF, Zhao JH, Shen GS, Dong L and Zhao FX helped to analyze the data; Ren DF, Ahmad R, Zheng FC and Zhao JD revised the manuscript; Zhao JD provided financial support for this work; all authors read and approved the final manuscript.

Supported by the Thousand Talents of Program of High-end Innovation of Qinghai Province in China (For Jiuda Zhao); and the Clinical Oncology Medical Center of Qinghai Province in China, No. 2018-SF-113.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Affiliated Hospital of Qinghai University, People's Hospital of Qinghai Province, Qinghai Red Cross Hospital, and Cancer Institute & Hospital, Chinese Academy of Medical Sciences.

Informed consent statement: All patients or their legal guardians signed informed consent statements.

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

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Jiu-Da Zhao, MD, Professor, Department of Medical Oncology, Affiliated Hospital of Qinghai University, Affiliated Cancer Hospital of Qinghai University, No. 29, Tongren Road, Xining 810000, Qinghai Province,

China. jiudazhao@126.com
Telephone: +86-791-6162732
Fax: +81-971-6155740

Received: June 8, 2018
Peer-review started: June 8, 2018
First decision: July 3, 2018
Revised: July 17, 2018
Accepted: August 27, 2018
Article in press: August 28, 2018
Published online: September 26, 2018

Abstract

AIM

To investigate the safety and efficacy of S-1 plus oxaliplatin (SOX) as an adjuvant chemotherapy regimen in gastric cancer (GC) after D2 dissection.

METHODS

GC Patients who underwent D2 gastrectomy from September 2009 to December 2011 in four Chinese institutions were enrolled. Patients with stage I B-III C GC, who received adjuvant SOX treatment were matched by propensity scores with those who underwent surgery alone and those who conducted capecitabine plus oxaliplatin (XELOX) regimen. Disease-free survival (DFS) and overall survival (OS) were compared among the groups. In addition, adverse events in SOX patients were analyzed.

RESULTS

Of 1944 GC patients who underwent D2 dissection, 867 were included for analysis. One hundred and seventeen patients treated with SOX were matched to 234 patients who conducted surgery alone. Fifty-seven patients treated with SOX were matched to 57 patients who received XELOX. The estimated five-year DFS was 57.5% in the adjuvant SOX group which was higher than that (44.6%) in the surgery alone group ($P = 0.001$); and the estimated five-year OS was 68.3% which was higher than that (45.8%) of surgery alone group ($P < 0.001$). Survival benefit was also revealed in stage III and > 60 years old subgroups ($P < 0.001$ and $P = 0.015$, respectively). Compared with XELOX regimen, SOX showed no significant difference in DFS ($P = 0.340$) and OS ($P = 0.361$). The most common ≥ 3 grade adverse events of SOX regimen were neutropenia (22.6%), leukopenia (8.9%) and thrombocytopenia (5.6%).

CONCLUSION

Compared with surgery alone, SOX regimen significantly improves the long-term survival and has acceptable toxicity in patients with stage I B-III C GC after D2 dissection. It may be a novel adjuvant chemotherapy regimen in GC patients.

Key words: Gastric cancer; D2 gastrectomy; Adjuvant chemotherapy; S-1; Oxaliplatin; Capecitabine

© The Author(s) 2018. Published by Baishideng Publishing

Group Inc. All rights reserved.

Core tip: Based on the therapeutic efficacy of both S-1 mono-therapy and oxaliplatin plus capecitabine regimen in ACTS-gastric cancer (GC) and CLASSIC study, we conducted the multi-institutional research using propensity score-matched analysis to evaluate whether patients after D2 resection benefit from adjuvant chemotherapy with S-1 plus oxaliplatin (SOX). Here, we firstly report that SOX adjuvant chemotherapy, compared with surgery alone, significantly improves disease-free survival and overall survival in stage I B-III C GC patients undergoing D2 resection with accepted side effects.

Ren DF, Zheng FC, Zhao JH, Shen GS, Ahmad R, Zhang SS, Zhang Y, Kan J, Dong L, Wang ZY, Zhao FX, Zhao JD. Adjuvant chemotherapy with S-1 plus oxaliplatin improves survival of patients with gastric cancer after D2 gastrectomy: A multicenter propensity score-matched study. *World J Clin Cases* 2018; 6(10): 373-383 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/373.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.373>

INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies with high morbidity and mortality worldwide^[1]. Adequate surgical resection is the only curative therapeutic option for GC. In East Asia, gastrectomy with D2 lymphadenectomy is the standard surgical treatment^[2,3]. In fact, based on the results of the Dutch D1D2 trial^[4], the European and United States guidelines have likewise recommended the procedure^[5,6]. However, even with a potentially curative resection, approximately 50% of patients develop recurrence within 5 years after surgery^[7,8], and 50%-90% of patients die of tumor relapses^[9].

To decrease the risk of postoperative recurrence, various regimens for adjuvant chemotherapy have been implemented over the past 40 years. Results of two large randomized phase 3 trials, which are the ACTS-GC and CLASSIC trials, have shown survival benefit from adjuvant chemotherapy in patients who underwent D2 radical resection for stage II -III disease^[7,8]. In the ACTS-GC study, intake of S-1 treatment for one year after D2 gastrectomy increased the five-year relapse-free survival (RFS) and overall survival (OS) by 12.3% and 10.6%, respectively^[7]. In CLASSIC trial, 6 mo of capecitabine plus oxaliplatin (XELOX) therapy improved the estimated five-year disease-free survival (DFS) and OS by 15% and 9%, respectively^[8].

To date, only the two adjuvant chemotherapy regimens mentioned above have been proven to be significantly efficient in stage II -III GC patients who underwent D2 dissection. However, some aspects in the previous two studies on adjuvant chemotherapy

need to be improved. In the ACTS-GC trial, patients had a low compliance (65.8%) in taking S-1 for one year and a subgroup analysis showed that the effect was insufficient in the elderly or stage III patients^[7]. In the CLASSIC study, patients also had a low treatment completion rate (67%)^[8]. Therefore, new adjuvant chemotherapy regimens need to be explored.

Considering that both S-1 mono-therapy and combination therapy with oxaliplatin plus capecitabine have become the standard treatment for GC patients after D2 gastrectomy, a phase 2, single-arm study that investigated the safety of adjuvant chemotherapy regimen with S-1 plus oxaliplatin (SOX) in Japanese patients showed better toxicity profiles and relatively high completion rate (74.2%)^[10]. Therefore, adjuvant chemotherapy with SOX for GC is most likely reasonable and efficacious. Based on the aforementioned, we conducted this multicenter retrospective study to evaluate the safety and efficacy of SOX as adjuvant chemotherapy in stage I B–III C GC after D2 gastrectomy.

MATERIALS AND METHODS

Study population

The study included GC patients who underwent D2 gastrectomy at the Affiliated Hospital of Qinghai University, People's Hospital of Qinghai Province, Qinghai Red Cross Hospital, and Cancer Institute and Hospital, Chinese Academy of Medical Sciences from September 2009 to December 2011. Patients were selected if they met the following eligibility criteria: (1) histologically confirmed adenocarcinoma of the stomach; and (2) stage I B (pT1N1M0) or I B (pT2N0M0) with high-risk features including poorly differentiated or higher grade cancer, lymphovascular invasion, neural invasion, or < 50 years of age, stage II, or III disease according to the Seventh Edition of the American Joint Committee on Cancer (AJCC) classification. The following patients were excluded: (1) stage I A or I B (pT2N0M0) disease without aforementioned high-risk features; (2) those who received radiotherapy before or after surgery; (3) those who received neo-adjuvant chemotherapy; and (4) those who received adjuvant chemotherapy except for SOX or XELOX regimens.

We analyzed the clinicopathologic characteristics of the enrolled patients, including age, sex, tumor location, tumor grade, p-TNM stage (based on the Seventh AJCC classification), lymphatic and venous invasion, and perineural invasion. All eligible patients were divided into three parts, patients who treated with surgery alone, patients who received postoperative SOX adjuvant chemotherapy, and those who received XELOX adjuvant chemotherapy.

The study was approved by the institutional review boards of the Affiliated Hospital of Qinghai University, People's Hospital of Qinghai Province, Qinghai Red Cross Hospital, and Cancer Institute and Hospital, Chinese Academy of Medical Sciences.

Adjuvant chemotherapy regimens

SOX adjuvant chemotherapy was started within 3–6 wk after D2 gastrectomy. In all 3-wk cycles, S-1 was given orally twice daily for 2 wk at a dose of 80 mg/d for patients with a body surface area (BSA) < 1.25 m², 100 mg/d for patients with a BSA of 1.25 m² to < 1.5 m², and 120 mg/d for patients with a BSA of ≥ 1.5 m². On day 1 of each chemotherapy cycle, oxaliplatin was infused intravenously for 2–4 h at a dose of 130 mg/m². The Common Toxicity Criteria of the National Cancer Institute (version 4.0) was used to assess the adverse effects of chemotherapy. XELOX adjuvant chemotherapy was also started within 3–6 wk after D2 dissection. Capecitabine was given orally at a dose of 1000 mg/m² twice daily on days 1 to 14 of each 3-wk cycle. Oxaliplatin at 130 mg/m² was infused intravenously for 2–4 h on day 1 of each chemotherapy cycle.

Postoperative follow-up

Patients in the surgery alone group did not receive any antineoplastic agent until there was a confirmed recurrence. All the enrolled patients underwent hematologic tests, physical examination, and computed tomography every three months for the first two years after surgery, every six months from the third year to the fifth year, and annually thereafter.

Data, including tumor relapse, death from any cause and the last follow-up date were collected. DFS was defined as the time from surgery to tumor recurrence or the last follow-up date. OS was defined as the time from surgery to death or the last follow-up date.

Statistical analysis

To compare the baseline clinicopathologic characteristics between the adjuvant SOX and the surgery alone groups, the adjuvant SOX and the adjuvant XELOX groups, the χ^2 test or Fisher's exact test was used for categorical variables, whereas the Mann-Whitney *U* test was used for continuous variables. Survival outcomes were estimated using the Kaplan-Meier method, and the differences in survival between the treatment groups were compared using the log-rank test. An unadjusted Cox proportional hazards model was used to calculate the hazard ratio (HR) with the 95% confidence interval (CI) for the survival outcomes in all groups. To determine the independent prognostic factors for OS, a multiple regression analysis using a Cox proportional hazards model was performed. All tests were two-sided, and *P* < 0.05 was considered statistically significant.

We used propensity score matching to reduce to the greatest extent the effects of selection bias and the possible confounding factors. Propensity scores were estimated by a logistic regression model of the following covariates: age, sex, tumor location, tumor grade, p-TNM stage, lymphatic and venous invasion, and perineural invasion. Patients in the adjuvant SOX group were matched in a 1:2 ratio with those in the

Table 1 Clinicopathologic features of the study population undergoing surgery alone and receiving adjuvant S-1 plus oxaliplatin chemotherapy before and after propensity score-matching *n* (%)

Variable	Before PSM (<i>n</i> = 807)			After PSM (<i>n</i> = 351)		
	Surgery alone (<i>n</i> = 683)	Adjuvant SOX (<i>n</i> = 124)	<i>P</i> value	Surgery alone (<i>n</i> = 234)	Adjuvant SOX (<i>n</i> = 117)	<i>P</i> value
Age at diagnosis (yr)			0.099			0.443
< 35	9 (1.32)	3 (2.42)		3 (1.28)	0 (0.00)	
35-60	303 (44.36)	66 (53.23)		118 (50.43)	62 (52.99)	
> 60	371 (54.32)	55 (44.35)		113 (48.29)	55 (47.01)	
Gender			0.83			1
Female	177 (25.92)	31 (25.00)		60 (25.64)	30 (25.64)	
Male	506 (74.08)	93 (75.00)		174 (74.36)	87 (74.36)	
Tumor location			0.063			0.424
None-Cardia cancer	425 (62.23)	88 (70.97)		152 (64.96)	81 (69.23)	
Cardia cancer	258 (37.77)	36 (29.03)		82 (35.04)	36 (30.77)	
Tumor grade			0.006			0.725
Moderate to well	50 (7.32)	4 (3.23)		14 (5.98)	4 (3.42)	
Moderate	171 (25.04)	21 (16.94)		36 (15.38)	21 (17.95)	
Poor to moderate	427 (62.52)	97 (78.23)		180 (76.92)	90 (76.92)	
Early cancer or not reported	35 (5.12)	2 (1.61)		4 (1.71)	2 (1.71)	
Pathological stage			< 0.001			0.604
I B [†]	302 (44.22)	8 (6.45)		20 (8.55)	8 (6.84)	
II	127 (18.59)	29 (23.39)		48 (20.51)	29 (24.79)	
III	254 (37.19)	87 (70.16)		166 (70.94)	80 (68.38)	
Lymphatic and venous invasion			< 0.001			0.574
No	573 (83.89)	86 (69.35)		155 (66.24)	81 (69.23)	
Yes	110 (16.11)	38 (30.65)		79 (33.76)	36 (30.77)	
Perineural invasion			0.437			0.754
No	606 (88.73)	107 (86.29)		197 (84.19)	100 (85.47)	
Yes	77 (11.27)	17 (13.71)		37 (15.81)	17 (14.53)	

[†]Patients of stage I B (pT2N0M0) without high-risk features including poorly differentiated or higher grade cancer, lymphovascular invasion, neural invasion, or < 50 years of age were not included. SOX: S-1 plus oxaliplatin; PSM: Propensity score-matching.

surgery alone group and 1:1 ration with those in the adjuvant XELOX group using calculated propensity scores with a 0.05 caliper width. And only the patients matched with propensity scores were included in the time-to-event analyses. We performed the propensity score matching using the Matching package in R, version 3.3.1 (R Foundation)^[11].

Sensitivity analysis was conducted by adding comorbidity to our propensity score model before repeating the DFS and OS analyses between the adjuvant SOX group and the surgery alone group. Except for the propensity score matching, all statistical analyses were performed using SPSS software version 21.0 (SPSS Inc., Chicago, IL, United States).

RESULTS

Study population

From September 2009 to December 2011, there were 1944 GC patients who were treated by curative gastrectomy with D2 lymphadenectomy. Among them, 1077 patients were excluded for being in stage I A or I B (pT2N0M0) without high-risk features (*n* = 249), having received radiotherapy before or after surgery (*n* = 105), neo-adjuvant chemotherapy (*n* = 60), other adjuvant chemotherapy except for SOX or XELOX regimens after surgery (*n* = 663). A total of 867 patients were analyzed in this study; 124 patients

received SOX adjuvant chemotherapy, 60 patients received XELOX adjuvant therapy and 683 patients underwent surgery alone. After propensity score matching, 117 pairs of 1:2 matched patients (*i.e.*, 351 patients) and 57 pairs of 1:1 matched patients (*i.e.*, 114 patients) were generated (Figure 1).

The clinicopathologic characteristics of patients in adjuvant SOX group and surgery alone group before and after matching are shown in Table 1. Overall, compared with the surgery alone group, the adjuvant SOX group had more poor to moderate grade tumor (78.23% vs 62.52%), more pathologic stage III cancer (70.16% vs 37.19%), and more lymphatic and venous invasion (30.65% vs 16.11%). After matching, all the baseline clinicopathologic characteristics including age, sex, tumor location, tumor grade, p-TNM stage, lymphatic and venous invasion, and perineural invasion, were similar between the two groups. The clinicopathologic characteristics of patients in adjuvant SOX group and adjuvant XELOX group before and after matching are shown in Table 2.

Survival benefit of adjuvant SOX chemotherapy

In adjuvant SOX group, a median cycle of 4 (1-12 cycles) were received. After a median follow-up of 42 mo after gastrectomy, the number of patients who developed relapse and died was 42 (35.9%) and 36 (30.8%), respectively, in the adjuvant SOX group and

Table 2 Clinicopathologic features of the study population in adjuvant S-1 plus oxaliplatin group and adjuvant capecitabine plus oxaliplatin group before and after propensity score-matching *n* (%)

Variable	Before PSM (<i>n</i> = 184)			After PSM (<i>n</i> = 114)		
	Adjuvant SOX (<i>n</i> = 124)	Adjuvant XELOX (<i>n</i> = 60)	<i>P</i> value	Adjuvant SOX (<i>n</i> = 57)	Adjuvant XELOX (<i>n</i> = 57)	<i>P</i> value
Age at diagnosis (yr)			0.322			0.848
≤ 60	69 (55.65)	38 (63.33)		34 (59.65)	35 (61.40)	
> 60	55 (44.35)	22 (36.67)		23 (40.35)	22 (38.60)	
Gender			1			0.404
Female	31 (25.00)	15 (25.00)		18 (31.58)	14 (24.56)	
Male	93 (75.00)	45 (75.00)		39 (68.42)	43 (75.44)	
Tumor location			0.567			1
None-Cardia cancer	88 (70.97)	45 (75.00)		42 (73.68)	42 (73.68)	
Cardia cancer	36 (29.03)	15 (25.00)		15 (26.32)	15 (26.32)	
Tumor grade			0.521			0.233
Moderate to well	4 (3.23)	1 (1.67)		4 (7.02)	1 (1.75)	
Moderate	21 (16.94)	14 (23.33)		13 (22.81)	12 (21.05)	
Poor to moderate	97 (78.23)	45 (75.00)		38 (66.67)	44 (77.19)	
Early cancer or not reported	2 (1.61)	0 (0.00)		2 (3.51)	0 (0.00)	
Pathological stage			0.038			0.708
I B ¹	8 (6.45)	11 (18.33)		7 (12.28)	9 (15.79)	
II	29 (23.39)	10 (16.67)		12 (21.05)	9 (15.79)	
III	87 (70.16)	39 (65.00)		38 (66.67)	39 (68.42)	
Lymphatic and venous invasion			0.929			0.839
No	86 (69.35)	42 (70.00)		39 (68.42)	40 (70.18)	
Yes	38 (30.65)	18 (30.00)		18 (31.58)	17 (29.82)	
Perineural invasion			0.102			1
No	107 (86.29)	46 (76.67)		46 (80.70)	46 (80.70)	
Yes	17 (13.71)	14 (23.33)		11 (19.30)	11 (19.30)	

¹Patients of stage I B (pT2N0M0) without high-risk features including poorly differentiated or higher grade cancer, lymphovascular invasion, neural invasion, or < 50 years of age were not included. SOX: S-1 plus oxaliplatin; XELOX: Capecitabine plus oxaliplatin; PSM: Propensity score-matching.

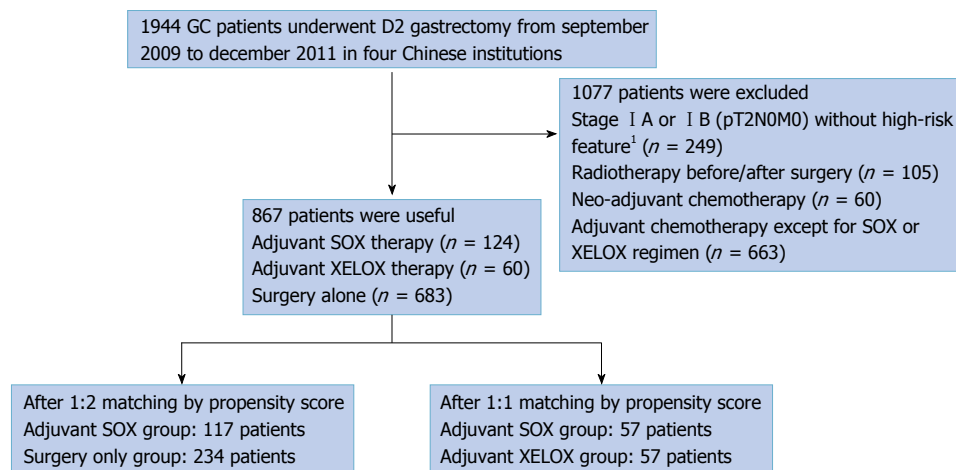


Figure 1 Flowchart of the study population. GC: Gastric cancer; SOX: S-1 plus oxaliplatin.¹High-risk features include poorly differentiated or higher grade cancer, lymphovascular invasion, neural invasion, or < 50 years of age.

122 (52.1%) and 117 (50.0%), respectively, in the surgery alone group. The estimated five-year DFS was 57.5% in the adjuvant SOX group and 44.6% in the surgery alone group (HR = 0.559; 95%CI: 0.393-0.794; *P* = 0.001; Figure 2A). The estimated five-year OS was 68.3% in the adjuvant SOX group and 45.8% in the surgery alone group (HR = 0.505; 95%CI: 0.348-0.734; *P* < 0.001; Figure 2B).

After addition of co-morbidity to the propensity score model in the sensitivity analysis, 116 pairs of

1:2 matched patients (*i.e.*, 348 patients) were generated. Repeat analyses showed that compared with the surgery alone group, the adjuvant SOX group had significantly better DFS (HR = 0.542; 95%CI: 0.377-0.779; *P* = 0.001) and OS (HR = 0.496; 95%CI: 0.338-0.728; *P* < 0.001).

Subgroup analysis

To further investigate whether stage III or elderly patients can benefit from SOX adjuvant chemotherapy,

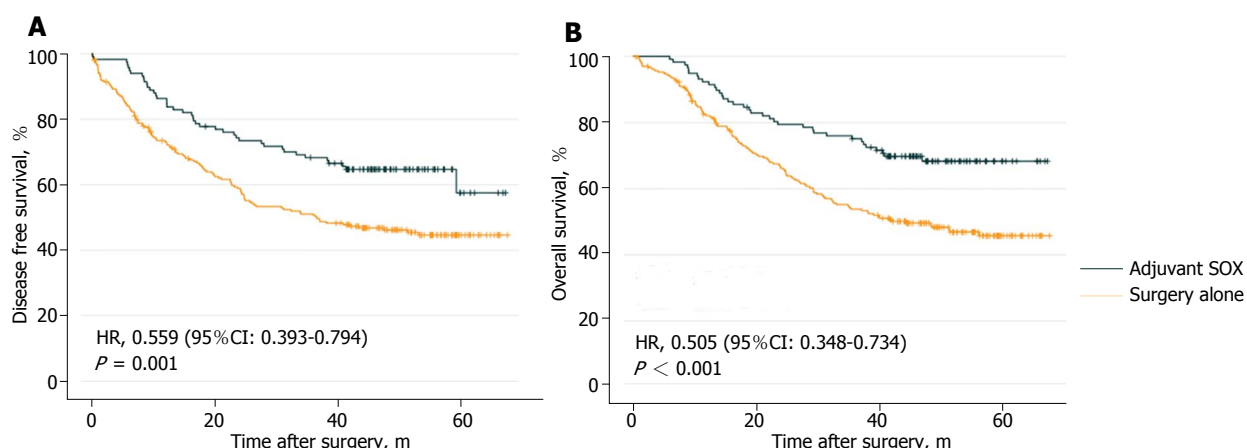


Figure 2 Kaplan-Meier curves for disease-free survival (A) and overall survival (B) between matched patients in the surgery alone group and adjuvant S-1 plus oxaliplatin group. SOX: S-1 plus oxaliplatin; HR: Hazard ratio.

exploratory subgroup analyses were performed among the 351 matched patients. In the stage III patients, the estimated five-year DFS rates were 33.4% in the surgery alone group and 49.1% in the adjuvant SOX group, with an HR of 0.530 (95%CI: 0.361-0.779; $P = 0.001$; Figure 3A). The estimated five-year OS rates were 34.1% in the surgery alone group and 62.5% in the adjuvant SOX group, with an HR of 0.458 (95%CI: 0.302-0.692; $P < 0.001$; Figure 3B).

In patients aged ≤ 60 years, the estimated five-year DFS rates were 54.5% in the surgery alone group and 61.6% in the adjuvant SOX group, with an HR of 0.553 (95%CI: 0.319-0.959; $P = 0.032$; Figure 3C). The estimated five-year OS rates were 55.2% in the surgery alone group and 77.2% in the adjuvant SOX group, with an HR of 0.435 (95%CI: 0.236-0.802; $P = 0.006$; Figure 3D).

For patients > 60 years old, the estimated five-year DFS rates were 34.2% in the surgery alone group and 54.4% in the adjuvant SOX group, with an HR of 0.557 (95%CI: 0.353-0.879; $P = 0.011$; Figure 3E). The estimated five-year OS rates were 36.0% in the surgery alone group and 58.0% in the adjuvant SOX group, with an HR of 0.559 (95%CI: 0.348-0.897; $P = 0.015$; Figure 3F).

Evaluation of prognostic factors

The multivariate Cox proportional hazards model showed that age (HR, 1.629; 95%CI: 1.155-2.297; $P = 0.005$), p-TNM stage III (HR = 10.258; 95%CI: 2.202-47.783; $P = 0.003$), perineural invasion (HR = 1.637; 95%CI: 1.056-2.538; $P = 0.028$), and SOX adjuvant chemotherapy (HR = 0.481; 95%CI: 0.329-0.702; $P < 0.001$) were the independent prognostic factors for OS of GC patients after D2 gastrectomy (Table 3).

Safety of adjuvant SOX chemotherapy

Out of 124 patients who received SOX adjuvant chemotherapy, 122 patients (98.4%) developed different grades of adverse events. Table 4 shows all

the grades of adverse events reported by $\geq 10\%$ of patients. Grade 3 or 4 adverse events were reported by 47 (37.9%) patients, and the most common adverse events were neutropenia (22.6%), leukopenia (8.9%) and thrombocytopenia (5.6%). Among all grades of adverse events, neutropenia (75.0%), leukopenia (60.5%) and peripheral sensory neuropathy (52.4%) had the highest event rate. In addition, one patient developed febrile neutropenia during the chemotherapy period. Fifty-six patients (45.2%) experienced reduction of S-1 or/and oxaliplatin dose mainly because of the adverse events of neutropenia, leukopenia and peripheral sensory neuropathy. Seventy-eight patients (62.9%) had a delay in the subsequent treatment and the most common reason is the adverse event of neutropenia.

Efficacy between SOX regimen and XELOX regimen

In adjuvant SOX group (57 patients), patients received 250 cycles chemotherapy in total with median cycles of 4. In adjuvant XELOX group (57 patients), patients received 258 cycles chemotherapy in total with median cycles of 5. After a median follow-up of 42 mo after gastrectomy, 47 patients developed relapse (21 in the SOX group and 26 in the XELOX group), 42 patients died (18 in the SOX group and 24 in the XELOX group). The estimated five-year DFS was 63.1% in the adjuvant SOX group and 54.0% in the adjuvant XELOX group (HR = 0.658; 95%CI: 0.360-1.203; $P = 0.340$; Figure 4A). The estimated five-year OS was 67.0% in the adjuvant SOX group and 56.5% in the adjuvant XELOX group (HR = 0.714; 95%CI: 0.382-1.334; $P = 0.361$; Figure 4B).

DISCUSSION

To the best of our knowledge, this was the first study to report that adjuvant chemotherapy with SOX can significantly improve the long-term survival of patients with GC after D2 radical gastrectomy, compared with surgery alone. After adjustment for confounders in

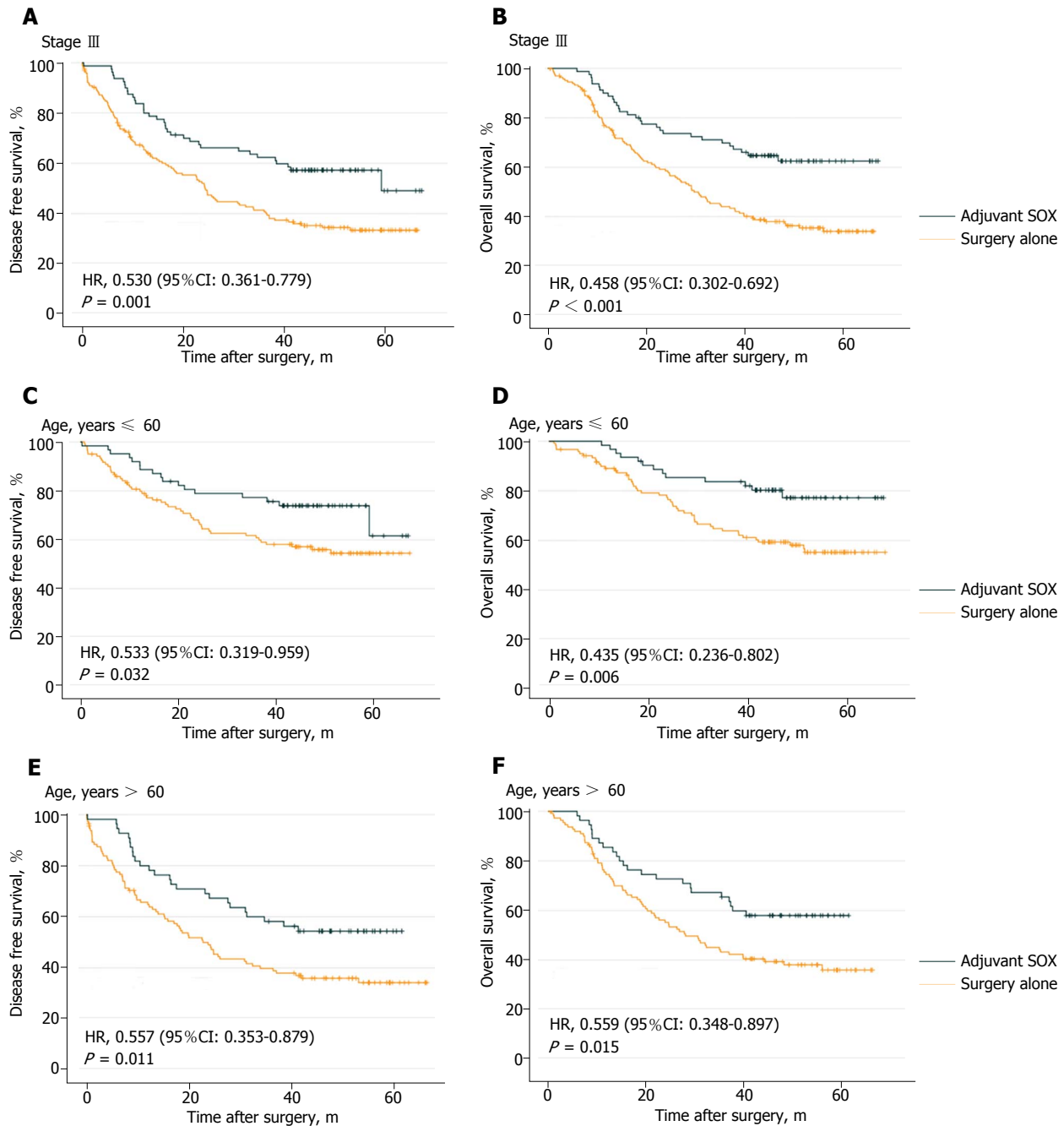


Figure 3 Kaplan-Meier curves for disease-free survival and overall survival of subgroups between matched patients in the surgery alone group and adjuvant S-1 plus oxaliplatin group. A: Disease-free survival (DFS) in matched patients with stage III; B: Overall survival (OS) in matched patients with stage III; C: DFS in matched patients who ≤ 60 years old; D: OS in matched patients who ≤ 60 years old; E: DFS in matched patients who > 60 years old; F: OS in matched patients who > 60 years old. SOX: S-1 plus oxaliplatin; HR: Hazard ratio.

the propensity score-matched analysis, adjuvant chemotherapy with SOX, compared with surgery alone, improved the estimated five-year DFS and OS by approximately 12.9% ($P = 0.001$) and 22.5% ($P < 0.001$), respectively, with mild and well-tolerated toxicities. The results were similar in the sensitivity analysis after addition of co-morbidity to the propensity score model; the estimated five-year DFS and OS improved by 13.9% ($P = 0.001$) and 22.2% ($P < 0.001$), respectively. Moreover, our research showed that SOX regimen was as effective as XELOX for stage

I B-III C GC patients after D2 dissection. These results strongly suggest that SOX is very likely to become a novel adjuvant chemotherapy regimen in patients with GC after D2 radical resection.

D2 gastrectomy is the standard of surgical procedure in patients with GC in East Asia^[12,13]. Moreover, the European and United States treatment guidelines have suggested such procedure in resectable patients, based on the Dutch D1D2 clinical study, which showed that D2 gastrectomy reduced the number of cancer-related deaths compared with D1^[4-6,14,15]. Two recent,

Table 3 Prognostic factors of overall survival in 351 matched patients with gastric cancer after D2 dissection

Variable	Hazard ratio	95%CI	P value
Age at diagnosis (yr)			
≤ 60	1		
> 60	1.629	(1.155-2.297)	0.005
Gender			
Female	1		
Male	1.073	(0.731-1.576)	0.718
Tumor location			
None-Cardia cancer	1		
Cardia cancer	0.862	(0.605-1.227)	0.409
Tumor grade			0.888
Moderate to well	1		
Moderate	1.37	(0.558-3.363)	0.492
Poor to moderate	1.283	(0.551-2.983)	0.563
Early cancer or not reported	1.972	(0.205-18.948)	0.557
Pathological stage			< 0.001
I B ¹	1		
II	4.691	(0.994-22.128)	0.051
III	9.857	(2.174-44.686)	0.003
Lymphatic and venous invasion			
No	1		
Yes	0.963	(0.670-1.384)	0.837
Perineural invasion			
No	1		
Yes	1.679	(1.091-2.585)	0.019
Adjuvant chemotherapy			
Surgery alone	1		
SOX	0.475	(0.326-0.693)	< 0.001

¹Patients of stage I B (pT2N0M0) without high-risk features including poorly differentiated or higher grade cancer, lymphovascular invasion, neural invasion, or < 50 years of age were not included. SOX: S-1 plus oxaliplatin.

excellent, and large-scale randomized trials have shown that adjuvant chemotherapy can improve both DFS and OS in patients with resectable GC after D2 gastrectomy^[7,8].

The ACTS-GC trial revealed that one year of adjuvant chemotherapy with S-1 for stage II/III GC patients after D2 dissection increased the five-year RFS and OS rates from 53.1% to 65.4% and 61.1% to 71.7%, respectively^[7]. The phase 3 CLASSIC study reported that six months of adjuvant chemotherapy with capecitabine and oxaliplatin after curative D2 gastrectomy in stage II to IIIB GC patients improved the estimated five-year DFS and OS rates from 53% to 68% and 69% to 78%, respectively^[8]. However, it should be noted that in the ACTS-GC study, the effect of adjuvant chemotherapy with S-1 in GC was stage-dependent. In particular, a superior treatment effect was observed in stage II cases (HR = 0.509), but it was rather ineffective for stage IIIA (HR = 0.708) and stage IIIB (HR = 0.791) disease^[7]. These results suggested that S-1 treatment was insufficient in eliminating micrometastatic cancer cells in cases with high p-TNM stage. Furthermore, in their subgroup analysis, S-1 treatment was shown to be not beneficial in elderly patients (≥ 60 years) and could not be sustained up to one year, with a 12-mo completion rate of only 65.8%^[16]. In the CLASSIC

study, only 67% received the planned eight cycles of adjuvant capecitabine and oxaliplatin chemotherapy; 56% experienced grade 3 or 4 adverse events; and 90% needed dose modifications because of adverse events^[17]. Therefore, novel adjuvant chemotherapy regimen with high efficiency and mild side effect needs to be explored for GC patients undergoing D2 dissection.

In this study, patients who received adjuvant chemotherapy with SOX had significantly better survival than those who underwent surgery alone. In the ACTS-GC trial and CLASSIC studies, both S-1 mono-therapy and oxaliplatin combined with capecitabine were confirmed to have a survival benefit for patients with GC after D2 dissection^[7,8]. Moreover, SOX was shown to have a high response rate (55.7%) and disease control rate (85.2%) in advanced GC^[18]. Therefore, adjuvant chemotherapy with SOX is reasonable for GC. A single-arm, phase 2 study revealed that adjuvant SOX treatment was manageable and safe with optimal dose reduction or delay in the initiation of a subsequent cycle in stage III GC patients undergoing D2 or more extensive lymphadenectomy^[10]. Most recently, Wang *et al.*^[19] reported DFS (75.9%) and OS (85.2%) for 3 years by adjuvant SOX chemotherapy for Chinese patients in GC. However, there had been no study that evaluated the survival benefit of adjuvant SOX chemotherapy over surgery alone in GC patients after D2 gastrectomy.

In this study, the survival rates of propensity score-matched patients were compared between adjuvant SOX chemotherapy and surgery alone, adjuvant SOX and XELOX chemotherapy. The results showed that compared with surgery alone, adjuvant SOX chemotherapy had survival benefit in terms of DFS and OS. The 12.9% estimated five-year DFS benefit rate in this study was almost similar to the results of the ACTS-GC trial and CLASSIC studies. In this present study, the 22.5% significant improvement in the estimated five-year OS with SOX regimen was probably related to the adjuvant chemotherapy itself and to the fact that some patients with relapse in the surgery alone group declined further antineoplastic therapy, whereas the patients in the adjuvant chemotherapy group remained to receive palliative therapy after relapse or due to more other comorbidities or competing causes of death in surgery alone patients. Our exploratory subgroup analysis showed the same survival benefits of adjuvant SOX chemotherapy in stage III and elderly patients. These results were similar to those of the CLASSIC study, but were not consistent with those of the ACTS-GC clinical trial. These differences might suggest that adjuvant chemotherapy with a doublet regimen containing S-1 was superior to mono-therapy with S-1 in these patients.

In this study, the adverse events documented with SOX were similar with the reported safety profiles of SOX in a phase 2 adjuvant therapy study and a phase 3 palliative treatment study on GC^[10,18]. The most

Table 4 Adverse events reported by $\geq 10\%$ of patients who received adjuvant S-1 plus oxaliplatin chemotherapy

Event	Adjuvant SOX (<i>n</i> = 124)					
	Grade 1	Grade 2	Grade 3	Grade 4	All grade	Grade 3 or 4
	No. of patients				%	
Leukopenia	46	18	8	3	60.5	8.9
Neutropenia	34	31	19	9	75	22.6
Anemia	35	3	1	1	32.3	1.6
Thrombocytopenia	22	18	7	0	37.9	5.6
Elevated total serum bilirubin level	23	2	1	0	21	0.8
Elevated AST/ALT level	53	5	1	1	48.4	1.6
Elevated ALP level	12	3	1	0	12.9	0.8
Nausea	41	19	4	-	51.6	3.2
Vomiting	17	16	4	0	29.8	3.2
Diarrhea	33	7	1	0	33.1	0.8
Peripheral sensory neuropathy	61	4	0	-	52.4	0

Grades of adverse events were defined according to the Common Terminology Criteria for Adverse Events (Version 4.0). SOX: S-1 plus oxaliplatin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; -: Not available.

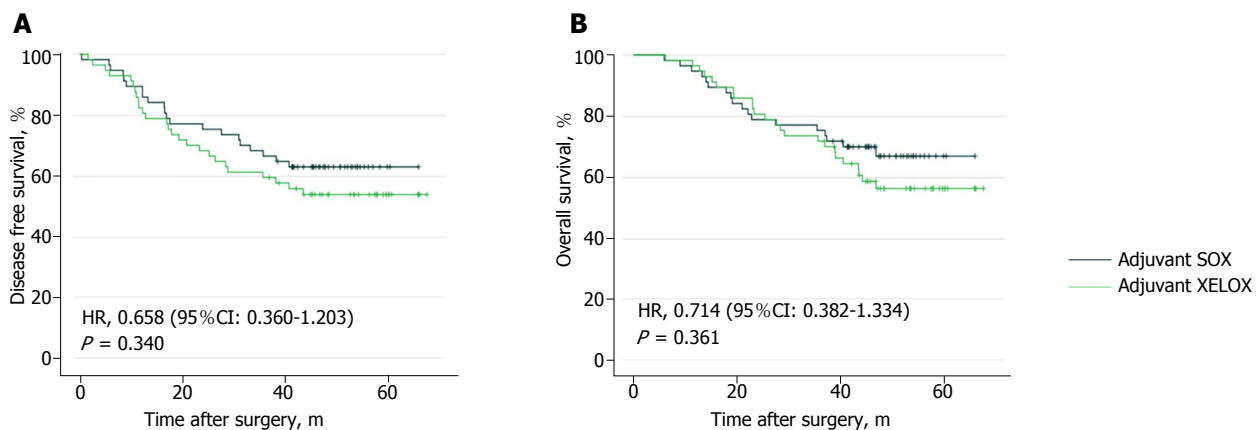


Figure 4 Kaplan-Meier curves for disease-free survival (A) and overall survival (B) between matched patients in the adjuvant S-1 plus oxaliplatin group and adjuvant capecitabine plus oxaliplatin group. SOX: S-1 plus oxaliplatin; XELOX: Capecitabine plus oxaliplatin; HR: Hazard ratio.

common adverse events were neutropenia, leukopenia, nausea, peripheral sensory neuropathy, and mild elevation of hepatic transaminases. Overall, the frequency of adverse events \geq grade 3 was less than 40%, suggesting that adjuvant SOX chemotherapy for GC after D2 radical gastrectomy was well tolerated.

The present study had several limitations. First, the baseline characteristics of the patients were different between both groups. Although we performed propensity score-matched analysis and multivariate regression to reduce biases, remnant heterogeneity between groups cannot be excluded. Second, although the entire study population was relatively large, the sample size of patients receiving adjuvant SOX or XELOX chemotherapy was not adequate for subgroup analyses according to each variable. Third, a majority of GC patients after D2 dissection in China were stage III disease which can't benefit from S-1 monotherapy according to ACTS-GC trial; most of the patients in our study didn't receive S-1 monotherapy. We didn't analyze data about patients only receiving S-1 in our study. Forth, the definite relapse locations were not

clear for part of the patients in our study, we didn't analyse the data about site of first relapse between patients received SOX or XELOX chemotherapy and those underwent surgery alone. Fifth, considering this study comprised Chinese patients, the dose of adjuvant SOX in other populations, especially Caucasians, remains to be further investigated because of the differences in the pharmacokinetics and toxicities of S-1 between Caucasian and Asian patients^[20]. Moreover, the role of SOX in patients undergoing D1 dissection needs to be confirmed.

In conclusion, compared with surgery alone, adjuvant SOX regimen significantly improved the long-term survival of Chinese patients with stage I B-III C GC after D2 radical gastrectomy, with accepted side effects. It showed the similar DFS and OS outcomes with XELOX regimen which had become the standard adjuvant therapy nowadays. Therefore, SOX is likely to become a novel adjuvant chemotherapy regimen in GC. Several ongoing studies on the role of SOX for adjuvant chemotherapy in GC are expected to convey new and definite proofs in future^[21-24].

ARTICLE HIGHLIGHTS

Research objectives

The main objectives of this retrospective study were to evaluate the safety and efficacy of S-1 plus oxaliplatin (SOX) as adjuvant chemotherapy for gastric cancer (GC) after D2 dissection.

Research methods

We collected patients with GC who underwent D2 gastrectomy from September 2009 to December 2011 in four Chinese institutions. Patients with stage IB-IIIc GC, who received adjuvant SOX treatment were matched by propensity scores with those who underwent surgery alone and those who conducted adjuvant capecitabine plus oxaliplatin (XELOX) regimen. We compared the estimated 5-year disease-free survival (DFS) and 5-year overall survival (OS) between the groups and analyzed adverse events in SOX patients.

Research results

In total, 867 GC patients were included for analysis. Among 124 patients treated with SOX regimen, 117 patients were matched to 234 patients who conducted surgery alone, and 57 patients were matched to 57 patients who received XELOX regimen. The estimated five-year DFS was 57.5% in the adjuvant SOX group and 44.6% in the surgery alone group ($P = 0.001$); and the estimated five-year OS was 68.3% and 45.8% ($P < 0.001$), respectively. Compared with XELOX regimen, SOX showed no significant difference in DFS and OS. The most common ≥ 3 grade adverse events of SOX regimen were neutropenia (22.6%), leukopenia (8.9%) and thrombocytopenia (5.6%).

Research conclusions

This study showed that compared with surgery alone, adjuvant SOX regimen significantly improves the long-term survival and have acceptable toxicity in patients with stage I B-IIIc GC after D2 dissection.

REFERENCES

- 1 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87-108 [PMID: 25651787 DOI: 10.3322/caac.21262]
- 2 Park JM, Kim YH. Current approaches to gastric cancer in Korea. *Gastrointest Cancer Res* 2008; **2**: 137-144 [PMID: 19259291]
- 3 Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2014 (ver. 4). *Gastric Cancer* 2017; **20**: 1-19 [PMID: 27342689 DOI: 10.1007/s10120-016-0622-4]
- 4 Songun I, Putter H, Kranenbarg EM, Sasako M, van de Velde CJ. Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. *Lancet Oncol* 2010; **11**: 439-449 [PMID: 20409751 DOI: 10.1016/S1470-2045(10)70070-X]
- 5 Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D; ESMO Guidelines Committee. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2016; **27**: v38-v49 [PMID: 27664260 DOI: 10.1093/annonc/mdw350]
- 6 National Comprehensive Cancer Network. Gastric Cancer (Version 5. 2017). Available from: URL: http://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf
- 7 Sasako M, Sakuramoto S, Katai H, Kinoshita T, Furukawa H, Yamaguchi T, Nashimoto A, Fujii M, Nakajima T, Ohashi Y. Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. *J Clin Oncol* 2011; **29**: 4387-4393 [PMID: 22010012 DOI: 10.1200/JCO.2011.36.5908]
- 8 Noh SH, Park SR, Yang HK, Chung HC, Chung IJ, Kim SW, Kim HH, Choi JH, Kim HK, Yu W, Lee JI, Shin DB, Ji J, Chen JS, Lim Y, Ha S, Bang YJ; CLASSIC trial investigators. Adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): 5-year follow-up of an open-label, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1389-1396 [PMID: 25439693 DOI: 10.1016/S1470-2045(14)70473-5]
- 9 GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group., Paoletti X, Oba K, Burzykowski T, Michiels S, Ohashi Y, Pignon JP, Rougier P, Sakamoto J, Sargent D, Sasako M, Van Cutsem E, Buyse M. Benefit of adjuvant chemotherapy for resectable gastric cancer: a meta-analysis. *JAMA* 2010; **303**: 1729-1737 [PMID: 20442389 DOI: 10.1001/jama.2010.534]
- 10 Shitara K, Chin K, Yoshikawa T, Katai H, Terashima M, Ito S, Hirao M, Yoshida K, Oki E, Sasako M, Emi Y, Tsujinaka T. Phase II study of adjuvant chemotherapy of S-1 plus oxaliplatin for patients with stage III gastric cancer after D2 gastrectomy. *Gastric Cancer* 2017; **20**: 175-181 [PMID: 26626800 DOI: 10.1007/s10120-015-0581-1]
- 11 Sekhon JS. Multivariate and propensity score matching software with automated balance optimization: the Matching package for R. *J Stat Softw* 2011; **42**: 1-52 [DOI: 10.18637/jss.v042.i07]
- 12 Sasako M, Inoue M, Lin JT, Khor C, Yang HK, Ohtsu A. Gastric Cancer Working Group report. *Jpn J Clin Oncol* 2010; **40** Suppl 1: i28-i37 [PMID: 20870917 DOI: 10.1093/jjco/hyq124]
- 13 Japanese Gastric Cancer Society. Guidelines for Diagnosis and Treatment of Carcinoma of the Stomach. Available from: URL: http://www.jgca.jp/pdf/Guidelines2004_eng.pdf
- 14 Allum WH, Blazeby JM, Griffin SM, Cunningham D, Jankowski JA, Wong R; Association of Upper Gastrointestinal Surgeons of Great Britain and Ireland, the British Society of Gastroenterology and the British Association of Surgical Oncology. Guidelines for the management of oesophageal and gastric cancer. *Gut* 2011; **60**: 1449-1472 [PMID: 21705456 DOI: 10.1136/gut.2010.228254]
- 15 Van Cutsem E, Dico M, Geva R, Arber N, Bang Y, Benson A, Cervantes A, Diaz-Rubio E, Ducreux M, Glynne-Jones R, Grothey A, Haller D, Haustermans K, Kerr D, Nordlinger B, Marshall J, Minsky BD, Kang YK, Labianca R, Lordick F, Ohtsu A, Pavlidis N, Roth A, Rougier P, Schmoll HJ, Sobrero A, Tabernero J, Van de Velde C, Zalcberg J. The diagnosis and management of gastric cancer: expert discussion and recommendations from the 12th ESMO/World Congress on Gastrointestinal Cancer, Barcelona, 2010. *Ann Oncol* 2011; **22** Suppl 5: v1-v9 [PMID: 21633049 DOI: 10.1093/annonc/mdr284]
- 16 Chang SH, Kim SN, Choi HJ, Park M, Kim RB, Go SI, Lee WS. Adjuvant Chemotherapy for Advanced Gastric Cancer in Elderly and Non-elderly Patients: Meta-Analysis of Randomized Controlled Trials. *Cancer Res Treat* 2017; **49**: 263-273 [PMID: 27384158 DOI: 10.4143/crt.2016.054]
- 17 Bang YJ, Kim YW, Yang HK, Chung HC, Park YK, Lee KH, Lee KW, Kim YH, Noh SI, Cho JY, Mok YJ, Kim YH, Ji J, Yeh TS, Button P, Sirzén F, Noh SH; CLASSIC trial investigators. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): a phase 3 open-label, randomised controlled trial. *Lancet* 2012; **379**: 315-321 [PMID: 22226517 DOI: 10.1016/S0140-6736(11)61873-4]
- 18 Yamada Y, Higuchi K, Nishikawa K, Gotoh M, Fuse N, Sugimoto N, Nishina T, Amagai K, Chin K, Niwa Y, Tsuji A, Imamura H, Tsuda M, Yasui H, Fujii H, Yamaguchi K, Yasui H, Hironaka S, Shimada K, Miwa H, Hamada C, Hyodo I. Phase III study comparing oxaliplatin plus S-1 with cisplatin plus S-1 in chemotherapy-naïve patients with advanced gastric cancer. *Ann Oncol* 2015; **26**: 141-148 [PMID: 25316259 DOI: 10.1093/annonc/mdu472]
- 19 Wang G, Zhao J, Song Y, Zhang W, Sun Y, Zhou A, Huang J, Du F, Yang L. Phase II study of adjuvant chemotherapy with S1 plus oxaliplatin for Chinese patients with gastric cancer. *BMC Cancer* 2018; **18**: 547 [PMID: 29743043 DOI: 10.1186/s12885-018-4480-9]
- 20 Comets E, Ikeda K, Hoff P, Fumoleau P, Wanders J, Tanigawara Y. Comparison of the pharmacokinetics of S-1, an oral anticancer agent, in Western and Japanese patients. *J Pharmacokinet Pharmacodyn* 2003; **30**: 257-283 [PMID: 14650374 DOI: 10.1023/A:1026142601822]
- 21 Hu X, Chen L, Du Y, Fan B, Bu Z, Wang X, Ye Y, Zhang Z, Xiao G, Li F, He Q, Li G, Shen X, Xiong B, Zhu L, Liu J, Liu L, Wu T,

- Zhou J, Zhang J, Zhao G, Wang X, Liang P, Wang X, Zhang Y, Wu X, Zhang J, Ji X, Zong X, Fu T, Jia Z, Ji J. Postoperative chemotherapy with S-1 plus oxaliplatin versus S-1 alone in locally advanced gastric cancer (RESCUE-GC study): a protocol for a phase III randomized controlled trial. *Chin J Cancer Res* 2017; **29**: 144-148 [PMID: 28536493 DOI: 10.21147/j.issn.1000-9604.2017.02.07]
- 22 **Shen L.** Phase III Study to Compare Perioperative Chemotherapy of Oxaliplatin Combined With S-1(SOX) Versus SOX or Oxaliplatin With Capecitabine (XELOX) as Post-operative Chemotherapy in Locally Advanced Gastric Adenocarcinoma With D2 Dissection. [accessed 2012 Feb 16]. In: ClinicalTrials.gov [Internet]. Beijing: Peking University. Available from: <http://clinicaltrials.gov/ct2/show/NCT01534546> ClinicalTrials.gov Identifier: NCT01534546
- 23 **Lin Y.** SOX as Adjuvant Chemotherapy for Resectable Gastric Cancer. [accessed 2012 Mar 2]. In: ClinicalTrials.gov [Internet]. Beijing: Chinese Academy of Medical Sciences. Available from: <http://clinicaltrials.gov/ct2/show/NCT01542294> ClinicalTrials.gov Identifier: NCT01542294
- 24 **Shen L.** A Phase II Study: SOX vs SP in Adjuvant Chemotherapy After D2 Surgery. [accessed 2012 Sep 6]. In: ClinicalTrials.gov [Internet]. Beijing: Peking University. Available from: <http://clinicaltrials.gov/ct2/show/NCT01679340> ClinicalTrials.gov Identifier: NCT01679340

P- Reviewer: Hashimoto N, Noshiro H **S- Editor:** Dou Y
L- Editor: A **E- Editor:** Wu YXJ



Rectal perforation by inadvertent ingestion of a blister pack: A case report and review of literature

Francesco Fleres, Antonio Ieni, Edoardo Saladino, Giuseppe Speciale, Michele Aspromonte, Antonio Cannà, Antonio Macrì

Francesco Fleres, Michele Aspromonte, Department of Human Pathology of the Adult and Evolutive Age “Gaetano Barresi”, Section of General Surgery, University of Messina, Messina 98125, Italy

Antonio Ieni, Giuseppe Speciale, Department of Human Pathology of the Adult and Evolutive Age “Gaetano Barresi”, Section of Anatomic Pathology, University of Messina, Messina 98125, Italy

Edoardo Saladino, General and Oncologic Surgery Unit, Clinica Cappellani-GIOMI, Messina 98168, Italy

Antonio Cannà, Messina University Medical School Hospital, Messina 98125, Italy

Antonio Macrì, Peritoneal Surface Malignancy and Soft Tissue Sarcoma Program, Messina University Medical School Hospital, Messina 98125, Italy

ORCID number: Francesco Fleres (0000-0002-1092-8975); Antonio Ieni (0000-0003-2878-3572); Edoardo Saladino (0000-0002-4528-6626); Giuseppe Speciale (0000-0001-5875-0785); Michele Aspromonte (0000-0001-7132-9493); Antonio Cannà (0000-0001-5198-1222); Antonio Macrì (0000-0003-2671-5314).

Author contributions: Fleres F, Saladino E, Aspromonte M and Macrì A participated in the conception and design of the report; Fleres F and Macrì A drafted the paper and analyzed the report; Macrì A performed the surgical procedure; Macrì A was involved in the diagnosis, surgical management and follow-up of the patient; Cannà A was involved in the patient's surgical management; Ieni A and Speciale G carried out the histological procedures.

Conflict-of-interest statement: All authors have no conflicts of interest to report.

Informed consent statement: Written informed consent was obtained from the patient ahead of the publication of this Case Report and its accompanying images. A copy of the written informed consent is available for review by the Editor-in-Chief of this journal.

CARE Checklist (2013) statement: The authors have read the CARE Checklist (2013), and the manuscript was prepared and revised according to the CARE Checklist (2013).

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Francesco Fleres, MD, Doctor, Medical Assistant, Surgeon, Department of Youth and Adulthood Human Pathology “Gaetano Barresi”, General Surgery Unit, University of Messina, Via Consolare Valeria, Messina 98125, Italy. franz.fleres@gmail.com
Telephone: +39-090-2212678
Fax: +39-090-2213524

Received: May 16, 2018

Peer-review started: May 16, 2018

First decision: May 24, 2018

Revised: July 26, 2018

Accepted: August 26, 2018

Article in press: August 27, 2018

Published online: September 26, 2018

Abstract

The accidental ingestion of a foreign body (FB) is a relatively common condition. In the present study, we report a peculiar case of rectal perforation, the first to our knowledge, caused by the inadvertent ingestion of a blister pill pack. The aim of this report is to illustrate the difficulties of the case from a diagnostic and therapeutic viewpoint as well as its unusual

presentation. A 75-year-old woman, mentally impaired, arrived at our emergency department in critical condition. The computed tomography scan revealed a substantial abdominopelvic peritoneal effusion and free perigastric air. The patient was therefore submitted to an urgent exploratory laparotomy; a 2-cm long, full-thickness lesion was identified in the anterior distal part of the intraperitoneal rectum. Hence, we performed a Hartmann's procedure. Because of her critical condition, the patient was eventually transferred to the Intensive Care Unit, where she died after 10 d, showing no surgical complication. The ingestion of FBs is usually treated with observation or endoscopic removal. Less than 1% of FBs are likely to cause an intestinal perforation. The intestinal perforation resulting from the unintentional ingestion of an FB is often a difficult challenge when it comes to treatment, due to its late diagnosis and the patients' deteriorated clinical condition.

Key words: Foreign body; Acute abdomen syndrome; Ingestion; Rectal perforation; Blister pill pack

© The Author(s) 2018. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Ingestion of a foreign body (FB) is usually treated with observation or endoscopic removal. Less than 1% of FBs can cause an intestinal perforation. Diverticular disease and FB may be associated with pathological processes, including inflammation, perforation, abscess and fistula. The diagnosis of intestinal perforation following the unknown ingestion of a FB is a clinical challenge, first of all because it happens often in patients with intellectual disability or among the psychiatric population and secondly because it is not reported during questioning. Caregivers should be cautious and aware of the cutting of drug blisters.

Fleres F, Ieni A, Saladino E, Speciale G, Aspromonte M, Cannao A, Macri A. Rectal perforation by inadvertent ingestion of a blister pack: A case report and review of literature. *World J Clin Cases* 2018; 6(10): 384-392 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/384.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.384>

INTRODUCTION

The accidental ingestion of a foreign body (FB) is a relatively common condition, affecting patients of every age. However, the two age extremities, *i.e.* children and elderly people, are at a higher risk of inadvertently ingesting FBs, as are alcoholic, psychiatric or mentally impaired patients as well as patients affected by intellectual disability or neurological disorder^[1].

Most FBs pass through the gastrointestinal (GI) tract without any complications. On the other hand, FBs such as fish bones, chicken bones and toothpicks can

cause perforation of the GI tract. As it turns out, in less than 1% of the cases, FB ingestion can result in acute surgical abdomen for intestinal perforation which needs emergency surgery^[2].

In the present paper, we report the first case, to our knowledge, of rectal perforation caused by the inadvertent ingestion of a blister pill pack (BPP). BPPs are commonly used for drug storage, as they provide a protection barrier and ensure preservation from the damage.

In some countries, a BPP is known as a "push-through pack". Push-through packs consist of two main features: (1) the cover foil being resistant but breaking easily, so that the drug can be pressed out by easily breaking the cover foil; and (2) the semirigid formed cavity can be folded to dispense the drug by pressing it out with a thumb. In both cases, breaking the cover foil with a fingernail will make the pressing-out easier.

The aim of this report is to illustrate the difficulties of a FB ingestion case from diagnostic and therapeutic viewpoints as well as its unusual presentation.

CASE REPORT

A 75-year-old woman was admitted to hospital for fever (39 °C) and vomiting. The medical history of the patient showed arterial hypertension, diabetes mellitus type II, uncontrolled hepatic cryptogenic cirrhosis with last MELD-score 19 and CHILD-score C-11, chronic metabolic failure, chronic heart failure, bilateral pulmonary thickening, urinary tract infections, previous uterine cervix carcinoma, chronic cerebral vasculopathy, major ischemic stroke and subarachnoid hemorrhage due to an accidental trauma. Since July 2017 the patient had been admitted to a rehabilitation center due to a pertrochanteric fracture of her left femur, which could not be treated surgically because of high operative risk.

The patient was admitted at the Department of Medicine in poor clinical condition, awake, noncollaborative, oriented only in space, dehydrated, with fever (39 °C) and hypotension (80/50 mmHg). At physical examination, the abdomen appeared distended, without tenderness, and peristalsis was present. Due to persistent hypotension and oliguria (150 mL/24 h), the patient was treated with noradrenalin (4 phials in 40 mL of NaCl solution 0.9% at 0.2 mL/h). After 24 h, the hypotension persisted (85/40 mmHg) and the abdomen was becoming hyper-tympanic with a mild pain in the left hypochondrium accompanied by intestinal borborygmi. Forty-eight hours after hospitalization, the patient manifested an acute abdominal syndrome characterized by diffuse abdominal pain at superficial palpation with resistance in hypogastrium, pelvic pain and meteorism. The digital rectal examination revealed an empty ampulla recti, with traces of bright red blood.

The laboratory data revealed the following: white blood cell count of 25920 mmc, with 87% neutrophils;

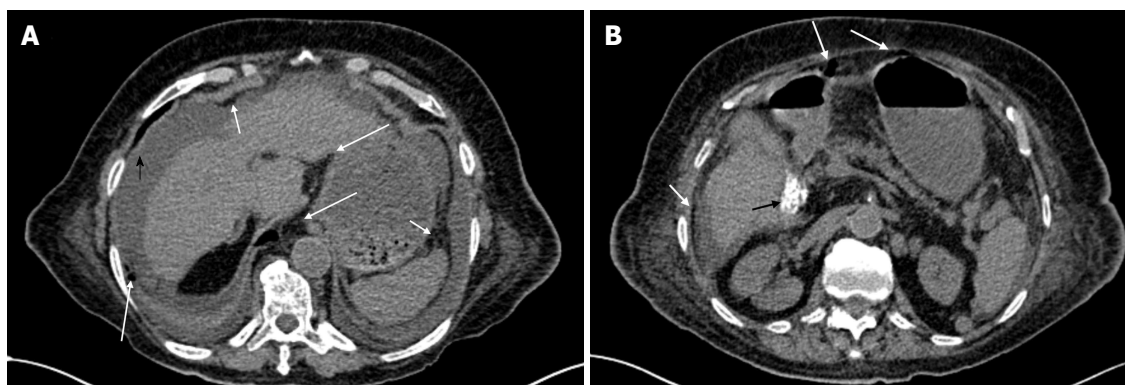


Figure 1 CT scan findings. A: Evidence of an important abdominopelvic peritoneal effusion (black arrowhead), perigastric free air along the gastrocolic ligament and under the anterior abdominal wall (white arrows), and pericardial and bilateral pleural effusions; B: In addition to perigastric free air and under the abdominal wall (white arrows), a microlithiasis (black arrow) of the gallbladder could also be observed.

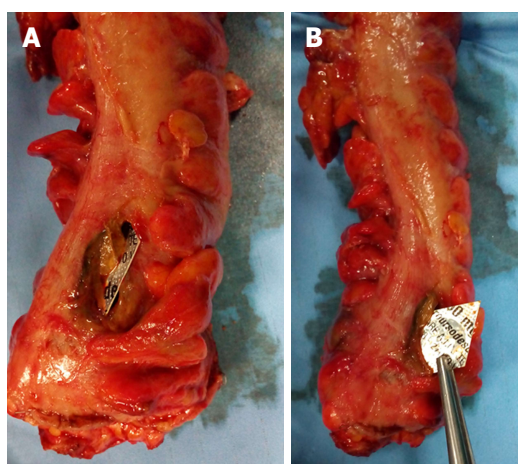


Figure 2 Postoperative findings. A, B: Blister pill pack with rectal perforation.

platelet count of 90000 mmc; hemoglobin of 10.9 gr%; C-reactive protein of 10.5 mg/dL; and procalcitonin of 34.9 mg/dL.

A rectal probe was inserted and a phial of trimebutin was administered without any benefit. The patient was therefore submitted to an abdominal X-ray and computed tomography (CT) (Figure 1), which revealed an abundant abdominopelvic peritoneal effusion and free perigastric air (along the gastrocolic ligament and under the anterior abdominal wall). Moreover, microlithiasis of the gallbladder as well as pericardial and bilateral pleural effusions were also present. Based on the CT findings, the origin of the perforation was suspected to be gastric.

On the basis of this clinical picture, the patient was transferred to our unit and we submitted her to an urgent exploratory laparotomy. The general clinical conditions were very bad, showing hypotension and oliguria. The abdomen exploration revealed a cirrhotic liver, an abundant amount of purulent intraperitoneal liquid, and a fecaloid collection in the pelvic pouch, which was buffered by the uterus. In the anterior distal part of the intraperitoneal rectum, a 2-cm long, full-thickness lesion was evident. The lesion was

surrounded by a necrotic wall, from which a part of a BPP with the pill inside could be seen (Figure 2). In addition, at sigmoid level, some diverticula were filled with coprolites and the wall was rather thin. Therefore, we performed a Hartmann's procedure, positioning three intraperitoneal drainage routes (Douglas' pouch, right and left paracolic gutters).

Due to the patient's worsened condition, she was transferred to the Intensive Care Unit, where she died after 10 d without any surgical complication.

The histological exam (Figure 3) showed a diverticular structure consisting of mucosa and submucosa, with a small rim of longitudinal muscle; superficial ulcerations were evident in the pathological area, with full-thickness mucosal necrosis associated with a massive inflammatory cell infiltrate having transmural pattern and involving serosal surface with partial necrosis.

The exploratory laparotomy allowed us to identify the BPP that had been initially missed on the CT scan images; it had appeared as a radiopaque intraluminal body, located in the high rectum and without any evidence of collection or air leakage (Figure 4).

DISCUSSION

Taking into consideration the diagnostic and management difficulties of this exceptional clinical case, we have performed a systemic review of the literature to evaluate the diagnostic role of imaging procedures and the management of a GI perforation caused by the inadvertent ingestion of a BPP. Indeed, the goal of this analysis was to identify in the literature the most relevant information about cases of perforation caused by the inadvertent ingestion of a BPP.

For this purpose, we carried out a comprehensive search of citations from PubMed between January 1st 1988 to January 31st 2018, starting from the first article concerning bowel perforation related to a BPP, using the key words "intestinal perforation blister", "diagnosis", "CT blister pill pack" and "rectal perforation blister". Our search yielded 20 articles, in which 23

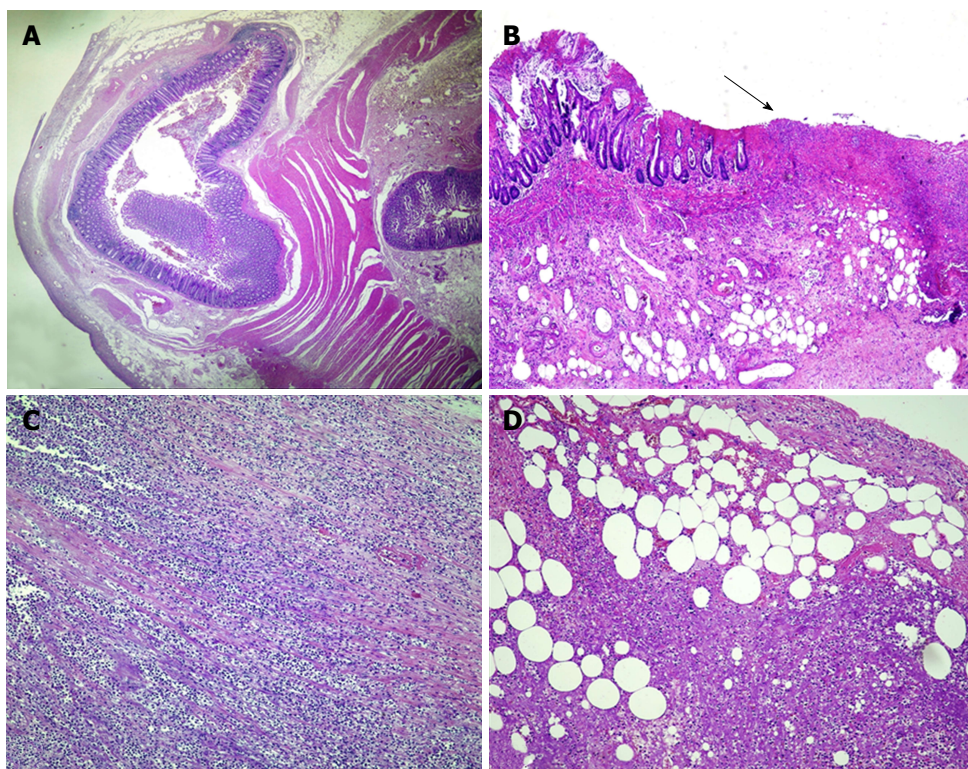


Figure 3 Histological findings. A: The whole histological section shows a diverticular structure consisting of mucosa and submucosa with a small rim of longitudinal muscle ($\times 5$); B: Evidence of superficial ulcerations (arrow) with full-thickness mucosal necrosis in the pathological area ($\times 10$); C: Massive inflammatory cell infiltration having a transmurular pattern and involving the serosal surface ($\times 20$); D: Massive inflammatory cell infiltration having transmurular pattern and involving the serosal surface with partial necrosis ($\times 20$). Hematoxylin and eosin staining.



Figure 4 Upon re-reading of the computed tomography imaging, a radiopaque intraluminal body (white arrow) without any evidence of collection or air leakage was visible in the high rectum.

cases of GI perforation were reported, but none of rectal perforation. We excluded articles concerning GI perforation by other types of FBs. In the case of multiple publications on the same group of patients, only the most recent and complete paper was retained, including all types of study design. The following data were analyzed: year of publication; patient's sex and age (years); location of the perforation; diagnostic modalities; treatment applied; and mortality. We also added our case, in order to compare it with the literature findings.

Based on our search criteria, we identified 23 GI

perforations by BPP in the literature; including our case, we had data on a total of 24 cases (Table 1). The patients were composed of 10 males and 14 females, with a median age of 70 and a standard deviation of 12.9. In particular, the perforations were located as follows: 5 in the esophagus; 1 in the stomach; 1 in the duodenum; 15 in the ileum; 1 in the sigmoid; and 1 in the rectum (represented by our case). In 18 patients, the clinical presentation was characterized by abdominal pain and acute abdomen syndrome, with 1 case presenting only vomiting, 1 presenting diarrhea and vomiting of pieces of plastic, 3 presenting chest pain (1 with right-sided pyopneumothorax), and 1 showing pneumomediastinum.

In this case series, the diagnosis was reached by CT in 7 patients, chest X-rays with gastrograffin study of fistula in 1 patient, endoscopy in 2 patients, surgery (laparotomy) in 13 patients (2 with positive findings in CT images upon postsurgery revision 1 of which being our case), and postmortem examination in 1 patient.

With regard to treatment, endoscopic removal was performed in 3 patients (in 1 of them, bilateral cervicotomy, abscess drainage and tracheostomy were also performed), palliative care was undertaken in 2 patients, radiological drainage and conservative medical treatment were adopted in 1 patient, and laparotomy was performed in 18 patients.

The mortality was not reported for 1 patient; for the remaining cases, 15 patients survived and 8 patients

Table 1 Reported cases of gastrointestinal perforation caused by blister packs

Ref.	Year of publication	Age	Gender	Main symptoms	Location	Diagnostic modality	Management	Exitus
Crowley and Bretzke ^[33]	1988	68	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	Yes
Fernando ^[34]	1989	43	F	Diarrhea and pieces of plastic sheeting in vomit	Sigmoid colon	Postmortem examination	Palliative care	Yes
Sato <i>et al</i> ^[35]	1992	50	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	No
Norstein <i>et al</i> ^[36]	1995	68	M	Abdominal pain and severe tenderness right iliac fossa	Distal ileum (15 cm from ileocecal valve)	Laparotomy	Laparotomy	No
Lurton <i>et al</i> ^[37]	1996	63	M	Abdominal pain	Stomach	Laparotomy	Laparotomy	No
Fulford <i>et al</i> ^[38]	1996	80	M	Generalized peritonitis	Antimesenteric border of the ileum 5 cm proximal to the ileocecal valve	Laparotomy	Laparotomy	Yes
Kansal and Agrawal ^[39]	2000	65	M	Abdominal pain	Ileum	Laparotomy	Laparotomy	No
Dutta <i>et al</i> ^[40]	2001	50	M	Severe retrosternal pain and dysphagia, 10 d after: right-sided pyopneumothorax	Esophagus fistula (right posterolateral wall of the lower third)	Chest X-rays, intercostal drain, gastrografin study of fistula	Radiological drainage, conservative medical treatment	No
Gupta <i>et al</i> ^[41]	2002	84	M	Chest pain	Esophagus	Endoscopy	Endoscopic removal	Yes
Gupta <i>et al</i> ^[42]	2002	58	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	No
Ishikura <i>et al</i> ^[43]	2003	85	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	-
Fierens <i>et al</i> ^[44]	2007	75	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	No
Domen <i>et al</i> ^[45]	2011	90	F	Abdominal pain	Ileum	Laparotomy	Laparotomy	No
Purnak <i>et al</i> ^[41]	2011	73	F	Vomiting	Esophagus	Endoscopy	Endoscopic removal	No
Sasko <i>et al</i> ^[46]	2012	70	F	Acute abdominal pain	Ileum	CT	Laparotomy	No
Orry <i>et al</i> ^[29]	2014	57	F	Abdominal pain	Ileum	CT	Laparotomy	No
Orry <i>et al</i> ^[29]	2014	90	M	Abdominal pain	Ileum	CT	Laparotomy	No
Coulter <i>et al</i> ^[4]	2014	84	F	Abdominal pain	Ileum	CT	Laparotomy	No
Coulter <i>et al</i> ^[4]	2014	85	M	Chest pain	Esophagus	CT	Palliative care	Yes
Yao <i>et al</i> ^[47]	2015	72	M	Abdominal pain	Duodenum	Laparotomy	Laparotomy	Yes
Al-Ramahi <i>et al</i> ^[3]	2015	66	F	Severe, colicky abdominal pain and bloating of 1-wk duration	Terminal ileum (25 cm proximal to the ileocecal junction)	Laparotomy (CT positive at revision after surgery)	Laparotomy	No
Prokop <i>et al</i> ^[48]	2016	61	M	Sore throat and hoarseness, sepsis, pneumomediastinum	Esophagus	CT	Endoscopic removal, bilateral cervicotomy and abscess drainage, tracheostomy	Yes
Prokop <i>et al</i> ^[48]	2016	68	F	Acute abdominal pain	Ileum	CT	Laparotomy	No
Our case	2018	75	F	Acute abdominal pain	Rectal	Laparotomy (CT positive at revision after surgery)	Laparotomy	Yes due to sepsis

CT: Computed tomography.

died (among them, 1 is our case, although the patient's mortality was not related to the surgery procedure but to sepsis).

As shown in Table 1, imaging exams (like the CT scan) often fail to identify the GI perforation by BPP and the definitive diagnosis is reached, in most cases, during emergency surgery. Therefore, to the best of our knowledge, the present case represents the first report in the literature about a rectal perforation by BPP.

Generally, the ingestion of FBs is seen in emergency departments and it is typically treated by observation or endoscopic removal. Approximately 80%-90% of ingested FBs pass through the GI tract with no complication, whereas the remaining 10%-20% fail to progress. Less than 1% of FBs can determine an intestinal perforation^[3]. Small

orthodontic appliances and dentures account for 73% of the FBs accidentally ingested by the elderly. Other common FBs are toys, jewelry, nails, gravel, needles/pins, staples, thumbtacks, wire bristles and magnetic objects.

Particularly, the elderly population is exposed to multiple risk factors that make accidental ingestion of BPPs more likely to happen; these include poor vision, presence of dentures, and polypharmacy. Dentures, in particular, produce a lack of normal palatal and gingival sensation, playing an important role in the accidental ingestion of BPPs^[4,5]. In addition, in elderly patients, diverticular disease is very common in Western countries and predominately affects the distal colon. This condition produces structural abnormalities such as tortuous lumen, strictures and mural pockets that could aggravate an FB-caused injury and the FB itself can be “marooned-in”. Consequently, diverticular disease and FBs may be associated with pathological processes, including inflammation, perforation, abscess and fistula^[6].

In practical terms, a BPP appears as an object with sharp and pointed edges and it can produce a bowel perforation. Therefore, sharp, thin, stiff, pointed or long FBs can cause perforation by direct penetration or by remaining stuck within the lumen, thus provoking necrosis of the bowel wall^[3].

However, FB intestinal perforation represents a challenging clinical scenario, mainly in patients with intellectual disability or psychiatric disorders characterized by a noncollaborative attitude during medical questioning. Unfortunately, the serious deteriorating symptoms and signs in these patients are the primary cause of admission to hospital.

Frequently, FB ingestion can be asymptomatic or with unspecific symptoms and can vary depending on the sites where the FB arrives and in the related complications. Moreover, the FB ingestion signs can mimic those of other surgical conditions, such as appendicitis, diverticulosis or colonic perforation^[7]. Hence, the preoperative diagnosis is frequently a surgical acute abdomen of unknown origin^[8].

The various clinical symptoms include dysphagia, odynophagia, chest pain, respiratory distress, hematemesis, GI bleeding, acute abdominal pain, intestinal perforation, bowel obstruction, localized abscess formation, peritonitis, inflammatory masses or sepsis^[9-11], perineal and scrotal abscess, and enterobladder fistulas^[12,13].

Many studies have suggested that FBs may stop in areas of anatomical narrowing (*i.e.*, cricopharyngeal ring, lower esophageal sphincter, pylorus, duodenal sweep, ileocecal valve, and anus), physiological angling (curvature of the duodenum) or pathological stricture or adhesion presence, mainly in patients with previous bowel pathology (*e.g.*, intestinal stricture, Crohn’s disease)^[14-17]. Usually, FBs with size exceeding 2-2.5 cm pass with difficulty through the pyloric canal and those having a dimension of 6-10 cm or greater cannot move

through the duodenum. Even if the most frequent sites of perforation are the lower esophagus and the terminal ileum, a small percentage of perforations can occur at any level, from mouth to anus^[14-16,18]. GI mucosa injury can produce digestive hemorrhage^[19], while other potential complications may arise as a result of FB migration to the liver and pancreas, with pancreatitis, gastric varices development, splenic artery pseudoaneurysm, or even aspects mimicking locally advanced pancreatic carcinoma^[20-23].

Typically, the time from ingestion to perforation is very long; it has been demonstrated that 10.4 d represents the median time^[8]. Most of these patients occur in the straits and the angles of the GI tract. Therefore, distal ileum, cecum and left colon are other common sites of perforation, although some authors have reported an increased incidence of perforation in association with Meckel’s diverticulum and diverticular disease^[13,24-26].

In obese and bedridden patients, the identification of a low calcium opaque FB can be hindered by a large amount of soft tissue or simply by a high level of liquid in the viscera^[27]. Nevertheless, the radiographic appearances of BPPs are typically identifiable by their aluminum foil backing and plastic blister surrounding the pill or tablet along with a thin rim of air. Their lateral appearance has been compared to that of a “UFO”^[28]. It was also observed that the density of the pill itself could be extremely variable, some pills being completely radiolucent^[4]. The most direct assessment for FB perforation on CT is the identification of the FB near extraluminal gas. Other findings include localized wall thickening, fat stranding and abscess. Multidetector CT, through the possibilities of high-quality multiplanar three-dimensional reconstructions and maximum intensity projection, can reach the definitive diagnosis of perforated intestinal structures caused by ingested FBs, often with demonstration of the responsible FB^[27,29].

Treatment depends upon patient age, the anatomical location, type and nature of the material ingested along with the presenting symptoms. Therefore, early diagnosis and prompt removal *via* either endoscopic or surgical measure are necessary. The management may indeed consist of conservative or interventional methods; in almost 20% of the cases, endoscopic treatment is required or possibly laparoscopy or exploratory laparotomy^[30]. The last of these can prove especially risky in psychiatric patients with a history of ingesting multiple FBs and who have already undergone multiple surgeries^[31]. If the FB arrives in the stomach or in the duodenum, endoscopic removal should be attempted immediately, in order to avoid the risk of perforation of the ileocecal valve, which is approximately 35%^[14,15,17]. If the pointed object moves past the duodenum, the patient should be monitored daily through a series of radiographs under close observation. Surgery becomes necessary if the object is no longer progressing radiographically after a 72-h observation. Emergency laparotomy is required if the patient develops clinical

signs of acute peritonitis^[14,15].

The case reported herein represents the first case in literature describing a rectal perforation caused by a BBP. The lack of similar situations underlines how difficult it is to reach a correct early diagnosis because of nonspecific and vague symptoms. Moreover, the patient was initially treated for a septic state suspected of having originated from the abdomen. Most certainly, an immediate CT scan would have helped to reach the diagnosis, but it was deemed unnecessary when the patient was taken to the emergency department for observation. Based on an abdomen and thorax X-ray, the hypothesis of acute abdominal perforation had actually been excluded. It is quite hard to establish whether the general outcome would have been different had a CT scan been performed, for the patient's conditions appeared highly critical since her first day of admission and only rarely does a severe abdominal sepsis in a critically ill patient, as in this case, give survival chance.

Thus, as can easily be understood, the diagnosis of our patient was delayed due to her mental impairment, while the CT scan could neither reveal the presence of the BPP nor any air leakage or collection in the proximity of the rectum. Based on the CT findings, our first hypothesis was of a pneumoperitoneum caused by a gastric-duodenal perforation. Probably, as described in the literature, our difficulty in identifying the BPP had to do with the radiolucent characteristics of the BPP itself. In light of the exploratory laparotomy findings, we decided to review the CT scan images. The BPP initially missed on CT scan was identified as a radiopaque intraluminal body in the high rectum, without any evidence of collection or air leakage (Figure 4).

Moreover, the damage caused by the BPP derived both from the BPP itself and from the presence of sigmoid-rectum diverticula. The definitive diagnosis was only reached after exploratory laparotomy and prompt treatment was applied, which included the removal of the causative agent through a Hartmann's procedure, peritoneal lavage and drainage. Our patient presented the diagnostic criteria of severe sepsis and septic shock. The mortality from severe sepsis and septic shock is now closer to 20%-30% in many series^[32], and indeed our patient died after 10 d without surgical complications.

The diagnosis of intestinal perforation following the unknown ingestion of an FB is often a difficult challenge in terms of treatment, due to its late diagnosis and a deteriorated clinical condition. Caregivers should be cautious, they should avoid cutting drug blisters in order to not administer the pill with its blister pack to patients. Intraoperative exploration remains critical in most cases.

condition, awake, noncollaborative, oriented only in space, dehydrated, with fever (39 °C) and vomiting, oliguria and hypotension (80/50 mmHg). After 48 h, she presented an acute abdominal pain.

Clinical diagnosis

The diagnostic hypothesis was firstly sepsis of unknown origin.

Differential diagnosis

Based on the computed tomography (CT) findings, the origin of the perforation was suspected to be gastric.

Laboratory diagnosis

The laboratory data revealed white blood cell count of 25920 mmc, with 87% neutrophils, platelet count of 90000 mmc, hemoglobin of 10.9 gr%, C-reactive protein of 10.5 mg/dL and procalcitonin of 34.9 mg/dL.

Imaging diagnosis

The abdominal X-ray and CT revealed an abundant abdominopelvic peritoneal effusion, free perigastric air (along the gastrocolic ligament and under the anterior abdominal wall).

Pathological diagnosis

The abdomen exploration revealed a cirrhotic liver, an abundant amount of purulent intraperitoneal liquid, and a fecaloid collection in the pelvic pouch, which was buffered by the uterus. In the anterior distal part of the intraperitoneal rectum, a 2-cm long, full-thickness lesion was evident. The lesion was surrounded by a necrotic wall from which appeared a part of the blister pill pack (BPP) with the pill inside. In addition, at sigmoid level, some diverticula were filled with coprolites and the wall was rather thin. Exploratory laparotomy findings allowed us to identify the BPP initially missed on CT scan images. Review of the images revealed that it had appeared as a radiopaque intraluminal body in the high rectum, without any evidence of collection or air leakage.

Treatment

The patient was submitted to an urgent exploratory laparotomy. The general clinical conditions were very bad and included hypotension and oliguria. Hence, we performed a Hartmann's procedure and positioned three intraperitoneal drainage routes. Due to her worsening condition, the patient was transferred to the Intensive Care Unit, where she died after 10 d without any surgical complication.

Related reports

As can easily be understood, the diagnosis was delayed due to the patient's mental impairment, while the CT scan could neither reveal the presence of the BPP nor any air leakage or collection in the proximity of the rectum. Based on the CT findings, our first hypothesis was of a pneumoperitoneum caused by a gastric-duodenal perforation. Probably, as described in the literature, our difficulty in identifying the BPP had to do with the radiolucent characteristics of the BPP itself.

Term explanation

In some countries, a BPP is known as a "push-through pack". Push-through packs consist of two main features: (1) the cover foil being resistant but breaking easily, so that the drug can be pressed out by easily breaking the cover foil; and (2) the semirigid formed cavity can be folded to dispense the drug by pressing it out with a thumb; in both cases, breaking the cover foil with a fingernail will make the pressing-out easier.

Experiences and lessons

To the best of our knowledge, this is the first case report describing a rectal perforation caused by a BBP. In light of the exploratory laparotomy findings, we decided to review the CT scan images. The BPP initially missed on CT scan was identified as a radiopaque intraluminal body in the high rectum, without any evidence of collection or air leakage. The diagnosis of intestinal perforation following the unknown ingestion of an FB is often a difficult challenge in terms of treatment, due to its late diagnosis and a deteriorated clinical condition

ARTICLE HIGHLIGHTS

Case characteristics

A 75-year-old woman arrived at our emergency department in poor clinical

ACKNOWLEDGEMENTS

The authors thank Lucia Lo Conti for proofreading the

English.

REFERENCES

- 1 **Laeq SM**, Rai AA, Tasneem AA, Luck NH, Majid Z. Pill in the blister pack: a rare cause of dysphagia in an elderly adult. *Pan Afr Med J* 2015; **22**: 176 [PMID: 26918072 DOI: 10.11604/pamj.2015.22.176.8031]
- 2 **Goh BK**, Chow PK, Quah HM, Ong HS, Eu KW, Ooi LL, Wong WK. Perforation of the gastrointestinal tract secondary to ingestion of foreign bodies. *World J Surg* 2006; **30**: 372-377 [PMID: 16479337 DOI: 10.1007/s00268-005-0490-2]
- 3 **Al-Ramahi G**, Mohamed M, Kennedy K, McCann M. Obstruction and perforation of the small bowel caused by inadvertent ingestion of a blister pill pack in an elderly patient. *BMJ Case Rep* 2015; **2015**: [PMID: 26475885 DOI: 10.1136/bcr-2015-212822]
- 4 **Coulier B**, Rubay R, Van den Broeck S, Azar AR, Maldague P, Mailleux P, Lismonde Y, Bueres I. Perforation of the gastrointestinal tract caused by inadvertent ingestion of blister pill packs: report of two cases diagnosed by MDCT with emphasis on maximal intensity and volume rendering reformations. *Abdom Imaging* 2014; **39**: 685-693 [PMID: 24643854 DOI: 10.1007/s00261-014-0120-2]
- 5 **Gunn A**. Intestinal perforation due to swallowed fish or meat bone. *Lancet* 1966; **1**: 125-128 [PMID: 4158955 DOI: 10.1016/S0140-6736(66)91262-1]
- 6 **Ross E**, McKenna P, Anderson JH. Foreign bodies in sigmoid colon diverticulosis. *Clin J Gastroenterol* 2017; **10**: 491-497 [PMID: 29030789 DOI: 10.1007/s12328-017-0786-4]
- 7 **Yao CC**, Yang CC, Liew SC, Lin CS. Small bowel perforation caused by a sharp bone: laparoscopic diagnosis and treatment. *Surg Laparosc Endosc Percutan Tech* 1999; **9**: 226-227 [PMID: 10804008 DOI: 10.1097/00129689-199906000-00017]
- 8 **Rodríguez-Hermosa JI**, Codina-Cazador A, Sirvent JM, Martín A, Gironès J, Garsot E. Surgically treated perforations of the gastrointestinal tract caused by ingested foreign bodies. *Colorectal Dis* 2008; **10**: 701-707 [PMID: 18005196 DOI: 10.1111/j.1463-1318.2007.01401.x]
- 9 **Schwartz JT**, Graham DY. Toothpick perforation of the intestines. *Ann Surg* 1977; **185**: 64-66 [PMID: 318821 DOI: 10.1097/00000658-197701000-00010]
- 10 **Maleki M**, Evans WE. Foreign-body perforation of the intestinal tract. Report of 12 cases and review of the literature. *Arch Surg* 1970; **101**: 475-477 [PMID: 5457244 DOI: 10.1001/archsurg.1970.01340280027008]
- 11 **Purnak T**, Ozaslan E, Efe C. Concomitant oesophageal perforation and bleeding due to a tiny pill with its blister pack. *Age Ageing* 2011; **40**: 645-646 [PMID: 21771743 DOI: 10.1093/ageing/afq081]
- 12 **Moreira CA**, Wongpakdee S, Gennaro AR. A foreign body (chicken bone) in the rectum causing extensive perirectal and scrotal abscess: report of a case. *Dis Colon Rectum* 1975; **18**: 407-409 [PMID: 1097218 DOI: 10.1007/BF02587433]
- 13 **Akhtar S**, McElvanna N, Gardiner KR, Irwin ST. Bowel perforation caused by swallowed chicken bones--a case series. *Ulster Med J* 2007; **76**: 37-38 [PMID: 17288304]
- 14 **Pavlidis TE**, Marakis GN, Triantafyllou A, Psarras K, Kontoulis TM, Sakantamis AK. Management of ingested foreign bodies. How justifiable is a waiting policy? *Surg Laparosc Endosc Percutan Tech* 2008; **18**: 286-287 [PMID: 18574418 DOI: 10.1097/SLE.0b013e31816b78f5]
- 15 **Eisen GM**, Baron TH, Dominitz JA, Faigel DO, Goldstein JL, Johanson JF, Mallory JS, Raddawi HM, Vargo JJ 2nd, Waring JP, Fanelli RD, Wheeler-Harborough J; American Society for Gastrointestinal Endoscopy. Guideline for the management of ingested foreign bodies. *Gastrointest Endosc* 2002; **55**: 802-806 [PMID: 12024131 DOI: 10.1016/S0016-5107(02)70407-0]
- 16 **Abraham B**, Alao AO. An unusual foreign body ingestion in a schizophrenic patient: case report. *Int J Psychiatry Med* 2005; **35**: 313-318 [PMID: 16480246 DOI: 10.2190/7AE8-3AV0-W3UA-TKV4]
- 17 **Murshid KR**, Khairy GE. Laparoscopic removal of a foreign body from the intestine. *J R Coll Surg Edinb* 1998; **43**: 109-111 [PMID: 9621536]
- 18 **Puia IC**, Puia VR, Andreescu A, Cristea PG. [Ascending colon perforation by ingested fruit stones]. *Chirurgia (Bucur)* 2011; **106**: 825-827 [PMID: 22308923]
- 19 **Blaho KE**, Merigian KS, Winbery SL, Park LJ, Cockrell M. Foreign body ingestions in the Emergency Department: case reports and review of treatment. *J Emerg Med* 1998; **16**: 21-26 [PMID: 9472755 DOI: 10.1016/S0736-4679(97)00229-1]
- 20 **Goh BK**, Jeyaraj PR, Chan HS, Ong HS, Agasthian T, Chang KT, Wong WK. A case of fish bone perforation of the stomach mimicking a locally advanced pancreatic carcinoma. *Dig Dis Sci* 2004; **49**: 1935-1937 [PMID: 15628728 DOI: 10.1007/s10620-004-9595-y]
- 21 **Goh BK**, Yong WS, Yeo AW. Pancreatic and hepatic abscess secondary to fish bone perforation of the duodenum. *Dig Dis Sci* 2005; **50**: 1103-1106 [PMID: 15986862 DOI: 10.1007/s10620-005-2712-8]
- 22 **Kim KH**, Woo EY, Rosato EF, Kochman ML. Pancreatic foreign body: Ingested toothpick as a cause of pancreatitis and hemorrhage. *Gastrointest Endosc* 2004; **59**: 147-150 [PMID: 14722574 DOI: 10.1016/S0016-5107(03)02364-2]
- 23 **Rahalkar MD**, Pai B, Kukade G, Al Busaidi SS. Sewing needles as foreign bodies in the liver and pancreas. *Clin Radiol* 2003; **58**: 84-86 [PMID: 12565211 DOI: 10.1053/crad.2002.1118]
- 24 **Pinero Madrona A**, Fernández Hernández JA, Carrasco Prats M, Riquelme Riquelme J, Parrila Paricio P. Intestinal perforation by foreign bodies. *Eur J Surg* 2000; **166**: 307-309 [PMID: 10817327 DOI: 10.1080/110241500750009140]
- 25 **Mcpherson RC**, Karlan M, Williams RD. Foreign body perforation of the intestinal tract. *Am J Surg* 1957; **94**: 564-566 [PMID: 13458636 DOI: 10.1016/0002-9610(57)90580-9]
- 26 **Gómez N**, Roldós F, Andrade R. [Intestinal perforation caused by chicken bone mimicking perforated colonic diverticulitis]. *Acta Gastroenterol Latinoam* 1997; **27**: 329-330 [PMID: 9460513]
- 27 **Coulier B**, Tancredi MH, Ramboux A. Spiral CT and multidetector-row CT diagnosis of perforation of the small intestine caused by ingested foreign bodies. *Eur Radiol* 2004; **14**: 1918-1925 [PMID: 15378256 DOI: 10.1007/s00330-004-2430-1]
- 28 **Tai AW**, Sodickson A. Foreign body ingestion of blister pill pack causing small bowel obstruction. *Emerg Radiol* 2007; **14**: 105-108 [PMID: 17342467 DOI: 10.1007/s10140-007-0582-4]
- 29 **Orry X**, Balaj C, Lecocq S, Blum A, Delvaux M, Régent D, Claudon M, Laurent V. CT diagnosis of small bowel perforation by ingestion of a blister pack: two case reports. *Diagn Interv Imaging* 2014; **95**: 101-103 [PMID: 23726172 DOI: 10.1016/j.diii.2013.04.001]
- 30 **Athanassiadi K**, Gerazounis M, Metaxas E, Kalantzi N. Management of esophageal foreign bodies: a retrospective review of 400 cases. *Eur J Cardiothorac Surg* 2002; **21**: 653-656 [PMID: 11932163 DOI: 10.1016/S1010-7940(02)00032-5]
- 31 **Wu C**, Hungness ES. Laparoscopic removal of a pancreatic foreign body. *JSLs* 2006; **10**: 541-543 [PMID: 17575779]
- 32 **Angus DC**, van der Poll T. Severe sepsis and septic shock. *N Engl J Med* 2013; **369**: 840-851 [PMID: 23984731 DOI: 10.1056/NEJMr1208623]
- 33 **Crowley LV**, Bretzke ML. Bowel perforation from ingested unit dose blister-pak. *Am J Gastroenterol* 1988; **83**: 1011-1012 [PMID: 3414640]
- 34 **Fernando GC**. Colonic perforation following ingestion of plastic sheeting. *Med Sci Law* 1989; **29**: 263-264 [PMID: 2770479 DOI: 10.1177/002580248902900313]
- 35 **Sato H**, Endo T, Tajima K, Sanada Y. A rare case of perineal pain: intestinal perforation caused by a press-through package. *Anesth Analg* 1992; **75**: 456-457 [PMID: 1510270 DOI: 10.1213/00000539-199209000-00025]
- 36 **Norstein J**, Krajci P, Bergan A, Geiran O. Intestinal perforation after ingestion of a blister-wrapped tablet. *Lancet* 1995; **346**: 1308

- [PMID: 7475762 DOI: 10.1016/S0140-6736(95)91918-X]
- 37 **Lurton A**, Ntiruhungwa J, Saillant H, Surugue J. Stomach perforation by a blister-wrapped capsule. *N Engl J Med* 1996; **335**: 754 [PMID: 8786776 DOI: 10.1056/NEJM199609053351020]
 - 38 **Fulford S**, Tooley AH. Intestinal perforation after ingestion of a blister-wrapped tablet. *Lancet* 1996; **347**: 128-129 [PMID: 8538332 DOI: 10.1016/S0140-6736(96)90259-7]
 - 39 **Kansal G**, Agrawal V. Intestinal perforation--a unique cause. *J Indian Med Assoc* 2000; **98**: 184, 186 [PMID: 11016184]
 - 40 **Dutta U**, Gupta NM, Nagi B, Singh K. Blister pack ingestion resulting in esophago-pleural fistula. *Indian J Gastroenterol* 2001; **20**: 79-80 [PMID: 11305505]
 - 41 **Gupta NM**, Gupta V, Gupta R, Sudhakar V. Esophageal perforation caused by a blister-wrapped tablet. *Asian Cardiovasc Thorac Ann* 2002; **10**: 87-88 [PMID: 12079986 DOI: 10.1177/021849230201000127]
 - 42 **Gupta V**, Manikyam SR, Gupta R, Gupta NM. Pelvic abscess after ingestion of blister-wrapped tablet. *Am J Gastroenterol* 2002; **97**: 2142-2143 [PMID: 12190196 DOI: 10.1111/j.1572-0241.2002.05940.x]
 - 43 **Ishikura H**, Sakata A, Sakaki Y, Kimura S, Sumi T, Ichimori T, Uyama K. Intestinal perforation due to ingestion of blister-wrapped tablet in a press-through package. *Am J Gastroenterol* 2003; **98**: 1665-1666 [PMID: 12873609 DOI: 10.1111/j.1572-0241.2003.07560.x]
 - 44 **Fierens K**, Van Outryve L, Kint M. Bowel perforation from ingested blister-pack. *Acta Chir Belg* 2007; **107**: 564-565 [PMID: 18074922 DOI: 10.1080/00015458.2007.11680125]
 - 45 **Domen H**, Ohara M, Noguchi M, Nakanishi Y, Komuro K, Iwashiro N, Ishizaka M. Inadvertent Ingestion of a Press-Through Package Causing Perforation of the Small Intestine within an Incisional Hernia and Panperitonitis. *Case Rep Gastroenterol* 2011; **5**: 391-395 [PMID: 21792348 DOI: 10.1159/000330290]
 - 46 **Sasko B**, Butz T, Winnekendonk G, Plehn G, Prull M, Liermann D, Trappe HJ. [Bowel perforation because of ingestion of a blister-wrapped tablet after post-interventional coronary perforation]. *Dtsch Med Wochenschr* 2012; **137**: 2637-2640 [PMID: 23225187 DOI: 10.1055/s-0032-1327336]
 - 47 **Yao SY**, Matsui Y, Shiotsu S. An unusual case of duodenal perforation caused by a blister pack: A case report and literature review. *Int J Surg Case Rep* 2015; **14**: 129-132 [PMID: 26263453 DOI: 10.1016/j.ijscr.2015.07.013]
 - 48 **Prokop A**, Stepper H, Koll S, Chmielnicki M. [No Blister-Wrapped Pills: Perforation after Ingestion a Blister-Pack]. *Z Orthop Unfall* 2016; **154**: 299-302 [PMID: 27351163 DOI: 10.1055/s-0042-101962]

P- Reviewer: Desai DJ, Kai K, Sergi CM, Vento S, Virk JS

S- Editor: Ma YJ **L- Editor:** A **E- Editor:** Wu YXJ



Unusual complication in patient with Gardner's syndrome: Coexistence of triple gastrointestinal perforation and lower gastrointestinal bleeding: A case report and review of literature

Sami Akbulut, Cemalettin Koc, Abuzer Dirican

Sami Akbulut, Cemalettin Koc, Abuzer Dirican, Department of Surgery and Liver Transplant Institute, Inonu University Faculty of Medicine, Malatya 44280, Turkey

ORCID number: Sami Akbulut (0000-0002-6864-7711); Cemalettin Koc (0000-0002-5676-6772); Abuzer Dirican (0000-0002-8647-3268).

Author contributions: Akbulut S and Dirican A performed surgical procedure; Akbulut S and Koc C collected the patient's clinical data; Akbulut S and Koc C analyzed the data and wrote the paper.

Informed consent statement: Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Conflict-of-interest statement: The author declares no potential conflict of interest

CARE Checklist (2013) statement: The authors have read the guidelines of the CARE Checklist (2013) and the manuscript adheres to CARE Checklist guidelines.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Invited manuscript

Correspondence to: Sami Akbulut, MD, Associate Professor, Department of Surgery and Liver Transplant Institute, Inonu University Faculty of Medicine, Elazig Yolu 10. Km, Malatya 44280, Turkey. akbulutsami@gmail.com
Telephone: +90-422-3410660

Fax: +90-422-3410036

Received: May 18, 2018

Peer-review started: May 18, 2018

First decision: June 14, 2018

Revised: June 19, 2018

Accepted: June 28, 2018

Article in press: June 28, 2018

Published online: September 26, 2018

Abstract

Gardner's syndrome (GS) is a rare syndrome with autosomal dominant inheritance, which is characterized by multiple intestinal polyps, dental anomalies, desmoid tumors, and soft tissue tumors. All gastrointestinal symptoms seen in GS are associated with the underlying familial adenomatous polyposis and abdominal desmoid tumors, with the most common symptoms being anemia, lower gastrointestinal bleeding, abdominal pain, diarrhea, obstruction, and mucous defecation. To our best knowledge, no case of GS that has presented with gastrointestinal perforation and bleeding has ever been reported in the English language medical literature. A 37-year-old male who had been diagnosed with GS five years earlier was referred to our clinic for lower gastrointestinal bleeding. Despite the absence of a bleeding focus on conventional angiography, the patient was operated on with laparotomy, due to the persistence of both signs and symptoms of mild peritonitis. On the laparotomy, the patient was noted to have areas of perforation in the duodenum, splenic flexura, and mid-rectum. The third and fourth part of the duodenum, the proximal 15 cm segment of the jejunum, a 10 cm segment of the terminal ileum, the whole colon, and the upper and middle rectum were resected, and duodeno-jejunal side-to-side anastomosis and terminal ileostomy

were performed. The histopathological analysis of the large mass measuring 30 cm × 20 cm was reported as a desmoid tumor. The pathological examination of the tumor foci detected in the colonic specimen revealed poorly differentiated adenosquamous carcinoma.

Key words: Gastrointestinal perforation; Gastrointestinal bleeding; Adenosquamous carcinoma; Complications; Gardner's syndrome

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: Gardner's syndrome (GS) is a rare syndrome with autosomal dominant inheritance, which is characterized by multiple intestinal polyps, dental anomalies, desmoid tumors, and soft tissue tumors. All gastrointestinal symptoms that are seen in GS are associated with the underlying familial adenomatous polyposis and abdominal desmoid tumors, with the most common symptoms being anemia, lower gastrointestinal bleeding, abdominal pain, diarrhea, obstruction, and mucous defecation. To our best knowledge, no case of GS that has presented with gastrointestinal perforation and bleeding has ever been reported in the English language medical literature. Herein, we report a complicated GS case that we managed for multiple intestinal perforation and massive gastrointestinal bleeding.

Akbulut S, Koc C, Dirican A. Unusual complication in patient with Gardner's syndrome: Coexistence of triple gastrointestinal perforation and lower gastrointestinal bleeding: A case report and review of literature. *World J Clin Cases* 2018; 6(10): 393-397 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/393.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.393>

INTRODUCTION

Gardner's syndrome (GS) is a rare, autosomal dominant disorder characterized by gastrointestinal (colonic polyps, gastric polyps, duodenal polyps, and desmoid tumors) and extragastrointestinal (osteomas, dental abnormalities, epidermal cysts, fibromas, lipomas, pilomatricomas, congenital hypertrophies of retinal pigment epithelium, adrenal adenomas, and nasal angiofibromas) manifestations^[1,2]. GS was first considered to be a variant of familial adenomatous polyposis (FAP). However, the detection of adenomatous polyposis coli (APC) gene mutations in families with GS, as well as the extracolonic manifestations of GS in patients with FAP, have shown that GS and FAP are relatively similar to one another. The gastrointestinal symptoms seen in GS are usually closely related to the underlying FAP and abdominal desmoid tumors. The most common gastrointestinal symptoms are nonspecific abdominal pain, weight loss, palpable abdominal masses, diarrhea, obstruction, mucous defecation, and rare lower gastrointestinal bleeding^[1-3]. To our knowledge, no GS case

presenting with gastrointestinal perforation and massive lower gastrointestinal bleeding has ever been reported in the English language medical literature. In this study, we report a complicated GS case that we managed for multiple intestinal perforation and massive gastrointestinal bleeding.

CASE REPORT

A 37-year-old man (BMI = 19 kg/m²) who had been diagnosed with GS five years earlier was referred to our center with lower gastrointestinal bleeding. The patient's male sibling had died of colonic cancer, but we had no information as to whether he also had GS. This patient had been transfused with 12 units of whole blood suspension before being referred to our center, and had a final hemoglobin level of 8 g/dL, a creatinine value of 2.9 mg/dL, an albumin level of 2.1 g/dL and a total bilirubin level of 6.3 mg/dL. An endoscopic examination failed to detect any abnormalities, with the exception of duodenal polyposis. Colonoscopy was attempted, but no higher than the rectosigmoid region was reached due to bleeding. Upon physical examination, the patient was noted to have a mass with a size of 30 cm × 20 cm, which originated from the right flank region and was partially fixed to the abdominal wall. There were also multiple lesions (likely lipomas) with a size range between 3 cm - 7 cm on the abdomen, thoracic wall, and axilla (Figures 1-4). There was a mass lesion compatible with an osteoma in the left mandible (Figure 5). Conventional celiac and mesenteric angiographies that were performed to detect the hemorrhagic focus showed no extravasation that was indicative of bleeding. The patient's hemodynamic and biochemical parameters improved, but since he had persistent signs of mild peritonitis, a decision was made to proceed with surgery. Upon the request of the patient, the mass lesions on the abdominal and thoracic walls were the first to be excised. A midline incision was then performed, and approximately 500 cc of seropurulent fluid was drained. Upon exploration, there were perforated areas in the fourth part of the duodenum, the splenic flexura of the colon, and the middle part of the rectum. The perforations in the splenic flexura and middle part of the rectum were consistent with tumor perforation. Firstly, the resection included the proximal 15 cm section of the jejunum, as well as the third and fourth parts of the duodenum. Then, a side-to-side anastomosis was performed between the proximal jejunum and the second part of the duodenum. There were polypoid lesions detected in the mucosa from both of the anastomosed small intestinal segments. A tube was inserted from the distal jejunum to the proximal part of the anastomosis in order to protect the jejuno-duodenal anastomosis. After carrying out a total abdominal colectomy, which included 10 cm of distal ileum, the whole colon and the upper/middle part of the rectum, the distal rectal stump was closed and an end ileostomy was created. Additionally, multiple masses consistent



Figure 1 Desmoid tumor. Posterior view of a giant desmoid tumor located in the right flank area.



Figure 2 Epidermoid cysts. Anterior view of several epidermoid cysts together with a giant desmoid tumor localized in the right flank area.



Figure 3 Epidermoid cysts. Top view of several epidermoid cysts together with a giant desmoid tumor localized in the right flank area.

with metastases were detected in both lobes of the liver. The patient developed a postoperative pneumothorax and a chest tube was inserted. The patient then developed a severe lung infection during the intensive care unit follow-up, and attempts to extubate him failed. A tracheostomy was therefore opened. Despite having no gastrointestinal problems, the patient died due to pulmonary complications. Histopathological examination revealed the presence of three foci of poorly-differentiated adenosquamous carcinoma in

the duodenal serosa, the largest of which was 10 mm in diameter. These cancer foci were thought to have developed following diffuse lymphovascular invasion of the tumor into the splenic flexura. There were a total of 260 polypoid lesions in the duodenal mucosa, the largest of which measured 10 mm in diameter. Two foci of poorly-differentiated adenosquamous carcinoma (Pan-Cytokeratin: strongly positive, Cytokeratin 5/6: 40% positive, Ki67: 80% positive, Cytokeratin 20: negative, p53: negative, Synaptophysin: negative, CgA: negative) were present in the colon, the diameters of which were 80 mm and 30 mm. A total of 172 polypoid lesions were detected in the colonic and rectal mucosa, the largest of which had a diameter of 10 mm. Tumors were detected in both areas of colonic perforation. In the colon specimen, a tumor was detected in 5 of the 40 lymph nodes. The small lesions excised from the abdominal wall were reported to be epidermoid cysts. Pathological examination of the large mass that was excised from the right flank region was revealed to be a desmoid tumor (S100: negative, Desmin: focal positive, α -smooth muscle actin: positive, CD117: negative, DOG-1: negative, Ki67: 4%-5% positive).

DISCUSSION

In 1951, Gardner reported a significant association between colonic polyps with high malignant potential and some bone and soft tissue tumors^[1]. In 1952, Gardner and his colleagues reported that the co-occurrence of multiple bone tumors and colonic polyps is an autosomal dominant condition^[1]. In 1953, Gardner and his colleagues defined this syndrome, characterized by hereditary colonic polyposis that was associated with bone and soft tissue tumors, as GS^[1]. The gastrointestinal components of GS are colorectal polyps, gastric polyps, small bowel polyps, and mesenteric desmoid tumors. The extragastrointestinal components of GS include osteomas, dental anomalies (unerupted teeth, supernumerary teeth, dentigerous cysts, and odontomas), skin and subcutaneous lesions (epidermal cysts, fibromas, lipomas, pilomatricomas), desmoid tumors of the abdominal wall, congenital hypertrophy of the retinal pigment epithelium, adrenal adenomas, and nasal angiofibromas. The incidence of cancers affecting extracolonic sites, such as the duodenum, periampullary region, thyroid, pancreas, stomach, central nervous system, liver, small bowel distal to the duodenum, and adrenal gland, is one or several folds higher than that of the general population.

In 80% of patients with GS, a hereditary mutation is found in the APC gene (5q21-5q22), a tumor suppressor gene located in the 21st and 22nd loci of the long arm of the fifth chromosome^[1,2]. Twenty percent of GS cases occur as a result of sporadic, uninherited mutations in the APC gene. To date, around 1400 mutations have been detected in the APC gene^[1]. Hence, the diversity of both gastrointestinal and extragastrointestinal manifestations of GS results from the variability of APC mutation penetration. The number of colonic polyps depends on



Figure 4 Epidermoid cysts. Lateral view of several epidermoid cysts together with a giant desmoid tumor localized in the right flank area.



Figure 5 Osteoma-like lesion. View of an osteoma-like lesion originating from the left mandible.

the location of the *APC* mutation. Mutations occurring in the center of the *APC* gene result in about 5000 colonic polyps. When mutations affect the proximal or distal parts of the gene, however, approximately 1000 polyps develop in the colon. When mutations are found in the extreme ends of the *APC* gene, on the other hand, about 100 polyps develop, which is defined as attenuated FAP.

While GS and FAP incidences range between 0.62 and 2.38 per million people, their prevalence ranges between 0.88 and 46.5 per million people^[4-6]. GS occurs equally in both sexes. The symptoms mostly appear in the second decade of life^[1]. Bone and soft tissue tumors usually appear 10 years earlier than colonic polyps^[1]. While polyps begin to appear at puberty, GS is not diagnosed until the third decade of life in the majority of affected people^[1]. All untreated people with malignant GS transformations develop tumors in the fourth decade of life^[1].

The gold standard for the diagnosis of GS and FAP is identifying the *APC* gene mutation^[1]. This test is also very effective in revealing gene mutations in the relatives of GS patients^[1]. However, the detection of either multiple polyps in a colonoscopy or bone and soft tissue tumors during a physical examination are sufficient for diagnosis without a genetic diagnostic test^[1]. The different diagnoses of GS include Peutz–

Jeghers syndrome, Juvenile polyposis, multiple hamartoma syndrome, Basal-cell nevus syndrome and Cronkite-Canada syndrome^[2]. All of these syndromes involve gastrointestinal polypoid lesions that develop at varying rates. Importantly, however, these syndromes lack extraintestinal signs, such as osteomas, epidermal inclusion cysts, and multiple impacted permanent teeth^[2].

We believe that the case presented here will contribute several important aspects to the literature. Firstly, colorectal cancer shows an aggressive behavior. Despite being just under 40 years old, the patient suffered from an aggressive cancer that caused multiple perforations, duodenal invasion, and liver metastases. In our opinion, the role of squamous differentiation is an important contributor to this aggressive behavior. Adenosquamous cancers constitute 0.025% to 0.2% of all colorectal cancers, although the corresponding rate of FAP is quite low^[7,8]. The rates of both local and distant metastasis are moderately higher for colorectal adenosquamous cancers vs. adenocarcinomas. As extrapolated from the case presented here, colorectal adenosquamous cancers are highly aggressive cancers^[7,8]. To the best of our knowledge, no study in the literature has ever reported the development of colorectal adenosquamous cancer in patients with GS. This is because all of the polyps that develop in FAP and GS are adenomatous polyps, whereas all of the tumors that develop from these are adenocarcinomas. The second important contribution of this case is the mode of GS presentation. The gastrointestinal signs and symptoms of GS are almost always related to the underlying FAP and desmoid tumors. Hence, the most common symptoms are anemia, abdominal pain, intestinal obstruction, and hemorrhage. To the best of our knowledge, there has been no case of GS reported that has presented with massive lower gastrointestinal bleeding and multiple intestinal perforations. In the present case, we believe that the bleeding developed secondary to perforations. The detection of abundant blood in the perirectal pouch where the perforation opens supports this claim.

In conclusion, although GS is a rare condition, it should be taken seriously, as it may cause colorectal cancers until the age of 40. Therefore, close follow-up of GS patients and prophylactic surgery during early stages of GS are the most appropriate approaches. Patients who are diagnosed with upper gastrointestinal polyps should be closely examined by endoscopy. The case presented here clearly demonstrates that serious life-threatening complications like tumor perforation and massive bleeding may develop in neglected GS cases.

ARTICLE HIGHLIGHTS

Case characteristics

A 37-year old cachectic male patient was referred to our center with lower gastrointestinal bleeding.

Clinical diagnosis

Gardner's syndrome (GS), lower gastrointestinal bleeding due to gastro-

intestinal polyposis.

Differential diagnosis

Bleeding due to colorectal cancer, bleeding due to colonic polyposis.

Laboratory diagnosis

Hemoglobin: 8 g/dL, creatinine: 2.9 mg/dL, albumin: 2.1 g/dL, total bilirubin: 6.3 mg/dL.

Imaging diagnosis

Conventional celiac and mesenteric angiographies that were performed to detect the hemorrhagic focus showed no extravasation that was indicative of bleeding.

Pathological diagnosis

Poorly-differentiated adenosquamous carcinoma of the colon, colorectal polyposis, duodenal polyposis, desmoid tumor.

Treatment

Resection of the distal duodenum and proximal jejunum, latero-lateral duodenojejunal anastomosis, total abdominal colectomy with end ileostomy, distal rectal stump closure, resection of the desmoid tumor-like lesions.

Related reports

To the best of our knowledge, no case of GS has ever been reported in the English language medical literature that presents with gastrointestinal perforation and bleeding.

Experiences and lessons

Although GS is a rare condition, it should be taken seriously since it may cause colorectal cancers until the age of 40. Therefore, close follow-up of GS patients and prophylactic surgery during early stages of GS are the most appropriate

approaches. Patients who are diagnosed with upper gastrointestinal polyps should be closely examined by endoscopy. The case presented here clearly demonstrates that serious life-threatening complications like tumor perforation and massive bleeding may develop in neglected GS cases.

REFERENCES

- 1 **Fotiadis C**, Tsekouras DK, Antonakis P, Sfiniadakis J, Genetzakis M, Zografos GC. Gardner's syndrome: a case report and review of the literature. *World J Gastroenterol* 2005; **11**: 5408-5411 [PMID: 16149159 DOI: 10.3748/wjg.v11.i34.5408]
- 2 **Bhargava P**, Guttal K, Khan S, Shekhawat C. Polyps of malignancy: Gardner's syndrome. *J Indian Acad Oral Med Radiol* 2016; **28**: 470-473 [DOI: 10.4103/jiaomr.JIAOMR_19_16]
- 3 **Agrawal D**, Newaskar V, Shrivastava S, Nayak PA. External manifestations of Gardner's syndrome as the presenting clinical entity. *BMJ Case Rep* 2014; **2014**: pii: bcr2013200293 [PMID: 25139912 DOI: 10.1136/bcr-2013-200293]
- 4 **Varesco L**. Familial adenomatous polyposis: genetics and epidemiology. *Tech Coloproctol* 2004; **8** Suppl 2: s305-s308 [PMID: 15666112 DOI: 10.1007/s10151-004-0182-1]
- 5 **Järvinen HJ**. Epidemiology of familial adenomatous polyposis in Finland: impact of family screening on the colorectal cancer rate and survival. *Gut* 1992; **33**: 357-360 [PMID: 1314763 DOI: 10.1136/gut.33.3.357]
- 6 **Bülow S**. Results of national registration of familial adenomatous polyposis. *Gut* 2003; **52**: 742-746 [PMID: 12692062 DOI: 10.1136/gut.52.5.742]
- 7 **Kang DB**, Oh JT, Jo HJ, Park WC. Primary adenosquamous carcinoma of the colon. *J Korean Surg Soc* 2011; **80** Suppl 1: S31-S35 [PMID: 22066079 DOI: 10.4174/jkss.2011.80.Suppl1.S31]
- 8 **Sunkara T**, Caughey ME, Makkar P, John F, Gaduputi V. Adenosquamous Carcinoma of the Colon. *Case Rep Gastroenterol* 2018; **11**: 791-796 [PMID: 29606937 DOI: 10.1159/000485558]

P- Reviewer: Gurkan A, Zerem E **S- Editor:** Ji FF
L- Editor: Filipodia **E- Editor:** Song H



Laparoscopic repair *via* the transabdominal preperitoneal procedure for bilateral lumbar hernia: Three cases report and review of literature

Di-Yu Huang, Long Pan, Ming-Yu Chen, Jing Fang

Di-Yu Huang, Long Pan, Ming-Yu Chen, Jing Fang, Department of General Surgery, Sir Run-Run Shaw Hospital, Zhejiang University, Hangzhou 310016, Zhejiang Province, China

Long Pan, Jing Fang, Key Laboratory of Laparoscopic Technique Research of Zhejiang Province, Sir Run-Run Shaw Hospital, Zhejiang University, Hangzhou 310016, Zhejiang Province, China

ORCID number: Di-Yu Huang (0000-0002-6179-9763); Long Pan (0000-0003-1550-8280); Ming-Yu Chen (0000-0001-5113-754X); Jing Fang (0000-0001-5914-8163).

Author contributions: Huang DY and Pan L contributed equally to this work; Huang DY and Pan L designed the report; Chen MY and Fang J collected the clinical data of the patients; Huang DY and Pan L analyzed the data and wrote the paper.

Informed consent statement: The patients were not required to provide informed consent for this study because the study is retrospective and anonymous clinical data were collected after the patients had agreed to treatment *via* the laparoscopic technique and signed written surgical informed consent. The surgical informed consent has been uploaded with the manuscript.

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

CASE Checklist (2013): The authors have confirmed that the paper conforms to the guidelines of the CASE Checklist (2013).

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Manuscript source: Unsolicited manuscript

Correspondence to: Di-Yu Huang, MD, Attending Doctor, Surgeon, Department of General Surgery, Sir Run-Run Shaw Hospital, Zhejiang University, Jianggan District, East Qingchun Road No. 3, Hangzhou 310016, Zhejiang Province, China. 3199007@zju.edu.cn
Telephone: +86-571-86006617
Fax: +86-571-86044817

Received: April 19, 2018

Peer-review started: April 19, 2018

First decision: June 4, 2018

Revised: June 22, 2018

Accepted: June 28, 2018

Article in press: June 28, 2018

Published online: September 26, 2018

Abstract

A lumbar hernia is a rare entity, and a bilateral lumbar hernia is much rarer. From May 2015 to October 2017, we treated only three patients with bilateral lumbar hernias. One patient came to the hospital presenting with right-sided abdominal pain, and the other two patients presented with bilateral lumbar masses. The previous bilateral lumbar hernia reported in the literature was repaired by open surgery. The laparoscopic approach *via* the transabdominal preperitoneal (TAPP) procedure with the self-gripping Parietex ProGrip™ mesh was performed at our center. The laparoscopic repair was conducted by a skilled hernia surgeon, and was successfully performed in the three patients. The patients resumed a semi-liquid diet and had no activity restriction after six hours following the operation. No antibiotics were used after the surgery. The operative times of the three patients were 120 min, 85 min, and 130 min. The blood loss volumes of the three patients were 20 mL, 5 mL, and 5 mL. The visual analogue scale pain scores of the three patients were 1, 2, and 2 on postoperative day

1, and were 1, 2, and 1 on postoperative day 3. No perioperative complications, such as bulge, wound infection and hematoma, occurred after the surgery. All of the patients were discharged on the third day after the operation. There was no chronic pain and no hernia recurrence during the follow-up. This study showed that the laparoscopic TAPP approach with the self-gripping mesh is safe and feasible, and can be considered an alternative method for the treatment of bilateral lumbar hernias.

Key words: Bilateral lumbar hernia; Laparoscopic repair; Transabdominal preperitoneal; Self-gripping mesh

© **The Author(s) 2018.** Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This study reports the successful implementation of a transabdominal preperitoneal procedure with self-gripping mesh for the management of a bilateral lumbar hernia. There were no adverse events, chronic pain or recurrence occurring in any of the patients. The findings confirm the safety and feasibility of the technique, and support the application of the laparoscopic approach for bilateral lumbar hernia repair.

Huang DY, Pan L, Chen MY, Fang J. Laparoscopic repair *via* the transabdominal preperitoneal procedure for bilateral lumbar hernia: Three cases report and review of literature. *World J Clin Cases* 2018; 6(10): 398-405 Available from: URL: <http://www.wjgnet.com/2307-8960/full/v6/i10/398.htm> DOI: <http://dx.doi.org/10.12998/wjcc.v6.i10.398>

INTRODUCTION

Lumbar hernias, which are rare abdominal wall hernias, are the protrusion of the extraperitoneal fat or intra-abdominal contents through a defect in the posterolateral abdominal wall. The existence of lumbar hernias was first described by Cavallaro *et al*^[1], and the first case was reported by Moreno-Egea *et al*^[2] in 1731. The proportion of lumbar hernias to all abdominal hernias is less than 1.5%, and most of them are unilateral^[3,4]. To date, approximately 300 cases of lumbar hernias have been reported in the world^[2], and cases of bilateral lumbar hernias are even rarer^[5,6]. Open hernia repair, which is used as the conventional approach, has been proven to be a safe and effective treatment option. Currently, with the development of laparoscopic technology, laparoscopic herniorrhaphy is increasingly used for the treatment of lumbar hernias^[7,8]. Compared with the conventional approach, laparoscopic lumbar hernia repair has several advantages, such as mild postoperative pain, fewer perioperative complications, a shorter hospital stay, a faster recovery to normal activity, and a similar recurrence rate^[9]. Nevertheless, there is no relevant standard in the detailed procedures of laparoscopic

lumbar hernia surgery, nor in the type and fixation of meshes, due to the limited number of laparoscopic surgeries that have been performed. All of the currently reported bilateral lumbar hernias were repaired by open surgery^[10,11]. As with inguinal hernias, the laparoscopic technique in bilateral lumbar hernia repair is expected to be superior compared with open surgery, as long as the technique is available. Recently, we completed three cases of laparoscopic bilateral lumbar hernia repair *via* the transabdominal preperitoneal (TAPP) procedure using self-gripping mesh, and demonstrated the feasibility of this technique. To the best of our knowledge, this is the first report of the laparoscopic approach for the treatment of bilateral lumbar hernias. In addition, this report is followed by a literature review of lumbar hernias with regard to their etiologies, anatomical characteristics, clinical features, diagnoses, and surgical treatments.

CASE REPORT

From May 2015 to October 2017, three patients were diagnosed with bilateral lumbar hernias and treated. The baseline characteristics of the patients are shown in Table 1. Computed tomography imaging demonstrated the existence of bilateral lumbar hernias (Figure 1A). For the operation, the patient was placed under general anesthesia in a 60° lateral position. The first 10 mm trocar was introduced by a mini-incision above the umbilicus for insufflation of carbon dioxide and for a 30° angled laparoscope. The other trocars were placed under direct vision. The second 5 mm trocar was introduced at the midpoint of the xiphoid and umbilicus, and the third 5 mm trocar was introduced at the midpoint of the umbilicus and pubic symphysis (Figure 2A). The ascending colon or descending colon was clamped off with non-injury clamps to maintain tension. The peritoneum was cut above the paracolic sulci, and the extraperitoneal tissue was separated to fully expose the defect and hernia contents (Figure 2B). The hernia contents, such as the colon and mesentery from the defect, were carefully divided and then returned completely (Figure 2C). Subsequently, the preperitoneal space along the midline was freed to expose the psoas major. The head and tail sides of the psoas major were freed to approximately 6 cm in length (Figure 2D). A 15 cm × 9 cm self-gripping Parietex ProGrip™ mesh (Sofradim Production, Trévoux, France) was fully unfolded and placed onto the psoas major (Figure 2E). The long axis of the patch was consistent with the direction of the psoas major, and the center of the patch was consistent with the defect. The margin of the patch overlapped with the defect for at least 3 cm in every direction. Next, 2-0 Prolene was used to suture and reinforce the four corners of the patch when the defect was relatively large, and a 3-0 absorbable suture-line was used to close the peritoneum with a continuous suture (Figure 2F). Carbon dioxide was released, each trocar was pulled out, and the patient's position

Table 1 Baseline characteristics of the patients

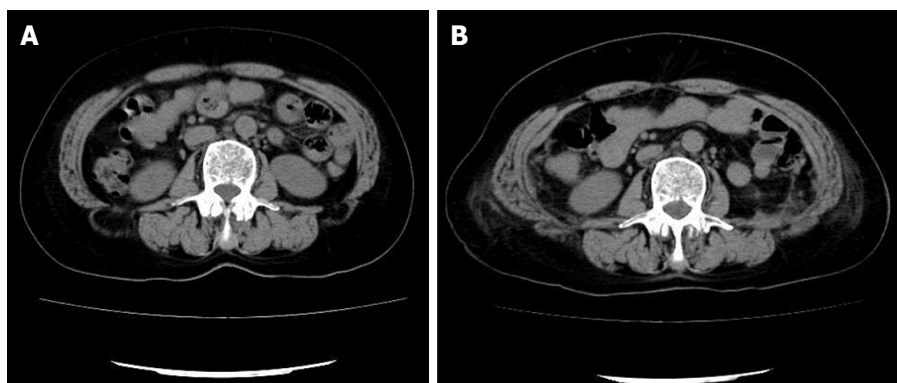
Case No.	Age (yr)	Sex	BMI (kg/m ²)	Chief complaint	Physical examination	Past history
1	70	F	23.3	Finding bilateral lumbar masses for approximately 1 mo	Bilateral lumbar masses (left: 5 cm × 4 cm; right: 4 cm × 4 cm), reducible	No surgery and/or trauma of the abdomen and waist
2	68	F	31.1	Right-sided abdominal pain for 1 mo	No abnormal signs	
3	69	F	24.0	Finding bilateral lumbar masses for approximately 8 mo	Bilateral lumbar masses (left and right: 3 cm × 2 cm), irreducible	

BMI: Body mass index.

Table 2 The perioperative and follow-up patient data

Case No.	ASA	Hernia content	Operative time (min)	Blood loss (mL)	VAS/POD1	VAS/POD3	Complications	Hospital stay (d)	Chronic pain	Recurrence
1	II	Colon, mesentery	120	20	1	1	No	3	No	No
2	II	Colon, mesentery	85	5	2	2	No	3	No	No
3	II	Mesentery	130	5	2	1	No	3	No	No

ASA: American Society of Anesthesiologists; VAS: Visual analogue scale; POD: Post-operative day.

**Figure 1** Preoperative vs postoperative imaging. Comparison of the preoperative (A) and postoperative (B) images in a patient with a bilateral lumbar hernia.

was adjusted to the contralateral position. Then, the contralateral lumbar hernia was repaired in the same manner. Finally, no intraperitoneal or retroperitoneal drain was placed. All of the operations were successful, and none of the patients required a conversion to laparotomy. The patients resumed a semi-liquid diet and had unlimited activity at six hours following the operation. No antibiotics were used either before or after the surgery. The relative perioperative data are shown in Table 2. All of the patients were discharged on the third day after the operation. There was no chronic pain and no hernia recurrence during the six month follow-up.

DISCUSSION

Here, we reported the first case of performing the laparoscopic approach for the treatment of bilateral spontaneous lumbar hernias. Considering the rarity of spontaneous lumbar hernias, we included a literature review of lumbar hernias to better understand the disease. We have conducted a literature search on

PubMed and found 14 papers about laparoscopic repair of spontaneous lumbar hernias, which are presented in Table 3.

According to the underlying cause, lumbar hernias can be divided into primary lumbar hernias and secondary lumbar hernias. Primary lumbar hernias mostly occur in infancy and are often associated with an abnormal development of bones and abdominal muscles^[24]. The majority of secondary lumbar hernias occur in middle-aged and elderly people, and the causes of secondary lumbar hernias include trauma, infection, surgery, etc. Surgery is the main cause of secondary lumbar hernias, especially surgeries using a back approach, such as nephrectomy, latissimus dorsi muscle transplantation and lumbar incision^[25]. If some important anatomical structures of the back are not protected well, or anatomical structures of man-made destruction are not closed during the operation, postoperative lumbar hernias form relatively easily^[19]. In addition, long-term cough, constipation, and other factors that cause increased intra-abdominal pressure are high-risk factors for secondary lumbar hernias^[26-28].

Table 3 Literature review: laparoscopic repair for spontaneous lumbar hernia

Ref.	Size	Technique	Mesh	Fixation
1997, Heniford <i>et al</i> ^[12]	4 × 3	TAPP	PTFE	Sutures
1997, Bickle <i>et al</i> ^[3]	3 × 3	TAPP	PPL	Tacks
2002, Postema <i>et al</i> ^[13]	-	TEP	PPL	Tacks
2003, Habib ^[14]	3 × 4	TAPP	PPL	Tacks
2004, Grauls <i>et al</i> ^[15]	3 × 5	TAPP	PPL	Tacks
2005, Ipek <i>et al</i> ^[16]	8 × 10	TAPP	PTFE	Tacks + sutures
2011, Lim <i>et al</i> ^[17]	5 × 6	TEP	PPL	Tacks + sutures
2011, Nam <i>et al</i> ^[18]	3 × 5	TAPP	PPL	Tacks
2013, Suarez <i>et al</i> ^[19]	-	TAPP	-	Tacks
2014, Wei <i>et al</i> ^[20]	3 × 3	TEP (Single incision)	PPL	Tacks
2015, Walgamage <i>et al</i> ^[4]	5 × 5	TAPP	PPL	Tacks
2016, Agresta <i>et al</i> ^[21]	-	TAPP	Composite PPL	Tacks
2017, Claus <i>et al</i> ^[22]	1.5 × 2	TAPP	PPL	Tacks
2018, Sarwal <i>et al</i> ^[23]	3 × 3	TAPP	PPL	Tacks + fibrin sealant

TAPP: Transabdominal preperitoneal; TEP: Extraperitoneal; PPL: Polypropylene; PTFE: Polytetrafluoroethylene.

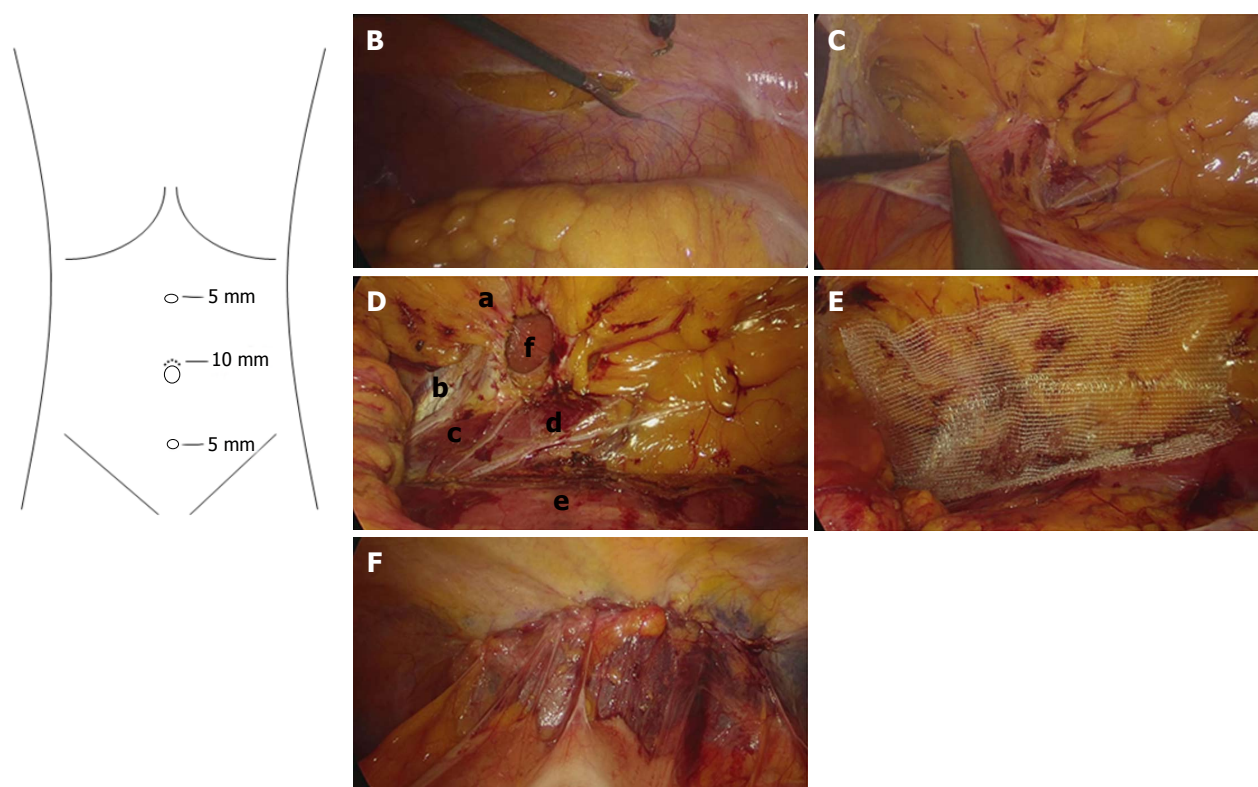


Figure 2 Surgical technique keynotes. A: The location of trocars; B: The incision into the peritoneum; C: The exposure of hernia contents; D: The structure of the defect area. a: The twelfth rib; b: Neurovascular bundles; c: The erector spinae; d: The ilioinguinal nerve; e: The psoas major; f: The defect; E: The implantation of the meshes; F: An overview upon finishing the repair.

Spontaneous lumbar hernias can be divided into the superior lumbar triangle hernias (Grynfeltt) and inferior lumbar triangle hernias (Petit), according to the location of the abdominal defect, both of which are naturally weakened areas in the dorsal abdominal wall^[1]. Grynfeltt's triangle is located below the 12th rib, and is limited laterally by the internal oblique muscle and medially by the erector spinae muscle, with the transversus abdominis aponeurosis serving as its floor^[29]. Sometimes, the serratus posterior inferior is also involved in the formation of the upper edge of

Grynfeltt's triangle. Petit's triangle is limited medially by the latissimus dorsi, laterally by the external abdominal oblique muscle, and inferiorly by the iliac crest, with the internal oblique aponeurosis serving as its floor^[29]. Due to the protective effect of internal oblique aponeurosis on Petit's triangle, the incidence of a Petit hernia is lower in comparison with a Grynfeltt hernia^[1]. Additionally, the hernia contents may be extraperitoneal adipose tissue or various intraperitoneal organs, such as the colon, small intestine, kidney, liver, stomach, *etc.*^[30,31]. During the operations, especially during fixation, more

attention should be paid to protecting nerves near the superior lumbar triangle, such as the ilioinguinal nerve, lateral femoral cutaneous nerve and genitofemoral nerve, to avoid chronic pain after surgery^[19].

The majority of patients arrive at the hospital because of a protruding bulge in the posterior abdominal wall without having any subjective symptoms. A small subgroup of patients has some atypical symptoms, such as a backache and intercostal neuralgia, due to the oppression of the inner contents of the lumbar hernia. Very few patients with lumbar hernias show specific symptoms, such as bowel obstruction, hydronephrosis and hydroureter, because the contents are relevant to the abdominal organ^[32-34]. In our study, two patients presented with a semispherical bulge in the back, while one patient presented with unexplained abdominal pain. A lumbar hernia should be distinguished from a lipoma, fibroma, hematoma, abscess and retroperitoneal tumor^[16]. The main differences are that a lumbar hernia is reducible and that it will become larger when patients hold their breath to increase intra-abdominal pressure, while the mass in patients with the other diseases mentioned above is commonly unchanged. Nowadays, with the rapid development of radiation technology, an abdominal CT scan is playing an increasing role in assessing the complexity, actual contents, site and size of the hernia to formulate a suitable operative plan, although the diagnosis of lumbar hernias is based on clinical manifestation^[2,35].

Although lumbar hernias are a rare clinical entity, the surgical procedure for lumbar hernias is constantly evolving and changing ever since lumbar hernia repair was first reported by Owen in 1888. Open surgery for a lumbar hernia involves the following stages: direct suture repair, the Dowd technique, and mesh repair. Direct suture repair: after the contents of the hernia are returned and the hernia sac is ligated, the adjacent fascia and muscle are directly overlapped with a nonabsorbable suture. Direct suture repair is suitable for lumbar hernias with a defect < 2.5 cm in diameter, and is mainly used for the repair of primary lumbar hernias in children^[36,37]. When the diameter of the defect is too large, a direct suture will create great tension, which can easily lead to the postoperative recurrence of hernias^[38].

In this method, the transversalis fascia is imbricated, and the defect is covered with a flap from the fascia and muscle^[39]. This method effectively relieves local tension and reduces the recurrence rate of large lumbar hernias. However, the disadvantages of this method are that the operation is pretty complicated and the damage to the surrounding tissue is large. Complications, such as local hematoma and ischemic necrosis of the flaps, easily occur and will affect the overall success of the operation^[38].

The creation of an artificial patch has changed the surgical method of traditional hernia repair. In 1963, Hafner *et al.*^[40] first used Marlex mesh to repair Petit's lumbar hernia with an uneventful postoperative course.

Mesh repair reduces the tension during the repair of large hernias and avoids flap transplantation, which is quite traumatic and complicated, thus improving the success rate of herniorrhaphy and decreasing the postoperative recurrence rate. Nevertheless, mesh repair also has its disadvantages. Synthetic materials do not have the ability to resist infection. When the strangulated hernia combines with intestinal necrosis, artificial patches easily cause infection. Once the infection occurs, the patches must be removed.

Upon reviewing the English literature, tension-free open herniorrhaphy with a synthetic mesh is currently the most widely-accepted surgery for lumbar hernia repair, and the optimal location for mesh placement is the preperitoneal space^[38]. As with most abdominal wall hernias, the same principles apply to a lumbar hernia. The mesh should overlap the defect by 3-5 cm and should be fixed with nonabsorbable sutures. Although laparoscopic techniques are increasingly being used to repair hernias, open surgery still has advantages. For example, when the defect is very large, open surgery can use the stitches to directly close the abdominal wall defect for reinforcement. In addition, two meshes can be respectively placed on the peritoneum and muscle. The mesh can be fixed onto the iliac crest to decrease recurrence.

With the development of the laparoscopic technique, surgeons began to use a minimally-invasive surgery approach to repair lumbar hernias. Since Burick *et al.*^[7] reported the first case of laparoscopic lumbar hernia repair in 1996, relevant reports are increasing in number^[41]. The patient is commonly placed in a lateral position with 45° elevation of the same side as the hernia to allow for the abdominal viscera to fall due to gravity to increase the exposure. As with open surgery, the placement of the patch through the laparoscopic approach is also in the preperitoneal space. Operating under direct vision can create a larger dissection area compared with open surgery, allowing the patch to be placed more accurately and flatter, and reducing the risk for injury to blood vessels and nerves. Laparoscopic lumbar hernia repair can be performed in a variety of ways. Currently, the TAPP and totally extraperitoneal techniques are the major laparoscopic procedures for lumbar hernias^[17,22]. Surgeons can directly observe the abdominal viscera *via* the TAPP approach, thus reducing the possibility of damaging these viscera. Meanwhile, TAPP provides a large operative space for ease of operation, can deal with relatively large defects, and is appropriate for the treatment of bilateral hernias. However, this procedure requires cutting and suturing the peritoneum. Totally extraperitoneal: under some conditions, for example, when the defect is small, totally extraperitoneal seems to be more convenient than TAPP because the incision and suturing of the peritoneum are not required, and because there is less interference with the abdominal cavity^[42]. However, when the defect is large, it is difficult to place and fix the patch due to insufficient working space, and if only three ports

are placed along the middle line, it is not suitable for bilateral lumbar hernias because of significant surgical injury. Sun *et al.*^[43] have performed the transabdominal partial extraperitoneal technique using an expanded polytetrafluoroethylene bilaminar mesh in laparoscopic lumbar hernia repair, which seems to be an alternative choice for the treatment of large lumbar hernias. Regardless of the type of surgical procedure, direct suturing of the defect *via* the laparoscopic approach is not recommended. The selection and fixation of meshes are currently inconclusive. In the case of ensuring peritoneal integrity, polypropylene meshes are used by most surgeons. When the peritoneum cannot be completely closed and the patch is exposed to the abdominal cavity, a mesh that can prevent intestinal adhesion, such as a composite mesh with expanded polytetrafluoroethylene, must be used. The ways to fix the mesh in lumbar hernia repair are similar to those for fixing anterior abdominal hernias, which include staples, sutures, transmural sutures, and combinations of these tools^[9]. Although these methods are effective based on the absence of recurrence, it is difficult to determine which one is the most superior because of the small number of cases. No matter what method is used for the fixation, special attention should be paid to the avoidance of nerve injury.

Bilateral primary lumbar hernias are rare because the bilateral superior and inferior lumbar triangles are generally asymmetrical. At present, all the bilateral lumbar hernias in the cases reported in the literature have been repaired by open surgery. To our knowledge, the laparoscopic procedure is superior for the management of bilateral inguinal hernias compared with open surgery^[44,45]. Based on the above consideration, we decided to use TAPP to repair bilateral lumbar hernias, despite the fact that there is no literature reporting the use of laparoscopic surgery for bilateral lumbar hernias. The operation was successfully performed in three patients, with mild postoperative pain, a shorter length of stay, and fewer complications compared with open surgery. We have observed the superiority of laparoscopic technique for bilateral lumbar hernias, its perioperative safety, and postoperative efficacy. The key points for this laparoscopic procedure are the location of the incision and the selection of the patient position. In the reported cases of lumbar hernia repair, the location of the observation ports was commonly in the umbilicus, while the location of the utility ports was not uniform. One method includes the two utility ports being placed along the middle line, approximately 5 cm from the observing port to reach both sides. Another method involves placing the ports along the midclavicular line on the ipsilateral side of the hernia. If the latter is used, five incisions must be performed for the bilateral lumbar hernia repair. Since none of the three patients were obese, we chose the first method in order to reduce the number of incisions. Our operations were successful, and the choice of incisions did not interfere with our surgeries. However,

we think that if the patient is obese, this option for the incisions may cause complications for the operation. For obese patients, five incisions may be more appropriate. As for the patient's position, if the patient is in a horizontal position, the lateral tilt of the operation table can also be used to partly achieve the effect of the lateral decubitus position, with no re-sterilization or drape required during the operation. However, if all of the trocars are located in the midline, exposing the preperitoneal space can be difficult, especially when the space is dissected toward the midline. Therefore, we decided to use the lateral position that is reported in most of the literature. After a one-sided lumbar hernia was repaired completely, we temporarily bandaged the incision and re-sterilized, draped and inserted trocars to repair the other lumbar hernia. Generally, there is no difference between our operation and the laparoscopic repair of a unilateral lumbar hernia.

In the currently reported laparoscopic lumbar hernia repair literature, meshes need to be fixed with sutures or staples, and in the process of fixing the meshes, the most important thing is to avoid damaging the nerves. Although nerves can be seen directly before the patches are placed, it is not easy to observe when the patches have been placed and partially fixed. At present, there are no reports of chronic pain after laparoscopic lumbar hernia repair, but the result is catastrophic if it does happen. To avoid nerve damage, we used self-gripping meshes in our study, which is also the first report to use this patch for lumbar hernia repair. Birk *et al.*^[46] performed TAPP using these self-gripping meshes for 220 inguinal hernias that had a follow-up of 12 mo, and confirmed that the mesh is safe and efficient with low pain and a low recurrence rate. The meshes do not require additional fixation for small defects, and we only needed to sew four stitches at the edges of the mesh for large defects. This not only reduces or avoids the possibility of nerve damage, but also makes the surgeries faster and easier. In addition, the patients in our study only had mild pain after the operation and had no chronic pain or recurrence at follow-up.

In conclusion, a lumbar hernia is considered to be a clinically rare entity, while a bilateral lumbar hernia is even rarer. As with bilateral inguinal hernia repair, laparoscopic bilateral lumbar hernia repair *via* TAPP is technically feasible. Meanwhile, the use of self-gripping mesh can reduce or even avoid nerve damage and simplify the operation. To the best of our knowledge, this study is the first report of bilateral lumbar hernias that were repaired by the laparoscopic approach using self-gripping mesh without any complications, implying that it is a safe and feasible surgical procedure.

ARTICLE HIGHLIGHTS

Case characteristics

Two patients presented with bilateral lumbar masses without any clinical symptoms. One patient presented with right-sided abdominal pain, but she did not show any abdominal signs.

Clinical diagnosis

Most people have a bilateral lumbar bulge with or without symptoms, such as abdominal pain. The bulge will become more apparent after a long period of standing, coughing, or holding one's breath. A contralateral bulge will shrink after patients are placed in a lateral position.

Differential diagnosis

Lumbar subcutaneous lipoma.

Imaging diagnosis

An abdominal computed tomography scan showed that the abdominal wall beside the two kidneys is weak, with part of the abdominal mesentery or colon included.

Treatment

Laparoscopic repair via the transabdominal preperitoneal procedure for a bilateral lumbar hernia.

Related reports

A bilateral lumbar hernia was previously repaired by open surgery, which is the first report of the use of laparoscopic technique for the repair of a bilateral lumbar hernia.

Experiences and lessons

Our study demonstrates that it is safe and efficient to use laparoscopic repair via transabdominal preperitoneal for a bilateral lumbar hernia, and provides an alternative way for the repair of bilateral lumbar hernias. Surgeons can use our method as long as they are proficient in laparoscopic repair of a unilateral lumbar hernia.

REFERENCES

- 1 **Cavallaro G**, Sadighi A, Paparelli C, Miceli M, D'Ermo G, Polistena A, Cavallaro A, De Toma G. Anatomical and surgical considerations on lumbar hernias. *Am Surg* 2009; **75**: 1238-1241 [PMID: 19999919]
- 2 **Moreno-Egea A**, Baena EG, Calle MC, Martínez JA, Albasini JL. Controversies in the current management of lumbar hernias. *Arch Surg* 2007; **142**: 82-88 [PMID: 17224505 DOI: 10.1001/archsurg.142.1.82]
- 3 **Bickel A**, Haj M, Eitan A. Laparoscopic management of lumbar hernia. *Surg Endosc* 1997; **11**: 1129-1130 [PMID: 9348391 DOI: 10.1007/s004649900547]
- 4 **Walgamage TB**, Ramesh BS, Alsawafi Y. Case report and review of lumbar hernia. *Int J Surg Case Rep* 2015; **6C**: 230-232 [PMID: 25555145 DOI: 10.1016/j.ijscr.2014.07.022]
- 5 **Okiemy E**, Mackoumbounkounka EL. Hernie lombaire bilatérale du triangle de petit: A propos d'un cas. *Médecine d'Afrique Noire* 2006
- 6 **Lazier J**, Mah JK, Nikolic A, Wei XC, Samedi V, Fajardo C, Brindle M, Perrier R, Thomas MA. Bilateral congenital lumbar hernias in a patient with central core disease--A case report. *Neuromuscul Disord* 2016; **26**: 56-59 [PMID: 26684984 DOI: 10.1016/j.nmd.2015.10.011]
- 7 **Burick AJ**, Parascandola SA. Laparoscopic repair of a traumatic lumbar hernia: a case report. *J Laparoendosc Surg* 1996; **6**: 259-262 [PMID: 8877746 DOI: 10.1089/lps.1996.6.259]
- 8 **Obregón L**, Ruiz-Castilla M, Binimelis MM, Guinot A, García V, Puig O, Barret JP. Laparoscopic repair of non-complicated lumbar hernia secondary to a latissimus dorsi flap. *J Plast Reconstr Aesthet Surg* 2014; **67**: 407-410 [PMID: 23910913 DOI: 10.1016/j.bjps.2013.07.022]
- 9 **Moreno-Egea A**, Alcaraz AC, Cuervo MC. Surgical options in lumbar hernia: laparoscopic versus open repair. A long-term prospective study. *Surg Innov* 2013; **20**: 331-344 [PMID: 22956401 DOI: 10.1177/1553350612458726]
- 10 **Radhakrishna V**, Tanga SM. Bilateral superior lumbar hernias: a case report and review of literature. *Int J Surg* 2017; **4**: 1472-1474 [DOI: 10.18203/2349-2902.isj20171163]
- 11 **Parreira JM**, Chibata M, Saucedo N, Colatusso RP, Paciornik R. Bilateral spiegelian hernia: case report and literature review. *Abcd Arqbrascirdig* 2007; **20**: 208-211 [DOI: 10.1590/S0102-67202007000300015]
- 12 **Heniford BT**, Iannitti DA, Gagner M. Laparoscopic inferior and superior lumbar hernia repair. *Arch Surg* 1997; **132**: 1141-1144 [PMID: 9336516 DOI: 10.1001/archsurg.1997.01430340095017]
- 13 **Postema RR**, Bonjer HJ. Endoscopic extraperitoneal repair of a Grynfeltt hernia. *Surg Endosc* 2002; **16**: 716 [PMID: 11972231 DOI: 10.1007/s00464-001-4218-6]
- 14 **Habib E**. Retroperitoneoscopic tension-free repair of lumbar hernia. *Hernia* 2003; **7**: 150-152 [PMID: 12942346 DOI: 10.1007/s10029-002-0109-6]
- 15 **Grauls A**, Lallemand B, Krick M. The retroperitoneoscopic repair of a lumbar hernia of Petit. Case report and review of literature. *Acta Chir Belg* 2004; **104**: 330-334 [PMID: 15285549 DOI: 10.1080/00015458.2004.11679566]
- 16 **Ipek T**, Eyuboglu E, Aydingoz O. Laparoscopic management of inferior lumbar hernia (Petit triangle hernia). *Hernia* 2005; **9**: 184-187 [PMID: 15614442 DOI: 10.1007/s10029-004-0269-7]
- 17 **Lim MS**, Lee HW, Yu CH, Yang DH. Laparoscopic total extraperitoneal repair of lumbar hernia. *J Korean Surg Soc* 2011; **81**: 287-290 [PMID: 22111086 DOI: 10.4174/jkss.2011.81.4.287]
- 18 **Nam SY**, Kee SK, Kim JO. Laparoscopic transabdominal extraperitoneal mesh repair of lumbar hernia. *J Korean Surg Soc* 2011; **81** Suppl 1: S74-S77 [PMID: 22319745 DOI: 10.4174/jkss.2011.81.Suppl1.S74]
- 19 **Suarez S**, Hernandez JD. Laparoscopic repair of a lumbar hernia: report of a case and extensive review of the literature. *Surg Endosc* 2013; **27**: 3421-3429 [PMID: 23636518 DOI: 10.1007/s00464-013-2884-9]
- 20 **Wei CT**, Chen YS, Sun CK, Hsieh KC. Single-incision laparoscopic total extraperitoneal repair for a Grynfeltt hernia: a case report. *J Med Case Rep* 2014; **8**: 16 [PMID: 24428946 DOI: 10.1186/1752-1947-8-16]
- 21 **Agresta F**, Marzetti A, Vigna S, Prando D, Porfidia R, Di Saverio S. Repair of primary and incisional hernias using composite mesh fixed with absorbable tackers: preliminary experience of a laparoscopic approach with a newly designed mesh in 29 cases. *Updates Surg* 2017; **69**: 493-497 [PMID: 28409440 DOI: 10.1007/s13304-017-0444-x]
- 22 **Claus CMP**, Nassif LT, Aguilera YS, Ramos EB, Coelho JCU. Laparoscopic repair of lumbar hernia (grynfeltt): technical description. *Arq Bras Cir Dig* 2017; **30**: 56-59 [PMID: 28489172 DOI: 10.1590/0102-6720201700010016]
- 23 **Sarwal A**, Sharma A, Khullar R, Soni V, Baijal M, Chowbey P. Primary lumbar hernia: A rare case report and a review of the literature. *Asian J Endosc Surg* 2018; [PMID: 29770607 DOI: 10.1111/ases.12603]
- 24 **Sharma A**, Pandey A, Rawat J, Ahmed I, Wakhlu A, Kureel SN. Congenital lumbar hernia: 20 years' single centre experience. *J Paediatr Child Health* 2012; **48**: 1001-1003 [PMID: 23039934 DOI: 10.1111/j.1440-1754.2012.02581.x]
- 25 **Reggio E**, Sette MJ, Lemos R, Timm O Jr, Junqueira RG. Lumbar hernia following percutaneous nephrolithotomy. *Clinics (Sao Paulo)* 2010; **65**: 1061-1062 [PMID: 21120313 DOI: 10.1590/S1807-59322010001000025]
- 26 **Xu T**, Zhang S, Wang H, Yu W. Lumbar hernia associated with chronic obstructive pulmonary disease (COPD). *Pak J Med Sci* 2013; **29**: 874-876 [PMID: 24353649 DOI: 10.12669/pjms.293.3232]
- 27 **Woolbert A**, Calasanz ER, Nazim M. Traumatic lumbar visceral herniation in a young woman. *Int J Surg Case Rep* 2013; **4**: 1061-1063 [PMID: 24211512 DOI: 10.1016/j.ijscr.2013.09.010]
- 28 **Stamatiou D**, Skandalakis JE, Skandalakis LJ, Mirilas P. Lumbar hernia: surgical anatomy, embryology, and technique of repair. *Am Surg* 2009; **75**: 202-207 [PMID: 19350853]
- 29 **Armstrong O**, Hamel A, Grignon B, NDoye JM, Hamel O, Robert

- R, Rogez JM. Lumbar hernia: anatomical basis and clinical aspects. *Surg Radiol Anat* 2008; **30**: 533-537; discussion 609-610 [PMID: 18553051 DOI: 10.1007/s00276-008-0361-2]
- 30 **Kim J**, Oh MM, Kim JJ, Moon D G. Delayed repair of a traumatic lumbar hernia with renal rupture. *Am Surg* 2012; **78**: E295-E296 [PMID: 22546113]
- 31 **Teo KA**, Burns E, Garcea G, Abela JE, McKay CJ. Incarcerated small bowel within a spontaneous lumbar hernia. *Hernia* 2010; **14**: 539-541 [PMID: 19890674 DOI: 10.1007/s10029-009-0581-3]
- 32 **Hide IG**, Pike EE, Uberoi R. Lumbar hernia: a rare cause of large bowel obstruction. *Postgrad Med J* 1999; **75**: 231-232 [PMID: 10715766 DOI: 10.1136/pgmj.75.882.231]
- 33 **Presti JC Jr**, Narayan P. Lumbar herniation of the kidney. *J Urol* 1988; **140**: 586-587 [PMID: 3411680 DOI: 10.1016/S0022-5347(17)41726-5]
- 34 **Willcox MJ**. Lumbar Herniation of Kidney following Iliac Crest Bone Harvest. *Case Rep Surg* 2016; **2016**: 5365647 [PMID: 28042490 DOI: 10.1155/2016/5365647]
- 35 **Chan K**, Towsey K, Cavallucci D, Green B. Traumatic lumbar hernia repair: experience at the Royal Brisbane and Women's Hospital. *Hernia* 2017; **21**: 317-322 [PMID: 26423294 DOI: 10.1007/s10029-015-1425-y]
- 36 **Wakhlu A**, Wakhlu AK. Congenital lumbar hernia. *Pediatr Surg Int* 2000; **16**: 146-148 [PMID: 10663870 DOI: 10.1007/s003830050048]
- 37 **Rattan KN**, Agarwal A, Dhiman A, Rattan A. Congenital Lumbar Hernia: A 15-Year Experience at a Single Tertiary Centre. *Int J Pediatr* 2016; **2016**: 7162475 [PMID: 27994626 DOI: 10.1155/2016/7162475]
- 38 **Cavallaro G**, Sadighi A, Miceli M, Burza A, Carbone G, Cavallaro A. Primary lumbar hernia repair: the open approach. *Eur Surg Res* 2007; **39**: 88-92 [PMID: 17283432 DOI: 10.1159/000099155]
- 39 **Dowd CN**. CONGENITAL LUMBAR HERNIA, AT THE TRIANGLE OF PETIT. *Ann Surg* 1907; **45**: 245-248 [PMID: 17861929 DOI: 10.1097/00000658-190702000-00007]
- 40 **Hafner CD**, WYLIE JH Jr, BRUSH BE. Petit's lumbar hernia: repair with Marlex mesh. *Arch Surg* 1963; **86**: 180-186 [PMID: 13951839 DOI: 10.1001/archsurg.1963.01310080004002]
- 41 **Links DJ**, Berney CR. Traumatic lumbar hernia repair: a laparoscopic technique for mesh fixation with an iliac crest suture anchor. *Hernia* 2011; **15**: 691-693 [PMID: 20803044 DOI: 10.1007/s10029-010-0716-6]
- 42 **Meinke AK**. Totally extraperitoneal laparoendoscopic repair of lumbar hernia. *Surg Endosc* 2003; **17**: 734-737 [PMID: 12618948 DOI: 10.1007/s00464-002-8557-8]
- 43 **Sun J**, Chen X, Li J, Zhang Y, Dong F, Zheng M. Implementation of the trans-abdominal partial extra-peritoneal (TAPE) technique in laparoscopic lumbar hernia repair. *BMC Surg* 2015; **15**: 118 [PMID: 26507827 DOI: 10.1186/s12893-015-0104-3]
- 44 **Feliu X**, Claveria R, Besora P, Camps J, Fernández-Sallent E, Viñas X, Abad JM. Bilateral inguinal hernia repair: laparoscopic or open approach? *Hernia* 2011; **15**: 15-18 [PMID: 20960019 DOI: 10.1007/s10029-010-0736-2]
- 45 **Wauschkuhn CA**, Schwarz J, Boekeler U, Bittner R. Laparoscopic inguinal hernia repair: gold standard in bilateral hernia repair? Results of more than 2800 patients in comparison to literature. *Surg Endosc* 2010; **24**: 3026-3030 [PMID: 20454807 DOI: 10.1007/s00464-010-1079-x]
- 46 **Birk D**, Hess S, Garcia-Pardo C. Low recurrence rate and low chronic pain associated with inguinal hernia repair by laparoscopic placement of Parietex ProGrip™ mesh: clinical outcomes of 220 hernias with mean follow-up at 23 months. *Hernia* 2013; **17**: 313-320 [PMID: 23412779 DOI: 10.1007/s10029-013-1053-3]

P- Reviewer: Akbulut S, Demetrashvili Z, Hussain A, Losanoff JE

S- Editor: Cui LJ **L- Editor:** Filipodia **E- Editor:** Wu YXJ





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>

