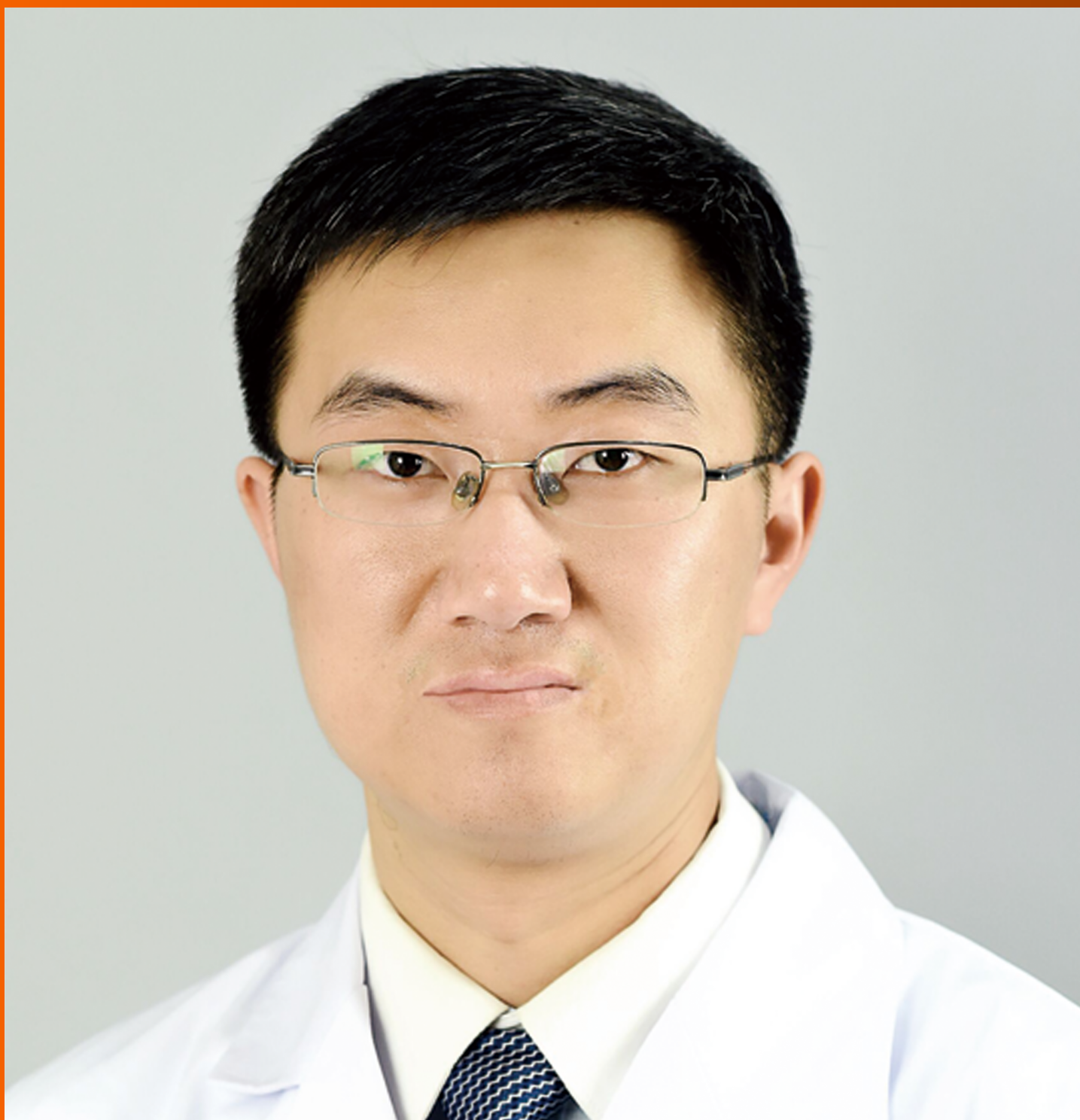


# World Journal of *Clinical Cases*

*World J Clin Cases* 2019 February 26; 7(4): 405-547



**MINIREVIEWS**

- 405** Immune checkpoint inhibitor-induced colitis: A comprehensive review  
*Som A, Mandaliya R, Alsaadi D, Farshidpour M, Charabaty A, Malhotra N, Mattar MC*

**ORIGINAL ARTICLE****Basic Study**

- 419** Formalin fixation on HER-2 and PD-L1 expression in gastric cancer: A pilot analysis using the same surgical specimens with different fixation times  
*Kai K, Yoda Y, Kawaguchi A, Minesaki A, Iwasaki H, Aishima S, Noshiro H*

**Case Control Study**

- 431** Nested case-control study of multiple serological indexes and Brighton pediatric early warning score in predicting death of children with sepsis  
*Xie X, Li M, Xiong TT, Wang R, Xiao L*

**Retrospective Study**

- 441** Intestinal endometriosis: Diagnostic ambiguities and surgical outcomes  
*Bong JW, Yu CS, Lee JL, Kim CW, Yoon YS, Park IJ, Lim SB, Kim JC*

**Randomized Controlled Trial**

- 452** Efficacy of 1.2 L polyethylene glycol plus ascorbic acid for bowel preparations  
*Tamaki H, Noda T, Morita M, Omura A, Kubo A, Ogawa C, Matsunaka T, Shibato M*

**CASE REPORT**

- 466** Congenital analbuminemia in a patient affected by hypercholesterolemia: A case report  
*Suppressa P, Carbonara C, Lugani F, Campagnoli M, Troiano T, Minchiotti L, Sabbà C*
- 473** Primary leiomyosarcoma of the thyroid gland with prior malignancy and radiotherapy: A case report and review of literature  
*Vujosevic S, Krnjecic D, Bogojevic M, Vuckovic L, Filipovic A, Dunđerović D, Sopta J*
- 482** Endoscopic resection for residual lesion of metastatic gastric cancer: A case report  
*Hayashi K, Suzuki S, Ikehara H, Okuno H, Irie A, Esaki M, Kusano C, Gotoda T, Moriyama M*
- 489** Peritoneal cavernous hemangiomatosis: A case report  
*Fu LY, Chen HY, Diao XL, Wang ZJ*
- 494** Recurrent acute liver failure associated with novel SCYL1 mutation: A case report  
*Li JQ, Gong JY, Knisely AS, Zhang MH, Wang JS*



- 500** Therapeutic plasma exchange and continuous renal replacement therapy for severe hyperthyroidism and multi-organ failure: A case report  
*Ba JH, Wu BQ, Wang YH, Shi YF*
- 508** Hydrochloric acid enhanced radiofrequency ablation for treatment of large hepatocellular carcinoma in the caudate lobe: Report of three cases  
*Deng HX, Huang JH, Lau WY, Ai F, Chen MS, Huang ZM, Zhang TQ, Zuo MX*
- 516** Long-term follow-up of a patient with venlafaxine-induced diurnal bruxism treated with an occlusal splint: A case report  
*Chen JM, Yan Y*
- 525** Primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization: A case report  
*Zhu KL, Cai XJ*
- 532** Anterior cervical corpectomy decompression and fusion for cervical kyphosis in a girl with Ehlers-Danlos syndrome: A case report  
*Fang H, Liu PF, Ge C, Zhang WZ, Shang XF, Shen CL, He R*
- 538** Rhombencephalitis caused by *Listeria monocytogenes* with hydrocephalus and intracranial hemorrhage: A case report and review of the literature  
*Liang JJ, He XY, Ye H*

**ABOUT COVER**

Editorial Board Member of *World Journal of Clinical Cases*, Xi Jin, PhD, Associate Professor, Doctor, Department of Gastroenterology, Institution of Gastroenterology, the First Affiliated Hospital, School of Medicine Zhejiang University, Zhejiang Province, Hangzhou 310003, China

**AIMS 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 Case Report, Clinical Management, Editorial, Field of Vision, Frontier, Medical Ethics, Original Articles, Meta-Analysis, Minireviews, and Review, in the fields of allergy, anesthesiology, cardiac medicine, clinical genetics, clinical neurology, critical care, dentistry, dermatology, emergency medicine, endocrinology, family medicine, gastroenterology and hepatology, *etc.*

**INDEXING/ABSTRACTING**

The *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).

**RESPONSIBLE EDITORS FOR THIS ISSUE**Responsible Electronic Editor: *Wen-Wen Tan*Proofing Editorial Office Director: *Jin-Lai Wang***NAME OF JOURNAL***World Journal of Clinical Cases***ISSN**

ISSN 2307-8960 (online)

**LAUNCH DATE**

April 16, 2013

**FREQUENCY**

Semimonthly

**EDITORS-IN-CHIEF**

Dennis A Bloomfield, Sandro Vento

**EDITORIAL BOARD MEMBERS**<https://www.wjgnet.com/2307-8960/editorialboard.htm>**EDITORIAL OFFICE**

Jin-Lai Wang, Director

**PUBLICATION DATE**

February 26, 2019

**COPYRIGHT**

© 2019 Baishideng Publishing Group Inc

**INSTRUCTIONS TO AUTHORS**<https://www.wjgnet.com/bpg/gerinfo/204>**GUIDELINES FOR ETHICS DOCUMENTS**<https://www.wjgnet.com/bpg/GerInfo/287>**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**<https://www.wjgnet.com/bpg/gerinfo/240>**PUBLICATION MISCONDUCT**<https://www.wjgnet.com/bpg/gerinfo/208>**ARTICLE PROCESSING CHARGE**<https://www.wjgnet.com/bpg/gerinfo/242>**STEPS FOR SUBMITTING MANUSCRIPTS**<https://www.wjgnet.com/bpg/GerInfo/239>**ONLINE SUBMISSION**<https://www.f6publishing.com>

## Immune checkpoint inhibitor-induced colitis: A comprehensive review

Aniruddh Som, Rohan Mandaliya, Dana Alsaadi, Maham Farshidpour, Aline Charabaty, Nidhi Malhotra, Mark C Mattar

**ORCID number:** Aniruddh Som (0000-0002-9031-8287); Rohan Mandaliya (0000-0002-0749-9022); Dana Alsaadi (0000-0003-0042-3671); Maham Farshidpour (0000-0001-6282-6148); Aline Charabaty (0000-0001-9810-3662); Nidhi Malhotra (0000-0001-7054-9961); Mark C Mattar (0000-0002-9339-1607).

**Author contributions:** All authors contributed to this paper.

**Conflict-of-interest statement:** None.

**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

**Received:** September 21, 2018

**Peer-review started:** September 21, 2018

**First decision:** October 16, 2018

**Revised:** January 21, 2019

**Accepted:** January 26, 2019

**Article in press:** January 26, 2019

**Published online:** February 26,

**Aniruddh Som**, Department of Internal Medicine, Medstar Washington Hospital Center, Washington, DC 20010, United States

**Rohan Mandaliya, Mark C Mattar**, Department of Gastroenterology, MedStar Georgetown University Hospital, Washington, DC 20007, United States

**Dana Alsaadi**, Department of Internal Medicine, MedStar Georgetown University Hospital, Washington, DC 20007, United States

**Maham Farshidpour**, Department of Internal Medicine, MedStar Union Memorial Hospital and Good Samaritan Hospital, Baltimore, MD 21218, United States

**Aline Charabaty**, Department of Gastroenterology, Sibley Memorial Hospital, Washington, DC 20007, United States

**Nidhi Malhotra**, Department of Gastroenterology, MedStar Washington Hospital Center, Washington, DC 20010, United States

**Corresponding author:** Mark C Mattar, FACG, MD, Attending Doctor, Gastroenterology Fellowship Program Director, Director of IBD Center, Department of Gastroenterology, MedStar Georgetown University Hospital, 3800 Reservoir Rd NW, Washington, DC 20007, United States. [mark.c.mattar@medstar.net](mailto:mark.c.mattar@medstar.net)  
**Telephone:** +1-202-4441039

### Abstract

Immune checkpoint inhibitors (ICIs) are monoclonal antibodies that target down-regulators of the anti-cancer immune response: Cytotoxic T-lymphocyte antigen-4, programmed cell death protein-1, and its ligand programmed death-ligand 1. ICIs have revolutionized the treatment of a variety of malignancies. However, many immune-related adverse events have also been described which mainly occurs as the immune system becomes less suppressed, affecting various organs including the gastrointestinal tract and causing diarrhea and colitis. The incidence of immune-mediated colitis (IMC) ranges from 1%-25% depending on the type of ICI and if used in combination. Endoscopically and histologically there is a significant overlap between IMC and inflammatory bowel disease, however more neutrophilic inflammation without chronic inflammation is usually present in IMC. Corticosteroids are recommended for grade 2 or more severe colitis while holding the immunotherapy. About one third to two thirds of patients are steroid refractory and benefit from infliximab. Recently vedolizumab has been found to be efficacious in steroid and infliximab refractory cases. While



2019

in grade 4 colitis, the immunotherapy is permanently discontinued, the decision is controversial in grade 3 colitis.

**Key words:** Immune checkpoint inhibitors; Immune-related adverse events; Cytotoxic T-lymphocyte-associated antigen 4; Programmed cell death protein 1; Programmed death-ligand 1; Immune-mediated colitis

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Immune-mediated colitis (IMC) is a common immune related adverse event associated with immune checkpoint inhibitors. It typically occurs 5 wk-10 wk after the 2<sup>nd</sup> or 3<sup>rd</sup> dose of treatment. Endoscopically, it is indistinguishable from inflammatory bowel disease with significant overlap in histology. Optimal management of IMC requires early recognition and timely use of corticosteroids. About one third to two thirds of patients are steroid refractory. Infliximab is the second line therapy in these patients. Recent reports have shown that Vedolizumab is more gut specific and efficacious in steroid and infliximab refractory cases. Fecal microbiota transplant has recently been reported to be successful in steroid refractory cases.

**Citation:** Som A, Mandaliya R, Alsaadi D, Farshidpour M, Charabaty A, Malhotra N, Mattar MC. Immune checkpoint inhibitor-induced colitis: A comprehensive review. *World J Clin Cases* 2019; 7(4): 405-418

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/405.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.405>

## INTRODUCTION

Scientists have long experimented with the idea that the immune system holds the potential to fight not only infections but also malignancy. In the early 19<sup>th</sup> century, Busch and Fehleisen, both noted that infecting tumors with erysipelas resulted in tumor regression<sup>[1,2]</sup>. Towards the late 1800s, sarcoma regression was seen after injecting tumors with heat-inactivated bacteria known as “Coley’s Toxins”<sup>[3]</sup>. The pivotal change in the history of immune checkpoint inhibitors (ICIs) began with the discovery of the T-cell receptor by Allison *et al*<sup>[4]</sup> in the early 1980s. This discovery was soon followed by the first ever reported human tumor antigen recognized by T-cells<sup>[5]</sup>. A study on tumors in animal models provided proof of concept that an antibody was successful in blocking the cytotoxic T-lymphocyte antigen 4 (CTLA-4)<sup>[6]</sup>. Such seminal work culminated with the Food and Drug Administration (FDA) approving the first ICI for use in metastatic melanoma in 2011<sup>[7]</sup>. To date there are 7 approved checkpoint inhibitors that target 3 main checkpoints, including cytotoxic T-lymphocyte associated protein 4 (CTLA-4; ipilimumab and tremelimumab), programmed cell death receptor 1 (PD-1; pembrolizumab and nivolumab), and programmed death ligand 1 (PD-L1; atezolizumab, avelumab, and durvalumab). Indeed, ICIs have become the standard of care for a number of cancers and resulted in the awarding of the 2018 Nobel Prize in Physiology or Medicine to Allison and Honjo in recognition of their contribution to the discovery of ICIs<sup>[8]</sup>. While representing a remarkable breakthrough in the treatment of several advanced malignancies, several ICI-related adverse events that affect multiple body systems (Table 1)<sup>[9-14]</sup> have been recognized, including immune-mediated colitis (IMC) and enteritis<sup>[9-16]</sup>. The incidence of IMC ranges from 0.3% to 7% and may be associated with other immune-related adverse events (irAEs)<sup>[17]</sup>. As a result, addressing GI irAEs has become a major clinical issue for physicians and patients alike. This review summarizes the current clinical information of IMC, its postulated mechanism of injury, endoscopic features, and the management strategies that are currently advocated.

### Mechanism of action

The immune system has an important role in recognizing and eliminating tumors. Transformed tumor cells express tumor-associated antigens (TAAs) that are not seen on normal cells<sup>[18]</sup>. These TAAs are recognized by the immune system, and T cells can be stimulated in response to cellular presentation of TAAs<sup>[19]</sup>. TAAs are presented along with the major histocompatibility complex (MHC) I or II by specialized antigen-

**Table 1** Percentage ranges of all grade immune-related common adverse events by checkpoint inhibitor class

Class of immune checkpoint inhibitors	Approved agents	Rash	Diarrhea	Colitis	Elevated ALT	Hypothyroidism	Hypophysitis
Anti CTLA-4	Ipilimumab, Tremelimumab	12%-68%	31%-49%	7%-11.6%	3%-9%	4%-4.2%	4%-6%
Anti PD-1	Nivolumab, Pembrolizumab	11.7%-24%	2.9%-11.5%	1.3%-2.9%	1.8%-7.1%	3.4%-8.5%	0.25%
Anti PD-L1	Atezolizumab, Durvalumab, Avelumab	7.4%	11.6%-23%	0.7%-19.7%	0.9%-4.0%	5.0%-9.6%	0.2%

CTLA-4: Cytotoxic T-lymphocyte-associated antigen 4; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; ALT: Alanine aminotransferase.

presenting cells (APCs) that bind with T-cell receptors (TCRs). Activation of T cells requires a co-stimulatory signal which includes the interaction of TCR with MHC along with the interaction of CD-28 (stimulatory checkpoint expressed on T cells) with B7 (CD-80) present on APCs. This leads to T-cell proliferation, cytokine secretion, changes in gene expression and metabolism<sup>[20]</sup>.

Tumors may use immune-checkpoint pathways as a mechanism of immune resistance, principally against T cells that are specific for TAAs<sup>[21]</sup>. Two well-studied immune-checkpoint receptors are CTLA-4 (CD152) and programmed cell death protein 1 (PD-1 or CD279). CTLA-4 is a negative regulator of T-cell-mediated anti-tumor responses. Expression of CTLA-4 is up-regulated upon TCR stimulation. This molecule competes with CD28 for binding to B7 on APCs, avoiding the costimulatory signal and blunting T-cell activation and proliferation<sup>[22]</sup>. PD-1 is also expressed on the surface of activated T cells. The interaction between PD-1 and programmed death ligand (PD-L1 and PD-L2), expressed on APCs, leads to T-cell inactivation. Additionally, PD-1 plays an important role to limit the activity of T cells in peripheral tissues through inflammatory response to infection and to limit autoimmunity<sup>[23]</sup>.

Checkpoint inhibitors are monoclonal antibodies that block these pathways. Ipilimumab was the first checkpoint inhibitor immunotherapy approved by the FDA, in March 2011, for the treatment of melanoma. Ipilimumab is a fully humanized monoclonal antibody that competitively binds to CTLA-4 more efficiently when compared to B7 while preserving CD28 signaling<sup>[24]</sup>. Blockade of CTLA-4 signaling extends T-cell activation and reestablishes T-cell proliferation<sup>[25]</sup>. As CTLA-4 plays a crucial role in regulating tolerance to self-antigens, CTLA-4 blockade may lead to autoimmune damage to various organ systems, resulting in irAEs<sup>[26]</sup>. CTLA-4 blockade can initiate dysregulation of GI mucosal immunity which results in irAEs that comprise the esophagus, duodenum, stomach, ileum and colon<sup>[27]</sup>. The various types of ICIs are described in [Table 1](#).

### Mechanism of irAEs

Mechanisms of IMC and other irAEs are not fully understood; however, CTLA-4 blockade removes CTLA4-mediated protection from autoimmunity and is responsible for a large spectrum of autoimmune-side effects<sup>[28]</sup>. Immune-related toxicities are mostly associated with the inflammatory reaction produced by immune system responses against specific organs and tissues<sup>[29]</sup>. Immune-related T-cell activation leads to the secretion of high levels of CD4 T-helper cell cytokines and cytolytic CD8 T-cell tissue infiltration<sup>[21]</sup>. Another potential mechanism for generating colitis following anti-CTLA4 antibody involves CD25+CD4+ regulatory T cells (Treg). These immunosuppressive regulatory cells constitutively express high levels of CTLA-4 and data show increased autoimmune diseases in mice lacking Treg cells<sup>[30,31]</sup>. Consequently, it has been hypothesized that an antibody to CTLA4 might diminish Treg cells and induce autoimmunity<sup>[32]</sup>.

The enterocolitis related to ipilimumab has features similar to graft-versus-host disease. It has been proposed that a contributing factor to enterocolitis in this setting may be intestinal microflora and bacterial antigens, representing an area of future research for prophylaxis of enterocolitis in patients treated with ipilimumab<sup>[33,34]</sup>.

### Clinical presentation

Diarrhea and enterocolitis lie along a clinical spectrum where diarrhea is defined as increased stool frequency, and enterocolitis is defined as abdominal pain, rectal

bleeding or the presence of mucus in stools with either clinical or radiologic objective evidence of entero-colonic inflammation, as defined by the American Society of Clinical Oncology (ASCO)<sup>[35]</sup>. The presence of enterocolitis increases the risk of other complications, including ileus, colonic distension, and toxic megacolon, intestinal perforation, or even death. The clinical severity of both diarrhea and colitis is graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (Table 2). Mild diarrhea (grade 1) is defined as less than 4 stools per day above baseline. Grade 2 diarrhea is defined as 4 to 6 stools per day above baseline, while grade 2 colitis is characterized by abdominal pain or blood or mucus in the stool. Severe diarrhea (grade 3) is defined as  $\geq 7$  stools per day above baseline, and grade 3 colitis is defined by the presence of peritoneal signs with ileus and fever consistent with bowel perforation. A grade 4 designation is distinct from grade 3, reflecting increased severity and the life-threatening nature of symptoms.

While irAEs can affect any portion of the GI tract, the lower GI tract is most commonly involved. Less commonly, the upper GI tract can be affected, manifesting as aphthous ulcers, esophagitis, and gastritis. IMC and diarrhea typically occur 5 wk-10 wk after the 2<sup>nd</sup> or 3<sup>rd</sup> doses of treatment, but they have also been documented to occur as late as 4 mo after the last dose and may even recur one to two years after discontinuation of treatment<sup>[13,36-38]</sup>.

## EPIDEMIOLOGY

More than two-thirds of patients who receive anti-CTLA-4 therapy develop an irAE, and one-third of patients who are treated with anti-CTLA-4 therapy experience irAEs of the gastrointestinal tract, such as aphthous ulcers, esophagitis, gastritis, and enterocolitis, which usually presents as diarrhea<sup>[39,40]</sup>. The incidence of diarrhea is higher in patients receiving anti-CTLA-4 agents, such as ipilimumab, compared to patients receiving anti-PD-1/PD-L1 agents, such as nivolumab or pembrolizumab<sup>[41]</sup>, with grade 3/4 diarrhea seen in 10% *vs* 1%-2% of patients, respectively<sup>[42]</sup>.

Beck *et al*<sup>[33]</sup> showed that enterocolitis, defined by the presence of grade 3 or 4 symptoms and/or proven by biopsy, was the most common irAE associated with ipilimumab use, occurring in 21% of treated melanoma patients. Kwon *et al*<sup>[43]</sup> reported a 5% incidence of grade 3/4 colitis among patients with prostate cancer who were treated with ipilimumab at the dose of 10 mg/kg. Slovin *et al*<sup>[44]</sup> demonstrated that the incidence of grade 3/4 colitis increased from 13% to 16% with an increase in the dose of ipilimumab from 5 mg/kg to 10 mg/kg in patients with prostate cancer. Similarly, the incidence of enterocolitis in patients with renal cell carcinoma receiving higher doses of ipilimumab was 35% compared to 14% in patients receiving lower doses<sup>[33]</sup>.

Overall, the risk of severe grade adverse events increased from 7% to 25% with an increase in the dose of ipilimumab from 3 mg/kg to 10 mg/kg<sup>[43]</sup>. Most of the increase in adverse effects was due to an increase in the episodes of diarrhea. However, the toxicity profile would not increase if the dosage of nivolumab or pembrolizumab were increased from FDA approved doses (2 mg/kg every 3 wk) to higher doses (10 mg/kg every 2 wk or 3 wk). It may be argued that toxicities due to anti-CTLA-4 antibodies are dose-dependent whereas toxicities with anti-PD-1/anti-PD-L1 antibodies are perhaps independent of a dose-related effect<sup>[45]</sup>.

### Combination therapy and risk of enterocolitis

Combination therapies have so far only been approved for metastatic melanoma. Use of combined anti-CTLA4 and anti PD-1 agents results in increased frequency and severity of diarrhea and colitis than with the use of either agent alone<sup>[46-48]</sup>. They can also cause rarer forms of toxicities like pancreatitis and small bowel enteritis which warrants discontinuation of ICI treatment and initiation of immunosuppressive therapy.

### Risk factors for ICI enterocolitis

**Gut microbiome:** Baseline microbiota composition may predict ipilimumab-induced colitis. In one prospective study of 34 patients whose pre-treatment fecal composition was analyzed, an increased baseline presence of *Bacteroidetes* species was found in patients who remained free of colitis after ipilimumab treatment<sup>[49]</sup>. Another study of 26 patients with metastatic melanoma treated with ipilimumab again showed that no-colitis related phylotypes were assigned to *Bacteroidetes*; most of the baseline colitis-associated phylotypes were related to *Firmicutes*<sup>[50]</sup>. Compared with those whose baseline microbiota was driven by *Bacteroides*, patients with a baseline microbiota enriched with *Faecalibacterium* and *Firmicutes* had longer progression-free survival and overall survival.



**Table 2 Grading the severity of immune checkpoint inhibitor-induced colitis and diarrhea based on Common Terminology Criteria for Adverse Events grade**

	Colitis	Diarrhea
Grade 1	Asymptomatic	Increase of < 4 stools/d over baseline
Grade 2	Abdominal pain, mucus, blood in stool	Increase of 4-6 stools/d
Grade 3	Severe pain, fever, peritoneal signs	Increase of ≥ 7 stools/d
Grade 4	Life-threatening consequences such as perforation, ischemia, necrosis, bleeding, toxic megacolon	Life-threatening consequences such as hemodynamic collapse
Grade 5	Death	Death

Adapted from the Cancer Therapy Evaluation Program, National Cancer Institute Common Terminology Criteria for Adverse Events v5.0 Program, Common Terminology Criteria for Adverse Events v5.0.

[https://ctep.cancer.gov/protocolDevelopment/electronic\\_applications/docs/CTCAE\\_v5\\_Quick\\_Reference\\_8.5x11.pdf](https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf).

**Autoimmune disorders:** Patients with a history of autoimmune disease, are at risk for worsening of their autoimmune disease while on immune checkpoint blockade, but immune-mediated toxicities are often mild and manageable without discontinuation of treatment<sup>[51]</sup>. Patients that have experienced irAEs with prior checkpoint inhibitor therapy are at risk of developing irAEs following treatment with a different class of ICI; irAE risk also increases with dual therapy<sup>[51,52]</sup>.

**Other risk factors:** A recent series demonstrated that non-steroidal anti-inflammatory drug use was associated with an increased risk of ipilimumab-induced enterocolitis<sup>[53]</sup>. Little data is available on the risk of immune-related colitis in patients with Crohn's disease and ulcerative colitis.

## DIAGNOSIS

Infectious causes of diarrhea should be ruled out with the first presentation of diarrhea or abdominal pain in patients treated with ICI therapy. Stool should be sent for standard microbiological examinations, including stool ova and parasites, bacterial culture, and *Clostridium difficile* testing<sup>[40]</sup>. ICI-induced colitis and infection can coexist, as demonstrated by two case reports of co-infection by CMV and *Salmonella* species<sup>[54,55]</sup>. Patients who are treated with antibiotics for infection may not experience complete resolution of symptoms, suggesting a concomitant ICI component to their colitis<sup>[56]</sup>.

While infections are more common, gastrointestinal metastases should also be ruled out as a potential etiology of symptoms. Patients with clinical signs of peritonitis such as fever, severe abdominal tenderness, distention, and rigidity should be evaluated with abdominal CT to rule out colonic perforation, which is a rare but well-documented adverse event that can be fatal<sup>[57-59]</sup>.

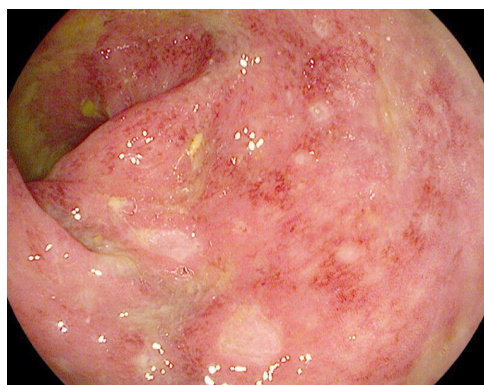
### Endoscopic features

Colonoscopy, with an exam of the terminal ileum and biopsies of the colon and ileal mucosa, is the gold standard diagnostic test for ICI-mediated colitis in patients with persistent grade 2 or higher diarrhea. Patients with upper GI symptoms such as nausea or vomiting should also undergo EGD with biopsies. A normal appearance of the mucosa on endoscopic examination does not exclude enterocolitis, and mucosal biopsies must always be attained<sup>[33]</sup>.

Some patients with immune-mediated diarrhea or colitis may demonstrate ulcerations, but others may demonstrate erosions, erythema, loss of vascular pattern, or even grossly normal appearing mucosa (Figure 1)<sup>[60]</sup>. Ipilimumab-induced colitis most often involves lesions of the rectum and sigmoid, so flexible sigmoidoscopy is usually sufficient for diagnosis. A majority of patients, however, also have endoscopic lesions proximal to the sigmoid. In more extensive colitis, inflammation can be either patchy or continuous<sup>[53,61]</sup>. Studies have shown no correlation between diarrheal grade or severity of abdominal pain and endoscopic appearance, but bloody stools have been correlated with higher endoscopic Mayo scores<sup>[56,62]</sup>.

### Histology

ICI colitis presents with an array of histologic findings ranging from focal active colitis with patchy crypt abscesses to diffuse mucosal inflammation<sup>[53,63]</sup>. Biopsies most commonly demonstrate features of acute colitis such as increased cellularity of the



**Figure 1** Patient who experienced immune-mediated colitis 4 wk after ipilimumab therapy. Colonoscopy showed a sigmoid colon with ulcers, diffuse erythema, and loss of vascularity.

lamina propria with mononuclear cells, intraepithelial neutrophilic infiltrates or crypt abscesses, and an increased number of apoptotic cells in crypts<sup>[56]</sup>.

Histologic findings may precede the onset of diarrhea or colitis. One study of asymptomatic patients who underwent colonoscopy 1 wk–2 wk following ipilimumab induction demonstrated that inflammatory changes were already present prior to symptoms (which occurred 3 wk later in the majority of patients)<sup>[63]</sup>. Biopsies most frequently showed focal neutrophilic cryptitis and neutrophilic infiltration in the lamina propria, and a subset of patients had excess plasma cells in the lamina propria and lymphocytic cryptitis. When a subset of these patients did eventually present with symptomatic diarrhea or colitis within 24 wk of induction, their biopsies showed more severe infiltration of the lamina propria with mixed inflammatory cells (neutrophils, lymphocytes, plasma cells, and eosinophils), neutrophilic cryptitis and crypt abscesses, as well as glandular destruction and mucosal erosions or ulcers. This study did not show a significant increase in the number of intraepithelial lymphocytes, apoptotic activity, or histologic evidence of chronicity, such as crypt architectural distortion, basal plasmacytosis, the presence of granulomas, Paneth cell metaplasia, or pyloric metaplasia. As several other studies have also shown a relative lack of chronic inflammation on histology, the pathogenesis of ICI-induced enterocolitis is generally considered distinct from that of IBD, although both diseases have similar clinical presentations.

However, the finding of chronic inflammation changes in some ICI-treated patients has caused speculation that their presence is consistent with the progression of acute inflammation or the development of IBD. More recent studies have shown that some patients do exhibit intraepithelial lymphocytes or basal lymphocytes and crypt architecture distortion, which is more consistent with findings in chronic colitis<sup>[62]</sup>. One study found features of chronic colitis in three out of nine patients with endoscopic or histological inflammation on biopsies that were taken several months after the onset of enterocolitis<sup>[53]</sup>.

Interestingly, one recent study demonstrated distinct immunological characteristics of colonic biopsies taken from patients with IMC and IBD that were analyzed by immunohistochemistry and flow cytometry. The lamina propria and epithelium of anti-PD-1-induced colitis was predominantly characterized by CD8<sup>+</sup> T-cells, whereas the lamina propria in anti-CTLA-4-induced colitis was predominantly characterized by CD4<sup>+</sup> T-cells and high mucosal TNF $\alpha$  concentrations. In IBD or anti-PD-1-induced colitis, Treg cells were predominant, whereas in anti-CTLA-4-induced colitis more conventional CD4<sup>+</sup> cells were found<sup>[63]</sup>.

Similarities between IMC and IBD still persist though these differences are helpful. As histologic features may overlap, clinical factors must be utilized to differentiate these distinct clinical entities, including disease onset soon after initiation of ICI or other associated symptoms. IMC is one of the most common irAEs, but patients often exhibit other concomitant irAEs such as hepatitis, hypophysitis, and hypothyroidism; if any of these also coincide in a patient with diarrhea, ICI colitis is much more likely than IBD.

### **Stool or serologic markers of ICI colitis**

Identification of serologic markers specific to IMC has been an important focus of research. Fecal calprotectin is a marker that has high sensitivity and specificity for intestinal inflammation. Calprotectin is a calcium-binding protein derived primarily from neutrophils and activated macrophages<sup>[64]</sup>. While calprotectin can be used to

distinguish inflammatory from noninflammatory diarrhea, it is not specific for ICI-induced colitis<sup>[65]</sup>. Further, while fecal calprotectin can be elevated in patients receiving ipilimumab, indicating active bowel inflammation, it does not predict the onset of colitis. There are few reports regarding the use of new novel markers to diagnose IMC. In one report by Callahan *et al*<sup>[66]</sup> patients with melanoma treated with ipilimumab who developed colitis had higher on-treatment serum concentrations of interleukin 17 compared with those without colitis. Another study reported that an increase in the number of eosinophils from baseline after treatment was associated with the advent of irAEs<sup>[67]</sup>. Whether these are predictors of who will develop colitis on treatment or just markers of active inflammation is still unclear. Plasma CRP and albumin may be helpful but again can be affected by other systemic inflammatory processes<sup>[68]</sup>. ICI-induced enterocolitis is one of the most commonly occurring irAEs, but the differential diagnosis for diarrhea in ICI-treated patients should also include other irAEs that manifest as diarrhea, such as ICI-induced hyperthyroidism or celiac disease.

## MANAGEMENT

Optimal management of immune-mediated enterocolitis requires early recognition and timely use of immunosuppressive agents which are chosen based on the severity of the colitis as determined by the CTCAE<sup>[69]</sup>. For mild grade 1 diarrhea, defined as less than 4 stools per day above baseline, patients may continue ICI therapy with symptomatic treatment of diarrhea with loperamide and electrolyte repletion<sup>[70]</sup>. For grade 2 diarrhea or colitis, defined as 4 to 6 stools per day above baseline, abdominal pain, or blood or mucus in the stool, infectious causes must be ruled out prior to initiation of immunosuppressive agents. Checkpoint inhibitor therapy should be withheld, and oral corticosteroids initiated at 0.5-1 mg/kg per day if symptoms persist for > 1 wk and tapered over 1 mo-2 mo<sup>[71]</sup>.

For severe grade 3 or 4 toxicity, defined as  $\geq 7$  stools above baseline per day or the presence of peritoneal signs with an ileus and fever consistent with bowel perforation, checkpoint inhibitor therapy should be permanently discontinued. Patients with grade 3 or 4 toxicity are usually hospitalized for intravenous fluid resuscitation and expedited initial workup and treatment. Systemic corticosteroids are initiated at 1-2 mg/kg per day (prednisone or equivalent) once bowel perforation is excluded and *Clostridium difficile* infection is ruled out.

Overall, one-third to two-thirds of patients either do not respond to high-dose intravenous steroids, or have a relapse requiring an increase in the corticosteroid dosage during the course of steroid tapering<sup>[53]</sup>. In a recent study of 92 patients who developed diarrhea/colitis on immunotherapy for melanoma or lung cancer, in 54 (56%) episodes, patients had corticosteroid-refractory colitis<sup>[56]</sup>. Recent studies suggest that the presence of colonic ulcers on endoscopic exam of patients with ipilimumab-induced colitis predict a steroid refractory course<sup>[56,61,62]</sup>. One study of 92 patients with ICI-colitis requiring corticosteroid therapy found that ulcers, pancolitis, and high Mayo scores or high van der Heide scores, which assess the severity of colitis based on endoscopic features, predict the likelihood that patients may be steroid-refractory and need infliximab<sup>[56,72,73]</sup>. Patients that require infliximab usually have an excellent response. If there is no improvement in symptoms within 3 d to 5 d of high-dose steroids, then immunosuppressive therapy with the anti-TNF $\alpha$  inhibitor infliximab is started at 5 mg/kg dosed every 2 wk until resolution of symptoms<sup>[74]</sup>. There are few differences in the management guidelines established by the Society for Immunotherapy of Cancer (SITC), ASCO, and European Society of Medical Oncology (ESMO) (Table 3)<sup>[10,36,75]</sup>.

All of the clinical guidelines recommend permanently discontinuing ICIs for grade IV colitis. For grade III colitis, recommendations vary. SITC recommends resuming ICI once corticosteroid is tapered to  $\leq 10$  mg/d and the patient remains symptom-free. ASCO recommends considering permanent discontinuation of CTLA-4 agents, while PD-1 or PD-L1 agents may be restarted if the patient can recover to grade 1 or less. ESMO does not make any clear recommendations regarding resuming ICI in grade 3 diarrhea/colitis. All of the societies agree that resuming ICI may be considered in grade II diarrhea once improvement is noted.

While infliximab has been successfully used to achieve clinical resolution in many patients with ICI-induced colitis, its use may be limited in those who are refractory or those who have contraindications to anti-TNF $\alpha$  therapy, including patients with a history of latent tuberculosis or chronic carriers of hepatitis B virus<sup>[76-78]</sup>. Historically infliximab use has notably been associated with skin cancers and with lymphomas (Hodgkin's, non-Hodgkin's, and hepatosplenic T-cell) in younger populations, but



**Table 3 Management of immune checkpoint inhibitor-induced colitis and diarrhea based on Common Terminology Criteria for Adverse Events grade, as summarized by the Society for Immunotherapy of Cancer, American Society of Clinical Oncology, and European Society of Medical Oncology**

Grade of colitis	Society for Immunotherapy of Cancer recommendations	American Society of Clinical Oncology recommendations	European Society for Medical Oncology recommendations
Grade 1	Continue ICI; Close follow up within 24 h-48 h; If symptoms persist, routine stool and blood tests; Bland diet during period of acute diarrhea; Anti-diarrheal medication is optional but not highly recommended but only if infectious work-up is negative	May continue ICI or hold ICI temporarily and resume if toxicity does not exceed grade 1. Monitor for dehydration and recommend dietary changes; Expedited phone contact with patient/caregiver; May obtain gastroenterology consult for prolonged grade 1 cases	Continue ICI; If symptomatic, treat with oral fluids, loperamide, avoid high fiber and lactose in diet; If persists > 14 d treat with Prednisolone 0.5 mg/kg-1 mg/kg or oral budesonide 9 mg daily; Blood tests: FBC, UEC, LFTs, CRP, TFTs; Stool microscopy for ova and parasites, culture, viral PCR, C difficile toxin and cryptosporidia
Grade 2	Hold ICI; Outpatient stool and blood work; CRP, ESR, fecal calprotectin, lactoferrin, imaging and endoscopy are optional; If diarrhea only, observe for 2 d-3 d. If no improvement start prednisone 1 mg/kg per day (or equivalent dose of methylprednisolone); anti-diarrheal medication is not recommended; If diarrhea and colitis symptoms (abdominal pain $\pm$ blood in BM), start prednisone 1 mg/kg per day (or equivalent dose of methylprednisolone) immediately; If no improvement in 48 h, increase corticosteroid dose to prednisone 2 mg/kg per day (or equivalent dose of methylprednisolone); If patient improves, Taper corticosteroid over 4 wk-6 wk; Resume ICI when corticosteroid is tapered to $\leq 10$ mg/d and patient remains symptom-free (grade $\leq 1$ ); Continue anti-PD-1 or anti-PD-L1 monotherapy; If using combination anti-CTLA-4/anti-PD-1 immunotherapy, continue anti-PD-1 agent only; ICI dose reduction is not recommended; If colitis returns on resuming ICI: Grade $\leq 2$ : Temporarily hold ICI Grade $\geq 3$ : permanently discontinue ICI	Hold ICI until symptoms recover to grade 1 or less; Consider permanently discontinuing CTLA-4 agents; may restart PD-1, PD-L1 agents if recovers to grade 1 or less. Concurrent immunosuppressant maintenance therapy ( $< 10$ mg prednisone equivalent dose) if clinically indicated in individual cases; Supportive care with loperamide if infection ruled out; Consult gastroenterology; Administer corticosteroids starting with an initial dose of 1 mg/kg per day prednisone or equivalent; When symptoms improve to grade 1 or less, taper corticosteroids over at least 4 wk to 6 wk before resuming treatment, although resuming treatment while on low-dose corticosteroid may also be an option after an evaluation of the risks and benefits; Endoscopic evaluation to stratify patients for early treatment with infliximab based on the endoscopic findings and to determine the safety of resuming PD-1, PD-L1 therapy; Testing for stool inflammatory markers, lactoferrin, or calprotectin to differentiate functional versus inflammatory diarrhea. Calprotectin testing may also be offered to monitor treatment response; Repeat colonoscopy is optional and may be offered for cases of grade 2 or higher for disease activity monitoring to achieve complete remission, especially if there is a plan to resume ICI	Hold ICI; Symptomatic management as above; If persists > 3 d or worsens, treat with Prednisolone 0.5 mg/kg-1 mg/kg or oral budesonide 9 mg daily; Schedule colonoscopy but do not wait for colonoscopy to start therapy; Baseline testing as above; consider abdominal X-ray for signs of colitis; Contact patient every 72 h; If no improvement in 72 h or worsening or absorption concern, treat as grade 3 with IV steroid

Grade 3	Grade 3: withhold ICI; consider resuming ICI when corticosteroid is tapered to $\leq 10$ mg/d and patient remains symptom-free (grade $\leq 1$ ). Consider hospitalization; Grade 4: Permanently discontinue ICI and hospitalize; Blood and stool infection work-up, inflammatory markers, imaging, endoscopy and GI consult; Start intravenous prednisone 1-2 mg/kg per day (or equivalent dose of methylprednisolone) immediately; If patient improves, follow instructions for "If patient improves" for grade 2; If refractory or no improvement on IV corticosteroid, start prednisone 2 mg/kg per day (or equivalent dose of methylprednisolone) for 3 d; Consider other anti-inflammatory agents <i>e.g.</i> , infliximab 5 mg/kg, which can be given again after two weeks if a second dose is needed. Vedolizumab may also be used	Should consider permanently discontinuing CTLA-4 agents and may restart PD-1, PD-L1 agents if patient can recover to grade 1 or less; Should administer corticosteroids (initial dose of 1 mg/kg to 2 mg/kg per day prednisone or equivalent); Should refer to hospitalization or outpatient facility for patients with dehydration or electrolyte imbalance; If symptoms persist $\geq 3$ d to 5 d or recur after improvement, may administer IV corticosteroid or noncorticosteroid ( <i>e.g.</i> , infliximab); May offer colonoscopy in cases where patients have been on immunosuppression and may be at risk for opportunistic infections as an independent cause for diarrhea ( <i>i.e.</i> , CMV colitis) and for those who are anti-TNF or corticosteroid refractory.	Hold ICI; Hospitalization until infection excluded; Gastroenterology consultation; Treat with IV methylprednisolone 1 mg/kg-2 mg/kg; If no improvement or worsening in 72 h, treat with infliximab 5 mg/kg (if no perforation, sepsis, TB, hepatitis, NYHA III/IV CHF); can repeat 2 wk later; Colonoscopy prior to initiation of infliximab; May consider other immunosuppressants: MMF 500-1000 mg BID or tacrolimus; Consider CT abdomen and pelvis; Review diet (NPO, clear fluids, TPN); Early surgical consultation if bleeding, pain or distension
Grade 4	Should permanently discontinue all ICI treatment; Rest same as grade 3	Permanently discontinue all ICI treatment; Should admit patient when clinically indicated. Patients managed as outpatients should be very closely monitored; Should administer IV corticosteroid until symptoms improve to grade 1 and then start taper over 4 wk to 6 wk; May offer early infliximab 5 mg/kg to 10 mg/kg if symptoms are refractory to corticosteroid within 2 d to 3 d; May offer lower GI endoscopy if	No distinction from Grade 3; Taper steroids over 2 wk-4 wk if moderate symptoms and 4 wk-8 wk if severe

ICI: Immune checkpoint inhibitors; FBC: Full blood count; UEC: Urea, electrolytes, creatinine; LFTs: Liver function tests; CRP: C-reactive protein; TFTs: Thyroid function tests; PCR: Polymerase chain reaction; CTLA-4: Cytotoxic T-lymphocyte-associated antigen 4; PD-1: Programmed cell death protein 1; PD-L1: Programmed death-ligand 1; NYHA: New York Heart Association; CHF: Congestive heart failure.

more recent studies suggest that these associations are confounded by prior thiopurine use and by overall increased risk of malignancies in the IBD population whose use of infliximab is the most studied<sup>[79,80]</sup>. One recent meta-analysis of 11702 patients with cancer history who were followed for 31258 person-years found that there was no increased risk of cancer recurrence in those exposed to anti-TNF $\alpha$  agents<sup>[81]</sup>. For patients who do not respond to the first or second dose of infliximab, drug trough levels should be obtained to determine whether dose escalation would be beneficial such as in those who have low trough levels<sup>[82]</sup>. Patients with adequate drug levels are likely primary non-responders and need to switch to a different drug class.

Treatment algorithms do not currently suggest the use of any immunosuppressants other than anti-TNF $\alpha$  agents in the treatment of irAEs such as colitis. There are few published reports in the literature demonstrating the use of vedolizumab in the treatment of ICI-induced colitis<sup>[38,68,77,83]</sup>. Vedolizumab, an antibody against the  $\alpha 4\beta 7$ -integrin on the surface of CD4<sup>+</sup> T cells, is approved for the treatment of inflammatory bowel diseases. Bergqvist *et al*<sup>[68]</sup> reported on 7 cases of steroid-dependent or steroid-refractory ICI-induced colitis successfully treated with vedolizumab, which has a uniquely gut-specific mechanism of action in that it prevents T cells from binding and homing into the inflamed bowel mucosa, which can explain its efficacy in ICI-mediated enterocolitis. Vedolizumab was administered with infusions of 300 mg at time-points 0 wk, 2 wk, and 6 wk or until clinical and laboratory regression was observed<sup>[68]</sup>. Vedolizumab's gut selectivity potentially allows minimization of the potential risk for cancer progression in patients known to have metastasis involving lymph nodes<sup>[83]</sup>. Vedolizumab use is thought to mitigate the antitumor effect of ICIs to a lesser extent potentially and to have the additional benefit of not heightening the risk of secondary malignancies in an already vulnerable patient as is a potential with infliximab use. Further studies are needed to establish the role of vedolizumab in the therapeutic algorithm for ICI-induced enterocolitis.

For patients that develop a colonic perforation, with or without intra-abdominal abscess, either initially or during the course of medical treatment, emergency colectomy is indicated. A subtotal colectomy may be recommended because colonic lesions are generally extensive and segmental colonic resection is generally followed

by severe inflammation of the remaining colon in the postoperative phase<sup>[53]</sup>.

### **Colitis relapse risk associated with ICI resumption**

In one recent study, four out of six patients who had an additional infusion of ipilimumab after going into enterocolitis remission relapsed<sup>[53]</sup>. Among them, three patients required a new steroid course, including one patient who had a severe steroid-refractory relapse requiring infliximab infusion. Reintroduction of anti-CTLA4 in patients, who had previously experienced enterocolitis, poses a high risk of relapse and should be discussed on an individual basis.

### **Prevention**

To date, there have been no studies that demonstrate effective measures to prevent ICI-induced colitis. One randomized, double-blind, placebo-controlled study of 115 patients receiving ipilimumab investigated the efficacy of concomitant budesonide as prophylaxis against colitis. The authors found that prophylactic steroids did not show a benefit in the tolerability of ipilimumab and did not alter the frequency of grade  $\geq 2$  diarrhea compared to placebo<sup>[65,84]</sup>.

While rare, bowel perforation is an irAE with fatal consequences. Smith *et al*<sup>[85]</sup> noted that in a cohort of 22 patients treated with ipilimumab followed by high-dose IL-2 therapy, 3 patients experienced perforation and subsequently required emergency laparotomy. This was found to be more significant compared to 8 perforations among 1797 patients treated with IL-2 alone and compared to 4 perforations among 198 patients treated with ipilimumab alone<sup>[85]</sup>. To reduce the risk of this complication, the authors recommended that patients who have received prior anti-CTLA4 therapy, and who plan to receive treatment with IL-2, undergo diagnostic colonoscopy before initiating IL-2 to rule out chronic active colitis even in the absence of symptoms<sup>[85]</sup>.

### **Prognosis of cancer in patients with IMC**

Current theories suggest that increased irAEs predict improved response to checkpoint inhibitors and improved overall survival<sup>[62]</sup>. One study of 117 patients treated with ICIs who experienced diarrhea found that diarrhea is an independent predictor of improved survival regardless of treatment requirement, and that immunosuppressive treatment for diarrhea did not significantly affect overall survival<sup>[13]</sup>.

## **CONCLUSION**

IMC is one of the most common adverse effects associated with checkpoint inhibitors. Those who experience persistent grade 2 or above diarrhea or abdominal pain should undergo infectious work up and colonoscopy with biopsies. Endoscopic features of IMC range from normal appearing mucosa to erosions, erythema, and loss of vascular pattern, to ulcerations. Symptom severity does not correspond with endoscopic appearance. While IMC shares some clinical characteristics with inflammatory bowel disease. IMC biopsies typically demonstrate findings of acute colitis such as increased cellularity of the lamina propria, intraepithelial neutrophilic infiltrates, and crypt abscesses rather than characteristics of chronic colitis such as intraepithelial or basal lymphocytes and crypt architecture distortion. Most patients with IMC respond to corticosteroids. Patients refractory to steroids are treated with short-term biologic therapy including infliximab or the gut-specific vedolizumab. Immunotherapy may be resumed in patients with IMC who respond to treatment except for those with grade 4 colitis. Multidisciplinary collaboration among gastroenterologists, oncologists and pathologists is necessary to better characterize this immune-mediated adverse event and improve upon current standard management algorithms.

## **REFERENCES**

1. Busch W. Aus der Sitzung der medicinischen Section vom 13 November 1867. *Berlin Klin Wochenschr* 1868; 5:137.
2. Fehleisen F. Ueber die Züchtung der Erysipelkokken auf künstlichem Nährboden und ihre Übertragbarkeit auf den Menschen. *Dtsch Med Wochenschr* 1882; 8: 553-554 [DOI: [10.1055/s-0029-1196806](https://doi.org/10.1055/s-0029-1196806)]
3. McCarthy EF. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop J* 2006; 26: 154-158 [PMID: [16789469](https://pubmed.ncbi.nlm.nih.gov/16789469/)]
4. Allison JP, McIntyre BW, Bloch D. Tumor-specific antigen of murine T-lymphoma defined with monoclonal antibody. *J Immunol* 1982; 129: 2293-2300 [PMID: [6181166](https://pubmed.ncbi.nlm.nih.gov/6181166/)]
5. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science*



- 1991; **254**: 1643-1647 [PMID: [1840703](#) DOI: [10.1126/science.1840703](#)]
- 6 **Leach DR**, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996; **271**: 1734-1736 [PMID: [8596936](#) DOI: [10.1126/science.271.5256.1734](#)]
- 7 **Cancer Research Institute**. FDA Approves New Immunotherapy for Metastatic Melanoma 2011. Available from: <http://www.cancerresearch.org/news/2011/fda-approves-new-immunotherapy-for-melanoma>
- 8 **Nobel Prize Organisation**. Press release: The Nobel Prize in Physiology or Medicine 2018. Available from: <https://www.nobelprize.org/prizes/medicine/2018/press-release>
- 9 **Brahmer JR**, Lacchetti C, Thompson JA. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline Summary. *J Oncol Pract* 2018; **14**: 247-249 [PMID: [29517954](#) DOI: [10.1200/JOP.18.00005](#)]
- 10 **Haanen JBAG**, Carbone F, Robert C, Kerr KM, Peters S, Larkin J, Jordan K; ESMO Guidelines Committee. Management of toxicities from immunotherapy: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2017; **28**: iv119-iv142 [PMID: [28881921](#) DOI: [10.1093/annonc/mdx225](#)]
- 11 **Nagai H**, Muto M. Optimal management of immune-related adverse events resulting from treatment with immune checkpoint inhibitors: a review and update. *Int J Clin Oncol* 2018; **23**: 410-420 [PMID: [29516216](#) DOI: [10.1007/s10147-018-1259-6](#)]
- 12 **González-Rodríguez E**, Rodríguez-Abreu D; Spanish Group for Cancer Immuno-Biotherapy (GETICA). Immune Checkpoint Inhibitors: Review and Management of Endocrine Adverse Events. *Oncologist* 2016; **21**: 804-816 [PMID: [27306911](#) DOI: [10.1634/theoncologist.2015-0509](#)]
- 13 **Wang Y**, Abu-Sbeih H, Mao E, Ali N, Ali FS, Qiao W, Lum P, Raju G, Shuttlesworth G, Strohlein J, Diab A. Immune-checkpoint inhibitor-induced diarrhea and colitis in patients with advanced malignancies: retrospective review at MD Anderson. *J Immunother Cancer* 2018; **6**: 37 [PMID: [29747688](#) DOI: [10.1186/s40425-018-0346-6](#)]
- 14 **Moore C**, Chen I. Immunotherapy in Cancer Treatment: A Review of Checkpoint Inhibitors. *US Pharm* 2018; **43**: 27-31
- 15 **Sharpe AH**. Introduction to checkpoint inhibitors and cancer immunotherapy. *Immunol Rev* 2017; **276**: 5-8 [PMID: [28258698](#) DOI: [10.1111/imr.12531](#)]
- 16 **Chascsa DM**, Rakela J. Knowns and Unknowns: The Safety and Efficacy of Cancer Immunotherapy in Chronic Liver Disease. *Curr Hepatology Rep* 2018; **17**: 153-155 [DOI: [10.1007/s11901-018-0408-8](#)]
- 17 **Prieux-Klotz C**, Dior M, Damotte D, Dreanic J, Brieau B, Brezault C, Abitbol V, Chaussade S, Coriat R. Immune Checkpoint Inhibitor-Induced Colitis: Diagnosis and Management. *Target Oncol* 2017; **12**: 301-308 [PMID: [28540478](#) DOI: [10.1007/s11523-017-0495-4](#)]
- 18 **Rivoltini L**, Carrabba M, Huber V, Castelli C, Novellino L, Dalerba P, Mortarini R, Arancia G, Anichini A, Fais S, Parmiani G. Immunity to cancer: attack and escape in T lymphocyte-tumor cell interaction. *Immunol Rev* 2002; **188**: 97-113 [PMID: [12445284](#) DOI: [10.1034/j.1600-065X.2002.18809.x](#)]
- 19 **Melero I**, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nat Rev Cancer* 2007; **7**: 95-106 [PMID: [17251916](#) DOI: [10.1038/nrc2051](#)]
- 20 **Nagorsen D**, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U. Natural T cell immunity against cancer. *Clin Cancer Res* 2003; **9**: 4296-4303 [PMID: [14555498](#) DOI: [10.1371/journal.pone.0128244](#)]
- 21 **Tarhini A**. Immune-mediated adverse events associated with ipilimumab ctla-4 blockade therapy: the underlying mechanisms and clinical management. *Scientifica (Cairo)* 2013; **2013**: 857519 [PMID: [24278787](#) DOI: [10.1155/2013/857519](#)]
- 22 **Engelhardt JJ**, Sullivan TJ, Allison JP. CTLA-4 overexpression inhibits T cell responses through a CD28-B7-dependent mechanism. *J Immunol* 2006; **177**: 1052-1061 [PMID: [16818761](#) DOI: [10.4049/jimmunol.177.2.1052](#)]
- 23 **Wang W**, Lau R, Yu D, Zhu W, Korman A, Weber J. PD1 blockade reverses the suppression of melanoma antigen-specific CTL by CD4+ CD25(Hi) regulatory T cells. *Int Immunol* 2009; **21**: 1065-1077 [PMID: [19651643](#) DOI: [10.1093/intimm/dxp072](#)]
- 24 **Weber J**. Ipilimumab: controversies in its development, utility and autoimmune adverse events. *Cancer Immunol Immunother* 2009; **58**: 823-830 [PMID: [19198837](#) DOI: [10.1007/s00262-008-0653-8](#)]
- 25 **Robert C**, Ghiringhelli F. What is the role of cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma? *Oncologist* 2009; **14**: 848-861 [PMID: [19648604](#) DOI: [10.1634/theoncologist.2009-0028](#)]
- 26 **Kaehler KC**, Piel S, Livingstone E, Schilling B, Hauschild A, Schadendorf D. Update on immunologic therapy with anti-CTLA-4 antibodies in melanoma: identification of clinical and biological response patterns, immune-related adverse events, and their management. *Semin Oncol* 2010; **37**: 485-498 [PMID: [21074064](#) DOI: [10.1053/j.seminoncol.2010.09.003](#)]
- 27 **Oble DA**, Mino-Kenudson M, Goldsmith J, Hodi FS, Seliem RM, Dranoff G, Mihm M, Hasserjian R, Lauwers GY. Alpha-CTLA-4 mAb-associated panenteritis: a histologic and immunohistochemical analysis. *Am J Surg Pathol* 2008; **32**: 1130-1137 [PMID: [18545145](#) DOI: [10.1097/PAS.0b013e31817150e3](#)]
- 28 **Della Vittoria Scarpati G**, Fusciello C, Perri F, Sabbatino F, Ferrone S, Carlomagno C, Pepe S. Ipilimumab in the treatment of metastatic melanoma: management of adverse events. *Onco Targets Ther* 2014; **7**: 203-209 [PMID: [24570590](#) DOI: [10.2147/OTT.S57335](#)]
- 29 **Bourke JM**, O'Sullivan M, Khattak MA. Management of adverse events related to new cancer immunotherapy (immune checkpoint inhibitors). *Med J Aust* 2016; **205**: 418-424 [PMID: [27809739](#) DOI: [10.5694/mja16.00586](#)]
- 30 **Brunkow ME**, Jeffery EW, Hjerrild KA, Paepel B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001; **27**: 68-73 [PMID: [11138001](#) DOI: [10.1038/83784](#)]
- 31 **Read S**, Malmström V, Powrie F. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25(+)CD4(+) regulatory cells that control intestinal inflammation. *J Exp Med* 2000; **192**: 295-302 [PMID: [10899916](#) DOI: [10.1084/jem.192.2.295](#)]
- 32 **Walker LS**. Treg and CTLA-4: two intertwining pathways to immune tolerance. *J Autoimmun* 2013; **45**: 49-57 [PMID: [23849743](#) DOI: [10.1016/j.jaut.2013.06.006](#)]
- 33 **Beck KE**, Blansfield JA, Tran KQ, Feldman AL, Hughes MS, Royal RE, Kammula US, Topalian SL, Sherry RM, Kleiner D, Quezado M, Lowy I, Yellin M, Rosenberg SA, Yang JC. Enterocolitis in patients

- with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol* 2006; **24**: 2283-2289 [PMID: [16710025](#) DOI: [10.1200/JCO.2005.04.5716](#)]
- 34 **Guthery SL**, Heubi JE, Filipovich A. Enteral metronidazole for the prevention of graft versus host disease in pediatric marrow transplant recipients: results of a pilot study. *Bone Marrow Transplant* 2004; **33**: 1235-1239 [PMID: [15077127](#) DOI: [10.1038/sj.bmt.1704474](#)]
- 35 **Postow MA**. Managing immune checkpoint-blocking antibody side effects. *Am Soc Clin Oncol Educ Book* 2015; **35**: 76-83 [PMID: [25993145](#) DOI: [10.14694/EdBook\\_AM.2015.35.76](#)]
- 36 **Puzanov I**, Diab A, Abdallah K, Bingham CO, Brogdon C, Dadu R, Hamad L, Kim S, Lacouture ME, LeBoeuf NR, Lenihan D, Onofrei C, Shannon V, Sharma R, Silk AW, Skondra D, Suarez-Almazor ME, Wang Y, Wiley K, Kaufman HL, Ernstoff MS; Society for Immunotherapy of Cancer Toxicity Management Working Group. Managing toxicities associated with immune checkpoint inhibitors: consensus recommendations from the Society for Immunotherapy of Cancer (SITC) Toxicity Management Working Group. *J Immunother Cancer* 2017; **5**: 95 [PMID: [29162153](#) DOI: [10.1186/s40425-017-0300-z](#)]
- 37 **Kumar V**, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Current Diagnosis and Management of Immune Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitor Therapy. *Front Pharmacol* 2017; **8**: 49 [DOI: [10.3389/fphar.2017.00049](#)]
- 38 **Abu-Sbeih H**, Ali FS, Alsaadi D, Jennings J, Luo W, Gong Z, Richards DM, Charabaty A, Wang Y. Outcomes of vedolizumab therapy in patients with immune checkpoint inhibitor-induced colitis: a multicenter study. *J Immunother Cancer* 2018; **6**: 142 [PMID: [30518410](#) DOI: [10.1186/s40425-018-0461-4](#)]
- 39 **Graziani G**, Tentori L, Navarra P. Ipilimumab: a novel immunostimulatory monoclonal antibody for the treatment of cancer. *Pharmacol Res* 2012; **65**: 9-22 [PMID: [21930211](#) DOI: [10.1016/j.phrs.2011.09.002](#)]
- 40 **Gupta A**, De Felice KM, Loftus EV, Khanna S. Systematic review: colitis associated with anti-CTLA-4 therapy. *Aliment Pharmacol Ther* 2015; **42**: 406-417 [PMID: [26079306](#) DOI: [10.1111/apt.13281](#)]
- 41 **Hofmann L**, Forschner A, Loquai C, Goldinger SM, Zimmer L, Ugurel S, Schmidgen MI, Gutzmer R, Utikal JS, Göppner D, Hassel JC, Meier F, Tietze JK, Thomas I, Weishaupt C, Leverkus M, Wahl R, Dietrich U, Garbe C, Kirchberger MC, Eigentler T, Berking C, Gesierich A, Krackhardt AM, Schadendorf D, Schuler G, Dummer R, Heinzerling LM. Cutaneous, gastrointestinal, hepatic, endocrine, and renal side-effects of anti-PD-1 therapy. *Eur J Cancer* 2016; **60**: 190-209 [PMID: [27085692](#) DOI: [10.1016/j.ejca.2016.02.025](#)]
- 42 **Naidoo J**, Page DB, Li BT, Connell LC, Schindler K, Lacouture ME, Postow MA, Wolchok JD. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. *Ann Oncol* 2016; **27**: 1362 [PMID: [27072927](#) DOI: [10.1093/annonc/mdw141](#)]
- 43 **Kwon ED**, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, Krainer M, Houede N, Santos R, Mahammedi H, Ng S, Maio M, Franke FA, Sundar S, Agarwal N, Bergman AM, Ciuleanu TE, Korbenfeld E, Sengeløv L, Hansen S, Logothetis C, Beer TM, McHenry MB, Gagnier P, Liu D, Gerritsen WR; CA184-043 Investigators. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol* 2014; **15**: 700-712 [PMID: [24831977](#) DOI: [10.1016/S1470-2045\(14\)70189-5](#)]
- 44 **Slovin SF**, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, Scher HI, Chin K, Gagnier P, McHenry MB, Beer TM. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. *Ann Oncol* 2013; **24**: 1813-1821 [PMID: [23535954](#) DOI: [10.1093/annonc/mdt107](#)]
- 45 **Kumar V**, Chaudhary N, Garg M, Floudas CS, Soni P, Chandra AB. Corrigendum: Current Diagnosis and Management of Immune Related Adverse Events (irAEs) Induced by Immune Checkpoint Inhibitor Therapy. *Front Pharmacol* 2017; **8**: 49 [DOI: [10.3389/fphar.2017.00311](#)]
- 46 **Larkin J**, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, Ferrucci PF, Hill A, Wagstaff J, Carlino MS, Haanen JB, Maio M, Marquez-Rodas I, McArthur GA, Ascierto PA, Long GV, Callahan MK, Postow MA, Grossmann K, Sznol M, Dreno B, Bastholt L, Yang A, Rollin LM, Horak C, Hodi FS, Wolchok JD. Combined nivolumab and ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* 2015; **373**: 23-34 [PMID: [26027431](#) DOI: [10.1056/NEJMoa1504030](#)]
- 47 **Hodi FS**, Chesney J, Pavlick AC, Robert C, Grossmann KF, McDermott DF, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor DR, Salama AK, Taylor MH, Ott PA, Horak C, Gagnier P, Jiang J, Wolchok JD, Postow MA. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol* 2016; **17**: 1558-1568 [PMID: [27622997](#) DOI: [10.1016/S1470-2045\(16\)30366-7](#)]
- 48 **Postow MA**, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, Linette GP, Meyer N, Giguere JK, Agarwala SS, Shaheen M, Ernstoff MS, Minor D, Salama AK, Taylor M, Ott PA, Rollin LM, Horak C, Gagnier P, Wolchok JD, Hodi FS. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med* 2015; **372**: 2006-2017 [PMID: [25891304](#) DOI: [10.1056/NEJMoa1414428](#)]
- 49 **Dubin K**, Callahan MK, Ren B, Khanin R, Viale A, Ling L, No D, Gobourne A, Littmann E, Huttenhower C, Pamer EG, Wolchok JD. Intestinal microbiome analyses identify melanoma patients at risk for checkpoint-blockade-induced colitis. *Nat Commun* 2016; **7**: 10391 [PMID: [26837003](#) DOI: [10.1038/ncomms10391](#)]
- 50 **Chaput N**, Lepage P, Coutzac C, Soularue E, Le Roux K, Monot C, Boselli L, Routier E, Cassard L, Collins M, Vaysse T, Marthey L, Eggermont A, Asvatourian V, Lanoy E, Mateus C, Robert C, Carbonnel F. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol* 2017; **28**: 1368-1379 [PMID: [28368458](#) DOI: [10.1093/annonc/mdx108](#)]
- 51 **Menzies AM**, Johnson DB, Ramanujam S, Atkinson VG, Wong ANM, Park JJ, McQuade JL, Shoushtari AN, Tsai KK, Eroglu Z, Klein O, Hassel JC, Sosman JA, Guminski A, Sullivan RJ, Ribas A, Carlino MS, Davies MA, Sandhu SK, Long GV. Anti-PD-1 therapy in patients with advanced melanoma and preexisting autoimmune disorders or major toxicity with ipilimumab. *Ann Oncol* 2017; **28**: 368-376 [PMID: [27687304](#) DOI: [10.1093/annonc/mdw443](#)]
- 52 **Bowyer S**, Prithviraj P, Lorigan P, Larkin J, McArthur G, Atkinson V, Millward M, Khou M, Diem S, Ramanujam S, Kong B, Liniker E, Guminski A, Parente P, Andrews MC, Parakh S, Cebon J, Long GV, Carlino MS, Klein O. Efficacy and toxicity of treatment with the anti-CTLA-4 antibody ipilimumab in patients with metastatic melanoma after prior anti-PD-1 therapy. *Br J Cancer* 2016; **114**: 1084-1089 [PMID: [27124339](#) DOI: [10.1038/bjc.2016.107](#)]
- 53 **Marthey L**, Mateus C, Mussini C, Nachury M, Nancey S, Grange F, Zallot C, Peyrin-Biroulet L, Rahier

- JF, Bourdier de Beauregard M, Mortier L, Coutzac C, Soularue E, Lanoy E, Kapel N, Planchard D, Chaput N, Robert C, Carbonnel F. Cancer Immunotherapy with Anti-CTLA-4 Monoclonal Antibodies Induces an Inflammatory Bowel Disease. *J Crohns Colitis* 2016; **10**: 395-401 [PMID: [26783344](#) DOI: [10.1093/ecco-jcc/jjv227](#)]
- 54 **McCutcheon JL**, McClain CM, Puzanov I, Smith TA. Infectious Colitis Associated With Ipilimumab Therapy. *Gastroenterology Res* 2014; **7**: 28-31 [PMID: [27785266](#) DOI: [10.14740/gr594e](#)]
- 55 **Lankes K**, Hundorfean G, Harrer T, Pommer AJ, Agaimy A, Angelovska I, Tajmir-Riahi A, Göhl J, Schuler G, Neurath MF, Hohenberger W, Heinzerling L. Anti-TNF-refractory colitis after checkpoint inhibitor therapy: Possible role of CMV-mediated immunopathogenesis. *Oncoimmunology* 2016; **5**: e1128611 [PMID: [27471608](#) DOI: [10.1080/2162402X.2015.1128611](#)]
- 56 **Geukes Foppen MH**, Rozeman EA, van Wilpe S, Postma C, Snaebjornsson P, van Thienen JV, van Leerdam ME, van den Heuvel M, Blank CU, van Dieren J, Haanen JBAG. Immune checkpoint inhibition-related colitis: symptoms, endoscopic features, histology and response to management. *ESMO Open* 2018; **3**: e000278 [PMID: [29387476](#) DOI: [10.1136/esmoopen-2017-000278](#)]
- 57 **Dilling P**, Walczak J, Pikiel P, Kruszezski WJ. Multiple colon perforation as a fatal complication during treatment of metastatic melanoma with ipilimumab - case report. *Pol Przegl Chir* 2014; **86**: 94-96 [PMID: [24670341](#) DOI: [10.2478/pjs-2014-0017](#)]
- 58 **Burdine L**, Lai K, Laryea JA. Ipilimumab-induced colonic perforation. *J Surg Case Rep* 2014; **2014**: pii: rju010 [PMID: [24876393](#) DOI: [10.1093/jscr/rju010](#)]
- 59 **Shah R**, Witt D, Asif T, Mir FF. Ipilimumab as a Cause of Severe Pan-Colitis and Colonic Perforation. *Cureus* 2017; **9**: e1182 [PMID: [28533998](#) DOI: [10.7759/cureus.1182](#)]
- 60 **Verschuren EC**, van den Eertwegh AJ, Wonders J, Slangen RM, van Delft F, van Bodegraven A, Neefjes-Borst A, de Boer NK. Clinical, Endoscopic, and Histologic Characteristics of Ipilimumab-Associated Colitis. *Clin Gastroenterol Hepatol* 2016; **14**: 836-842 [PMID: [26748223](#) DOI: [10.1016/j.cgh.2015.12.028](#)]
- 61 **Jain A**, Lipson EJ, Sharfman WH, Brant SR, Lazarev MG. Colonic ulcerations may predict steroid-refractory course in patients with ipilimumab-mediated enterocolitis. *World J Gastroenterol* 2017; **23**: 2023-2028 [PMID: [28373768](#) DOI: [10.3748/wjg.v23.i11.2023](#)]
- 62 **Wang Y**, Abu-Sbeih H, Mao E, Ali N, Qiao W, Trinh VA, Zobniw C, Johnson DH, Samdani R, Lum P, Shuttlesworth G, Blechacz B, Bresalier R, Miller E, Thirumurthi S, Richards D, Raju G, Stroehlein J, Diab A. Endoscopic and Histologic Features of Immune Checkpoint Inhibitor-Related Colitis. *Inflamm Bowel Dis* 2018; **24**: 1695-1705 [PMID: [29718308](#) DOI: [10.1093/ibd/izy104](#)]
- 63 **Coutzac C**, Adam J, Soularue E, Collins M, Racine A, Mussini C, Boselli L, Kamsukom N, Mateus C, Charrier M, Cassard L, Planchard D, Ribrag V, Fizazi K, Lorient Y, Lepage P, Scoazec JY, Robert C, Carbonnel F, Chaput N. Colon Immune-Related Adverse Events: Anti-CTLA-4 and Anti-PD-1 Blockade Induce Distinct Immunopathological Entities. *J Crohns Colitis* 2017; **11**: 1238-1246 [PMID: [28967957](#) DOI: [10.1093/ecco-jcc/jjx081](#)]
- 64 **Poullis A**, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. *J Gastroenterol Hepatol* 2003; **18**: 756-762 [PMID: [12795745](#) DOI: [10.1046/j.1440-1746.2003.03014.x](#)]
- 65 **Berman D**, Parker SM, Siegel J, Chasalow SD, Weber J, Galbraith S, Targan SR, Wang HL. Blockade of cytotoxic T-lymphocyte antigen-4 by ipilimumab results in dysregulation of gastrointestinal immunity in patients with advanced melanoma. *Cancer Immunol* 2010; **10**: 11 [PMID: [21090563](#)]
- 66 **Callahan MK**, Yang A, Tandon S, Xu Y, Subudhi SK, Roman RA. Evaluation of serum IL-17 levels during ipilimumab therapy: correlation with colitis. *J Clin Oncol* 2011; **29**: 2505 [DOI: [10.1200/jco.2011.29.15\\_suppl.2505](#)]
- 67 **Schindler K**, Harmankaya K, Kuk D, Mangana J, Michielin O, Hoeller C, Dummer R, Pehamberger H, Wolchok JD, Postow A. Correlation of absolute and relative eosinophil counts with immune-related adverse events in melanoma patients treated with ipilimumab. *J Clin Oncol* 2014; **32**: 9096-9096 [DOI: [10.1200/jco.2014.32.15\\_suppl.9096](#)]
- 68 **Bergqvist V**, Hertervig E, Gedeon P, Kopljär M, Griph H, Kinhult S, Carneiro A, Marsal J. Vedolizumab treatment for immune checkpoint inhibitor-induced enterocolitis. *Cancer Immunol Immunother* 2017; **66**: 581-592 [PMID: [28204866](#) DOI: [10.1007/s00262-017-1962-6](#)]
- 69 **Linardou H**, Gogas H. Toxicity management of immunotherapy for patients with metastatic melanoma. *Ann Transl Med* 2016; **4**: 272 [PMID: [27563659](#) DOI: [10.21037/atm.2016.07.10](#)]
- 70 **Weber JS**, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol* 2012; **30**: 2691-2697 [PMID: [22614989](#) DOI: [10.1200/JCO.2012.41.6750](#)]
- 71 **Food and Drug Administration**. Highlights of prescribing information. 2011; 1–20 Available from: [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2011/125377s00001bl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/125377s00001bl.pdf)
- 72 **Schroeder KW**, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; **317**: 1625-1629 [PMID: [3317057](#) DOI: [10.1056/NEJM198712243172603](#)]
- 73 **van der Heide H**, van den Brandt-Gradel V, Tytgat GN, Endert E, Wiltink EH, Schipper ME, Dekker W. Comparison of beclomethasone dipropionate and prednisolone 21-phosphate enemas in the treatment of ulcerative proctitis. *J Clin Gastroenterol* 1988; **10**: 169-172 [PMID: [3047215](#) DOI: [10.1097/00004836-198804000-00013](#)]
- 74 **Johnston RL**, Lutzky J, Chodhry A, Barkin JS. Cytotoxic T-lymphocyte-associated antigen 4 antibody-induced colitis and its management with infliximab. *Dig Dis Sci* 2009; **54**: 2538-2540 [PMID: [19104936](#) DOI: [10.1007/s10620-008-0641-z](#)]
- 75 **Brahmer JR**, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, Chau I, Ernstoff MS, Gardner JM, Ginex P, Hallmeyer S, Holter Chakrabarty J, Leighl NB, Mammen JS, McDermott DF, Naing A, Nastoupil LJ, Phillips T, Porter LD, Puzanov I, Reichner CA, Santomaso BD, Seigel C, Spira A, Suarez-Almazor ME, Wang Y, Weber JS, Wolchok JD, Thompson JA; National Comprehensive Cancer Network. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2018; **36**: 1714-1768 [PMID: [29442540](#) DOI: [10.1200/JCO.2017.77.6385](#)]
- 76 **Hodi FS**, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Uria WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010; **363**: 711-723 [PMID: [20525992](#) DOI: [10.1056/NEJMoa1003466](#)]

- 77 **Robert C**, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, Hassel JC, Rutkowski P, McNeil C, Kalinka-Warzocha E, Savage KJ, Hernberg MM, Lebbé C, Charles J, Mihalcioiu C, Chiarion-Sileni V, Mauch C, Cognetti F, Arance A, Schmidt H, Schadendorf D, Gogas H, Lundgren-Eriksson L, Horak C, Sharkey B, Waxman IM, Atkinson V, Ascierto PA. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015; **372**: 320-330 [PMID: [25399552](#) DOI: [10.1056/NEJMoa1412082](#)]
- 78 **Rastogi P**, Sultan M, Charabaty AJ, Atkins MB, Mattar MC. Ipilimumab associated colitis: an IpiColitis case series at MedStar Georgetown University Hospital. *World J Gastroenterol* 2015; **21**: 4373-4378 [PMID: [25892889](#) DOI: [10.3748/wjg.v21.i14.4373](#)]
- 79 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Langholff W, Londhe A, Sandborn WJ. Drug therapies and the risk of malignancy in Crohn's disease: results from the TREAT<sup>TM</sup> Registry. *Am J Gastroenterol* 2014; **109**: 212-223 [PMID: [24394749](#) DOI: [10.1038/ajg.2013.441](#)]
- 80 **Cohn HM**, Dave M, Loftus EV. Understanding the Cautions and Contraindications of Immunomodulator and Biologic Therapies for Use in Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2017; **23**: 1301-1315 [PMID: [28708806](#) DOI: [10.1097/MIB.0000000000001199](#)]
- 81 **Shelton E**, Laharie D, Scott FI, Mamtani R, Lewis JD, Colombel JF, Ananthakrishnan AN. Cancer Recurrence Following Immune-Suppressive Therapies in Patients With Immune-Mediated Diseases: A Systematic Review and Meta-analysis. *Gastroenterology* 2016; **151**: 97-109.e4 [PMID: [27039969](#) DOI: [10.1053/j.gastro.2016.03.037](#)]
- 82 **Diana P**, Mankongpaisarnrungs C, Atkins MB, Zeck JC, Charabaty A. Emerging Role of Vedolizumab in Managing Refractory Immune Checkpoint Inhibitor-Induced Enteritis. *ACG Case Rep J* 2018; **5**: e17 [PMID: [29516018](#) DOI: [10.14309/crj.2018.17](#)]
- 83 **Hsieh AH**, Ferman M, Brown MP, Andrews JM. Vedolizumab: a novel treatment for ipilimumab-induced colitis. *BMJ Case Rep* 2016; **2016**: pii: bcr2016216641 [PMID: [27539137](#) DOI: [10.1136/bcr-2016-216641](#)]
- 84 **Weber J**, Thompson JA, Hamid O, Minor D, Amin A, Ron I, Ridolfi R, Assi H, Maraveyas A, Berman D, Siegel J, O'Day SJ. A randomized, double-blind, placebo-controlled, phase II study comparing the tolerability and efficacy of ipilimumab administered with or without prophylactic budesonide in patients with unresectable stage III or IV melanoma. *Clin Cancer Res* 2009; **15**: 5591-5598 [PMID: [19671877](#) DOI: [10.1158/1078-0432.CCR-09-1024](#)]
- 85 **Smith FO**, Goff SL, Klapper JA, Levy C, Allen T, Mavroukakis SA, Rosenberg SA. Risk of bowel perforation in patients receiving interleukin-2 after therapy with anti-CTLA 4 monoclonal antibody. *J Immunother* 2007; **30**: 130 [PMID: [17198092](#) DOI: [10.1097/01.cji.0000211334.06762.89](#)]

**P- Reviewer:** de Souza HSP, Hughes PAA, Martin-Villa JM

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Tan WW





## Basic Study

**Formalin fixation on HER-2 and PD-L1 expression in gastric cancer: A pilot analysis using the same surgical specimens with different fixation times**

Keita Kai, Yukie Yoda, Atsushi Kawaguchi, Akimichi Minesaki, Hironori Iwasaki, Shinichi Aishima, Hirokazu Noshiro

**ORCID number:** Keita Kai (0000-0003-1553-2598); Yukie Yoda (0000-0003-1469-3592); Atsushi Kawaguchi (0000-0002-8911-3321); Akimichi Minesaki (0000-0002-2127-9704); Hironori Iwasaki (0000-0001-7580-1265); Shinichi Aishima (0000-0002-1448-6510); Hirokazu Noshiro (0000-0003-3227-7816).

**Author contributions:** Kai K conceived and designed the study. Kai K, Yoda Y, Kawaguchi A, Minesaki A, Iwasaki H, Aishima S and Noshiro H researched and analyzed data, and wrote, edited and reviewed the manuscript. The manuscript is an original work of all authors. All authors gave final approval for publication. Kai K takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript.

**Institutional review board**

**statement:** This study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine at Saga University (approval no. 29-74)

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

**Keita Kai, Shinichi Aishima,** Department of Pathology, Saga University Hospital, Saga 849-8501, Japan

**Yukie Yoda, Hironori Iwasaki, Hirokazu Noshiro,** Department of Surgery, Saga University Faculty of Medicine, Saga 849-8501, Japan

**Atsushi Kawaguchi,** Center for Comprehensive Community Medicine, Saga University Faculty of Medicine, Saga 849-8501, Japan

**Akimichi Minesaki, Shinichi Aishima,** Department of Pathology and Microbiology, Saga University Faculty of Medicine, Saga 849-8501, Japan

**Akimichi Minesaki,** Department of Otolaryngology - Head and Neck Surgery, Saga University Faculty of Medicine, Saga 849-8501, Japan

**Corresponding author:** Keita Kai, MD, PhD, Associate Professor, Department of Pathology, Saga University Hospital, 5-1-1 Nabeshima, Saga City, Saga 849-8501, Japan.

[kaikeit@cc.saga-u.ac.jp](mailto:kaikeit@cc.saga-u.ac.jp)

**Telephone:** +81-952-343264

**Fax:** +81-952-342055

**Abstract****BACKGROUND**

The needs for human epidermal growth factor receptor 2 (HER-2) and/or programmed death-ligand 1 (PD-L1) evaluations in gastric cancer are dramatically increasing. Although the importance of standardization of sample fixation has been widely recognized, most of the evidence regarding the fixation duration or type of fixing solution are based on breast cancer.

**AIM**

To investigate the real effects of fixation conditions on HER-2 testing or PD-L1 testing for gastric cancer using gastrectomy specimens.

**METHODS**

Thirty-two patients who underwent gastrectomy for gastric cancer were enrolled. Their resected specimens were each divided into four pieces and fixed in four strictly controlled different durations (6 h, 24 h, and 48 h, and 1 wk) by 10% formalin ( $n = 22$ ) or 10% neutral buffered formalin (NBF) ( $n = 10$ ).



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

**Received:** November 25, 2018

**Peer-review started:** November 26, 2018

**First decision:** December 15, 2018

**Revised:** December 24, 2018

**Accepted:** December 29, 2018

**Article in press:** December 30, 2018

**Published online:** February 26, 2019

Immunohistochemistry (IHC) of HER-2 and PD-1 was performed, and a pathology examination was conducted. In the HER-2-immunoreactive cases, all four specimens were subjected to dual-color in situ hybridization (DISH). Five cases were assessed as HER-2-positive by IHC and DISH. We used the cut-off values of 1%, 10%, and 50% to assess the IHC findings of PD-L1.

## RESULTS

No significant difference was observed in comparisons between the shorter fixation period groups (6 h, 24 h, and 48 h) and the prolonged fixation period (1 wk) group in the HER-2 and PD-L1 analyses. Although no significant difference was observed between 10% formalin and 10% NBF within 1 wk of fixation, the superiority of 10% NBF was confirmed in a long-term (> 3 mo) fixation in both the HER-2 and PD-L1 analyses.

## CONCLUSION

In this small-numbered pilot study, prolonged fixation within 1 wk showed no inferiority in HER-2 or PD-L1 testing. However, a large-numbered prospective study is needed to obtain conclusive results.

**Key words:** Gastric cancer; Programmed death-ligand 1; Human epidermal growth factor receptor 2; Neutral buffered formalin; Fixation time

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** We prospectively investigated the real effects of fixation conditions on human epidermal growth factor receptor 2 (HER-2) or programmed death-ligand 1 (PD-L1) testing for gastric cancer using 32 cases of gastrectomy specimens. We analyzed these resected specimens dividing into four pieces and fixed in four strictly controlled different durations (6 h, 24 h, and 48 h, and 1 wk) by 10% formalin or 10% neutral buffered formalin. In this small-numbered pilot study, the prolonged fixation within 1 wk showed no inferiority in HER-2 or PD-L1 testing. These results will provide supporting information for the interpretation of HER-2 and PD-L1 testing for prolonged fixation cases due to unavoidable circumstances.

**Citation:** Kai K, Yoda Y, Kawaguchi A, Minesaki A, Iwasaki H, Aishima S, Noshiro H. Formalin fixation on HER-2 and PD-L1 expression in gastric cancer: A pilot analysis using the same surgical specimens with different fixation times. *World J Clin Cases* 2019; 7(4): 419-430

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/419.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.419>

## INTRODUCTION

After the results of 2010 international prospective randomized phase III trial (the ToGA study)<sup>[1]</sup>, trastuzumab therapy combined with chemotherapy became the standard treatment for human epidermal growth factor receptor 2 (HER-2)-positive advanced gastric cancer. The needs for HER-2 evaluations in gastric cancer by immunohistochemistry (IHC) and/or in situ hybridization (ISH) has dramatically increased and the importance of standardization of HER-2 testing has been widely recognized.

It was noted that fixation procedures affect the results of HER-2 testing<sup>[2]</sup>. The updated 2013 American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guideline recommends the fixation of HER-2 testing specimens in 10% neutral buffered formalin (NBF) for 6 to 72 h<sup>[3,4]</sup>. However, in some cases it may be difficult to strictly control the formalin fixation time due to institutional constraints, and the fixation time may exceed 72 h. Moreover, some institutions do not use 10% NBF as a standard fixation solution. Even though some institutions strictly control the formalin fixation times and use 10% NBF in their routine work, it is sometimes necessary to perform HER-2 testing using previous formalin-fixed paraffin-embedded (FFPE) gastric cancer tissues for which the fixation solution and/or duration have not been managed.

There is not enough evidence regarding the interpretation of the results of HER-2 testing for gastric cancer conducted using fixed solutions other than 10% NBF or prolonged-fixation specimens. Most of the evidence regarding the relationship between HER-2 testing and the fixation duration or type of fixing solution was obtained in studies of breast cancer<sup>[5-11]</sup>. Although it seems reasonable that the findings obtained for breast cancer may be applicable to gastric cancer, the data that are specific to gastric cancer are needed, because characteristics of HER-2 overexpression (*e.g.*, positivity and heterogeneity) are quite different between breast cancer and gastric cancer<sup>[12-14]</sup>. We could find only one study using a xenograft model of gastric cancer cell lines<sup>[15]</sup> and a single clinical study of gastric cancer<sup>[16]</sup> that analyzed the association between HER-2 test results and the formalin fixation status.

A significant therapeutic effect of a humanized monoclonal antibody against programmed death-1 (PD-1) was confirmed for non-small-cell lung cancer (NSCLC), which has shown a high level of programmed death-ligand 1 (PD-L1) expression<sup>[17]</sup>, after which the need for the immunohistochemical evaluations of PD-L1 in NSCLC specimens has greatly increased. In gastric cancer, a multicenter, open-label, phase 1b trial recently reported the safety and efficacy of a humanized monoclonal antibody against PD-1 for patients with PD-L1-positive advanced gastric cancer<sup>[18]</sup>. Therefore, although the utility of PD-L1 IHC for the prediction of the effectiveness of anti-PD-1 therapy is under investigation<sup>[19]</sup>, it is expected that the need for PD-L1 IHC in gastric cancer will increase in the near future.

In the present study, we sought to verify the effect of the duration of formalin fixation on both HER-2 and PD-L1 testing, using surgically resected gastric cancer specimens. We also attempted to determine whether prolonged fixation (we considered 1 wk as an appropriate maximum prolonged-fixation duration in a daily pathology practice) is truly inferior to a more standard fixation period. We also examined the differences between the uses of 10% NBF and 10% formalin in HER-2 and PD-L1 testing.

## MATERIALS AND METHODS

### *Patients and formalin fixation of resected specimens*

This study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine at Saga University (approval No. 29-74), and written informed consent for the study was obtained from all of the patients. From 2014 to 2016, a total of 32 consecutive patients with gastric cancer clinically diagnosed as a Type 2 or Type 3 tumor and who underwent a gastrectomy without neoadjuvant chemotherapy were enrolled in this study. The resected tumor specimen from each patient was immediately treated by a pathologist (K.K.). Each patient's specimen was divided into four pieces and fixed by fixing solution for a strictly controlled duration (6 h, 24 h, 48 h, or 1 wk) (Figure 1). For the first 22 cases, 10% formalin was used as the fixing solution. For the remaining 10 cases, 10% NBF was used.

### *IHC and dual-color in situ hybridization (DISH) for HER-2 expression*

Paraffin-embedded specimens were sectioned at a thickness of 3 µm and subjected to IHC and DISH. The HER-2 IHC was performed using the HercepTest II (Agilent Technologies, Santa Clara, CA) on an Autostainer Link 48 platform (Agilent Technologies) per the manufacturer's instructions. We outsourced the DISH to a clinical test company (BML, Kurume Laboratory, Saitama, Japan) and they performed DISH using a Ventana INFORM Dual ISH HER2 kit (Roche Diagnostics, Tokyo).

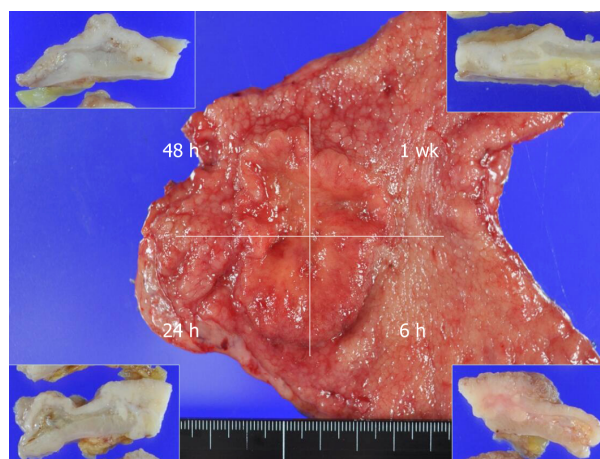
### *Evaluation of HER-2 expression*

Our evaluation of the IHC results (score 0, 1+, 2+, and 3+) and DISH results were based on the Updated 2013 ASCO/CAP guideline<sup>[3,4]</sup>. If at least one specimen was score 1+ or more for all four specimen-pieces with different fixation times, all four pieces were subjected to the DISH analysis.

The assessment of the HER-2 IHC and DISH results was performed by consensus of two pathologists (K.K. and S.A.). When at least one specimen-piece was revealed to be positive by IHC or by DISH among the same specimen's four pieces examined with different fixation times, we considered the result HER-2-positive.

### *Construction of tissue microarray (TMA), IHC, and evaluation of PD-L1 expression*

For the PD-L1 IHC, we prepared a TMA using a JF-4 Tissue Microarrayer (Sakura Finetek Japan, Tokyo). A tissue core (2.0 mm) from each of a patient specimen's four fixation pieces was selected and used for the TMA. We performed IHC of PD-L1 using the PD-L1 IHC 22C3 pharmDx kit (Agilent Technologies) on an Autostainer Link 48 platform (Agilent Technologies) per the manufacturer's instructions. Membranous



**Figure 1** Representative image of a resected gastric cancer specimen. Each patient's resected specimen was immediately divided into four pieces that were individually fixed in a solution for 6 h, 24 h, or 48 h or 1 wk. Insets: Cut surfaces of the specimen after fixation for each duration.

expression of tumor cells irrespective of its intensity was considered PD-L1-positivity. The same two pathologists (K.K. and S.A.) assessed the IHC results and achieved a consensus when necessary. The IHC specimens were categorized into 0%, 1%-9%, and every 10% of PD-L1-positive cells. We set cut-offs as 1%, 10%, and 50% PD-L1-positive cells. When at least one of a specimen's four pieces was positive at a given cut-off in a fixation time, we considered this result PD-L1 positivity.

### Statistical analyses

All statistical analyses were performed using JMP ver. 12.2 software (SAS, Cary, NC) or R version 3.4.4 (free software). The findings from pairs of groups were compared by Student's *t*-test or Pearson's chi-square test, as appropriate. We compared the "proper" fixation periods (6 h, 24 h, and 48 h) and the prolonged fixation period (1 wk) was performed by simultaneous tests for linear hypotheses (the difference of the average of the proper fixation period and the prolonged fixation period) based on a linear mixed effects model. In this analysis, we used the HER2/CEP17 ratio for the assessment of DISH findings, and we regarded the finding of "0%-9%" of PD-L1 as "5%". *P*-values < 0.05 were accepted as significant. All statistical analyses were supervised by the co-author statistician (A. K.).

## RESULTS

### Clinicopathological characteristics and comparison of the 10% formalin-fixed and 10% NBF-fixed groups

The clinicopathological features of the 32 gastric cancer cases are summarized in Table 1. The mean age of all patients is 74.5 years old; 18 males and 14 females were enrolled. Based on pathological examinations, six cases were classified as T1b2, nine cases were T2, five cases were T3, and 12 cases were T4a according to TNM classification. Eighteen cases were classified as intestinal type, and the other 14 cases were classified as diffuse/mixed type by Lauren's classification. No significant difference was observed between the 10% formalin-fixed group (*n* = 22) and the 10% NBF-fixed group (*n* = 10) in the comparisons of age, gender, T-stage, Lauren's classification, HER-2 positivity, or PD-L1 positivity.

### Whole IHC data

The whole data of IHC, *i.e.*, both the HER-2 and PD-L1 results, are summarized in Table 2. Of the 32 cases, 24 (75%) were completely negative (score 0) for HER-2 IHC, and the remaining eight cases showed positive HER-2 staining of score 1+ or more in at least one of the four specimens pieces (subjected to the four different fixation times). No significant difference was observed in the comparisons between the three proper fixation periods (6 h, 24 h, and 48 h) and the prolonged fixation period (1 wk) groups (*P* = 0.7713).

Twelve cases (37.5%) were completely negative for PD-L1 IHC, and the remaining 20 cases showed positive staining of  $\geq 1\%$  tumor cells in at least one of the four specimen pieces. No significant difference was revealed in our comparisons between

**Table 1 Clinicopathological characteristics and immunohistochemistry results according to used formalin solution *n* (%)**

	All cases ( <i>n</i> = 32)	10% formalin ( <i>n</i> = 22)	1% NBF ( <i>n</i> = 10)	<i>P</i>
Age (mean ± SD)	74.5 ± 7.8	73.3 ± 8.0	77.1 ± 7.0	0.2020
Gender				0.7731
Male	18 (56.3)	12 (54.6)	6 (60.0)	
Female	14 (43.7)	10 (45.4)	4 (40.0)	
T-Stage				0.6793
T1b2	6 (18.8)	3 (13.6)	3 (30.0)	
T2	9 (28.1)	6 (27.3)	3 (30.0)	
T3	5 (15.6)	4 (18.2)	1 (10.0)	
T4a	12 (37.5)	9 (40.9)	3 (30.0)	
Lauren's classification				
Intestinal	18 (56.2)	12 (54.6)	6 (60.0)	0.7731
Diffuse / mixed	14 (43.8)	10 (45.4)	4 (40.0)	
HER-2 IHC				
Complete negative <sup>1</sup>	24 (75)	18 (81.8)	6 (60.0)	0.1966
Final positive by DISH	5 (15.6)	2 (9.1)	3 (30.0)	0.1311
PD-L1 positivity				
Cut off 1%	20 (62.5)	14 (63.4)	6 (60.0)	0.8439
Cut off 10%	13 (40.6)	8 (36.4)	5 (50.0)	0.4666
Cut off 50%	7 (21.9)	4 (18.2)	3 (30.0)	0.4535

<sup>1</sup>The cases that all four specimens of different fixation time were assessed as score 0.

IHC: Immunohistochemistry; NBF: Neutral buffered formalin; DISH: Dual color in situ hybridization; HER-2: Human epidermal growth factor receptor 2; PD-L1: Programmed death-ligand 1.

the proper fixation period group and the prolonged-fixation group ( $P = 0.46$ ).

### **The HER-2-immunoreactive cases, and their DISH findings**

The details of the HER-2 IHC of the eight cases which showed HER-2 immunoreactivity and the DISH results are summarized in Table 3. As the HER-2 amplification could not be confirmed by DISH analysis, three cases (case nos. 14, 20 and 25) were assessed as “HER-2-negative”. A final total of five cases were assessed as “HER-2-positive”. Among these five cases, two cases (case nos. 12 and 31) diffusely expressed HER-2 irrespective of the fixation duration. No significant difference was observed in the comparisons between the proper fixation period groups and the prolonged-fixation group in the analysis of HER-2 IHC ( $P = 1.000$ ) or the HER2/CEP17 ratio in the DISH analysis ( $P = 0.6989$ ).

Among the five HER-2-positive cases, two showed a discrepant expression of HER-2. In case 22, the 6 h-fixation piece was confirmed as negative by both IHC (1+) and DISH, and the 24 h-fixation piece was indicated as negative by IHC (1+) but equivocal by DISH (HER2/CEP17: 1.9, average copy number: 4.4), and both the 48 h- and 1 wk-fixed pieces were equivocal by IHC but positive by DISH.

In case 27, only the 1 wk-fixed piece was assessed as positive by both IHC (3+) and DISH; the other three pieces (6 h-, 24 h-, and 48 h-fixed) were negative or equivocal by IHC and all negative by DISH. We speculate that these discrepant results could be explained by the heterogeneity of the HER-2 expression of the tumor tissues.

### **The PD-L1 immunoreactive cases**

The details of the PD-L1 IHC of the 20 cases (excluding the 12 completely PD-1-negative cases) are summarized in Table 4. In the assessment using the 1% cut-off, all 20 cases were determined as PD-L1-positive. With the 10% cut-off, 13 cases were PD-L1-positive, and with the 50% cut-off, seven cases were PD-L1-positive. No significant difference was revealed by our comparisons of the three proper fixation period groups and the prolonged-fixation group in the analysis of PD-L1 IHC ( $P = 0.4605$ ).

### **IHC of HER-2 and PD-L1 in the long-fixation cases**

We re-assessed the five cases that received the final assessment of HER-2-positivity and the three of the seven cases assessed as PD-L1-positive with the 50% cut-off (long-fixed tissue of four cases were unavailable) by HER-2 or PD-L1 IHC using cancer

**Table 2** Whole data of immunohistochemistry according to fixation time

Case No.	Formalin	HER-2 IHC score; $P = 0.7713$ (6 h-48 h vs 1 wk) <sup>1</sup>				PD-L1 expression (%); $P = 0.46$ (6 h-48 h vs 1 wk) <sup>1</sup>			
		6 h	24 h	48 h	1 wk	6 h	24 h	48 h	1 wk
1	10% F	0	0	0	0	0	0	0	0
2	10% F	0	0	0	0	10	10	10	1-9
3	10% F	0	0	0	0	0	0	0	0
4	10% F	0	0	0	0	80	80	90	90
5	10% F	0	0	0	0	0	0	0	1-9
6	10% F	0	0	0	0	0	0	0	0
7	10% F	0	0	0	0	0	0	0	0
8	10% F	0	0	0	0	0	0	1-9	1-9
9	10% F	0	0	0	0	0	1-9	0	1-9
10	10% F	0	0	0	0	40	30	30	40
11	10% F	0	0	0	0	1-9	0	10	10
12	10% F	3+	3+	3+	3+	1-9	1-9	1-9	0
13	10% F	0	0	0	0	0	0	0	0
14	10% F	1+	2+	0	0	100	1-9	30	60
15	10% F	0	0	0	0	0	0	0	0
16	10% F	0	0	0	0	1-9	10	1-9	1-9
17	10% F	0	0	0	0	0	0	0	0
18	10% F	0	0	0	0	20	60	20	40
19	10% F	0	0	0	0	1-9	1-9	1-9	0
20	10% F	2+	0	0	0	80	80	20	1-9
21	10% F	0	0	0	0	1-9	0	1-9	1-9
22	10% F	1+	1+	2+	2+	0	0	0	0
23	10% NBF	0	0	0	0	0	0	0	0
24	10% NBF	0	0	0	0	0	0	0	0
25	10% NBF	0	1+	2+	1+	10	0	10	0
26	10% NBF	2+	2+	2+	2+	0	0	0	0
27	10% NBF	2+	1+	1+	3+	20	70	30	1-9
28	10% NBF	0	0	0	0	0	0	0	0
29	10% NBF	0	0	0	0	1-9	1-9	0	1-9
30	10% NBF	0	0	0	0	100	100	100	100
31	10% NBF	3+	3+	3+	3+	0	1-9	10	1-9
32	10% NBF	0	0	0	0	70	80	90	90

<sup>1</sup>Simultaneous tests for linear hypotheses based on a linear mixed effects model.

F: Formalin; NBF: Neutral buffered formalin; HER-2: Human epidermal growth factor receptor 2; PD-L1: Programmed death-ligand 1.

tissue that had been fixed for very long durations, *i.e.*, 3 mo to 28 mo. The fixation durations and the IHC results for each case are summarized in Table 5.

All three heterogeneous cases (cases 22, 26 and 27) completely lost HER-2 expression after long fixation by both 10% formalin and 10% NBF. Of note, the HER-2 expression of case 12, which diffusely and strongly expressed HER-2 (Figure 2A), was significantly weakened (assessed as 2+) after long fixation by 10% formalin (Figure 2B). In contrast, the HER-2 expression of case 31, which also diffusely and strongly expressed HER-2 (Figure 2C), maintaining strong expression (3+) even after long fixation by 10% NBF (Figure 2D).

In case 20, which expressed PD-L1 at the maximum 80% of cancer cells (Figure 2E), the PD-L1 expression was completely lost after long fixation by 10% formalin (Figure 2F). In contrast, the PD-L1 expression was maintained even after long fixation in cases 30 and 32, which were fixed by 10%NBF (Figure 2G and H).



**Table 3** Human epidermal growth factor receptor 2 immunohistochemistry and dual color in situ hybridization results in Human epidermal growth factor receptor 2-immunoreactive cases (*n* = 8)

Case No.	Formalin	HER-2 IHC score; <i>P</i> = 1.000 (6 h-48 h vs 1 wk) <sup>†</sup>				DISH; HER2/CEP17ratio (average HER2 copy number); <i>P</i> = 0.6989 (6 h-48 h vs 1 wk) <sup>†</sup>				Final assessment
		6 h	24 h	48 h	1 wk	6 h	24 h	48 h	1 wk	
12	10% F	3+	3+	3+	3+	11.4 (33.7)	10.9 (28.5)	7.1 (20.4)	4.8 (17.4)	+
14	10% F	1+	2+	0	0	1.2 (3.0)	1.3 (3.3)	1.3 (2.2)	1.3 (2.2)	-
20	10% F	2+	0	0	0	0.8 (2.1)	1.2 (2.8)	1.3 (2.1)	1.3 (2.0)	-
22	10% F	1+	1+	2+	2+	1.6 (3.4)	1.9 (4.4)	2.1 (2.5)	2.1 (2.8)	+
25	10% NBF	0	1+	2+	1+	1.0 (1.9)	1.1 (2.2)	1.8 (3.1)	1.4 (2.5)	-
26	10% NBF	2+	2+	2+	2+	2.6 (5.2)	3.7 (6.5)	3.5 (6.6)	3.3 (5.7)	+
27	10% NBF	2+	1+	1+	3+	1.1 (2.9)	1.4 (2.8)	1.3 (2.1)	2.2 (4.9)	+
31	10% NBF	3+	3+	3+	3+	4.7 (19.8)	6.7 (21.2)	4.7 (15.7)	7.2 (21.2)	+

<sup>†</sup>Simultaneous tests for linear hypotheses based on a linear mixed effects model.

F: Formalin; NBF: Neutral buffered formalin; DISH: Dual color in situ hybridization; HER-2: Human epidermal growth factor receptor 2; PD-L1: Programmed death-ligand 1.

## DISCUSSION

In 2007, the ASCO/CAP guideline recommended the fixation duration of 6 h to 48 h using 10%NBF for HER-2 testing in breast cancer<sup>[20]</sup>, and this recommendation was changed to 6 h-72 h in the updated 2013 ASCO/CAP guideline<sup>[3]</sup> based on the accumulated data and to conform with the ASCO/CAP estrogen receptor (ER)/progesterone receptor (PgR) testing guidelines<sup>[21]</sup>.

Several studies reported that HER-2 expression shown by IHC was comparatively resistant to prolonged fixation and provided stable results, especially in positive (3+) cases<sup>[4-8]</sup>. Regarding fixatives, although the superiority of 10%NBF in IHC<sup>[22]</sup> and in the preservation of DNA<sup>[23]</sup> had been reported, Hashizume *et al*<sup>[7]</sup> reported that the HER-2 overexpression rate was not significantly different among 10% NBF, 15% NBF, and 20% formalin. In addition, Moatamed *et al*<sup>[6]</sup> analyzed the HER-2 amplification by IHC and ISH using mastectomy samples subjected to various fixation times with various fixatives, and they concluded that HER-2 testing results remain accurate beyond the ASCO/CAP recommendation.

Considering the results of breast cancer studies, the ASCO/CAP guideline regarding fixation duration seems too strict. However, that guideline was based on the concept of mitigating false-negative results of HER-2 testing and on the conclusions made in consensus meetings<sup>[24,25]</sup>, and thus that guideline's recommendation is considered an ideal goal for HER-2 testing. The findings of previous studies regarding fixation durations may be good references for the interpretation of HER-2 testing beyond the ASCO/CAP recommendation in unavoidable circumstances.

It is well known that the positivity or heterogeneity of HER-2 expression in gastric cancer is quite different from that of breast cancer<sup>[12-14]</sup>. The HER-2 positivity in patients with gastric cancer varied widely (3.8% to 36.6%) in a study using a different method for HER-2 determination<sup>[26]</sup>. Many studies have demonstrated the heterogeneity of HER-2 expression in gastric cancer, and the percentages of heterogeneity in these studies varied from a minimum of 5% to a maximum of 69%, although the definition of heterogeneity was not universal in the studies<sup>[27-33]</sup>.

As the HER-2 expression of gastric cancer is unique, the effects of the fixative conditions on the determination of HER-2 results in gastric cancer may differ from those of breast cancer. However, the amount of relevant evidence is insufficient. We could find only one study using a xenograft model of gastric cancer cell lines<sup>[15]</sup> and a clinical study of gastric cancer<sup>[16]</sup> that analysed the association between HER-2 test results and the formalin fixation status. To the best of our knowledge, no previous study has prospectively analyzed the effect of formalin fixation on HER-2 expression using the same gastrectomy specimens under different fixation times, as in the present study.

Our analyses did not confirm the phenomenon in which the expression was attenuated in proportion to the fixation duration (within 1 wk), in either our HER-2 or PD-L1 testing. Rather, there were cases that showed stronger expression in the specimen-pieces fixed for longer durations. We speculate that the reason for these results is based on the heterogenous expression of HER-2 and PD-L1 in gastric cancer.

**Table 4** Programmed death-ligand 1 expression results of the programmed death-ligand 1 immunoreactive cases (*n* = 20)

Case No.	Used formalin	PD-L1 expression; <i>P</i> = 0.4605 (6 h-48 h vs 1 wk) <sup>1</sup>				Assessment (Cut-off 1%)	Assessment (Cut-off 10%)	Assessment (Cut-off 50%)
		6 h	24 h	48 h	1 wk			
2	10% F	10	10	10	1-9	+	+	-
4	10% F	80	80	90	90	+	+	+
5	10% F	0	0	0	1-9	+	-	-
8	10% F	0	0	1-9	1-9	+	-	-
9	10% F	0	1-9	0	1-9	+	-	-
10	10% F	40	30	30	40	+	+	-
11	10% F	1-9	0	10	10	+	+	-
12	10% F	1-9	1-9	1-9	0	+	-	-
14	10% F	100	1-9	30	60	+	+	+
16	10% F	1-9	10	1-9	1-9	+	+	-
18	10% F	20	60	40	40	+	+	+
19	10% F	1-9	1-9	1-9	0	+	-	-
20	10% F	80	80	20	1-9	+	+	+
21	10% F	1-9	0	1-9	1-9	+	-	-
25	10% NBF	10	0	10	0	+	+	-
27	10% NBF	20	70	30	1-9	+	+	+
29	10% NBF	1-9	1-9	0	1-9	+	-	-
30	10% NBF	100	100	100	100	+	+	+
31	10% NBF	0	1-9	10	1-9	+	+	-
32	10% NBF	70	80	90	90	+	+	+

<sup>1</sup>Simultaneous tests for linear hypotheses based on a linear mixed effects model.

F: Formalin; NBF: Neutral buffered formalin.

It may be difficult to overcome this problem of heterogeneity, because it is too expensive to perform HER-2 testing in multiple specimens.

Regarding fixatives, although we observed no significant difference between 10% formalin and 10% NBF within 1 wk of fixation, the superiority of 10% NBF was apparent in the very-long-term fixation.

Many studies have described the PD-L1 expression in gastric cancer, and a meta-analysis of 15 studies<sup>[34]</sup> concluded that (1) the expression of PD-L1 was associated with the overall survival of gastric cancer patients; and (2) Epstein-Barr virus infection and microsatellite instability cases are more likely to express PD-L1. Although the need for PD-L1 IHC in gastric cancer is expected to increase, the method of assessment for PD-L1 IHC is not yet standardized. The cut-off values of previous studies varied from 1% to 50% positivity of tumor cells<sup>[34]</sup>.

We assessed the results of our PD-L1 IHC by using cut-off values of 1%, 10%, and 50% because the cut-off values of earlier studies varied widely. Like the results of our HER-2 IHC, the heterogeneous expression of PD-L1 influenced the results, and no significant influence of fixative duration on the assessment of PD-L1 IHC findings in gastric cancer was revealed within 1 wk of fixation. Although we also observed no significant difference between 10% formalin and 10% NBF within 1 wk of fixation in our assessment of PD-L1 expression, a significant difference was apparent in the long-term fixation, and the superiority of 10% NBF was reconfirmed.

The small number of cases (especially HER-2-positive cases) is a limitation of the present study. We could not perform the IHC/DISH of all of the whole-sectioned specimens, and we were not able to unify the durations of the very-long-term fixation. In addition, our results were probably affected by tumor heterogeneity. We therefore consider that our results were not conclusive. However, our results provide the interpretation of the cases beyond fixation duration of the ASCO / CAP recommendation that these cases are also worth HER-2 / PD-L1 examination. This is clinically very important.

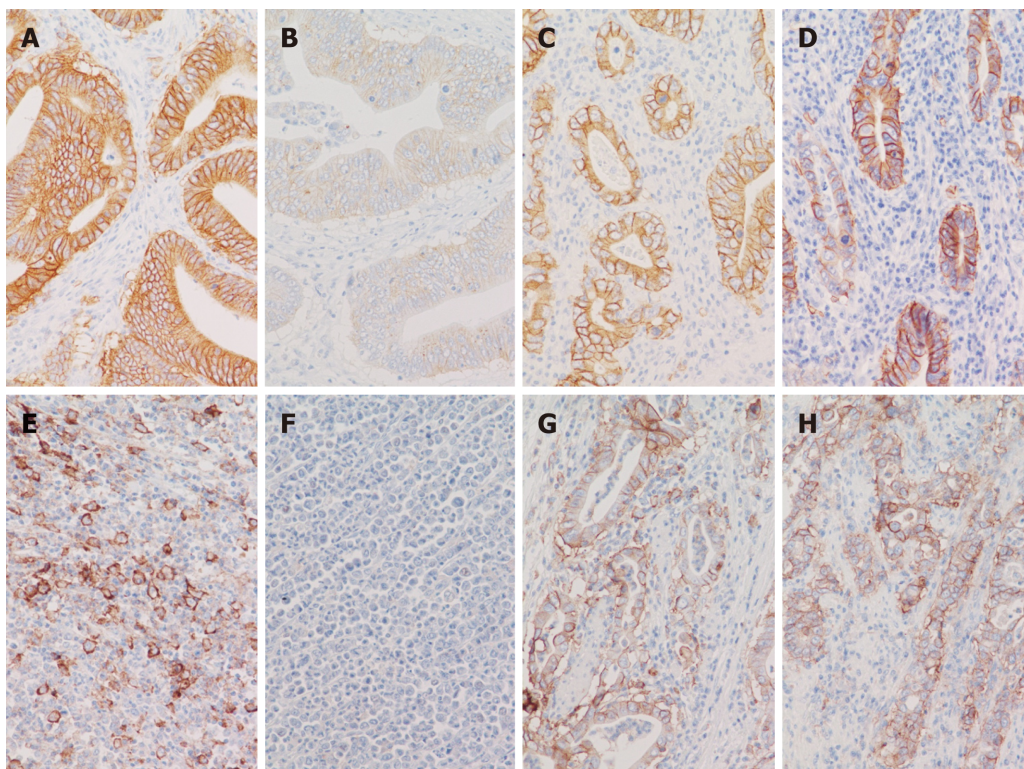
In conclusion, we performed a prospective analysis to identify the effects of formalin fixation on the assessment of HER-2 and PD-L1 expression. Our analyses revealed that prolonged fixation did not show inferiority within 1 wk of duration. Although no significant difference was observed between 10% formalin and 10% NBF

Table 5 Details of long-fixation cases

Case No.	Formalin	Min. expression (6 h - 1 wk fixation)	Max. expression (6 h - 1 wk fixation)	Fixation period (mo)	Results after long fixation
HER-2:					
12	10% F	Score 3+	Score 3+	19	Score 2+
22	10% F	Score 1+	Score 2+	3	Score 0
26	10% NBF	Score 2+	Score 2+	20	Score 0
27	10% NBF	Score 1+	Score 3+	18	Score 0
31	10% NBF	Score 3+	Score 3+	16	Score 3+
PD-L1:					
20	10% F	1%-9%	80%	28	0%
30	10% NBF	100%	100%	16	90%
32	10% NBF	70%	90%	14	70%

F: Formalin; NBF: Neutral buffered formalin; HER-2: Human epidermal growth factor receptor 2; PD-L1: Programmed death-ligand 1.

within a week of fixation, the superiority of 10% NBF was confirmed in the very-long-term (> 3 mo) fixation. To minimize the risk of false-negative results, it is important to comply with the ASCO/CAP recommendation as much as possible. Our present findings provide supporting information for the interpretation of HER-2 testing and PD-L1 testing for cases beyond the ASCO/CAP fixation recommendation due to unavoidable circumstances.



**Figure 2 Representative immunohistochemistry images from very-long-fixation cases.** A, B: Human epidermal growth factor receptor 2 (HER-2) immunohistochemistry (IHC) of case 12. Diffuse and strong HER-2 expression (score 3+) was observed in the specimen piece fixed by 10% formalin for 1 week (A). The HER-2 expression was significantly weakened (assessed as 2+) after very-long fixation (19 mo) by 10% formalin (B); C, D: HER-2 IHC of case 31. Diffuse and strong HER-2 expression (score 3+) was observed in the piece fixed by 10% NBF for 1 wk (C), and this expression was maintained (score 3+) even after very-long fixation (16 mo) by 10% NBF (D); E, F: PD-L1 IHC of case 20. The PD-L1 expression by tumor cells was observed in the piece that underwent 24-h fixation by 10% formalin (E) but it completely disappeared after very-long fixation (28 mo) by 10% formalin (F); G, H: PD-L1 IHC of case 30. The PD-L1 expression by tumor cells was observed in the piece that underwent 1-wk fixation by 10% NBF (G) and was maintained even after very-long fixation (16 mo) by 10% NBF (H).

## ARTICLE HIGHLIGHTS

### Research background

The needs for human epidermal growth factor receptor 2 (HER-2) and/or programmed death-ligand 1 (PD-L1) evaluations in gastric cancer are dramatically increasing. However, most of the evidences regarding the fixation duration or type of fixing solution are based on breast cancer.

### Research motivation

As the HER-2 expression of gastric cancer is unique, we speculate that the effects of the fixative conditions on the determination of HER-2 results in gastric cancer may differ from those of breast cancer.

### Research objectives

To investigate the real effects of fixation conditions on HER-2 testing or PD-L1 testing for gastric cancer using gastrectomy specimens.

### Research methods

Resected gastric specimens were each divided into four pieces and fixed in four strictly controlled different durations (6 h, 24 h, and 48 h, and 1 wk) by 10% formalin or 10% neutral buffered formalin (NBF). Immunohistochemistry (IHC) of HER-2 and PD-1 was performed, and a pathology examination was conducted.

### Research results

Prolonged fixation did not show inferiority within the 1-wk period for the assessment of both HER-2 and PD-L1 expressions. The superiority of 10% NBF was confirmed in the long-term (> 3 mo) fixation.

### Research conclusions

In this pilot study, prolonged fixation within 1 wk showed no inferiority in HER-2 or PD-L1 testing. However, a large-numbered prospective study is needed to obtain conclusive results.

### Research perspectives



Our findings provide supporting information for the interpretation of HER-2 and/or PD-L1 testing for cases beyond the ASCO/CAP fixation recommendation due to unavoidable circumstances.

## REFERENCES

- Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: [20728210](#) DOI: [10.1016/S0140-6736\(10\)61121-X](#)]
- Rüschoff J**, Hanna W, Bilous M, Hofmann M, Osamura RY, Penault-Llorca F, van de Vijver M, Viale G. HER2 testing in gastric cancer: a practical approach. *Mod Pathol* 2012; **25**: 637-650 [PMID: [2222640](#) DOI: [10.1038/modpathol.2011.198](#)]
- Wolff AC**, Hammond ME, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF; American Society of Clinical Oncology; College of American Pathologists. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med* 2014; **138**: 241-256 [PMID: [24099077](#) DOI: [10.5858/arpa.2013-0953-SA](#)]
- Bartley AN**, Washington MK, Ventura CB, Ismaila N, Colasacco C, Benson AB, Carrato A, Gulley ML, Jain D, Kakar S, Mackay HJ, Streutker C, Tang L, Troxell M, Ajani JA. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline From the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *Am J Clin Pathol* 2016; **146**: 647-669 [PMID: [28077399](#) DOI: [10.1093/ajcp/aqw206](#)]
- Selvarajan S**, Bay BH, Choo A, Chuah KL, Sivaswaren CR, Tien SL, Wong CY, Tan PH. Effect of fixation period on HER2/neu gene amplification detected by fluorescence in situ hybridization in invasive breast carcinoma. *J Histochem Cytochem* 2002; **50**: 1693-1696 [PMID: [12486093](#) DOI: [10.1177/002215540205001215](#)]
- Arber DA**. Effect of prolonged formalin fixation on the immunohistochemical reactivity of breast markers. *Appl Immunohistochem Mol Morphol* 2002; **10**: 183-186 [PMID: [12051639](#) DOI: [10.1097/00129039-200206000-00015](#)]
- Hashizume K**, Hatanaka Y, Kamihara Y, Kato T, Hata S, Akashi S, Kato T, Koyatsu J, Tani Y, Tsujimoto M, Tsuda H. Interlaboratory comparison in HercepTest assessment of HER2 protein status in invasive breast carcinoma fixed with various formalin-based fixatives. *Appl Immunohistochem Mol Morphol* 2003; **11**: 339-344 [PMID: [14663361](#) DOI: [10.1097/00129039-200312000-00011](#)]
- Moatamed NA**, Nanjangud G, Pucci R, Lowe A, Shintaku IP, Shapourifar-Tehrani S, Rao N, Lu DY, Apple SK. Effect of ischemic time, fixation time, and fixative type on HER2/neu immunohistochemical and fluorescence in situ hybridization results in breast cancer. *Am J Clin Pathol* 2011; **136**: 754-761 [PMID: [22031314](#) DOI: [10.1309/AJCP99WZGBPKCXOQ](#)]
- Ibarra JA**, Rogers LW. Fixation time does not affect expression of HER2/neu: a pilot study. *Am J Clin Pathol* 2010; **134**: 594-596 [PMID: [20855640](#) DOI: [10.1309/AJCPAIIJPSNA49MJI](#)]
- Tong LC**, Nelson N, Tsurugiannis J, Mulligan AM. The effect of prolonged fixation on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast cancer: a prospective study. *Am J Surg Pathol* 2011; **35**: 545-552 [PMID: [21358301](#) DOI: [10.1097/PAS.0b013e31820e6237](#)]
- Kao KR**, Hasan T, Baptista A, Truong T, Gai L, Smith AC, Li S, Gonzales P, Voisey K, Eriwvo P, Power J, Denic N. Effect of fixation time on breast biomarker expression: a controlled study using cell line-derived xenografted (CDX) tumours. *J Clin Pathol* 2017; **70**: 832-837 [PMID: [28341657](#) DOI: [10.1136/jclinpath-2017-204381](#)]
- Grillo F**, Fassan M, Sarocchi F, Fiocca R, Mastracci L. HER2 heterogeneity in gastric/gastroesophageal cancers: From benchside to practice. *World J Gastroenterol* 2016; **22**: 5879-5887 [PMID: [27468182](#) DOI: [10.3748/wjg.v22.i26.5879](#)]
- Fusco N**, Bosari S. HER2 aberrations and heterogeneity in cancers of the digestive system: Implications for pathologists and gastroenterologists. *World J Gastroenterol* 2016; **22**: 7926-7937 [PMID: [27672288](#) DOI: [10.3748/wjg.v22.i35.7926](#)]
- Wada R**, Hirabayashi K, Ohike N, Morii E. New guidelines for HER2 pathological diagnostics in gastric cancer. *Pathol Int* 2016; **66**: 57-62 [PMID: [26814046](#) DOI: [10.1111/pin.12390](#)]
- Yamashita-Kashima Y**, Shu S, Yoroza K, Hashizume K, Moriya Y, Fujimoto-Ouchi K, Harada N. Importance of formalin fixing conditions for HER2 testing in gastric cancer: immunohistochemical staining and fluorescence in situ hybridization. *Gastric Cancer* 2014; **17**: 638-647 [PMID: [24414131](#) DOI: [10.1007/s10120-013-0329-8](#)]
- Matsusaka S**, Nashimoto A, Nishikawa K, Miki A, Miwa H, Yamaguchi K, Yoshikawa T, Ochiai A, Morita S, Sano T, Kodera Y, Kakeji Y, Sakamoto J, Saji S, Yoshida K. Clinicopathological factors associated with HER2 status in gastric cancer: results from a prospective multicenter observational cohort study in a Japanese population (JFMC44-1101). *Gastric Cancer* 2016; **19**: 839-851 [PMID: [26265390](#) DOI: [10.1007/s10120-015-0518-8](#)]
- Reck M**, Rodríguez-Abreu D, Robinson AG, Hui R, Csösz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, O'Brien M, Rao S, Hotta K, Leiby MA, Lubiniecki GM, Shentu Y, Rangwala R, Brahmer JR; KEYNOTE-024 Investigators. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* 2016; **375**: 1823-1833 [PMID: [27718847](#) DOI: [10.1056/NEJMoa1606774](#)]
- Muro K**, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtress B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang YJ. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; **17**: 717-726 [PMID: [27157491](#) DOI: [10.1016/S1470-2045\(16\)00175-3](#)]
- Tran PN**, Sarkissian S, Chao J, Klempner SJ. PD-1 and PD-L1 as emerging therapeutic targets in gastric cancer: current evidence. *Gastrointest Cancer* 2017; **7**: 1-11 [PMID: [28757801](#) DOI: [10.1016/S1470-2045\(16\)00175-3](#)]



- 10.2147/GICTT.S113525]
- 20 **Wolff AC**, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van de Vijver M, Wheeler TM, Hayes DF; American Society of Clinical Oncology/College of American Pathologists. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007; **131**: 18-43 [PMID: 19548375 DOI: 10.1043/1543-2165(2007)131[18:ASOCCO]2.0.CO;2]
  - 21 **Hammond ME**, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, Hicks DG, Lester S, Love R, Mangu PB, McShane L, Miller K, Osborne CK, Paik S, Perlmutter J, Rhodes A, Sasano H, Schwartz JN, Sweep FC, Taube S, Torlakovic EE, Valenstein P, Viale G, Visscher D, Wheeler T, Williams RB, Wittliff JL, Wolff AC. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med* 2010; **134**: 907-922 [PMID: 20524868]
  - 22 **Arnold MM**, Srivastava S, Fredenburgh J, Stockard CR, Myers RB, Grizzle WE. Effects of fixation and tissue processing on immunohistochemical demonstration of specific antigens. *Biotech Histochem* 1996; **71**: 224-230 [PMID: 8896794 DOI: 10.3109/10520299609117164]
  - 23 **Baloglu G**, Haholu A, Kucukodaci Z, Yilmaz I, Yildirim S, Baloglu H. The effects of tissue fixation alternatives on DNA content: a study on normal colon tissue. *Appl Immunohistochem Mol Morphol* 2008; **16**: 485-492 [PMID: 18594471 DOI: 10.1097/PAI.0b013e31815dffa6]
  - 24 **Hammond ME**, Barker P, Taube S, Gutman S. Standard reference material for Her2 testing: report of a National Institute of Standards and Technology-sponsored Consensus Workshop. *Appl Immunohistochem Mol Morphol* 2003; **11**: 103-106 [PMID: 12777990 DOI: 10.1097/00129039-200306000-00001]
  - 25 **Yaziji H**, Taylor CR, Goldstein NS, Dabbs DJ, Hammond EH, Hewlett B, Floyd AD, Barry TS, Martin AW, Badve S, Baehner F, Cartun RW, Eisen RN, Swanson PE, Hewitt SM, Vyberg M, Hicks DG; Members of the Standardization Ad-Hoc Consensus Committee. Consensus recommendations on estrogen receptor testing in breast cancer by immunohistochemistry. *Appl Immunohistochem Mol Morphol* 2008; **16**: 513-520 [PMID: 18931614 DOI: 10.1097/PAI.0b013e31818a9d3a]
  - 26 **Boku N**. HER2-positive gastric cancer. *Gastric Cancer* 2014; **17**: 1-12 [PMID: 23563986 DOI: 10.1007/s10120-013-0252-z]
  - 27 **Hofmann M**, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; **52**: 797-805 [PMID: 18422971 DOI: 10.1111/j.1365-2559.2008.03028.x]
  - 28 **Grabsch H**, Sivakumar S, Gray S, Gabbert HE, Müller W. HER2 expression in gastric cancer: Rare, heterogeneous and of no prognostic value - conclusions from 924 cases of two independent series. *Cell Oncol* 2010; **32**: 57-65 [PMID: 20208134 DOI: 10.3233/CLO-2009-0497]
  - 29 **Lee S**, de Boer WB, Fermoy S, Platten M, Kumarasinghe MP. Human epidermal growth factor receptor 2 testing in gastric carcinoma: issues related to heterogeneity in biopsies and resections. *Histopathology* 2011; **59**: 832-840 [PMID: 22092394 DOI: 10.1111/j.1365-2559.2011.04017.x]
  - 30 **Van Cutsem E**, Bang YJ, Feng-Yi F, Xu JM, Lee KW, Jiao SC, Chong JL, López-Sánchez RI, Price T, Gladkov O, Stoss O, Hill J, Ng V, Lehle M, Thomas M, Kiermaier A, Rüschoff J. HER2 screening data from ToGA: targeting HER2 in gastric and gastroesophageal junction cancer. *Gastric Cancer* 2015; **18**: 476-484 [PMID: 25038874 DOI: 10.1007/s10120-014-0402-y]
  - 31 **Lee HE**, Park KU, Yoo SB, Nam SK, Park DJ, Kim HH, Lee HS. Clinical significance of intratumoral HER2 heterogeneity in gastric cancer. *Eur J Cancer* 2013; **49**: 1448-1457 [PMID: 23146959 DOI: 10.1016/j.ejca.2012.10.018]
  - 32 **Wang T**, Hsieh ET, Henry P, Hanna W, Streutker CJ, Grin A. Matched biopsy and resection specimens of gastric and gastroesophageal adenocarcinoma show high concordance in HER2 status. *Hum Pathol* 2014; **45**: 970-975 [PMID: 24656529 DOI: 10.1016/j.humpath.2013.12.010]
  - 33 **Ahn S**, Ahn S, Van Vrancken M, Lee M, Ha SY, Lee H, Min BH, Lee JH, Kim JJ, Choi S, Jung SH, Choi MG, Lee JH, Sohn TS, Bae JM, Kim S, Kim KM. Ideal number of biopsy tumor fragments for predicting HER2 status in gastric carcinoma resection specimens. *Oncotarget* 2015; **6**: 38372-38380 [PMID: 26460823 DOI: 10.18632/oncotarget.5368]
  - 34 **Gu L**, Chen M, Guo D, Zhu H, Zhang W, Pan J, Zhong X, Li X, Qian H, Wang X. PD-L1 and gastric cancer prognosis: A systematic review and meta-analysis. *PLoS One* 2017; **12**: e0182692 [PMID: 28796808 DOI: 10.1371/journal.pone.0182692]

P- Reviewer: Abadi ATB, Economescu M

S- Editor: Dou Y L- Editor: A E- Editor: Tan WW





Case Control Study

# Nested case-control study of multiple serological indexes and Brighton pediatric early warning score in predicting death of children with sepsis

Xiong Xie, Ming Li, Tian-Tian Xiong, Rui Wang, Liang Xiao

**ORCID number:** Xiong Xie (0000-0001-8731-4114); Ming Li (0000-0002-9391-7285); Tian-Tian Xiong (0000-0001-6753-4567); Rui Wang (0000-0002-8263-7542); Liang Xiao (0000-0001-4824-5481).

**Author contributions:** Xie X, Li M, and Xiong TT designed the research; Xie X, Li M, and Wang R performed the research; Xiao L and Wang R contributed new reagents; Xie X, Li M, and Xiong TT analyzed the data; and Xie X, Li M, Xiong TT, Wang R, and Xiao L wrote the paper.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of Gezhouba Group Central Hospital.

**Informed consent statement:** All patients gave informed consent to this study.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE Statement, and the manuscript was prepared and revised according to the STROBE Statement.

**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

**Xiong Xie, Ming Li, Tian-tian Xiong, Rui Wang, Liang Xiao,** Department of Pediatrics, Third Clinical Hospital, China Three Gorges University, Gezhouba Central Hospital, Yichang 443002, Hubei Province, China

**Corresponding author:** Ming Li, MD, Doctor, Department of Pediatrics, Third Clinical Hospital, China Three Gorges University, Gezhouba Central Hospital, No. 60, Qiaohu No. 1 Road, Yichang 443002, Hubei Province, China. [150501028@qq.com](mailto:150501028@qq.com)

**Telephone:** +86-717-6715660

## Abstract

### BACKGROUND

Currently, it is difficult to predict the complications of children at the early stage of sepsis. Brighton pediatric early warning score (PEWS) is a disease risk assessment system that is simple and easy to operate, which has good sensitivity and specificity in disease recognition among children. Because detection indicators vary widely in children, a single indicator is difficult to assess the post-treatment status of children with sepsis.

### AIM

To investigate the relationship between serological markers, Brighton PEWS, and death in children with sepsis after treatment.

### METHODS

A total of 205 children diagnosed with sepsis at our hospital were enrolled. The baseline data, serum scores, and PEWS scores were recorded. In the nested case-control study, children who died during the study period were included in an observation group. According to the matching principle, the children who were not dead in the same cohort were included in a control group. The influencing factors of death in children with sepsis after treatment and the value of each evaluation index in predicting the prognosis of children were analyzed.

### RESULTS

A total of 96 children were enrolled in the study, including 48 each in the observation group and the control group. Multivariate logistic regression analysis indicated that antibacterial treatments within 1 h ( $P = 0.017$ ), shock ( $P = 0.044$ ), multiple organ dysfunction syndrome (MODS) ( $P = 0.027$ ), serum procalcitonin (PCT) ( $P = 0.047$ ), serum albumin (ALB) ( $P = 0.024$ ), and PEWS ( $P = 0.012$ ) were independent risk factors for the death of children with sepsis. The area under the

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

**Received:** November 27, 2018

**Peer-review started:** November 27, 2018

**First decision:** December 15, 2018

**Revised:** December 25, 2018

**Accepted:** December 29, 2018

**Article in press:** December 30, 2018

**Published online:** February 26, 2019

curve of the combination of ALB, PCT, and PEWS to predict the death in children with sepsis was the highest (0.908).

## CONCLUSION

Antibacterial treatments within 1 h, shock, MODS, PCT, ALB, and PEWS are independent risk factors for the death of children with sepsis. The predictive accuracy of the combination of PCT, ALB, and PEWS for the prognosis of children with sepsis is the best.

**Key words:** Serological indicators; Pediatric early warning score; Sepsis; Nested case-control study; Systemic inflammatory reaction syndrome

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Currently, it is difficult to predict the complications of children at the early stage of sepsis. Brighton pediatric early warning score (PEWS) is a risk assessment system that is simple and easy to operate, which has good sensitivity and specificity in disease recognition among children. In this study, the nested case-control study found that the use of antimicrobial agents within 1 h, shock, number of organs with dysfunction, serum procalcitonin (PCT), serum albumin (ALB), and PEWS were independent risk factors for death of children with sepsis. The combination of ALB, PCT, and PEWS can predict the prognosis of children with sepsis with good accuracy and can improve the sensitivity of prediction.

**Citation:** Xie X, Li M, Xiong TT, Wang R, Xiao L. Nested case-control study of multiple serological indexes and Brighton pediatric early warning score in predicting death of children with sepsis. *World J Clin Cases* 2019; 7(4): 431-440

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/431.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.431>

## INTRODUCTION

Sepsis is a complicated systemic inflammatory reaction syndrome and also the main cause of death in children's intensive care unit. Sepsis can be caused by a variety of pathogenic bacteria and can cause severe complications such as shock and multiple organ dysfunction syndrome (MODS), resulting in higher mortality in children<sup>[1-4]</sup>. Some non-specific inflammatory markers such as serum procalcitonin (PCT), C-reactive protein (CRP), and serum albumin (ALB) have certain value in the evaluation of post-treatment complications in children with sepsis, but they are inaccurate. Currently, it is difficult to make more accurate predictions of complications in children with sepsis at an early stage<sup>[5-7]</sup>. Therefore, the assessment of death in children with sepsis is still a difficult problem to be solved clinically. Brighton pediatric early warning score (Brighton PEWS) is a simple and easy-to-use disease risk assessment system based on adult early warning scores<sup>[8,9]</sup>. It has good sensitivity and specificity in condition recognition among children. Because detection indicators vary widely in children, a single indicator is difficult to assess the post-treatment status of children with sepsis. Therefore, the present study intended to assess the value of serological markers combined with Brighton PEWS in predicting the prognosis of children with sepsis by performing a nested case-control study.

## MATERIALS AND METHODS

### Research subjects

A total of 205 children with sepsis/severe sepsis were enrolled as the study subjects (sepsis:  $n = 135$ , severe sepsis:  $n = 70$ ). They were all diagnosed at Gezhouba Group Central Hospital from October 2015 to December 2017. Among them, 143 were male and 62 were female. They were aged from 6 mo to 9 years and the average age was  $5.5 \pm 3.3$  years. The diagnostic criteria for sepsis/severe sepsis in children were based on the definition of 2005 international pediatric sepsis<sup>[10]</sup>. The inclusion criteria and exclusion criteria are shown in Table 1. All patients and their families signed an

informed consent form and the study was approved by the Ethics Committee of Gezhouba Group Central Hospital.

### **Treatment**

After admission, the children began to accept broad-spectrum antibiotic treatment and retained bacterial culture. The central venous catheter was left in place and fluid was given early to prevent shock. Endotracheal intubation for mechanical ventilation was given when severe sepsis occurs. Once hypotension or lactic acidosis was detected, fluid resuscitation was given, which made the central venous pressure reach 8 to 10 mmHg within 6 h, the mean arterial pressure > 65 mmHg, the urine volume  $\geq$  0.5 mL/kg per hour, and the central venous mixed oxygen saturation  $\geq$  70%. Vasopressors or inotropes such as dopamine may be used when volume expansion was poorly treated.

### **Groups**

This study began with patients admitted to hospital for sepsis/severe sepsis and ended with their death or discharge. The nested case-control study was used to define the child who died during the study period as an observation group. In addition, whenever a child died in the cohort, one child with similar or identical conditions such as age, sex, and infection site was enrolled in a control group by 1:1 matching in the cohort (Figure 1)<sup>[11]</sup>.

### **Research methods**

**Blood sample collection:** At 8 to 12 h after admission, three tubes of fasting venous blood were taken from the subjects in the early morning using vacuum blood collection tubes. There was 3 mL of blood in each tube. One of the tubes was centrifuged (3000 r/min) for 10 min to obtain the supernatant, which was frozen in a refrigerator at -20 °C.

**Indicator determination:** White blood cell (WBC), platelet (PLT), and hemoglobin (Hb) counts were measured using a XE-2100 automatic hematology analyzer. PCT was measured with a Roche E170 Electrochemiluminometer and supporting PCT kit. CRP was measured by the rate scattering turbidimetric method using an IMMAGE800 Automated Immunoassay Analyzer (Beckman, USA). ALB was measured using a BECK MAN LX20 automatic biochemical analyzer. Hemoglobin scavenger receptor (sCD163) was measured by enzyme-linked immunosorbent assay; the kit was supplied by THERMO of Finland and the instrument was a DENLEY DRAGON Wellscan MK3 microplate reader. Serum D-dimer (DD) was determined using a fully automated coagulation analyzer; the kits and associated control products were manufactured by SIEMENS. Serum lactic acid value (Lac) was measured using a Hitachi 7600-120 fully automated biochemical analyzer; the reagent was provided by Beijing Lederman Biochemical Co., Ltd. Creatinine (Cr) was measured using a Bio-Rad automatic biochemical analyzer.

**Scoring criteria:** Brighton PEWS consists of behavioral, respiratory, and circulatory parameters. The higher the score, the heavier the condition (Table 2).

### **Data collection**

Baseline data of the two groups were collected and the treatment status of the children was recorded, including the number of antibacterial treatments within 1 h, duration of mechanical ventilation, and duration of vasoactive drug maintenance. The patient's complications were recorded, including acute respiratory distress syndrome (ARDS), number of organs with dysfunction, and shock. Laboratory diagnostic indicators and PEWS scores were recorded for both groups.

### **Statistical analysis**

Statistical analyses were performed using SPSS 19.0 software. The measurement data are expressed as the mean  $\pm$  SD, and the count data are expressed as numbers (percentages). The *t*-test was used to compare the measurement data between the two groups. The comparison of the count data was performed by the  $\chi^2$  test. Conditional logistic regression was used to further screen out the independent influencing factors of the death of children. A receiver operating characteristic (ROC) curve was established to analyze the ability of potential indicators to assess death in children with sepsis. The difference was considered statistically significant at  $P < 0.05$ .

## **RESULTS**

**Table 1** The inclusion criteria and exclusion criteria

Inclusion criteria	Exclusion criteria
Data completed within 24 h after admission	Previously diagnosed with tumor disease
Age > 28 d	Previously diagnosed with autoimmune disease

**Patient baseline data**

According to the nested case-control study design, a total of 48 children died during the study period, with a total case fatality rate of 23.4%. They were defined as the observation group. A total of 48 children with similar conditions were recruited as the control group. Among the 96 children with sepsis, 64 were male and 32 were female, aged 9 mo to 5 years, with an average age of ( $2.5 \pm 1.8$ ) years. The underlying diseases in the 96 patients included: 65 cases of blood system diseases (67.7%), 19 cases of congenital heart disease (19.8%), 7 cases of nephrotic syndrome (7.3%), and 5 cases of neuromuscular diseases (5.2%). The location of the primary infection included: 54 cases of pulmonary infection (56.3%), 28 cases of digestive system infection (29.2%), and 14 cases of blood system infection (14.5%).

**Comparison between two groups of patients**

There was no significant difference between the observation group and the control group in the baseline data, primary infection site, underlying disease, duration of vasoactive drug maintenance, ARDS, WBC, Hb, CRP, or Cr ( $P > 0.05$ ). The duration of mechanical ventilation, shock, MODS, PCT, sCD163, DD, Lac, and PEWS in the observation group were significantly higher than those in the control group ( $P < 0.05$ ), and the number of antibacterial treatments within 1 h, PLT, and ALB were significantly lower than those in the control group ( $P < 0.05$ ) (Table 3).

**Multivariate logistic regression analysis of death after treatment in children with sepsis**

Further multivariate logistic regression analysis showed that antibacterial treatments within 1 h ( $P = 0.017$ ), shock ( $P = 0.044$ ), number of organs with dysfunction ( $P = 0.027$ ), PCT ( $P = 0.047$ ), ALB ( $P = 0.024$ ) and PEWS ( $P = 0.012$ ) were independent influencing factors for the death of children with sepsis (Table 4).

**Analysis of the value of PCT, ALB, and PEWS in evaluating the outcome of children with sepsis**

The ROC curve was used to further analyze the predictive value of PCT, ALB, and PEWS for the death of children with sepsis, as shown in Figure 2. The area under the curve (AUC) of ALB was 0.761, and the best diagnostic cutoff value was 35.20 g/L. The sensitivity was 57.45%, and the specificity was 85.11%. The AUC of PCT was 0.730, and the best diagnostic cutoff value was 59.65  $\mu\text{g/L}$ . The sensitivity was 53.20%, and the specificity was 85.10%. The AUC of PEWS was 0.771, and the best diagnosis cutoff value was 6.5 points. The sensitivity was 74.50%, and the specificity was 68.10%. The AUC of the combination of ALB, PCT, and PEWS predicting the death in children with sepsis was 0.908. The sensitivity was 87.23%, and the specificity was 85.11%.

**DISCUSSION**

Sepsis is an inflammatory reaction caused by infections, which are mainly caused by bacteria in the clinic<sup>[12]</sup>. Early detection, diagnosis, and treatment have significant effects on post-treatment complications in children<sup>[13,14]</sup>. In the early stage of the disease, the clinical manifestations in children are nonspecific. However, once the inflammatory cascade reaction is triggered, the condition of children will decrease sharply. Even if the treatment is delayed for several hours, the mortality rate of children will be significantly increased<sup>[15]</sup>. Therefore, early accurate assessment of the prognosis of children with sepsis is the most effective way to improve the complications in children with sepsis. However, there is no specific laboratory test to predict the prognosis of children with sepsis. In addition, some clinical symptoms of sepsis in children are often similar to other diseases, which increases the misdiagnosis rate of sepsis undoubtedly. Current studies show that PLT, CRP, PCT, ALB, sCD163, and PEWS can be used to assess the recovery of children with sepsis after treatment<sup>[16-18]</sup>. However, throughout the disease cycle, children's indicators vary



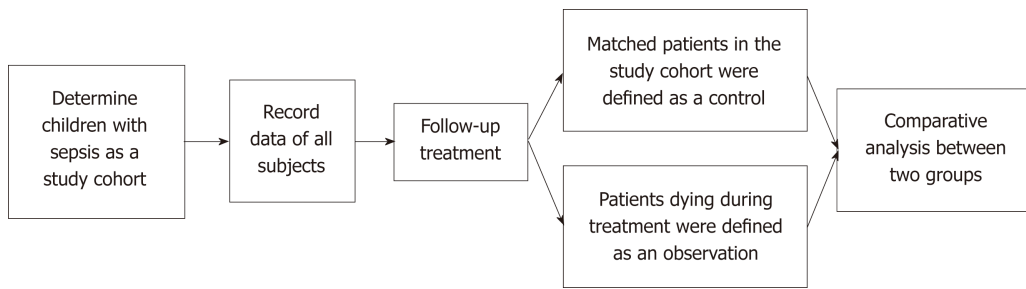


Figure 1 Schematic diagram of the design principle of a nested case-control study.

widely by individual and environmental influences. Current assessment methods are not sufficient to accurately assess the degree of recovery after treatment in children with sepsis. In order to find a more accurate method of assessing the prognosis of children with sepsis, this nested case-control study was conducted to evaluate serologic markers in combination with PEWS in predicting death in children with sepsis.

Epidemiological data from the United States showed that the mortality rate of children with sepsis was 10.3%. The mortality rate of children with underlying diseases increased to 12.8%. In the present study, 48 of 205 children died and the mortality rate was 23.4%, which was relatively high. The potential reason might be related to the late diagnosis. As the clinical symptoms of sepsis were mainly fever, the disease was difficult to identify early so that it was easy to misdiagnose and mistreat. Because the clinical symptoms of sepsis are mainly caused by fever, it is difficult to pay attention to the early stage of the disease. Hence, sepsis in children is easy to be misdiagnosed or mistreated.

Sepsis in children develops rapidly after infection occurs because children have weaker immune systems. Studies have revealed that immediate antibacterial treatment within 1 h could clear the lesion promptly, which preventing further deterioration<sup>[19]</sup>. According to the pathogenesis of sepsis, sepsis is most often associated with insufficient tissue perfusion pressure and tissue hypoxia<sup>[20]</sup>. Therefore, correcting shock and appropriate ventilation should also be stressed in the early treatment. When the infection control of children with sepsis is not ideal or the condition deteriorates, it may appears as shock and MODS<sup>[21]</sup>, which often indicate a poor prognosis of sepsis, especially when the number of organs with dysfunction increases. Serological indicators can objectively indicate changes in the sepsis condition and provide a direct basis for assessing the outcome of the disease after treatment. For example, CRP and PCT are clinically widely used indicators of inflammation<sup>[22,23]</sup>. They have a high specificity in the diagnosis of the degree of inflammation<sup>[24]</sup>. In addition, blood system disease is the most common underlying disease in children with sepsis. Therefore, the degree of thrombocytopenia and changes in DD can reflect the disorders of the blood system and abnormal coagulation function, suggesting the regression of the disease<sup>[25]</sup>.

Based on the analysis of existing studies on the death of children with sepsis, this study analyzed WBC, PLT, Hb, CRP, PCT, ALB, sCD163, DD, Lar, Cr, and PEWS between two groups and performed a multivariate logistic regression analysis for screening the impact factors of the prognosis of sepsis. It is shown that the antibacterial treatments within 1 h, shock, MODS, PCT, ALB, and PEWS were independent influencing factors of death in children. The reason why PEWS is an independent factor for the death of children may be because the scoring system is easy to operate and can monitor children's condition continuously and dynamically without using special equipment<sup>[26]</sup>. PEWS is not affected by age and is suitable for general wards. It can identify children who need intervention in the early stage, and there is good repeatability between different operators. Studies have shown that it has a sensitivity of 70% and a specificity of 90% in critically ill children who need to be transferred to the ICU for emergency care<sup>[27]</sup>. This allows doctors to assess children's condition more objectively and accurately, and avoids empirical subjective judgment. Therefore, early PEWS scores on admission can achieve early intervention, thereby improving the complications of death in children. PCT is a calcitonin precursor protein secreted by thyroid C cells<sup>[28]</sup>. Inflammatory mediators such as bacterial endotoxin and interleukin can stimulate the secretion of PCT by neuroendocrine cells of the liver, kidney, and spleen. When the level is raised, the degree of inflammatory reaction of the body can be more accurately indicated. During the process of sepsis, capillary vascular endothelial cells are destroyed due to the release of a large number

**Table 2 Brighton pediatric early warning score scoring criteria**

Project	0 Points	1 Point	2 Points	3 Points
Behavior	Normal	Somnolence	Irritability	Lethargy/coma Reduced pain response
Cardiovascular system	Pink skin CRT 1-2 s	Pale skin CRT 3 s	Gray skin CRT 4 s Heart rate increased 20 times/min	Cold skin, clammy skin CRT ≥ 5 s Heart rate increased 20 times/min or bradycardia
Respiratory system	Normal Air-free depression	Normal respiratory frequency Increased 10 times/min, FiO <sub>2</sub> 0.3, or oxygen inhalation flow 4 L/min	Normal respiratory frequency Increased 20 times/min, inspiratory depression, FiO <sub>2</sub> 0.4, or oxygen inhalation flow 4 L/min	Normal respiratory frequency Reduced 5 times/min, with sternal inspiratory depression, moaning, FiO <sub>2</sub> 0.5, or oxygen inhalation flow 8 L/min

CRT: Cardiac resynchronization therapy.

of inflammatory factors in the blood, resulting in increased capillary permeability of the whole body, and hypoproteinemia caused by leakage of intravascular albumin. Hypoproteinemia can cause a decrease in plasma osmotic pressure, a reduction in effective circulating blood volume, and multiple organ dysfunction<sup>[29]</sup>. Therefore, a decrease in ALB levels can indirectly indicate the extent of inflammatory infections<sup>[30]</sup>.

The detection indicators vary widely among children and the children's compensation mechanism is more complicated than that of adults, which will affect the accuracy of PCT, ALB, and PEWS in predicting the prognosis of children<sup>[31]</sup>. Therefore, the accuracy of a single indicator in predicting the death of children with sepsis is poor, and the AUC is lower. The results of this study showed that the AUC was the largest when PCT and ALB were combined with PEWS, and the sensitivity was significantly higher compared to any of the single indicators. Therefore, the combined diagnosis of the three indicators can reduce the impact of differences in organization and body compensation and improve the accuracy.

In summary, the present nested case-control study demonstrates that antibacterial treatments within 1 h, shock, MODS, PCT, ALB, and PEWS are independent risk factors for death in children with sepsis. The combined of PCT, ALB, and PEWS for predicting the prognosis of children with sepsis is better than any of the single detection indicators.

**Table 3 Comparison between the observation group and control group at the time of admission *n* (%)**

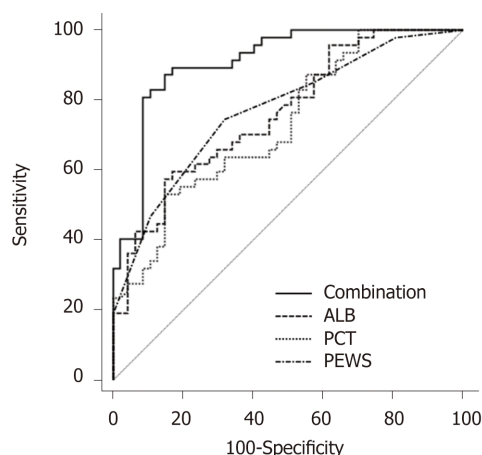
	Control group ( <i>n</i> = 48)	Observation group ( <i>n</i> = 48)	<i>t</i> / $\chi^2$	<i>P</i> -value
Baseline data				
Age (yr)	2.48 ± 1.71	2.51 ± 1.79	0.084	0.933
Gender (male/female)	32/16	32/16	0.000	1.000
Temperature (°C)	37.58 ± 1.03	38.26 ± 1.45	1.597	0.114
Weight (kg)	15.24 ± 2.68	14.39 ± 1.47	1.927	0.057
Primary infection site				
Pulmonary infection	28 (58.3)	31 (64.6)	0.396	0.529
Digestive system infections	17 (35.4)	16 (33.3)	0.046	0.830
Blood system infection	10 (20.8)	7 (14.6)	0.643	0.423
Basic disease				
Blood system diseases	33 (70.2)	32 (66.7)	0.048	0.827
Congenital heart disease	11 (22.9)	8 (16.7)	0.591	0.442
Treatment measures				
Antibacterial treatments within 1 h	40 (83.3)	33 (68.8)	2.802	0.094
Duration of mechanical ventilation (h)	83.41 ± 35.17	144.36 ± 52.55	6.678	0.000
Duration of vasoactive drug maintenance (h)	74.02 ± 31.90	78.16 ± 42.56	0.539	0.591
Complication				
ARDS	13 (27.1)	15 (31.3)	0.202	0.653
Shock	21 (43.8)	35 (72.9)	8.400	0.004
Number of organs with dysfunction	1.76 ± 0.78	3.42 ± 1.51	6.767	0.000
Laboratory examination				
WBC ( $\times 10^9$ /L)	13.96 ± 2.85	12.74 ± 3.21	1.969	0.052
PLT ( $\times 10^9$ /L)	107.38 ± 33.70	85.26 ± 45.35	2.712	0.008
Hb (g/L)	105.03 ± 20.25	104.42 ± 22.19	0.141	0.888
PCT (ng/mL)	7.48 ± 4.51	19.97 ± 6.38	11.177	0.000
CRP (ng/mL)	115.67 ± 66.23	135.14 ± 77.62	1.324	0.189
ALB (g/L)	40.11 ± 2.57	33.57 ± 4.97	8.098	0.000
sCD163 (mg/L)	165.31 ± 175.26	253.66 ± 216.81	2.196	0.031
DD ( $\mu$ g/L)	802.16 ± 269.08	1648.93 ± 502.55	10.282	0.000
Lac (mmol/L)	3.22 ± 2.13	7.69 ± 5.24	5.475	0.000
Cr ( $\mu$ mol/L)	452.56 ± 348.72	336.15 ± 356.80	1.617	0.109
Children's score				
PEWS	5.35 ± 1.89	7.22 ± 1.43	5.467	0.000

ARDS: Acute respiratory distress syndrome; WBC: White blood cells; PLT: Platelets; Hb: Hemoglobin; PCT: Serum procalcitonin; CRP: C-reactive protein; ALB: Serum albumin; DD: D-dimer; Lac: serum lactic acid value; Cr: Creatinine; PEWS: Pediatric early warning score.

**Table 4 Multivariate logistic analysis of death in children with sepsis**

	Assignment	<i>P</i>	OR	95%CI
Antibacterial treatments within 1 h	Yes = 0, No = 1	0.017	1.654	1.620-1.892
Duration of mechanical ventilation	-	0.182	1.679	0.848-2.675
Shock	No = 0, Yes = 1	0.044	1.574	1.388-3.043
Number of organs with dysfunction	-	0.027	2.101	1.615-2.013
PLT ( $10^9$ /L)	-	0.126	1.086	0.821-1.678
PCT (ng/mL)	-	0.047	1.283	1.434-1.792
ALB (g/L)	-	0.024	0.842	0.323-2.806
sCD163 (mg/L)	-	0.563	1.558	0.466-3.850
DD ( $\mu$ g/L)	-	0.180	1.149	0.709-1.251
Lac (mmol/L)	-	0.115	1.435	0.562-1.785
PEWS	-	0.012	2.476	1.153-2.617

PLT: Platelets; PCT: Serum procalcitonin; ALB: Serum albumin; DD: D-dimer; Lac: serum lactic acid value; PEWS: Pediatric early warning score.



**Figure 2** Receiver operating characteristic curve analysis of serum albumin, serum procalcitonin, pediatric early warning score, and their combination to assess death in children with sepsis. ALB: Serum albumin; PCT: Serum procalcitonin; PEWS: Pediatric early warning score.

## ARTICLE HIGHLIGHTS

### Research background

Sepsis is an inflammatory reaction caused by infection, and the microorganisms that cause infection are mainly bacteria. In the early stage of the disease, the clinical manifestations in children are nonspecific. However, once the inflammatory reaction is stimulated, even if the treatment is delayed several hours, the mortality of children can be significantly increased. Therefore, accurate early assessment of the prognosis of children with sepsis is the most effective way to improve the complications of children with sepsis.

### Research motivation

There are currently no specific laboratory tests or markers that can early predict the prognosis of children with sepsis. Moreover, some clinical symptoms of children with sepsis are often similar to those of other diseases, which increases the difficulty in diagnosing sepsis. Currently, studies have shown that platelet, C-reactive protein, serum procalcitonin (PCT), serum albumin (ALB), hemoglobin scavenger receptor, and pediatric early warning score (PEWS) can assess the recovery of children with sepsis after treatment, but the children's various indicators are affected by individual and environmental changes. Therefore, the clinic needs a method to make a more accurate prediction of the complications of children with sepsis at an early stage.

### Research objectives

The present study intended to conduct a nested case-control study to assess the value of serum markers in combination with Brighton PEWS in predicting the prognosis of children with sepsis, in order to explore whether it is a more accurate means of assessing the prognosis of children with sepsis or not.

### Research methods

A total of 205 children diagnosed with sepsis were enrolled. After admission, the patient began broad-spectrum antibiotic treatment and retained bacterial culture. The central venous catheter was indwelled and early rehydration was given to prevent shock. In the nested case-control study, patients who died during the study cohort were included in a study group, and children who did not die in the same cohort were defined as a control group. Baseline data, serological markers, and PEWS scores were recorded for the subjects. Conditional logistic regression was used to analyze the influencing factors of death in children with sepsis after treatment. Receiver operating characteristic (ROC) curves were established to evaluate the value of the indicators to predict the prognosis of the children.

### Research results

There were 48 children each in the experimental group and the control group. Multivariate logistic regression analysis indicated that antibacterial treatments within 1 h, shock, multiple organ dysfunction syndromes (MODS), PCT, ALB, and PEWS were independent influencing factors of death in children. ROC curve analysis found that the area under the curve of the combination of ALB, PCT, and PEWS in predicting the death in children with sepsis was the highest, and the sensitivity was significantly higher than those of the three individuals. Therefore, the combination of the three indicators can reduce the impact of differences in organizational and physical compensation on the assessment results and improve the accuracy.

### Research conclusions

The present study found that antibacterial treatments within 1 h, shock, MODS, PCT, ALB, and PEWS were independent risk factors for the death of children with sepsis. The combination of PCT, ALB, and PEWS for predicting the prognosis of children with sepsis can improve the accuracy of the prediction.

### Research perspectives

The results of the study need further evaluation because the sample size was limited. However, the results suggest that PEWS score combined with serological indicators can improve the accuracy of prognosis prediction in children with sepsis, which provides useful information for the treatment of children with sepsis.

## REFERENCES

- Zonneveld R**, Martinelli R, Shapiro NI, Kuijpers TW, Plötz FB, Carman CV. Soluble adhesion molecules as markers for sepsis and the potential pathophysiological discrepancy in neonates, children and adults. *Crit Care* 2014; **18**: 204 [PMID: 24602331 DOI: 10.1186/cc13733]
- Cuenca AG**, Joiner DN, Gentile LF, Cuenca AL, Wynn JL, Kelly-Scumpia KM, Scumpia PO, Behrns KE, Efron PA, Nacionales D, Lui C, Wallet SM, Reeves WH, Mathews CE, Moldawer LL. TRIF-dependent innate immune activation is critical for survival to neonatal gram-negative sepsis. *J Immunol* 2015; **194**: 1169-1177 [PMID: 25548220 DOI: 10.4049/jimmunol.1302676]
- Bogale TN**, Worku AG, Bikis GA, Kebede ZT. Why gone too soon? Examining social determinants of neonatal deaths in northwest Ethiopia using the three delay model approach. *BMC Pediatr* 2017; **17**: 216 [PMID: 29282018 DOI: 10.1186/s12887-017-0967-9]
- Sankar MJ**, Natarajan CK, Das RR, Agarwal R, Chandrasekaran A, Paul VK. When do newborns die? A systematic review of timing of overall and cause-specific neonatal deaths in developing countries. *J Perinatol* 2016; **36** Suppl 1: S1-S11 [PMID: 27109087 DOI: 10.1038/jp.2016.27]
- van Paridon BM**, Sheppard C, G GG, Joffe AR; Alberta Sepsis Network. Timing of antibiotics, volume, and vasoactive infusions in children with sepsis admitted to intensive care. *Crit Care* 2015; **19**: 293 [PMID: 26283545 DOI: 10.1186/s13054-015-1010-x]
- Hahn WH**, Song JH, Kim H, Park S. Is procalcitonin to C-reactive protein ratio useful for the detection of late onset neonatal sepsis? *J Matern Fetal Neonatal Med* 2018; **31**: 822-826 [PMID: 28277917 DOI: 10.1080/14767058.2017.1297410]
- Yang AP**, Liu J, Yue LH, Wang HQ, Yang WJ, Yang GH. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. *Clin Chem Lab Med* 2016; **54**: 345-351 [PMID: 26351925 DOI: 10.1515/cclm-2015-0277]
- Agulnik A**, Méndez Aceituno A, Mora Robles LN, Forbes PW, Soberanis Vasquez DJ, Mack R, Antillon-Klussmann F, Kleinman M, Rodriguez-Galindo C. Validation of a pediatric early warning system for hospitalized pediatric oncology patients in a resource-limited setting. *Cancer* 2017; **123**: 4903-4913 [PMID: 28881451 DOI: 10.1002/cncr.30951]
- Miranda JOF**, Camargo CL, Nascimento CL, Sobrinho, Portela DS, Monaghan A. Accuracy of a pediatric early warning score in the recognition of clinical deterioration. *Rev Lat Am Enfermagem* 2017; **25**: e2912 [PMID: 28699997 DOI: 10.1590/1518-8345.1733.2912]
- Brilli RJ**, Goldstein B. Pediatric sepsis definitions: past, present, and future. *Pediatr Crit Care Med* 2005; **6**: S6-S8 [PMID: 15857561 DOI: 10.1097/01.PCC.0000161585.48182.69]
- Ernst VL**. Nested case-control studies. *Prev Med* 1994; **23**: 587-590 [PMID: 7845919 DOI: 10.1006/pmed.1994.1093]
- DeAngelo AJ**, Bell DG, Quinn MW, Long DE, Ouellette DR. Erythropoietin response in critically ill mechanically ventilated patients: a prospective observational study. *Crit Care* 2005; **9**: R172-R176 [PMID: 15987387 DOI: 10.1186/cc3480]
- Chun K**, Syndergaard C, Damas C, Trubey R, Mukindaraj A, Qian S, Jin X, Breslow S, Niemz A. Sepsis Pathogen Identification. *J Lab Autom* 2015; **20**: 539-561 [PMID: 25631157 DOI: 10.1177/2211068214567345]
- Plunkett A**, Tong J. Sepsis in children. *BMJ* 2015; **350**: h3017 [PMID: 26060188 DOI: 10.1136/bmj.h3017]
- Gotts JE**, Matthay MA. Sepsis: pathophysiology and clinical management. *BMJ* 2016; **353**: i1585 [PMID: 27217054 DOI: 10.1136/bmj.i1585]
- Weiss SL**, Fitzgerald JC, Balamuth F, Alpern ER, Lavelle J, Chilutti M, Grundmeier R, Nadkarni VM, Thomas NJ. Delayed antimicrobial therapy increases mortality and organ dysfunction duration in pediatric sepsis. *Crit Care Med* 2014; **42**: 2409-2417 [PMID: 25148597 DOI: 10.1097/CCM.0000000000000509]
- Sapa A**, Rak A, Wybieralska M, Machoń J, Krzywonos-Zawadzka A, Zawadzki K, Welna M, Woźniak M. Diagnostic usefulness of sCD163, procalcitonin and neopterin for sepsis risk assessment in critically ill patients. *Adv Clin Exp Med* 2017; **26**: 101-108 [PMID: 28397440 DOI: 10.17219/acem/63251]
- Lu Q**, Duan H, Yu J, Yao Y. Are Global Coagulation and Platelet Parameters Useful Markers for Predicting Late-Onset Neonatal Sepsis? *Clin Lab* 2016; **62**: 73-79 [PMID: 27012035]
- Vincent JL**, De Backer D, Wiedermann CJ. Fluid management in sepsis: The potential beneficial effects of albumin. *J Crit Care* 2016; **35**: 161-167 [PMID: 27481753 DOI: 10.1016/j.jcrc.2016.04.019]
- Schlapbach LJ**, Straney L, Alexander J, MacLaren G, Festa M, Schibler A, Slater A; ANZICS Paediatric Study Group. Mortality related to invasive infections, sepsis, and septic shock in critically ill children in Australia and New Zealand, 2002-13: a multicentre retrospective cohort study. *Lancet Infect Dis* 2015; **15**: 46-54 [PMID: 25471555 DOI: 10.1016/S1473-3099(14)71003-5]
- Castillo L**. High elevated ferritin levels and the diagnosis of HLH/Sepsis/SIRS/MODS/MAS. *Pediatr Blood Cancer* 2008; **51**: 710; author reply 710-710; author reply 711 [PMID: 18615508 DOI: 10.1002/pbc.21681]
- Carcillo JA**, Simon DW, Podd BS. How We Manage Hyperferritinemic Sepsis-Related Multiple Organ Dysfunction Syndrome/Macrophage Activation Syndrome/Secondary Hemophagocytic Lymphohistiocytosis. *Pediatr Crit Care Med* 2015; **16**: 598-600 [PMID: 26154908 DOI: 10.1097/PCC.0000000000000460]



- 23 **Garnacho-Montero J**, Huici-Moreno MJ, Gutiérrez-Pizarra A, López I, Márquez-Vácaro JA, Macher H, Guerrero JM, Puppo-Moreno A. Prognostic and diagnostic value of eosinopenia, C-reactive protein, procalcitonin, and circulating cell-free DNA in critically ill patients admitted with suspicion of sepsis. *Crit Care* 2014; **18**: R116 [PMID: [24903083](#) DOI: [10.1186/cc13908](#)]
- 24 **Hedegaard SS**, Wisborg K, Hvas AM. Diagnostic utility of biomarkers for neonatal sepsis--a systematic review. *Infect Dis (Lond)* 2015; **47**: 117-124 [PMID: [25522182](#) DOI: [10.3109/00365548.2014.971053](#)]
- 25 **Meisner M**, Tschaikowsky K, Palmaers T, Schmidt J. Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. *Crit Care* 1999; **3**: 45-50 [PMID: [11056723](#) DOI: [10.1186/cc306](#)]
- 26 **Larkin CM**, Santos-Martinez MJ, Ryan T, Radomski MW. Sepsis-associated thrombocytopenia. *Thromb Res* 2016; **141**: 11-16 [PMID: [26953822](#) DOI: [10.1016/j.thromres.2016.02.022](#)]
- 27 **Fuijkschot J**, Vernhout B, Lemson J, Draaisma JM, Loeffen JL. Validation of a Paediatric Early Warning Score: first results and implications of usage. *Eur J Pediatr* 2015; **174**: 15-21 [PMID: [24942238](#) DOI: [10.1007/s00431-014-2357-8](#)]
- 28 **Rivero-Martín MJ**, Prieto-Martínez S, García-Solano M, Montilla-Pérez M, Tena-Martín E, Ballesteros-García MM. [Results of applying a paediatric early warning score system as a healthcare quality improvement plan]. *Rev Calid Asist* 2016; **31** Suppl 1: 11-19 [PMID: [27091366](#) DOI: [10.1016/j.cali.2016.03.005](#)]
- 29 **Liu Y**, Yang W, Wei J. Guiding Effect of Serum Procalcitonin (PCT) on the Antibiotic Application to Patients with Sepsis. *Iran J Public Health* 2017; **46**: 1535-1539 [PMID: [29167772](#)]
- 30 **Conner BJ**. Treating Hypoalbuminemia. *Vet Clin North Am Small Anim Pract* 2017; **47**: 451-459 [PMID: [27890435](#) DOI: [10.1016/j.cvsm.2016.09.009](#)]
- 31 **Seo MH**, Choa M, You JS, Lee HS, Hong JH, Park YS, Chung SP, Park I. Hypoalbuminemia, Low Base Excess Values, and Tachypnea Predict 28-Day Mortality in Severe Sepsis and Septic Shock Patients in the Emergency Department. *Yonsei Med J* 2016; **57**: 1361-1369 [PMID: [27593863](#) DOI: [10.3349/ymj.2016.57.6.1361](#)]

**P- Reviewer:** Kantsevov S, Snyder J, Tokunaga Y

**S- Editor:** Wang JL **L- Editor:** Wang TQ **E- Editor:** Tan WW





## Retrospective Study

# Intestinal endometriosis: Diagnostic ambiguities and surgical outcomes

Jun Woo Bong, Chang Sik Yu, Jong Lyul Lee, Chan Wook Kim, Yong Sik Yoon, In Ja Park, Seok-Byung Lim, Jin Cheon Kim

**ORCID number:** Jun Woo Bong (0000-0002-9530-7069); Chang Sik Yu (0000-0001-9401-9981); Jong Lyul Lee (0000-0002-5878-8000); Chan Wook Kim (0000-0002-2382-0939); Yong Sik Yoon (0000-0002-3196-8423); In Ja Park (0000-0001-5355-3969); Seok-Byung Lim (0000-0001-8824-4808); Jin Cheon Kim (0000-0003-4823-8619).

**Author contributions:** Bong JW contributed to the manuscript writing, performing data collecting and analysis; Yu CS contributed to the drafting conception and design; Lee JL, Kim CW and Yoon YS contributed to the collecting data; Park IJ, Lim SB and Kim JC contributed to the confirmation results.

### Institutional review board

**statement:** This study was reviewed and approved by the Institutional Review Board of Asan Medical Center.

**Informed consent statement:** This study is retrospective study and informed consent is waived because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment.

### Conflict-of-interest statement:

There is no conflict of interest or no source of support.

**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

**Jun Woo Bong, Chang Sik Yu, Jong Lyul Lee, Chan Wook Kim, Yong Sik Yoon, In Ja Park, Seok-Byung Lim, Jin Cheon Kim,** Division of Colon and Rectal Surgery, Department of Surgery, University of Ulsan College of Medicine and Asan Medical Center, Seoul 05505, South Korea

**Corresponding author:** Chang Sik Yu, MD, PhD, Professor, Surgeon, Division of Colon and Rectal Surgery, Department of Surgery, University of Ulsan College of Medicine and Asan Medical Center, 88, Olympic-ro 43-gil, Songpa-gu, Seoul 05505, South Korea.

[csyu@amc.seoul.kr](mailto:csyu@amc.seoul.kr)

**Telephone:** +82-2-30103494

**Fax:** +82-2-30106701

## Abstract

### BACKGROUND

Endometriosis is a common disease for women of reproductive age. However, when it involves intestines, it is difficult to diagnose preoperatively because its symptoms overlap with other diseases and the results of evaluations can be unspecific. Thus it is important to know the clinical characteristics of intestinal endometriosis and how to exactly diagnose.

### AIM

To analyze patients in whom intestinal endometriosis was diagnosed after surgical treatments, and to evaluate the clinical characteristics of preoperatively misdiagnosed cases.

### METHODS

We retrospectively reviewed the pathologic reports of 30 patients diagnosed as having intestinal endometriosis based on surgical specimens between January 2000 and December 2017. We reviewed their clinical characteristics and surgical outcomes.

### RESULTS

Twenty-three (76.6%) patients showed symptoms associated with endometriosis, with dysmenorrhea being the most common ( $n = 9$ , 30.0%). Thirteen patients (43.3%) had a history of pelvic surgeries. Ten patients (33.3%) had a history of treatment for endometriosis. Only 4 patients (13.3%) had a diagnosis of endometriosis based on endoscopic biopsy findings. According to preoperative evaluations, 13 patients (43.3%) had an initial diagnosis of pelvic endometriosis and 17 patients (56.6%) were misdiagnosed as having other diseases. The most common misdiagnosis was submucosal tumor in the large intestine ( $n = 8$ , 26.7%),

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

**Received:** November 5, 2018

**Peer-review started:** November 5, 2018

**First decision:** December 20, 2018

**Revised:** January 9, 2019

**Accepted:** January 26, 2019

**Article in press:** January 26, 2019

**Published online:** February 26, 2019

followed by malignancies of the colon/rectum ( $n = 3$ , 10.0%) and ovary ( $n = 3$ , 10.0%). According to the Clavien-Dindo classification, 5 complications were grade I or II and 2 complications were grade IIIa. The median follow-up period was 26.9 (0.6-132.1) mo, and only 1 patient had a recurrence of endometriosis.

## CONCLUSION

Intestinal endometriosis is difficult to diagnose preoperatively because it mimics various intestinal diseases. Thus, if women of reproductive age have ambiguous symptoms and signs with nonspecific radiologic and/or endoscopic findings, intestinal endometriosis should be included in the differential diagnosis.

**Key words:** Endometriosis; Intestinal endometriosis; Diagnosis; Surgery; Treatment

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Intestinal endometriosis is difficult to diagnose preoperatively because it mimics various intestinal diseases. The aim of this study is to analyze patients in whom intestinal endometriosis was diagnosed after surgical treatments, and to evaluate the clinical characteristics of preoperatively misdiagnosed cases. According to preoperative evaluations, 13 patients (43.3%) had an initial diagnosis of pelvic endometriosis and 17 patients (56.6%) were misdiagnosed as having other diseases. Only 4 patients (13.3%) had a diagnosis of endometriosis based on endoscopic biopsy findings. Thirteen patients (43.3%) had a history of pelvic surgeries. Ten patients (33.3%) had a history of treatment for endometriosis. Thus, if women of reproductive age have ambiguous symptoms and signs with nonspecific radiologic and/or endoscopic findings, intestinal endometriosis should be included in the differential diagnosis.

**Citation:** Bong JW, Yu CS, Lee JL, Kim CW, Yoon YS, Park IJ, Lim SB, Kim JC. Intestinal endometriosis: Diagnostic ambiguities and surgical outcomes. *World J Clin Cases* 2019; 7(4): 441-451

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/441.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.441>

## INTRODUCTION

Endometriosis is defined as a disease of endometrial-like tissue outside the uterus inducing a chronic inflammatory reaction<sup>[1]</sup>. Endometriosis is a relatively common disease with a reported incidence of up to 15% in women of reproductive age<sup>[1]</sup>. The mechanism of endometriosis has not been known well; however, ectopic implantation of endometrial cells following retrograde menstruation *via* the Fallopian tube into the pelvis is accepted as the main cause of endometriosis. The clinical manifestations of endometriosis include pelvic pain, infertility, and a pelvic mass. Because endometrial cells are influenced by hormonal changes, symptoms of endometriosis often worsen during the menstrual period.

When endometrial-like glands and stroma infiltrate the bowel wall, reaching at least the subserous fat tissue or the adjacent subserous plexus, the condition is diagnosed as intestinal endometriosis<sup>[2]</sup>. The incidence of intestinal endometriosis is estimated to be from 3% to 37% of all endometriosis cases<sup>[3]</sup>. In most cases (> 90%), intestinal endometriosis involves the sigmoid colon or rectum and the posterior pelvic compartment peritoneum<sup>[4]</sup>. It presents with symptoms including diarrhea, constipation, tenesmus, and rectal bleeding. Pelvic pain and infertility can also occur with or without these symptoms. The aims of treatment are to relieve symptoms and recover fertility with minimal injury to other gynecologic organs. Medical treatments including nonsteroidal anti-inflammatory drugs, oral contraceptives, progesterone, and gonadotropin-releasing hormone analogues were reported to be effective in relieving symptoms and in eradicating microscopic disease and diseases of vital structures<sup>[2]</sup>. *En bloc* resection is preferred to completely remove the endometrial tissue because multifocality (another lesion within 2 cm from the main lesion) and multicentricity (another lesion beyond 2 cm from the main lesion) are common in intestinal endometriosis (incidence: 62% and 38%, respectively)<sup>[5]</sup>.

Many diseases can be included in the differential diagnosis of intestinal

endometriosis, such as irritable bowel syndrome, solitary rectal ulcer syndrome, inflammatory bowel disease, colorectal cancer, ischemic colitis, and metastatic tumor<sup>[6]</sup>. However, reaching a diagnosis of intestinal endometriosis is complicated because its symptoms overlap with those of other diseases. Additionally, because endoscopically obtained biopsy material has a superficial origin and endometriosis usually involves the deeper layers of the bowel wall, tissue obtained in an endoscopic manner may reflect chronic injury but lack endometriotic foci<sup>[7]</sup>. Lesions, especially if firm and obstructive, can also be mistaken intraoperatively for gastrointestinal carcinoma. Misdiagnosis inevitably contributes to diagnostic delay and increased economic burden because of inappropriate management<sup>[6]</sup>. In this study, we reviewed the clinical courses of patients in whom intestinal endometriosis was diagnosed after surgical treatments at our institute, to evaluate the clinical characteristics of preoperatively misdiagnosed cases and the surgical outcomes of intestinal endometriosis.

## MATERIALS AND METHODS

From the database about pathologic reports of our institution, a tertiary referral center, we searched and collected medical records of patients who had been diagnosed with intestinal endometriosis from their surgical specimens from January 2000 to December 2017. We reviewed the clinical characteristics of the patients, including age at surgical treatment and history of abdominal surgery and endometriosis. Clinical presentation and computed tomography (CT) imaging findings related to the diagnosis were obtained from the medical records. Endoscopic findings were collected and categorized according to mucosal change and eccentric wall thickening. Biopsy specimens were obtained from lesions with abnormal changes by using standard endoscopic forceps. Preoperative diagnosis, locations of lesions, types of bowel surgeries, and combined operations were analyzed. Additionally, we collected data associated with postoperative complications within 30 d after the surgery and categorized them according to the Clavien-Dindo classification (CDC) to evaluate surgical outcomes. This study was approved by the Institutional Review Board of Asan Medical Center (IRB approval number: S2017-2143-0001).

## RESULTS

### *Baseline characteristics and preoperative evaluations*

Fifty patients with histologically confirmed intestinal endometriosis were identified and we retrospectively reviewed their medical records. Among them, cases were excluded if the diagnosis of intestinal endometriosis was made incidentally during surgical resection for other pathologies, including colorectal cancer ( $n = 10$ ), ovarian cancer ( $n = 7$ ), uterine myoma ( $n = 2$ ), and Crohn's disease ( $n = 1$ ). Finally, a total of 30 patients were included in this study. The median age at surgery for intestinal endometriosis was 43 (29-53) years (Table 1). Twenty-three (76.7%) patients showed symptoms associated with endometriosis, with dysmenorrhea being the most common ( $n = 9$ , 30.0%), followed by hematochezia ( $n = 5$ , 16.7%) and abdominal pain ( $n = 4$ , 13.3%). In the remaining 7 patients (23.3%), intestinal endometriosis was incidentally diagnosed using endoscopic screening. Thirteen patients (43.3%) had a history of pelvic surgeries including cesarean section, transabdominal hysterectomy, and/or unilateral/bilateral salpingo-oophorectomy for uterine myoma, ovarian cyst, and pelvic endometriosis. Additionally, 10 patients (33.3%) had a history of treatment for endometriosis and 6 patients (20.0%) had been previously treated with both surgical and medical therapies. Figure 1 shows the finding of magnetic resonance imaging (MRI) of a patient who were diagnosed preoperatively with intestinal endometriosis at rectosigmoid colon. A nodule infiltrating the rectal wall from the outside is detectable. Preoperative CT images showed a mass in the intestine or other gynecologic organs in most patients ( $n = 23$ , 76.7%; Table 2). Figure 2A shows a CT image of a patient who had a diagnosis of rectal submucosal tumor preoperatively. An about 3-cm mass with mild wall thickening was identified at the upper rectum without any infiltration and luminal obstruction. Figure 2B is a CT image from another patient, showing wall thickness and infiltration at the rectosigmoid colon without a definite mass. This patient had a diagnosis of rectosigmoid colon cancer preoperatively. The most common endoscopic finding was eccentric wall thickening of the bowel mucosa ( $n = 16$ , 53.4%). Of these 16 patients, 8 patients (26.7%) showed no mucosal change in the colonic lumen. Moreover, in 4 patients (13.3%), no abnormal change was identified in colonoscopic findings. Only 4 patients (13.3%) had a

diagnosis of endometriosis based on endoscopic biopsies. **Figure 3A** demonstrates the endoscopic biopsy of a patient who was diagnosed as having rectal endometriosis. Severe luminal obstruction with extrinsic compression and hyperemic change was identified in the mucosa. **Figure 3B** shows the endoscopic findings of another patient who was diagnosed as having submucosal tumor at the sigmoid colon. A mass was appeared to be in the submucosal layer without abnormal change in the colonic mucosa, and the diagnosis based on endoscopic biopsy was nonspecific colitis.

### **Preoperative diagnosis of patients with intestinal endometriosis**

Thirteen patients (43.3%) had an initial diagnosis of pelvic endometriosis according to the results of preoperative evaluations (**Table 3**). The remaining 17 patients (56.3%) were misdiagnosed as having other diseases. The most common misdiagnosis was submucosal tumor in the large intestine ( $n = 8$ , 26.7%), followed by malignancies of the colon/rectum ( $n = 3$ , 10.0%) and ovary ( $n = 3$ , 10.0%).

### **Location and types of surgeries for intestinal endometriosis**

Intestinal endometriosis most frequently occurred in the sigmoid colon/rectum ( $n = 25$ , 83.3%) (**Table 4**). *En bloc* resections were performed including the bowel and other involved pelvic organs. Anterior resection and low anterior resection of the colon and rectum were performed in most patients ( $n = 25$ , 83.3%), whereas gynecologic operations were also performed in 15 patients (50.0%). Ureteroureterostomy was performed in 1 patient because of ureteric invasion of endometriosis inducing hydroureteronephrosis (**Table 5**). **Figure 4A** shows the gross specimen sections from a patient with endometriosis at the rectosigmoid colon. The endometriotic nodule caused thickening of the bowel wall and mucosal changes. **Figure 4B** shows the microscopic findings of this patient. Several endometrial glands were embedded in the submucosal layer, and foci of dense fibrosis surround the glands. The glands infiltrate the muscularis propria with an irregular margin. CD10 and ER (estrogen receptor) were positive as results of immunohistochemical staining and these results supported the diagnosis of this patient (**Figure 4C** and **D**).

Complications within 30 d after the operation occurred in 7 (23.3%) patients: Pelvic abscess in 3 patients, paralytic ileus in 3 patients, and acute pyelonephritis in 1 patient (**Table 6**). Of these patients, 5 (16.7%) were treated with conservative management (CDC grade I-II) and 2 (6.7%) required intervention to manage the complication of fluid collection in the pelvic cavity (CDC grade IIIa). No patient had severe complications of CDC grade IIIb or more. Only 1 patient experienced recurrence at 3 years and 4 mo after the operation, and underwent bilateral salpingo-oophorectomy for recurrence of endometrioma of the ovary. Median follow-up period after operation for intestinal endometriosis was 24 mo (0-128).

## **DISCUSSION**

Intestinal endometriosis mostly presents with symptoms mimicking other diseases; thus, it is difficult to diagnose preoperatively. Screening techniques including CT and colonoscopy have limited values in the diagnosis because the disease invades inwards from the serosa and the mucosa remains uninvolved in the majority of cases. CT is an important modality as the first-line screening tool for identifying endometriomas and mapping multifocal lesions. However, our data showed that the incidence of intestinal endometriosis without a mass on CT images was 23.3%, and all of these cases were misdiagnosed as other diseases. In addition, although CT might reveal bowel wall thickening with the main lesions, it is not sufficient to diagnose intestinal endometriosis because evaluations of the invaded bowel wall or the characteristics of the mass cannot be exactly performed with CT images.

MRI is one of the most commonly used techniques for this purpose. A contrast-enhanced mass or hyperintense foci on T1-weighted MRI strongly suggest the presence of hemorrhagic foci secondary to endometriosis<sup>[1]</sup>. The sensitivity and specificity of MRI in detecting pelvic endometriosis is around 90%<sup>[1,2]</sup>. Transvaginal ultrasound (TVUS) may identify the presence of pelvic endometriosis with a relatively high accuracy (sensitivity, 83%; specificity, 94%) and help in estimating the depth of infiltration of the nodules in the intestinal wall<sup>[8]</sup>. In our cases initially diagnosed as pelvic endometriosis (13 cases), MRI and TVUS were used in 6 cases (46.1%) and 11 cases (84.6%), respectively. On the other hand, in our misdiagnosed cases (17 cases), MRI and TVUS were performed in only 3 cases (17.6%) and 4 cases (23.5%), respectively. Thus, in cases that intestinal endometriosis is suspicious, further evaluation using MRI and TVUS is important to diagnose intestinal endometriosis preoperatively. In our institute, we prefer MRI plus TVUS because they are relatively convenient modalities to understand the extent of disease and depth of invasion.



**Table 1** Baseline characteristic of patients with intestinal endometriosis

Characteristics	n (%)
Age, yr, median (range)	43 (29-53)
Previous abdominal surgery	15 (50.0)
Pelvic surgery	13 (43.3)
Cesarean section	9 (30.0)
TAH ± BSO	4 (13.3)
Splenectomy	1 (3.3)
History of endometriosis	10 (33.3)
Surgical treatment	9 (30.0)
Ovarian cystectomy	3 (10.0)
TAH ± SO	2 (6.6)
Oophorectomy	2 (6.6)
Adhesiolysis	2 (6.6)
Medical treatment	7 (23.3)
Both	6 (20.0)
Initial symptom	
Dysmenorrhea	9 (30.0)
Hematochezia	5 (16.7)
Abdominal pain	4 (13.3)
Constipation	2 (6.7)
Abdominal mass	1 (3.3)
Urinary discomfort	1 (3.3)
Diarrhea	1 (3.3)
No symptom	7 (23.3)

TAH: Total abdominal hysterectomy; BSO: Bilateral salpingo-oophorectomy.

However, since TVUS depends on the experience of the operator, other modalities such as MR-enterography and red-blood cell scintigraphy can be good alternatives with more than 90% of sensitivity<sup>[9,10]</sup>. MR-enterography especially enables to figure out details of small bowel involvement. Thus, tailored choice of modality is also important to diagnosis intestinal endometriosis preoperatively.

In our cases, the diagnostic accuracy of colonoscopy was very low (13.3%). Although colonoscopy is the first-line test in the evaluation of colonic bleeding, it is of little use in the diagnosis of intestinal endometriosis, because infiltration of the lesion into the mucosa is rare<sup>[11]</sup>. The endoscopic findings of colorectal endometriosis were narrowing, bulging into the lumen, and sometimes polyps or mucosal change with erythema and granularity<sup>[12]</sup>. These nonspecific findings of colonoscopy and biopsy of only the superficial layers make the diagnosis of intestinal endometriosis more difficult.

Evaluation of the operative history of patients has an important role in the diagnosis of intestinal endometriosis. In our study, about 13 patients (41.9%) had a history of pelvic surgery including cesarean section and hysterectomy. The positive correlation between a previously operated pelvis and endometriosis has been reported previously in other studies<sup>[13]</sup>. Although the mechanism is not known well, endometrial cells are believed to be incidentally implanted into the peritoneum during pelvic surgery, which can develop into pelvic endometriosis. In our study, intestinal endometriosis occurred after hysterectomy in 4 cases. Endometriosis can occur even after hysterectomy if the function of the ovary is preserved or hormone replacement therapy is used after hysterectomy because the remnant endometriosis of microscopic foci or deeply infiltrated lesions may develop into the disease<sup>[14]</sup>. In our cases, the ovary was preserved in all 4 patients with a history of hysterectomy at the time of diagnosis of intestinal endometriosis.

Previous treatment for endometriosis is also an important factor in diagnosing intestinal endometriosis. The overall recurrence rate of endometriosis is reported to be up to 67%<sup>[15]</sup>. The recurrence of endometriosis after bowel resection has been reported in 4.7%-25% of cases during the follow-up period of > 2 years<sup>[5]</sup>. In our cases, 10 patients (33.3%) had a history of treatments for endometriosis at the time of diagnosis of intestinal endometriosis. In addition, 1 patient underwent reoperation for pelvic



**Figure 1** Magnetic resonance imaging, T2 weighted sagittal image. A mass-like lesion of about 2 cm in diameter on the rectosigmoid colon junction appears to grow from the outside to the inside of colonic wall (arrow).

endometriosis (bilateral salpingo-oophorectomy) at 39 mo after bowel surgery for intestinal endometriosis. Several studies demonstrated that the recurrence risk increases if the lesions are not completely removed during the initial surgery, and that recurrence tends to occur at the same location<sup>[16]</sup>. Particularly in deep infiltrating endometriosis, the recurrence rate is higher with lymph node involvement or lymphovascular invasion<sup>[17]</sup>. Thus, intestinal endometriosis should be considered if patients presenting with abdominal symptoms have a history of endometriosis.

Previous treatments for other diseases can also mislead the diagnosis of patients with intestinal endometriosis. In our cases, a patient with acute abdominal symptoms and a stricture of the terminal ileum in CT images was diagnosed as having an acute aggravation of Crohn's disease due to the history of treatment for Crohn's disease. The patient underwent ileocecal resection, and the pathologic diagnosis was intestinal endometriosis at the terminal ileum. Another patient with repeated pelvic surgeries showed symptoms of obstruction. Small-bowel obstruction was identified on CT images, and postoperative adhesion of the small intestine was diagnosed. However, after small-bowel resection and anastomosis, endometriosis was proven to be the main cause of the abdominal symptoms.

We also examined postoperative complications of patients. Paralytic ileus and pelvic abscess were the main complications after surgery, and most of them were managed conservatively. Complications related to anastomosis, such as rectovaginal fistula, anastomotic leakage, and pelvic abscess, are reported as the major postoperative complications after surgical treatment for intestinal endometriosis, and their prevalence was highly variable among studies<sup>[5]</sup>. Most of the complications were related to combined resection of other pelvic organs such as the bladder, ureter, ovary, and uterus. Additionally, functional problems including voiding difficulty and sexual dysfunction were also reported as postoperative complications after surgical treatment for rectal endometriosis<sup>[18]</sup>. Thus, a diverting stoma should be considered in case of *en bloc* resection of other pelvic organs with the rectum to avoid anastomotic problems. Moreover, autonomic nerve preservation is also required during dissection of the rectum from the pelvis to prevent functional problems related to pelvic denervation.

As we mentioned above, the recurrence rate of endometriosis is relatively high. To prevent recurrence, it is important to remove all endometriotic tissue while preserving fertility<sup>[19]</sup>. There are various surgical treatment methods for intestinal endometriosis, including resection and anastomosis, discoid resection, and superficial shaving<sup>[5]</sup>. No study about the postoperative results of different surgical methods has been reported yet. Many authors have demonstrated that incomplete excision is a major cause of clinical recurrence; thus, *en bloc* resection with anastomosis is recommended to minimize recurrence<sup>[20]</sup>. Hormonal treatments for intestinal endometriosis cannot be offered to all women because they inhibit ovulation and may not be effective in cases with > 60% stenosis of the bowel lumen<sup>[2]</sup>. Postoperative hormonal therapy is considered effective in prolonging the interval between surgery and the first recurrence by maintaining the minimal disease state<sup>[21]</sup>. However, it is known to be ineffective in eliminating residual disease. In our study, it was not possible to examine the incidence of malignant transformation of endometriosis, because patients diagnosed with intestinal endometriosis incidentally after surgeries for malignancy were excluded. It is rare but endometriosis is known to have a malignant potential in less than 1%<sup>[22]</sup>. More careful surveillance should be considered to the patients with potentials such as repeated or rapid progression or mural nodules

**Table 2 Preoperative computed tomography and colonoscopic findings**

Findings	n (%)
Computed tomography	
Eccentric intestinal wall thickening with a mass	15 (50.0)
Mass in the ovary or uterus	8 (26.7)
Intestinal stricture without a mass	4 (13.3)
Not definite	3 (10.0)
Colonoscopy	
Eccentric wall thickening without mucosal change	8 (26.7)
Eccentric wall thickening with mucosal change	8 (26.7)
Normal	4 (13.3)
Not evaluated	10 (33.3)
Colonoscopic biopsy	
Inflammation	6 (20.0)
Endometriosis	4 (13.3)
Adenoma	2 (6.7)
Normal mucosa	1 (3.3)
Not evaluated	17 (56.7)

to monitor a malignant transformation of endometriosis<sup>[23]</sup>.

There are several limitations to this study coming from a retrospective review of pathologic reports. In our institution, the number of total patients who underwent surgical treatments for endometriosis was 1205 during the study period, thus the rate of intestinal endometriosis was 2.5% (30/1205). This is relatively low compared with other studies about intestinal endometriosis. Actually, this study mainly focused on the surgical cases of intestinal endometriosis, thus this rate reflected only surgical treatments for intestinal endometriosis. However, as previously mentioned above, intestinal endometriosis without severe symptoms can be initially managed with medical treatments and these patients were omitted from our study. Additionally, although *en bloc* resection is a principle for surgical treatment of pelvic endometriosis, the gynecologic surgeons in our institution showed a tendency to avoid surgical resection of the intestinal tract in some cases of superficial involvement of intestinal tract. These cases could not be included in our study. Therefore, the prevalence of intestinal endometriosis could not be exactly assessed with our data. Secondly, exact definitions of preoperative symptoms could not be established because of the insufficient description of preoperative symptoms in our electrical medical records. We tried to gather exact information about initial symptoms, however, some of them were not enough to identify details of symptoms, such as degree, duration and location. Nevertheless, this study can be meaningful, because it suggests to colorectal surgeons that the intestinal endometriosis should be considered if female patients with ambiguous results of preoperative evaluations show severe symptoms requiring surgical treatment.

In conclusion, the clinical characteristics of intestinal endometriosis can mimic those of various intestinal diseases. Intestinal endometriosis should be included in the differential diagnosis of women of reproductive age who have ambiguous symptoms and signs, as well as nonspecific radiologic and/or endoscopic findings.

**Table 3 Categories of preoperative diagnosis**

Preoperative diagnosis	n (%)
Pelvic endometriosis	13 (43.3)
Rectosigmoid endometriosis	9 (30.0)
Rectovaginal endometriosis	2 (6.7)
Ovarian endometrioma	2 (6.7)
Malignancy	7 (23.3)
Colorectal cancer	3 (10.0)
Ovarian cancer	3 (10.0)
Small intestinal cancer	1 (3.3)
Other disease	10 (33.3)
Colorectal submucosal tumor	8 (26.7)
Crohn's disease with intestinal stricture	1 (3.3)
Postoperative adhesion of the small intestine	1 (3.3)

**Table 4 Locations of intestinal endometriosis in surgical specimens**

Location	n (%)
Rectum	19 (63.3)
Sigmoid colon	6 (20.0)
Ileum	3 (10.0)
Cecum	2 (6.7)

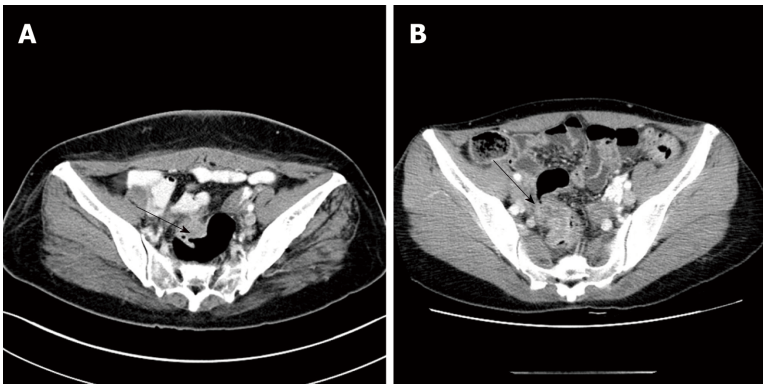
**Table 5 Types of operations for intestinal endometriosis**

Operations	n (%)
Type of intestinal operation	
Low anterior resection	15 (50.0)
Anterior resection	10 (33.3)
Ileocecal resection	3 (10.0)
Cecectomy	1 (3.3)
Resection and anastomosis of the small intestine	1 (3.3)
Type of combined operation	
TAH ± SO	7 (23.3)
Ovarian cystectomy	4 (13.3)
SO	3 (10.0)
Posterior vaginectomy	1 (3.3)
Ureteroureterostomy	1 (3.3)
None	14 (46.7)

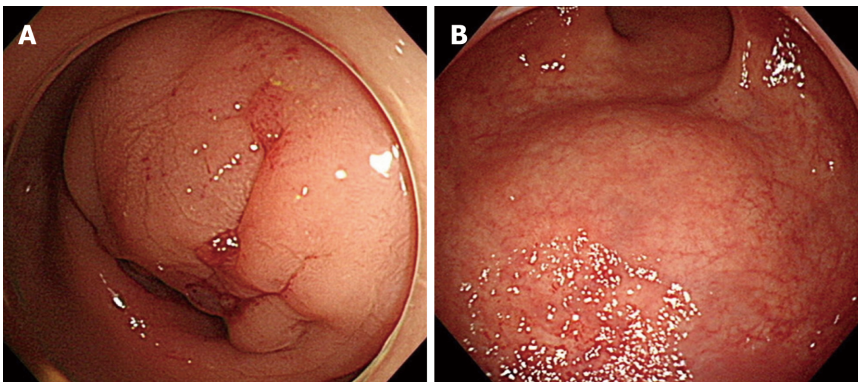
TAH: Total abdominal hysterectomy; BSO: Bilateral salpingo-oophorectomy.

**Table 6 Postoperative complications within 30 d after the operation**

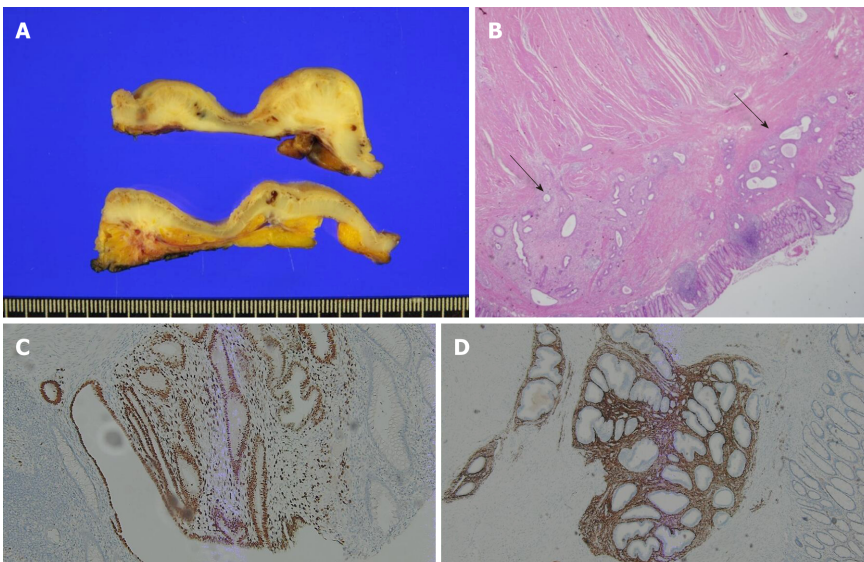
Complication	n (%)	Clavien-Dindo classification			
		I	II	IIIa	IIIb
Pelvic abscess	3 (10.0)	0	1	2	0
Paralytic ileus	3 (10.0)	3	0	0	0
Acute pyelonephritis	1 (3.3)	0	1	0	0
Total	7 (23.3)	3	2	2	0



**Figure 2 Computed tomography scans.** A: Upper rectal mass (arrow) without luminal obstruction and submucosal tumor diagnosed preoperatively; B: Colonic wall thickness (arrow) and infiltration and rectosigmoid colon cancer diagnosed preoperatively.



**Figure 3 Colonoscopic findings.** A: Severe luminal obstruction with extrinsic compression and mucosal change. Biopsy revealed endometriosis in the rectum; B: Extrinsic compression without mucosal change by a mass located at the submucosal layer.



**Figure 4 Pathologic and histologic findings.** A: Gross specimen sections showing endometriotic nodules infiltrating from the outer layers; B: Endometrial gland (arrow) in the submucosal layer, infiltrating to the muscularis mucosa (hematoxylin and eosin stain); C: Immunohistochemical examination for endometrial gland expressing ER; D: Immunohistochemical examination for the stroma expressing CD10.

## ARTICLE HIGHLIGHTS

### Research background



The intestinal endometriosis is a disease that endometrial tissue involves the small or large intestine. Endometriosis is relatively common disease, but intestinal endometriosis is very rare and it is difficult to diagnose preoperatively.

### Research motivation

A young woman who was diagnosed with Crohn's disease and under medical treatment for about 3 years at our hospital had suffered severe abdominal pain at right lower quadrant. We had found severe stricture of terminal ileum at computed tomography (CT) images. We had misdiagnosed her as acute phase of Crohn's disease, but the pathologic result was intestinal endometriosis. After that, we reviewed similar cases in our institution and identified there were several misdiagnosed cases before operations. We had also searched similar studies, but not enough information was acquired with their clinical characteristics.

### Research objectives

We aimed to evaluate the clinical characteristics of misdiagnosed cases before surgery and tried to suggest ways to reduce those cases.

### Research methods

We retrospectively reviewed medical records of patients who had been diagnosed with intestinal endometriosis from their surgical specimens. Fifty patients were identified and 20 cases were excluded because the diagnosis of intestinal endometriosis was made incidentally during surgical resection for other pathologies. A total of 30 patients were included in this study and their clinical characteristics including age, history of abdominal surgery or endometriosis were evaluated. Clinical presentation, CT imaging, endoscopic findings were also evaluated and preoperative diagnosis, locations of lesions, types of bowel surgeries, and combined operations were analyzed.

### Research results

According to preoperative evaluations, 13 patients (43.3%) had an initial diagnosis of pelvic endometriosis and 17 patients (56.6%) were misdiagnosed as having other diseases. Only 4 patients (13.3%) had a diagnosis of endometriosis based on endoscopic biopsy findings. The most common misdiagnosis was submucosal tumor in the large intestine ( $n = 8$ , 26.7%), followed by malignancies of the colon/rectum ( $n = 3$ , 10.0%) and ovary ( $n = 3$ , 10.0%).

### Research conclusions

Symptoms of intestinal endometriosis mimic various intestinal diseases, thus it is difficult to diagnose preoperatively. Intestinal endometriosis should be considered when women of reproductive age have ambiguous symptoms and signs with preoperative evaluations.

### Research perspectives

It will be meaningful to study about more long term results of patients with intestinal endometriosis and their potency of malignant formation.

## REFERENCES

- 1 **Wolthuis AM**, Meuleman C, Tomassetti C, D'Hooghe T, de Buck van Overstraeten A, D'Hoore A. Bowel endometriosis: colorectal surgeon's perspective in a multidisciplinary surgical team. *World J Gastroenterol* 2014; **20**: 15616-15623 [PMID: 25400445 DOI: 10.3748/wjg.v20.i42.15616]
- 2 **Ferrero S**, Camerini G, Maggiore ULR, Venturini PL, Biscaldi E, Remorgida V. Bowel endometriosis: Recent insights and unsolved problems. *World J Gastrointest Surg* 2011; **3**: 31-38 [DOI: 10.4240/wjgs.v3.i3.31]
- 3 **Remorgida V**, Ferrero S, Fulcheri E, Ragni N, Martin DC. Bowel endometriosis: presentation, diagnosis, and treatment. *Obstet Gynecol Surv* 2007; **62**: 461-470 [PMID: 17572918 DOI: 10.1097/01.ogx.0000268688.55653.5c]
- 4 **Campagnacci R**, Perretta S, Guerrieri M, Paganini AM, De Sanctis A, Ciavattini A, Lezoche E. Laparoscopic colorectal resection for endometriosis. *Surg Endosc* 2005; **19**: 662-664 [PMID: 15759190 DOI: 10.1007/s00464-004-8710-7]
- 5 **Meuleman C**, Tomassetti C, D'Hoore A, Van Cleynenbreugel B, Penninckx F, Vergote I, D'Hooghe T. Surgical treatment of deeply infiltrating endometriosis with colorectal involvement. *Hum Reprod Update* 2011; **17**: 311-326 [PMID: 21233128 DOI: 10.1093/humupd/dmq057]
- 6 **Seaman HE**, Ballard KD, Wright JT, de Vries CS. Endometriosis and its coexistence with irritable bowel syndrome and pelvic inflammatory disease: findings from a national case-control study--Part 2. *BJOG* 2008; **115**: 1392-1396 [PMID: 18715239 DOI: 10.1111/j.1471-0528.2008.01879.x]
- 7 **Yantiss RK**, Clement PB, Young RH. Endometriosis of the intestinal tract: a study of 44 cases of a disease that may cause diverse challenges in clinical and pathologic evaluation. *Am J Surg Pathol* 2001; **25**: 445-454 [PMID: 11257618 DOI: 10.1097/0000478-200104000-00003]
- 8 **Goncalves MO**, Podgaec S, Dias JA, Gonzalez M, Abrao MS. Transvaginal ultrasonography with bowel preparation is able to predict the number of lesions and rectosigmoid layers affected in cases of deep endometriosis, defining surgical strategy. *Hum Reprod* 2010; **25**: 665-671 [PMID: 20023291 DOI: 10.1093/humrep/dep433]
- 9 **Rousset P**, Peyron N, Charlot M, Chateau F, Golfier F, Raudrant D, Cotte E, Isaac S, Réty F, Valette PJ. Bowel endometriosis: preoperative diagnostic accuracy of 3.0-T MR enterography--initial results. *Radiology* 2014; **273**: 117-124 [PMID: 24828001 DOI: 10.1148/radiol.14132803]
- 10 **Demirel F**, Koca G, Demirel K, Aydogmus H, Korkmaz M, Gokmen B. Labeled red blood cell scintigraphy in the non-invasive diagnostics of endometriosis. *Fertil Steril* 2010; **94**: S202 [DOI: 10.1016/j.fertnstert.2010.05.000]

- 10.1016/j.fertnstert.2010.07.784]
- 11 **Kim KJ**, Jung SS, Yang SK, Yoon SM, Yang DH, Ye BD, Byeon JS, Myung SJ, Kim JH. Colonoscopic findings and histologic diagnostic yield of colorectal endometriosis. *J Clin Gastroenterol* 2011; **45**: 536-541 [PMID: 21030871 DOI: 10.1097/MCG.0b013e3181fd297b]
  - 12 **Bergqvist A**. Different types of extragenital endometriosis: a review. *Gynecol Endocrinol* 1993; **7**: 207-221 [PMID: 8291459 DOI: 10.3109/09513599309152504]
  - 13 **Peterson CM**, Johnstone EB, Hammoud AO, Stanford JB, Varner MW, Kennedy A, Chen Z, Sun L, Fujimoto VY, Hediger ML, Buck Louis GM; ENDO Study Working Group. Risk factors associated with endometriosis: importance of study population for characterizing disease in the ENDO Study. *Am J Obstet Gynecol* 2013; **208**: 451.e1-451.11 [PMID: 23454253 DOI: 10.1016/j.ajog.2013.02.040]
  - 14 **Rizk B**, Fischer AS, Lotfy HA, Turki R, Zahed HA, Malik R, Holliday CP, Glass A, Fishel H, Soliman MY, Herrera D. Recurrence of endometriosis after hysterectomy. *Facts Views Vis Obgyn* 2014; **6**: 219-227 [PMID: 25593697]
  - 15 **Selçuk İ, Bozdağ G**. Recurrence of endometriosis; risk factors, mechanisms and biomarkers; review of the literature. *J Turk Ger Gynecol Assoc* 2013; **14**: 98-103 [PMID: 24592083 DOI: 10.5152/jtgga.2013.52385]
  - 16 **Guo SW**. Recurrence of endometriosis and its control. *Hum Reprod Update* 2009; **15**: 441-461 [PMID: 19279046 DOI: 10.1093/humupd/dmp007]
  - 17 **Randall GW**, Gantt PA, Poe-Zeigler RL, Bergmann CA, Noel ME, Strawbridge WR, Richardson-Cox B, Hereford JR, Reiff RH. Serum antiendometrial antibodies and diagnosis of endometriosis. *Am J Reprod Immunol* 2007; **58**: 374-382 [PMID: 17845208 DOI: 10.1111/j.1600-0897.2007.00523.x]
  - 18 **Landi S**, Ceccaroni M, Perutelli A, Allodi C, Barbieri F, Fiaccavento A, Ruffo G, McVeigh E, Zanolli L, Minelli L. Laparoscopic nerve-sparing complete excision of deep endometriosis: is it feasible? *Hum Reprod* 2006; **21**: 774-781 [PMID: 16449312 DOI: 10.1093/humrep/dei324]
  - 19 **Chopin N**, Vieira M, Borghese B, Foulot H, Dousset B, Coste J, Mignon A, Fauconnier A, Chapron C. Operative management of deeply infiltrating endometriosis: results on pelvic pain symptoms according to a surgical classification. *J Minim Invasive Gynecol* 2005; **12**: 106-112 [PMID: 15904612 DOI: 10.1016/j.jmig.2005.01.015]
  - 20 **Fedele L**, Bianchi S, Zanconato G, Bettoni G, Gotsch F. Long-term follow-up after conservative surgery for rectovaginal endometriosis. *Am J Obstet Gynecol* 2004; **190**: 1020-1024 [PMID: 15118634 DOI: 10.1016/j.ajog.2003.10.698]
  - 21 **Seong SJ**, Kim D, Lee KH, Kim TJ, Chung HH, Chang SJ, Lee EJ. Role of Hormone Therapy After Primary Surgery for Endometrioma: A Multicenter Retrospective Cohort Study. *Reprod Sci* 2016; **23**: 1011-1018 [PMID: 26763524 DOI: 10.1177/1933719115625841]
  - 22 **Heaps JM**, Nieberg RK, Berek JS. Malignant neoplasms arising in endometriosis. *Obstet Gynecol* 1990; **75**: 1023-1028 [PMID: 2188180]
  - 23 **Takeuchi M**, Matsuzaki K, Uehara H, Nishitani H. Malignant transformation of pelvic endometriosis: MR imaging findings and pathologic correlation. *Radiographics* 2006; **26**: 407-417 [PMID: 16549606 DOI: 10.1148/rg.262055041]

**P- Reviewer:** Osawa S, Seow-Choen F, Sipos F, Jiang QP  
**S- Editor:** Wang JL **L- Editor:** A **E- Editor:** Tan WW



## Randomized Controlled Trial

## Efficacy of 1.2 L polyethylene glycol plus ascorbic acid for bowel preparations

Hiroyuki Tamaki, Teruyo Noda, Masahiro Morita, Akina Omura, Atsushi Kubo, Chikara Ogawa, Toshihiro Matsunaka, Mitsushige Shibatoge

**ORCID number:** Hiroyuki Tamaki (0000-0001-6116-2175); Teruyo Noda (0000-0001-8879-4594); Masahiro Morita (0000-0003-4550-3691); Akina Omura (0000-0003-1116-5815); Atsushi Kubo (0000-0002-6136-0099); Chikara Ogawa (0000-0002-4534-6692); Toshihiro Matsunaka (0000-0001-9419-0201); Mitsushige Shibatoge (0000-0002-0800-0393).

**Author contributions:** Tamaki H was fully involved in the patient management, acquisition and interpretation of data, statistics, drafting, and preparation of final manuscript version; Shibatoge M was contributed to make the conception, study design, interpretation of data and critical review of the final manuscript version; all authors contributed to correction of the clinical data.

**Institutional review board**

**statement:** The protocol of this study was approved by the Investigational Review Board of Takamatsu Red Cross Hospital.

**Informed consent statement:** All patients provided written informed consent for their participation.

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

**Data sharing statement:** All authors agree that if this manuscript is finally accepted for publication, the Copyright License Agreement will become effective immediately.

**Hiroyuki Tamaki, Teruyo Noda, Masahiro Morita, Akina Omura, Atsushi Kubo, Chikara Ogawa, Toshihiro Matsunaka, Mitsushige Shibatoge,** Department of Gastroenterology, Takamatsu Red Cross Hospital, Takamatsu, Kagawa 760-0017, Japan

**Corresponding author:** Mitsushige Shibatoge, MD, PhD, Department of Gastroenterology, Takamatsu Red Cross Hospital, 4-1-3 Ban-cho, Takamatsu, Kagawa 760-0017 Japan.

[shibatoge-mitsushige@takamatsu.jrc.or.jp](mailto:shibatoge-mitsushige@takamatsu.jrc.or.jp)

**Telephone:** +81-87-8317101

**Fax:** +81-87-8347809

## Abstract

## BACKGROUND

A low-volume polyethylene glycol (PEG) solution that combines ascorbic acid with PEG-based electrolyte solution (PEG-ASC) is gaining mainstream acceptance for bowel preparation due to reduced volume and improved taste. Although several reports showed that bowel preparation with PEG-ASC volume lower than 2.0 L with laxative agents could be an alternative to traditional preparation regimen, the cleansing protocols have not been fully investigated.

## AIM

To evaluate the cleansing efficacy of 1.2 L PEG-ASC solution comparing with 2.0 L PEG electrolyte (PEG-ELS) for bowel preparations.

## METHODS

A randomized, single-blinded, open-label, single-center, non-inferiority study was conducted. In total, 312 Japanese adult patients (aged > 18 years) who underwent colonoscopy were enrolled. Patients were randomly allocated to bowel lavage with either 1.2 L of PEG-ASC solution with at least 0.6 L of an additional clear fluid (1.2 L PEG-ASC group) or 2.0 L of PEG-ELS (PEG-ELS group). Then, 48 mg of sennoside was administered at bedtime on the day before colonoscopy, and the designated drug solution was administered at the hospital on the day of colonoscopy. Bowel cleansing was evaluated using the Boston Bowel Preparation Scale (BBPS). The volume of fluid intake and required time for bowel preparation were evaluated. Furthermore, compliance, patient tolerance, and overall acceptability were evaluated using a patient questionnaire, which was assessed using a visual analog scale.

## RESULTS

In total, 291 patients (1.2 L PEG-ASC group, 148; PEG-ELS group, 143) completed

**CONSORT 2010 statement:** The authors have read the CONSORT 2010 Statement, and the manuscript was prepared and revised according to the CONSORT 2010 Statement.

**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

**Received:** October 25, 2018

**Peer-review started:** October 26, 2018

**First decision:** November 29, 2018

**Revised:** December 24, 2018

**Accepted:** January 23, 2019

**Article in press:** January 24, 2019

**Published online:** February 26, 2019

the study. There was no significant difference in successful cleansing, defined as a BBPS score  $\geq 2$  in each segment, between the two groups (1.2 L PEG-ASC group, 91.9%; PEG-ELS group, 90.2%; 95% CI: -0.03-0.09). The required time for bowel preparation was significantly shorter (164.95 min  $\pm$  68.95 min *vs* 202.16 min  $\pm$  68.69 min,  $P < 0.001$ ) and the total fluid intake volume was significantly lower (2.23 L  $\pm$  0.55 L *vs* 2.47 L  $\pm$  0.56 L,  $P < 0.001$ ) in the 1.2 L PEG-ASC group than in the PEG-ELS group. Palatability, acceptability of the volume of solution, and overall acceptability evaluated using a patient questionnaire, which was assessed by the visual analog scale, were significantly better in the 1.2 L PEG-ASC group than in the PEG-ELS group (7.70 cm  $\pm$  2.57 cm *vs* 5.80 cm  $\pm$  3.24 cm,  $P < 0.001$ ). No severe adverse event was observed in each group.

## CONCLUSION

The 1.2 L PEG-ASC solution was non-inferior to the 2.0 L PEG-ELS solution in terms of cleansing efficacy and had better acceptability among Japanese patients.

**Key words:** Ascorbic acid; Bowel preparation; Colonoscopy; Efficacy; Polyethylene glycol; Tolerability

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Adequate bowel preparation is essential to improve colonoscopy quality. Volume and palatability of bowel cleansing agents are important determinants of tolerability, acceptability, and efficacy. This randomized study evaluated the non-inferiority of 1.2 L polyethylene glycol plus ascorbic acid (PEG-ASC) plus sennoside to 2.0 L PEG electrolyte (PEG-ELS) solutions plus sennoside for outpatient bowel preparation. The 1.2 L PEG-ASC and 2.0 L PEG-ELS solutions are clinically equivalent with respect to cleansing efficacy. Furthermore, the 1.2 L PEG-ASC solution was superior to 2.0 L PEG-ELS solution in terms of acceptability, and it was associated with a shorter required time for bowel preparation and a lower volume of fluid intake.

**Citation:** Tamaki H, Noda T, Morita M, Omura A, Kubo A, Ogawa C, Matsunaka T, Shibato M. Efficacy of 1.2 L polyethylene glycol plus ascorbic acid for bowel preparations. *World J Clin Cases* 2019; 7(4): 452-465

**URL:** <https://www.wjnet.com/2307-8960/full/v7/i4/452.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.452>

## INTRODUCTION

Colorectal cancer (CRC) remains one of the most challenging diseases to treat worldwide and it is one of the leading causes of cancer death<sup>[1]</sup>. Because it is widely accepted that the adenoma-carcinoma and serrated polyp pathways play critical roles in the development of CRC, the main targets for screening colonoscopy for the prevention of CRC occurrence and deaths are adenomas and sessile serrated polyps<sup>[2-4]</sup>. Moreover, superficial curable cancers including flat or non-polypoid precursors should be treated as well. Colonoscopic polypectomy is the best diagnostic and therapeutic method, and removal of such precursor lesions during screening colonoscopy prevents death from CRC<sup>[5,6]</sup>. However, the miss rate for neoplastic polyps is estimated to be 16.8%-28%<sup>[7-9]</sup>. Several factors such as poor bowel cleansing, areas of poor visualization, and inadequate colonoscope withdrawal time were suggested as reasons for the increasing miss rate<sup>[10-15]</sup>.

Inadequate bowel preparation is a serious matter on screening colonoscopy because it may result in a higher adenoma miss rate, prolonged procedure time, lower colonoscopy completion rate, and increased cost because of the need for an earlier repeat examination<sup>[16-18]</sup>. An ideal bowel preparation agent should achieve high-quality bowel preparation and should be inexpensive and well-tolerated by a high proportion of patients<sup>[19]</sup>. Furthermore, the cleansing protocol should be simple and suitable for inpatients and outpatients. However, no available agent has completely met these criteria.

Currently, polyethylene glycol-based electrolyte solution (PEG-ELS) is used most commonly for bowel preparation owing to its cleansing efficacy and safety<sup>[20]</sup>. Based

on meta-analysis, split-dose regimens of 4.0 L of PEG-ELS increase the quality of colon cleansing and have higher acceptability compared to day-before preparations<sup>[21-23]</sup>. However, oral intake of a high-volume cleansing solution results in reduced tolerability and low adherence and consequently low-quality bowel preparations.

Nowadays, a low-volume PEG solution that combines ascorbic acid with PEG-ELS (PEG-ASC) is gaining mainstream acceptance due to reduced volume and improved taste. In Western countries, approximately 2.0 L of PEG-ASC achieved non-inferior efficacy for bowel cleansing with better acceptability and fewer side effects than the standard-volume PEG-ELS<sup>[24-28]</sup>. In addition, recent reports from Japan and Korea suggested appropriate volumes of PEG-ASC to be lower than 2.0 and laxative agents combined with low-residue diet prior to bowel cleansing showed similar cleansing effect to traditional regimen<sup>[29-32]</sup>. Taking these results into consideration, bowel preparation with PEG-ASC volume lower than 2.0 L with laxative agents in the optimized protocol can be alternative to traditional preparation regimen.

However, cleansing protocols with reduced volume using PEG-ASC have not been fully investigated. Therefore, we conducted the current study to evaluate the cleansing efficacy, acceptability, and safety of the 1.2 L PEG-ASC plus sennoside regimen comparing with the 2.0 L PEG-ELS regimen as an outpatient bowel preparation for afternoon colonoscopy in a Japanese population.

## MATERIALS AND METHODS

### Study design

A randomized, single-blinded, open-label, single-center, non-inferiority study was conducted. This trial is registered at UMIN (UMIN000020904), and its protocol was approved by the Investigational Review Board of Takamatsu Red Cross Hospital. All patients provided written informed consent for their participation.

### Patients

A total of 312 Japanese adult patients [ $> 18$  years; 177 men, 135 women; mean age 63.0 (range 18-89) years] who underwent colonoscopy at Takamatsu Red Cross Hospital between December 2014 and March 2016 were enrolled in the study. Patients were excluded if they had known or suspected bowel obstruction, ileus, and perforation. Patients with history of bowel resection, significant gastroparesis or gastric outlet obstruction, toxic colitis or megacolon, severe chronic renal failure [estimated glomerular filtration rate (eGFR)  $< 30$  mL/min  $1.73$  m<sup>2</sup>], severe congestive heart failure (New York Heart Association class III or IV), sustained tachyarrhythmia, and uncontrolled hypertension (systolic blood pressure  $\geq 170$  mmHg, diastolic blood pressure  $\geq 100$  mmHg) were excluded. Patients requiring hospitalization and pregnant or lactating women were also excluded.

### Randomization

Before the start of the study, a randomization list was computer generated using a method of randomly permuted blocks of four patients. Eligible patients were randomly assigned to bowel lavage with either 1.2 L of PEG-ASC or 2.0 L of PEG-ELS solution in numerical order of acceptance into the study. The randomization number was strictly given according to the order of patient enrollment, with each patient assigned the first available number on the randomization list. The randomization number, or the reason for not enrolling the patient, was reported for each patient in the appropriate forms. In this single-blind randomized controlled trial, patients were aware of the bowel lavage assigned, and the investigator and assessors were blinded to group allocation.

The primary population was the intent-to-treat (ITT) population, which was defined as all randomly assigned patients who received the bowel lavage. The secondary population was the per-protocol (PP) population, which was defined as randomly assigned patients who completed the recommended total fluid intake.

### Study procedures

At the screening visit, the patient's baseline characteristics, including demographic information and past surgical and medical therapy, were obtained. Patients enrolled in the study received verbal and written instructions on bowel preparation, including how the product should be taken. They were also informed of the potential side effects of the preparation solution as well as the drawbacks of an aborted procedure. Furthermore, dietary instruction indicating foods recommended to be taken (*e.g.*, rice, noodles, bread, banana) and to be avoided (*e.g.*, uncooked vegetable, vegetable or fruits with seeds, seaweeds, konjac) were given.



This study had three protocols for bowel preparation: (1) low-residue diet was started on the day before colonoscopy; (2) 48 mg of sennoside was administered on the day before colonoscopy; and (3) 1.2 L PEG-ASC or 2 L PEG-ELS solution was administered the day of colonoscopy on the designated group. Specially, on the day before colonoscopy, all patients were instructed to ingest a low-residue food until 8:00 pm. Only clear fluids were allowed after 8:00 pm, and 48 mg of sennoside was administered at bedtime (8:00 pm to 12:00 pm).

On the day of the colonoscopy, patients received either PEG-ASC (Moviprep®; EA Pharma Co., Ltd., Tokyo, Japan, each liter contained 100.0 g of macrogol 4000, 7.5 g of sodium sulfate, 2.7 g of sodium chloride, 1.0 g of potassium chloride, 4.7 g of ascorbic acid, 5.9 g of sodium ascorbate, and lemon flavoring) or PEG-ELS (Niflec®; EA Pharma Co., Ltd., Tokyo, Japan, each liter contained 59.0 g of macrogol 4000, 5.7 g of sodium sulfate, 1.5 g of sodium chloride, 0.7 g of potassium chloride, 1.7 g of sodium bicarbonate, and lemon flavoring). Patients in the first arm received 1.2 L of PEG-ASC at a rate of 0.2 L every 10 min to 15 min followed by at least 0.6 L of an additional clear fluid [1.2 L of PEG-ASC (1.2 L PEG-ASC) group]. Patients could take clear fluid while taking the cleansing solution, and they were instructed to take additional clear fluid until bowel cleansing was completed. There was no limitation to the amount of additional clear fluid. Patients in the second arm received 2.0 L of PEG-ELS at a rate of 0.25 L every 15 min (PEG-ELS group).

Before the start of the colonoscopy, patients filled in the three-item questionnaire: (1) please evaluate the taste of the cleansing lavage [assessed by visual analog scale (VAS): terrible - good]; (2) please evaluate the volume of the cleansing lavage (assessed by VAS: difficult to ingest - easy to ingest); and (3) please make a comprehensive evaluation of the cleansing lavage (assessed by VAS: terrible - good). The volume of fluid intake, required time for bowel preparation, and the time interval between the completion of bowel preparation and the start of colonoscopy were recorded. All adverse events were documented, classified, and graded according to the World Health Organization recommendations for the evaluation of active and subjective toxicity.

The colonoscopies, performed by skillful endoscopists who have experienced at least 1000 colonoscopies, were scheduled between 1:00 pm and 4:30 pm according to the normal standard of care. Bowel cleansing was evaluated using the Boston Bowel Preparation Scale (BBPS), which is a validated scoring system with scores between 0 and 9, where 9 is the best score<sup>[33]</sup>. The score is composed of three sub-scores between 0 and 3, evaluating the cleansing effect in each colon segment: The right colon (including the cecum and ascending colon), the transverse colon (including the hepatic and splenic flexures), and the left colon (including the descending colon, sigmoid colon, and rectum). BBPS sub-score  $\geq 2$  in each segment was defined as successful cleansing according to previous report<sup>[34]</sup>.

### Endpoints

The primary endpoint was the successful cleansing rate defined as BBPS sub-score  $\geq 2$  in each segment. Secondary endpoints were cleansing quality evaluated by BBPS, frequency of cleansing operation to remove foam or bubbles, the time interval between the completion of bowel preparation and the start of colonoscopy, polyp and adenoma detection rate defined as proportion of the total number of polyps and adenomas divided by the number of colonoscopies (PDR and ADR, respectively), and advanced adenoma detection rate (AADR) calculated as the percentage of patients in each group who had at least one advanced adenoma defined as any adenoma or sessile serrated polyp  $\geq 10$  mm in diameter, with villous components or high-grade dysplasia regardless of size, and sessile serrated polyps with dysplasia<sup>[35]</sup>. Furthermore, volume of fluid intake, required time for bowel preparation, patient tolerance, and acceptability evaluated using patient questionnaire which consists of evaluation for palatability, volume, and overall acceptability were evaluated.

### Sample size

The patient sample size was determined by considering the results of phase I and II studies on bowel preparation with PEG-ASC or PEG-ELS conducted by Ajinomoto Pharmaceuticals Co., Ltd. The sample size was determined as 143 subjects per treatment group to have  $> 80\%$  power to detect the non-inferiority of the PEG-ASC to PEG-ELS with a two-sided significance value of 5% (95%CI for evaluation) and a non-inferiority margin of 10%. Considering possible dropouts, 150 subjects were targeted for recruitment for each treatment group.

### Statistical analysis

Baseline patient characteristics were compared using Student's *t*-test for independent samples or Pearson's  $\chi^2$  test, as appropriate. Successful cleansing rate, frequency of

cleansing operation to remove foam or bubbles, PDR, ADR, and AADR were compared using Pearson's  $\chi^2$  test, and BBPS score, total volume of fluid intake, required time for bowel preparation, and patient acceptability assessed by VAS were compared using Mann-Whitney *U*-test between the two groups. Continuous variables are expressed as mean  $\pm$  SE, and categorical data are expressed as percentages. *P* values  $< 0.05$  were considered significant. All analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, Illinois, United States).

## RESULTS

### Participant flow

A total of 312 Japanese patients (156 in the 1.2 L PEG-ASC group and 156 in the PEG-ELS group) were enrolled between December 2014 and March 2016. One patient in the 1.2 L PEG-ASC group and one patient in the PEG-ELS group withdrew from the study before examination. Three patients in the 1.2 L PEG-ASC group and eight in the PEG-ELS group cancelled the examination, and the remaining four patients in the 1.2 L PEG-ASC group and four patients in the PEG-ELS group were lost to follow-up. Finally, 291 patients (1.2 L PEG-ASC group, 148; PEG-ELS group, 143) completed the study (94.9% and 91.7%, respectively; ITT population). A total of 147 patients in the 1.2 L PEG-ASC group and 137 patients in the PEG-ELS group completed the recommended total fluid intake (99.3% and 95.8%, respectively; PP population, [Figure 1](#)).

### Clinical characteristics

The clinical characteristics of the enrolled patients in the two groups are shown in [Table 1](#). No significant differences were identified in terms of demographic characteristics (mean age, sex, constipation, experience of abdominal operation, hypertension, diabetes, and experience of colonoscopy) or indications for colonoscopy.

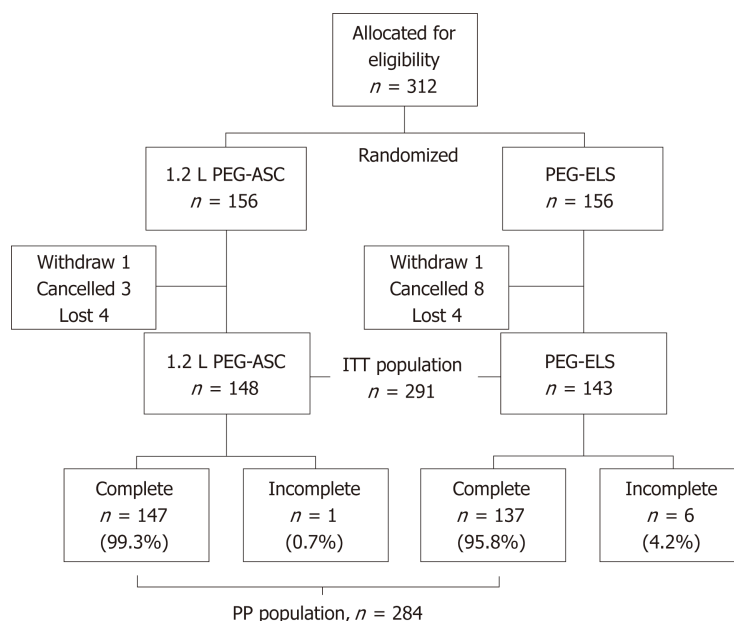
### Primary endpoint

There was no significant difference in successful cleansing, defined as a BBPS subscore  $\geq 2$  in each segment, between the two groups (1.2 L PEG-ASC group, 91.9%; PEG-ELS group, 90.2%; 95%CI: -0.03-0.09 in the ITT population, 1.2 L PEG-ASC group, 91.8%; PEG-ELS group, 90.5%; 95%CI: -0.02-0.08 in the PP population; [Figure 2](#)). [Table 2](#) shows the successful cleansing rate evaluated by BBPS according to the colonic segment. Using a segmental score of 2-3 as an indication of adequate cleansing, there was also no significant difference between preparations in each segment. Thus, the PEG-ASC demonstrated non-inferiority to the PEG-ELS with a two-sided significance value of 5% and a non-inferiority margin of 10%.

### Secondary endpoints

The total volume of fluid intake was significantly lower (2.23 L  $\pm$  0.55 L *vs* 2.47 L  $\pm$  0.56 L, *P*  $< 0.01$ ; [Figure 3A](#)), and the required time for bowel preparation was significantly shorter in the 1.2 L PEG-ASC group than in the PEG-ELS group (164.3 min  $\pm$  68.6 min *vs* 203.7 min  $\pm$  68.0 min, *P*  $< 0.01$ ; [Figure 3B](#)). The time interval was significantly longer in the 1.2 L PEG-ASC group than in the PEG-ELS group (147.3 min  $\pm$  66.2 min *vs* 115.9 min  $\pm$  54.7 min, *P*  $< 0.01$ ). The cleansing quality evaluated by BBPS, defined as the sum of each segmental score, was superior in the 1.2 L PEG-ASC group than in the PEG-ELS group (7.80  $\pm$  1.37 *vs* 7.30  $\pm$  1.40, *P*  $< 0.01$  in the ITT population, 7.76  $\pm$  1.35 *vs* 7.29  $\pm$  1.37, *P*  $< 0.01$  in the PP population; [Figure 4A](#)). Additionally, there was no significant difference in the successful cleansing rates according to various factors (age 70 years and older; female sex; constipation; diabetes; and history of abdominal operation) between the two groups ([Table 3](#)). However, foam or bubbles were observed more frequently in the 1.2 L PEG-ASC group than in the PEG-ELS group (35.7% *vs* 19.7%, *P*  $< 0.01$ ; [Figure 4B](#)). The PDR, ADR, and AADR in the 1.2 L PEG-ASC group were comparable to those in the PEG-ELS group (PDR, 42.6% *vs* 47.6%, *P* = 0.39; ADR, 34.5% *vs* 39.1%, *P* = 0.41; AADR, 10.8% *vs* 13.2%, *P* = 0.52; [Table 4](#)).

Although adherence with the recommended total fluid intake tended to be better in the 1.2 L PEG-ASC group than in the PEG-ELS group, this was not statistically different (99.3% *vs* 95.6%, *P* = 0.11). Regarding patient acceptability evaluated by the patient questionnaire assessed by VAS, patients randomized to the 1.2 L PEG-ASC group reported a significantly superior palatability and acceptability in the volume of the solution than those randomized to the PEG-ELS group (5.7 cm  $\pm$  2.2 cm *vs* 5.0 cm  $\pm$  2.6 cm, *P* = 0.02; 6.3 cm  $\pm$  2.1 cm *vs* 5.3 cm  $\pm$  2.5 cm, *P* = 0.03, respectively; [Figure 5A](#) and B). Furthermore, overall acceptability was significantly better in the 1.2 L PEG-



**Figure 1 Patient flow.** ITT: Intent-to-treat; PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution; PP: Per protocol.

ASC group than in the PEG-ELS group ( $7.70 \text{ cm} \pm 2.57 \text{ cm}$  *vs*  $5.80 \text{ cm} \pm 3.24 \text{ cm}$ ,  $P < 0.001$ ; [Figure 5C](#)).

There were no significant differences in the incidence and type of adverse events between the 1.2L PEG-ASC and the PEG-ELS groups. The most common reported adverse events were nausea and abdominal discomfort; however, no major adverse event was reported in either group ([Table 5](#)).

## DISCUSSION

In this study, the 1.2 L PEG-ASC solution showed non-inferiority to the 2.0 L PEG-ELS solution in terms of the cleansing efficacy. Moreover, the 1.2 L PEG-ASC solution was superior to the 2.0 L PEG-ELS solution in terms of patient acceptability, and it was associated with a shorter time for bowel preparation, lower volume of fluid intake, and superior palatability. Furthermore, no major adverse events were reported in either group. Overall, this study demonstrated that the 1.2 L PEG-ASC solution plus sennoside is comparable to the 2.0 L PEG-ELS solution plus sennoside in bowel cleansing efficacy.

Traditional 4 L PEG regimen is widely accepted as a first recommended regimen for its safety and efficacy. However, ingestion of the large volume of solution and its unpleasant taste may result in poor acceptability and adherence. To improve these limitations, low-volume regimens that combine PEG and osmotic agents (*e.g.*, ascorbic acid, sodium phosphate) or stimulant agents (*e.g.*, bisacodyl, sennoside) are developed. Several studies compared 2 L PEG-ASC and traditional 4 L PEG regimen and concluded that 2 L PEG-ASC had comparable cleansing efficacy with better acceptability<sup>[27,36]</sup>. In contrast, 2 L PEG regimen combined with bisacodyl was reported to have comparable cleansing effect to traditional 4 L PEG regimen<sup>[37,38]</sup>. Furthermore, several groups in East Asia recently reported that the combination of PEG-ASC and bisacodyl or sennoside achieved to reduce the volume of cleansing solution to 1 L or 1.5 L with comparative cleansing effect and improved patient acceptability to 2 L PEG regimen combined with laxative or split-dose 2-L PEG-ASC. Tajika *et al*<sup>[29]</sup> reported that the 1.5 L PEG-ASC solution plus sennoside was superior to the 2 L PEG-ELS solution plus sennoside with respect to patient acceptability of bowel preparation for colonoscopy, and it was comparable to the 2.0 L PEG-ELS in bowel cleansing efficacy, tolerability, and safety. Moreover, the efficacy of bowel preparation with the 1.0 L PEG-ASC solution was reported in a prospective study from Japan<sup>[31]</sup> and two randomized studies from South Korea<sup>[32,39]</sup>. Although their protocol had differences in terms of the kind of the laxative (sennoside or bisacodyl), these studies concluded that the 1.0 L PEG-ASC solution had similar efficacy with the 2.0 L PEG-ELS solution in bowel preparation. These results support that the efficacy of the reduced dose of PEG-ASC solution to less than 2.0 L plus laxative is comparable to the traditional PEG

**Table 1 Clinical characteristics**

	1.2 L PEG-ASC (n = 156)	PEG-ELS (n = 156)	Total (n = 312)	P value
Age (mean, range)	62.6 (19-89)	63.5 (24-89)	63.0 (19-89)	0.21
Sex (male, %)	93 (59.6)	84 (53.8)	177 (56.7)	0.30
Constipation, n (%)	39 (25.0)	38 (24.4)	77 (24.7)	0.89
Abdominal operation, n (%)	58 (37.2)	55 (35.3)	113 (36.2)	0.72
Hypertension, n (%)	36 (23.1)	26 (16.7)	62 (19.9)	0.16
Diabetes, n (%)	12 (7.7)	15 (9.6)	27 (8.7)	0.54
Experience of colonoscopy, n (%)	89 (57.0)	87 (55.8)	176 (56.4)	0.81
Indications for colonoscopy, n (%)				
Occult blood test-positive	76 (48.7)	70 (44.9)	146 (46.8)	0.50
Surveillance	30 (19.2)	27 (17.3)	57 (18.3)	0.66
Screening	21 (13.5)	22 (14.1)	43 (13.8)	0.87
Blood in stool	10 (6.4)	13 (8.3)	23 (7.4)	0.52
Abdominal pain	5 (3.2)	6 (3.9)	13 (4.2)	0.76
Constipation	4 (2.6)	5 (3.2)	9 (2.9)	0.74
Diarrhea	2 (1.3)	5 (3.2)	7 (2.2)	0.44
Other reason	8 (5.1)	8 (5.1)	16 (5.1)	0.80

PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.

regimen as bowel preparation. However, because there had not been specific criteria for adequate dosing, we performed a preliminary study by comparing the cleansing efficacy of 1.0 L, 1.2 L, and 1.5 L PEG-ASC solutions plus sennoside to determine the volume of PEG-ASC for our study ( $n = 25$  in each group, data not shown). According to the results of the preliminary study, we determined the regimen to be evaluated: an instruction for patients to take low-residue diet and an administration of 48 mg of sennoside on the day before colonoscopy, followed by bowel preparation with 1.2 L of PEG-ASC and at least 0.6 L of additional clear fluid during procedure on the day of colonoscopy. As we expected, the current study demonstrates the non-inferiority of 1.2 L PEG-ASC solution to 2.0 L PEG-ELS solution for successful cleansing, defined as BBPS sub-score  $\geq 2$  in each segment in the ITT and PP population. Moreover, the sum of each BBPS segmental score was significantly higher in the 1.2 L PEG-ASC group than in the 2.0 L PEG-ELS group.

Furthermore, our results demonstrate that the PDR, ADR, and AADR were comparable between the two groups, suggesting that similar visualization quality was achieved. ADR is recognized as a useful surrogate marker for CRC detection<sup>[40]</sup>, and for every 1% increase in the ADR, there is a 3% decrease in CRC incidence and a 5% decrease in CRC-related mortality<sup>[41]</sup>. Although it is still controversial whether bowel cleansing influences ADR, several studies, including meta-analyses, have demonstrated that adequate bowel cleansing is associated with a higher ADR<sup>[16,42]</sup>. The ACG/American Society for Gastrointestinal Endoscopy task force on quality colonoscopy recommended a minimum average risk screening ADR target of 25% in a combined male and female population (30% ADR in men and 20% ADR in women)<sup>[43]</sup>. The ADR in the current study, 34.5% in the 1.2L PEG-ASC group and 39.1% in the PEG-ELS group, is greater than the recommended value and suggests that both protocols have sufficient efficacy in bowel cleansing for screening colonoscopy.

The time interval between the bowel preparation and the start of colonoscopy was reported as one of the predicting factors affecting bowel cleansing effect as well as age, sex, diabetes, constipation, history of abdominal surgery, compliance with preparation instructions, and bowel preparation type. In the current study, the time interval was significantly longer in the 1.2 L PEG-ASC group than in the PEG-ELS group (147.3 min  $\pm$  66.2 min *vs* 115.9 min  $\pm$  54.7 min,  $P < 0.01$ ). This difference is considered to be due to the difference in the required time for bowel preparation between the two groups and fixed starting time of colonoscopy in both groups. Kim *et al*<sup>[44]</sup> reported the relationship in the time interval between the last PEG intake and the start of colonoscopy. Although they concluded that the optimal time interval was 5 h - 6 h for the full-dose PEG method, there was no significant difference in the cleansing effect between the time intervals under 3 h and 5 h - 6 h in the patients who received the PEG solution and colonoscopy on the same day. Therefore, we considered that the difference of 30 min in the time interval between the two groups in the current study

**Table 2 Successful cleansing rates according to colonic segment % (n)**

	1.2 L PEG-ASC (n = 148)	PEG-ELS (n = 143)	P value
Right	93.9 (139)	94.4 (135)	0.86
Transverse	95.9 (142)	95.1 (136)	0.73
Left	95.9 (142)	92.3 (132)	0.19
Over all	91.9 (136)	90.2 (129)	0.61

BBPS: Boston bowel preparation scale; PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.

did not have a potent influence on the evaluation of the cleansing effect.

The variant cleansing effect of PEG-ASC is considered to derive from the excessive ascorbic acid residues in the bowel lumen because its absorption mechanism saturates at a high dose<sup>[45]</sup>. Ascorbic acid residues can act as an osmotic laxative cooperating with PEG-ELS. In this respect, a risk of inducing intravascular volume depletion is alarming. Failure to maintain adequate hydration before, during, and after bowel preparation may increase the risk of severe and potentially fatal intravascular volume depletion-related complications such as fatal dysnatremia associated with PEG-ELS preparations or renal failure associated with sodium phosphate preparations<sup>[46-48]</sup>. Therefore, we excluded patients with renal dysfunction whose eGFR is < 30 mL/min 1.73 m<sup>2</sup> or those with severe congestive heart failure, and patients were encouraged to take additional clear fluid other than the required 0.6 L throughout the bowel-preparation process to maintain hydration. In this study, the minimum volume of clear fluid to be ingested during procedure was 0.6 L, which was in accordance with the instruction provided by the drug package insert: half of the volume of the ingested PEG-ASC solution. However, sufficient fluid replacement more than 0.6 L is considered to contribute to avoiding intravascular volume depletion-related complications. Essentially, the total volume of fluid intake amounted to 2.23 L ± 0.55 L suggesting that 1.03 L ± 0.55 L of additional clear fluid was ingested by patients in the 1.2 L PEG-ASC group. Consequently, no fatal dehydration-related complications were observed in the current study. In addition, there were no significant changes in eGFR before and after the procedure in the 1.2 L PEG-ASC group (82.9 mL/min 1.73 m<sup>2</sup> ± 1.9 mL/min 1.73 m<sup>2</sup> vs 81.5 mL/min 1.73 m<sup>2</sup> ± 1.6 mL/min 1.73 m<sup>2</sup>, *P* = 0.17; data not shown). These results suggested that the volume of fluid intake was sufficient to maintain hydration in the 1.2 L PEG-ASC group. Thus, our results demonstrated the safety of the bowel cleansing protocol with 1.2 L PEG-ASC solution with respect to intravascular volume depletion and renal dysfunction.

There are several limitations to this study. First, this study was conducted at a single center, limiting the generalizability of the results. Second, this study was performed in a single-blinded manner, and it may have possible influence on patient's rating on the acceptability evaluated by the patient questionnaire. Third, dietary regimen on the day before colonoscopy was not even because it depended on individual response after a dietary instruction. Finally, we have to take the difference between the races and the region into consideration when we discuss the efficacy of bowel cleansing regimens. They can vary in effectiveness depending on the racial or regional groups because body dimensions, diet habits, and bowel transit time. *etc.*, vary among population and are considered to affect the reactivity for cleansing agents. Although the efficacy of the combination of PEG-ASC lower than 2 L plus bisacodyl or sennoside was currently evaluated only in East Asia, they are thought to be effective in the population who are successfully treated with 2 L PEG-ELS plus laxative (*e.g.*, South Asia<sup>[37]</sup> or Canada<sup>[38]</sup>). In this point of view, further studies in various races and regions are required to confirm the efficacy of PEG-ASC lower than 2.0 L plus laxative.

In summary, this study demonstrated that 1.2 L of PEG-ASC and 2.0 L of PEG-ELS are clinically equivalent with respect to cleansing efficacy, including ADR. The 1.2 L PEG-ASC regimen was superior to the 2.0 L PEG-ELS regimen in terms of the required time for bowel preparation, palatability, and acceptability. These results support that the 1.2 L PEG-ASC solution plus sennoside with prior low-residue diet is a suitable alternative to the standard bowel preparation with PEG-ELS in outpatients for afternoon colonoscopy.



**Table 3 Successful cleansing rates according to various factors % (n)**

	1.2 L PEG-ASC	PEG-ELS	P-value
Age (70 years old and older)	89.8 (44)	89.6 (43)	0.77
Sex (Female)	93.2 (55)	87.7 (57)	0.46
Constipation	81.1 (30)	88.6 (31)	0.58
Diabetes	83.3 (10)	81.3 (13)	0.72
History of abdominal operation	93.1 (54)	92.7 (51)	0.77

PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.

**Table 4 The Polyp detection rate, the adenoma detection rate, and the advanced adenoma detection rate % (n)**

Variable	1.2 L PEG-ASC (n = 148)	PEG-ELS (n = 143)	P value
PDR	42.6 (63)	47.6 (68)	0.39
ADR	34.5 (51)	39.1 (56)	0.41
AADR <sup>1</sup>	10.8 (16)	13.2 (19)	0.52

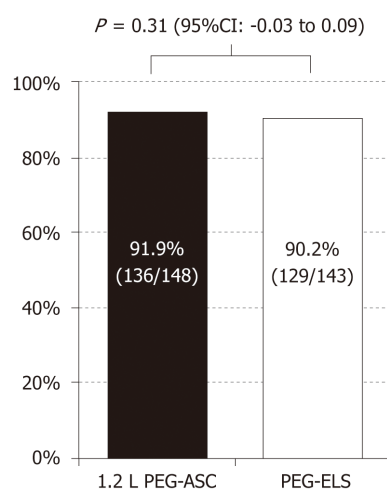
<sup>1</sup>Adenoma  $\geq 10$  mm in diameter, with villous components or high grade dysplasia.

PDR: Polyp detection rate; ADR: Adenoma detection rate; AADR: Advanced adenoma detection rate.

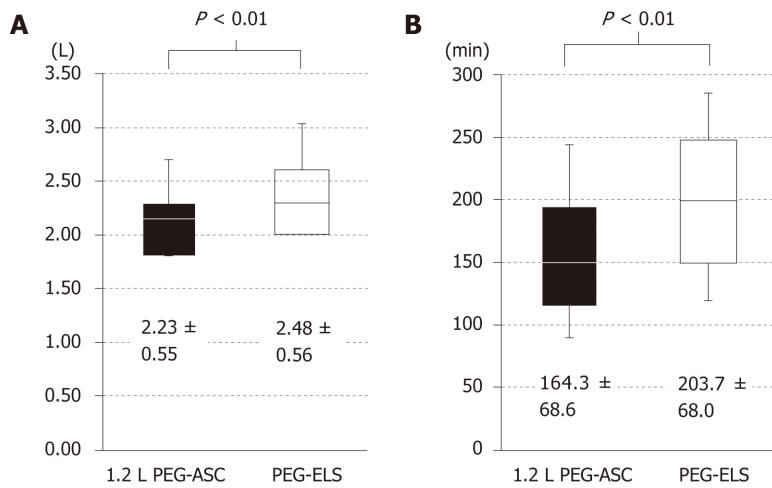
**Table 5 Adverse events % (n)**

	1.2 L PEG-ASC	PEG-ELS	P value
Nausea	6.1 (9)	12.6 (18)	0.087
Vomiting	0.7 (1)	2.8 (4)	0.34
Abdominal discomfort	9.5 (14)	7.7 (11)	0.59
Abdominal pain	2.7 (4)	3.5 (5)	0.96
Dizziness	0 (0)	2.1 (3)	0.23
Chill	1.4 (2)	2.1 (3)	0.97
No discomfort	81.8 (120)	76.2 (109)	0.31

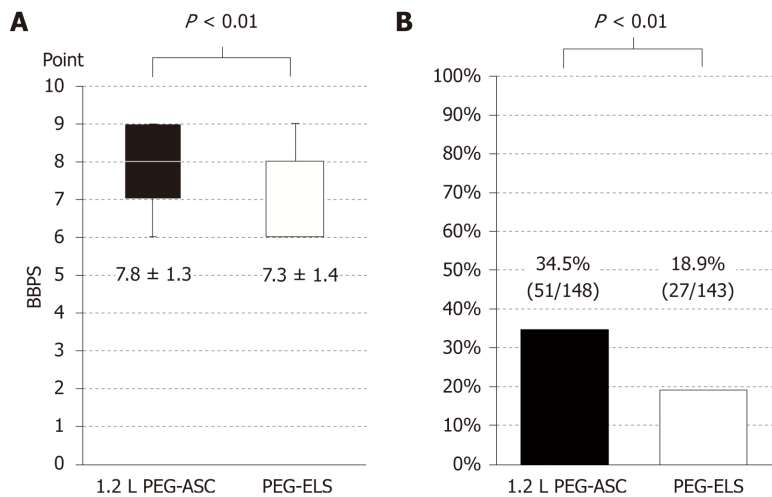
PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.



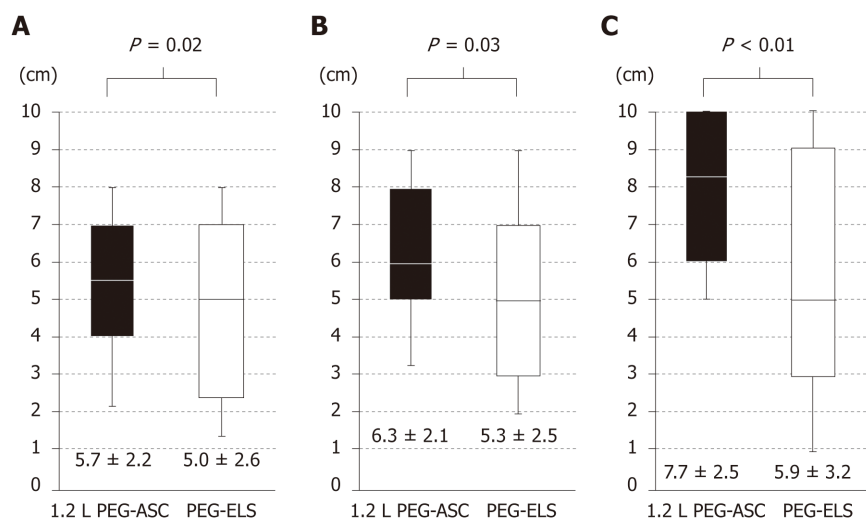
**Figure 2 Successful cleansing rate (BBPS score  $\geq 5$ ).** The 1.2 L PEG-ASC group was shown to be non-inferior to the PEG-ELS group in terms of successful cleansing rate with a two-sided significance value of 5% and a non-inferiority margin of 10% (1.2 L PEG-ASC group, 91.9%; PEG-ELS group, 90.2%; 95%CI: -0.03-0.09 in the intention-to-treat population). BBPS: Boston Bowel Preparation Scale; PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.



**Figure 3** Difference in the total volume of fluid intake and the required time for bowel preparation between the 1.2 L polyethylene glycol plus ascorbic acid group and the polyethylene glycol-based electrolyte solution group. A: Total volume of fluid intake. The total volume of fluid intake was significantly lower in the 1.2 L PEG-ASC group than in the PEG-ELS group (2.23 L ± 0.55 L vs 2.47 L ± 0.56 L,  $P < 0.01$ ); B: Required time for bowel preparation. The required time for bowel preparation was significantly shorter in the 1.2 L PEG-ASC group than in the PEG-ELS group (164.3 min ± 68.6 min vs 203.7 min ± 68.0 min,  $P < 0.01$ ). PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution.



**Figure 4** Difference in the cleansing quality and the frequency of cleansing operations to remove foam or bubbles between the 1.2 L polyethylene glycol plus ascorbic acid group and the polyethylene glycol-based electrolyte solution group. A: Cleansing quality evaluated by the BBPS. The sum of each segmental score of BBPS was higher in the 1.2 L PEG-ASC group than in the PEG-ELS group (7.80 ± 1.37 vs 7.30 ± 1.40,  $P < 0.01$  in ITT population, 7.76 ± 1.35 vs 7.29 ± 1.37,  $P < 0.01$  in the per-protocol population). B: Frequency of cleansing operations to remove foam or bubbles. Foam or bubbles were observed more frequently in the 1.2 L PEG-ASC group than in the PEG-ELS group (35.7% vs 19.7%,  $P < 0.01$ ). BBPS, Boston Bowel Preparation Scale; PEG-ASC, polyethylene glycol plus ascorbic acid; PEG-ELS, polyethylene glycol-based electrolyte solution.



**Figure 5 Patient acceptability of cleansing solution assessed by visual analog scale.** A: Patient acceptability for palatability. Patient acceptability for palatability was significantly better in the 1.2 L PEG-ASC group than in the PEG-ELS group (5.7 cm  $\pm$  2.2 cm vs 5.0 cm  $\pm$  2.6 cm,  $P = 0.02$ ); B: Patient acceptability for volume. Patient acceptability for volume was significantly better in the 1.2 L PEG-ASC group than in the PEG-ELS group (6.3 cm  $\pm$  2.1 cm vs 5.3 cm  $\pm$  2.5 cm,  $P = 0.03$ ); C: Overall acceptability. Overall acceptability was significantly better in the 1.2 L PEG-ASC group than in the PEG-ELS group (7.70 cm  $\pm$  2.57 cm vs 5.80 cm  $\pm$  3.24 cm,  $P < 0.001$ ). PEG-ASC: Polyethylene glycol plus ascorbic acid; PEG-ELS: Polyethylene glycol-based electrolyte solution; VAS: Visual analog scale.

## ARTICLE HIGHLIGHTS

### Research background

Inadequate bowel preparation is a serious matter on screening colonoscopy because it may result in a higher adenoma miss rate, prolonged procedure time, lower colonoscopy completion rate, and increased cost because of the need for an earlier repeat examination.

### Research motivation

Low-volume regimens that combine polyethylene glycol (PEG) and osmotic or stimulant agents are developed to improve acceptability. Although several reports showed that the combination of PEG plus ascorbic acid (PEG-ASC) solution lower than 2.0 L and laxative agents could be alternative to traditional preparation regimen, the cleansing protocols have not been fully investigated.

### Research objectives

We aimed to evaluate the cleansing efficacy of 1.2 L PEG-ASC comparing with 2.0 L PEG electrolyte (PEG-ELS) combined with sennoside as bowel preparations for afternoon colonoscopy.

### Research methods

A randomized, single-blinded, open-label, single-center, non-inferiority study was conducted. In total, 312 Japanese adult patients (aged  $> 18$  years) who underwent colonoscopy were enrolled. Patients were randomly allocated to bowel lavage with either 1.2 L of PEG-ASC solution with at least 0.6 L of an additional clear fluid (1.2L PEG-ASC group) or 2.0 L of PEG-ELS (PEG-ELS group). Then, 48 mg of sennoside was administered at bedtime on the day before colonoscopy, and the designated drug solution was administered at the hospital on the day of colonoscopy. Bowel cleansing was evaluated using the Boston Bowel Preparation Scale (BBPS). The volume of fluid intake and required time for bowel preparation were evaluated. Furthermore, compliance, patient tolerance, and overall acceptability were evaluated using a patient questionnaire, which was assessed using a visual analog scale.

### Research results

In total, 291 patients (1.2 L PEG-ASC group, 148; PEG-ELS group, 143) completed the study. There was no significant difference in successful cleansing, defined as a BBPS score  $\geq 2$  in each segment, between the two groups (1.2 L PEG-ASC group, 91.9%; PEG-ELS group, 90.2%; 95%CI: -0.03-0.09). The required time for bowel preparation was significantly shorter (164.95 min  $\pm$  68.95 min vs 202.16 min  $\pm$  68.69 min,  $P < 0.001$ ) and the total fluid intake volume was significantly lower (2.23 L  $\pm$  0.55 L vs 2.47 L  $\pm$  0.56 L,  $P < 0.001$ ) in the 1.2 L PEG-ASC group than in the PEG-ELS group. Palatability, acceptability of the volume of solution, and overall acceptability evaluated using a patient questionnaire, which was assessed by the visual analog scale, were significantly better in the 1.2 L PEG-ASC group than in the PEG-ELS group (7.70 cm  $\pm$  2.57 cm vs 5.80 cm  $\pm$  3.24 cm,  $P < 0.001$ ). No severe adverse event was observed in each group.

### Research conclusions

This study demonstrated that 1.2 L of PEG-ASC and 2.0 L of PEG-ELS are clinically equivalent with respect to cleansing efficacy, including ADR. Furthermore, the 1.2 L PEG-ASC regimen was superior to the 2.0 L PEG-ELS regimen in terms of the required time for bowel preparation, palatability, and acceptability. These results support that combination of 1.2 L PEG-ASC solution and sennoside with prior low-residue diet is a suitable alternative to the standard bowel preparation with PEG-ELS in outpatients for afternoon colonoscopy.

### Research perspectives

We have to take the difference between the races and the region into consideration when we discuss the efficacy of bowel cleansing regimens. They can vary in effectiveness depending on the racial or regional groups because body dimensions, diet habits, and bowel transit time, *etc.*, vary among population and are considered to affect the reactivity for cleansing agents. Although the efficacy of the combination of PEG-ASC lower than 2 L plus bisacodyl or sennoside was currently evaluated only in East Asia, they are thought to be effective in the population who are successfully treated with 2 L PEG-ELS plus laxative. In this point of view, further studies in various races and regions are required to confirm the efficacy of PEG-ASC lower than 2.0 L plus laxative.

## ACKNOWLEDGEMENTS

We wish to acknowledge the help of Mr. Hideki Hayashi as a statistical consultant.

## REFERENCES

- 1 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
- 2 Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996; **87**: 159-170 [PMID: 8861899 DOI: 10.1016/S0092-8674(00)81333-1]
- 3 Short MW, Layton MC, Teer BN, Domagalski JE. Colorectal cancer screening and surveillance. *Am Fam Physician* 2015; **91**: 93-100 [PMID: 25591210]
- 4 Winawer SJ, Zauber AG. The advanced adenoma as the primary target of screening. *Gastrointest Endosc Clin N Am* 2002; **12**: 1-9, v [PMID: 11916153 DOI: 10.1016/S1052-5157(03)00053-9]
- 5 Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaar I, van Ballegoijen M, Hankey BF, Shi W, Bond JH, Schapiro M, Panish JF, Stewart ET, Waye JD. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. *N Engl J Med* 2012; **366**: 687-696 [PMID: 22356322 DOI: 10.1056/NEJMoa1100370]
- 6 Shaikat A, Mongin SJ, Geisser MS, Lederle FA, Bond JH, Mandel JS, Church TR. Long-term mortality after screening for colorectal cancer. *N Engl J Med* 2013; **369**: 1106-1114 [PMID: 24047060 DOI: 10.1056/NEJMoa1300720]
- 7 Heresbach D, Barrioz T, Lapalus MG, Coumaros D, Bauret P, Potier P, Sautereau D, Boustière C, Grimaud JC, Barthélémy C, Sée J, Serraj I, D'Halluin PN, Branger B, Ponchon T. Miss rate for colorectal neoplastic polyps: a prospective multicenter study of back-to-back video colonoscopies. *Endoscopy* 2008; **40**: 284-290 [PMID: 18389446 DOI: 10.1055/s-2007-995618]
- 8 van Rijn JC, Reitsma JB, Stoker J, Bossuyt PM, van Deventer SJ, Dekker E. Polyp miss rate determined by tandem colonoscopy: a systematic review. *Am J Gastroenterol* 2006; **101**: 343-350 [PMID: 16454841 DOI: 10.1111/j.1572-0241.2006.00390.x]
- 9 Kim NH, Jung YS, Jeong WS, Yang HJ, Park SK, Choi K, Park DI. Miss rate of colorectal neoplastic polyps and risk factors for missed polyps in consecutive colonoscopies. *Intest Res* 2017; **15**: 411-418 [PMID: 28670239 DOI: 10.5217/ir.2017.15.3.411]
- 10 Lebwohl B, Kastrinos F, Glick M, Rosenbaum AJ, Wang T, Neugut AI. The impact of suboptimal bowel preparation on adenoma miss rates and the factors associated with early repeat colonoscopy. *Gastrointest Endosc* 2011; **73**: 1207-1214 [PMID: 21481857 DOI: 10.1016/j.gie.2011.01.051]
- 11 Cohen J, Grunwald D, Grossberg LB, Sawhney MS. The Effect of Right Colon Retroflexion on Adenoma Detection: A Systematic Review and Meta-analysis. *J Clin Gastroenterol* 2017; **51**: 818-824 [PMID: 27683963 DOI: 10.1097/MCG.0000000000000695]
- 12 Chang JY, Moon CM, Lee HJ, Yang HJ, Jung Y, Kim SW, Jung SA, Byeon JS. Predictive factors for missed adenoma on repeat colonoscopy in patients with suboptimal bowel preparation on initial colonoscopy: A KASID multicenter study. *PLoS One* 2018; **13**: e0195709 [PMID: 29698398 DOI: 10.1371/journal.pone.0195709]
- 13 Sulz MC, Kröger A, Prakash M, Manser CN, Heinrich H, Misselwitz B. Meta-Analysis of the Effect of Bowel Preparation on Adenoma Detection: Early Adenomas Affected Stronger than Advanced Adenomas. *PLoS One* 2016; **11**: e0154149 [PMID: 27257916 DOI: 10.1371/journal.pone.0154149]
- 14 Lee TJ, Blanks RG, Rees CJ, Wright KC, Nickerson C, Moss SM, Chilton A, Goddard AF, Patnick J, McNally RJ, Rutter MD. Longer mean colonoscopy withdrawal time is associated with increased adenoma detection: evidence from the Bowel Cancer Screening Programme in England. *Endoscopy* 2013; **45**: 20-26 [PMID: 23254403 DOI: 10.1055/s-0032-1325803]
- 15 Park JH, Kim SJ, Hyun JH, Han KS, Kim BC, Hong CW, Lee SJ, Sohn DK. Correlation Between Bowel Preparation and the Adenoma Detection Rate in Screening Colonoscopy. *Ann Coloproctol* 2017; **33**: 93-98 [PMID: 28761869 DOI: 10.3393/ac.2017.33.3.93]
- 16 Froehlich F, Wietlisbach V, Gonvers JJ, Burnand B, Vader JP. Impact of colonic cleansing on quality and diagnostic yield of colonoscopy: the European Panel of Appropriateness of Gastrointestinal Endoscopy European multicenter study. *Gastrointest Endosc* 2005; **61**: 378-384 [PMID: 15758907 DOI: 10.1016/S0016-5107(04)02776-2]
- 17 Rex DK, Imperiale TF, Latinovich DR, Bratcher LL. Impact of bowel preparation on efficiency and cost of colonoscopy. *Am J Gastroenterol* 2002; **97**: 1696-1700 [PMID: 12135020 DOI: 10.1016/S0016-5107(04)02776-2]

- 10.1111/j.1572-0241.2002.05827.x]
- 18 **Lee HS**, Byeon JS. Bowel preparation, the first step for a good quality colonoscopy. *Intest Res* 2014; **12**: 1-2 [PMID: 25349556 DOI: 10.5217/ir.2014.12.1.1]
- 19 **Michael KA**, DiPiro JT, Bowden TA, Tedesco FJ. Whole-bowel irrigation for mechanical colon cleansing. *Clin Pharm* 1985; **4**: 414-424 [PMID: 3899470]
- 20 **Connor A**, Tolan D, Hughes S, Carr N, Tomson C. Consensus guidelines for the safe prescription and administration of oral bowel-cleansing agents. *Gut* 2012; **61**: 1525-1532 [PMID: 22842619 DOI: 10.1136/gutjnl-2011-300861]
- 21 **Martel M**, Barkun AN, Menard C, Restellini S, Kherad O, Vanasse A. Split-Dose Preparations Are Superior to Day-Before Bowel Cleansing Regimens: A Meta-analysis. *Gastroenterology* 2015; **149**: 79-88 [PMID: 25863216 DOI: 10.1053/j.gastro.2015.04.004]
- 22 **Kilgore TW**, Abdinoor AA, Szary NM, Schowengerdt SW, Yust JB, Choudhary A, Matteson ML, Puli SR, Marshall JB, Bechtold ML. Bowel preparation with split-dose polyethylene glycol before colonoscopy: a meta-analysis of randomized controlled trials. *Gastrointest Endosc* 2011; **73**: 1240-1245 [PMID: 21628016 DOI: 10.1016/j.gie.2011.02.007]
- 23 **Enestvedt BK**, Tofani C, Laine LA, Tierney A, Fennerty MB. 4-Liter split-dose polyethylene glycol is superior to other bowel preparations, based on systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2012; **10**: 1225-1231 [PMID: 22940741 DOI: 10.1016/j.cgh.2012.08.029]
- 24 **Pontone S**, Angelini R, Standoli M, Patrizi G, Culasso F, Pontone P, Redler A. Low-volume plus ascorbic acid vs high-volume plus simethicone bowel preparation before colonoscopy. *World J Gastroenterol* 2011; **17**: 4689-4695 [PMID: 22180711 DOI: 10.3748/wjg.v17.i42.4689]
- 25 **Valiante F**, Pontone S, Hassan C, Bellumat A, De Bona M, Zullo A, de Francesco V, De Boni M. A randomized controlled trial evaluating a new 2-L PEG solution plus ascorbic acid vs 4-L PEG for bowel cleansing prior to colonoscopy. *Dig Liver Dis* 2012; **44**: 224-227 [PMID: 22119219 DOI: 10.1016/j.dld.2011.10.007]
- 26 **Ell C**, Fischbach W, Bronisch HJ, Dertinger S, Layer P, Rünzi M, Schneider T, Kachel G, Gröger J, Köllinger M, Nagell W, Goerg KJ, Wanitschke R, Gruss HJ. Randomized trial of low-volume PEG solution versus standard PEG + electrolytes for bowel cleansing before colonoscopy. *Am J Gastroenterol* 2008; **103**: 883-893 [PMID: 18190651 DOI: 10.1111/j.1572-0241.2007.01708.x]
- 27 **Ponchon T**, Boustière C, Heresbach D, Hagege H, Tarrerias AL, Halphen M. A low-volume polyethylene glycol plus ascorbate solution for bowel cleansing prior to colonoscopy: the NORMO randomised clinical trial. *Dig Liver Dis* 2013; **45**: 820-826 [PMID: 23769755 DOI: 10.1016/j.dld.2013.04.009]
- 28 **Xie Q**, Chen L, Zhao F, Zhou X, Huang P, Zhang L, Zhou D, Wei J, Wang W, Zheng S. A meta-analysis of randomized controlled trials of low-volume polyethylene glycol plus ascorbic acid versus standard-volume polyethylene glycol solution as bowel preparations for colonoscopy. *PLoS One* 2014; **9**: e99092 [PMID: 24902028 DOI: 10.1371/journal.pone.0099092]
- 29 **Tajika M**, Tanaka T, Ishihara M, Mizuno N, Hara K, Hijioka S, Imaoka H, Sato T, Yogi T, Tsutsumi H, Fujiyoshi T, Hieda N, Okuno N, Yoshida T, Bhatia V, Yatabe Y, Yamao K, Niwa Y. A Randomized Controlled Trial Evaluating a Low-Volume PEG Solution Plus Ascorbic Acid versus Standard PEG Solution in Bowel Preparation for Colonoscopy. *Gastroenterol Res Pract* 2015; **2015**: 326581 [PMID: 26649036 DOI: 10.1155/2015/326581]
- 30 **Tajika M**, Tanaka T, Ishihara M, Hirayama Y, Oonishi S, Mizuno N, Hara K, Hijioka S, Imaoka H, Fujiyoshi T, Hieda N, Okuno N, Yoshida T, Yamao K, Bhatia V, Ando M, Niwa Y. Optimal intake of clear liquids during preparation for afternoon colonoscopy with low-volume polyethylene glycol plus ascorbic acid. *Endosc Int Open* 2017; **5**: E416-E423 [PMID: 28573174 DOI: 10.1055/s-0043-106185]
- 31 **Kamei M**, Shibuya T, Takahashi M, Makino M, Haga K, Nomura O, Murakami T, Ritsuno H, Ueyama H, Kodani T, Ishikawa D, Matsumoto K, Sakamoto N, Osada T, Ogihara T, Watanabe S, Nagahara A. Efficacy and Acceptability of 1 Liter of Polyethylene Glycol with Ascorbic Acid vs. 2 Liters of Polyethylene Glycol Plus Mosapride and Sennoside for Colonoscopy Preparation. *Med Sci Monit* 2018; **24**: 523-530 [PMID: 29373569 DOI: 10.12659/MSM.908043]
- 32 **Kang SH**, Jeon YT, Lee JH, Yoo IK, Lee JM, Kim SH, Choi HS, Kim ES, Keum B, Lee HS, Chun HJ, Kim CD. Comparison of a split-dose bowel preparation with 2 liters of polyethylene glycol plus ascorbic acid and 1 liter of polyethylene glycol plus ascorbic acid and bisacodyl before colonoscopy. *Gastrointest Endosc* 2017; **86**: 343-348 [PMID: 27889546 DOI: 10.1016/j.gie.2016.10.040]
- 33 **Lai EJ**, Calderwood AH, Doros G, Fix OK, Jacobson BC. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. *Gastrointest Endosc* 2009; **69**: 620-625 [PMID: 19136102 DOI: 10.1016/j.gie.2008.05.057]
- 34 **Clark BT**, Protiva P, Nagar A, Imaeda A, Ciarleglio MM, Deng Y, Laine L. Quantification of Adequate Bowel Preparation for Screening or Surveillance Colonoscopy in Men. *Gastroenterology* 2016; **150**: 396-405; quiz e14-15 [PMID: 26439436 DOI: 10.1053/j.gastro.2015.09.041]
- 35 **Lieberman DA**, Rex DK, Winawer SJ, Giardiello FM, Johnson DA, Levin TR. Guidelines for colonoscopy surveillance after screening and polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer. *Gastroenterology* 2012; **143**: 844-857 [PMID: 22763141 DOI: 10.1053/j.gastro.2012.06.001]
- 36 **Kim MS**, Park J, Park JH, Kim HJ, Jang HJ, Joo HR, Kim JY, Choi JH, Heo NY, Park SH, Kim TO, Yang SY. Does Polyethylene Glycol (PEG) Plus Ascorbic Acid Induce More Mucosal Injuries than Split-Dose 4-L PEG during Bowel Preparation? *Gut Liver* 2016; **10**: 237-243 [PMID: 26260754 DOI: 10.5009/gnl14439]
- 37 **Jha AK**, Chaudhary M, Jha P, Kumar U, Dayal VM, Jha SK, Purkayastha S, Ranjan R, Mishra M, Sehrawat K. Polyethylene glycol plus bisacodyl: A safe, cheap, and effective regimen for colonoscopy in the South Asian patients. *JGH Open* 2018; **2**: 249-254 [PMID: 30619933 DOI: 10.1002/jgh3.12077]
- 38 **Brahmania M**, Ou G, Bressler B, Ko HK, Lam E, Telford J, Enns R. 2 L versus 4 L of PEG3350 + electrolytes for outpatient colonic preparation: a randomized, controlled trial. *Gastrointest Endosc* 2014; **79**: 408-416.e4 [PMID: 24206747 DOI: 10.1016/j.gie.2013.08.035]
- 39 **Kwon JE**, Lee JW, Im JP, Kim JW, Kim SH, Koh SJ, Kim BG, Lee KL, Kim SG, Kim JS, Jung HC. Comparable Efficacy of a 1-L PEG and Ascorbic Acid Solution Administered with Bisacodyl versus a 2-L PEG and Ascorbic Acid Solution for Colonoscopy Preparation: A Prospective, Randomized and Investigator-Blinded Trial. *PLoS One* 2016; **11**: e0162051 [PMID: 27588943 DOI: 10.1371/journal.pone.0162051]
- 40 **Aranda-Hernández J**, Hwang J, Kandel G. Seeing better--Evidence based recommendations on optimizing colonoscopy adenoma detection rate. *World J Gastroenterol* 2016; **22**: 1767-1778 [PMID:



- 26855536 DOI: [10.3748/wjg.v22.i5.1767](https://doi.org/10.3748/wjg.v22.i5.1767)]
- 41 **Corley DA**, Jensen CD, Marks AR, Zhao WK, Lee JK, Doubeni CA, Zauber AG, de Boer J, Fireman BH, Schottinger JE, Quinn VP, Ghai NR, Levin TR, Quesenberry CP. Adenoma detection rate and risk of colorectal cancer and death. *N Engl J Med* 2014; **370**: 1298-1306 [PMID: [24693890](https://pubmed.ncbi.nlm.nih.gov/24693890/) DOI: [10.1056/NEJMoa1309086](https://doi.org/10.1056/NEJMoa1309086)]
  - 42 **Clark BT**, Rustagi T, Laine L. What level of bowel prep quality requires early repeat colonoscopy: systematic review and meta-analysis of the impact of preparation quality on adenoma detection rate. *Am J Gastroenterol* 2014; **109**: 1714-1723; quiz 1724 [PMID: [25135006](https://pubmed.ncbi.nlm.nih.gov/25135006/) DOI: [10.1038/ajg.2014.232](https://doi.org/10.1038/ajg.2014.232)]
  - 43 **Rex DK**, Schoenfeld PS, Cohen J, Pike IM, Adler DG, Fennerty MB, Lieb JG 2nd, Park WG, Rizk MK, Sawhney MS, Shaheen NJ, Wani S, Weinberg DS. Quality indicators for colonoscopy. *Gastrointest Endosc* 2015; **81**: 31-53 [PMID: [25480100](https://pubmed.ncbi.nlm.nih.gov/25480100/) DOI: [10.1016/j.gie.2014.07.058](https://doi.org/10.1016/j.gie.2014.07.058)]
  - 44 **Kim TK**, Kim HW, Kim SJ, Ha JK, Jang HH, Hong YM, Park SB, Choi CW, Kang DH. Importance of the time interval between bowel preparation and colonoscopy in determining the quality of bowel preparation for full-dose polyethylene glycol preparation. *Gut Liver* 2014; **8**: 625-631 [PMID: [25368750](https://pubmed.ncbi.nlm.nih.gov/25368750/) DOI: [10.5009/gnl13228](https://doi.org/10.5009/gnl13228)]
  - 45 **Fujita I**, Akagi Y, Hirano J, Nakanishi T, Itoh N, Muto N, Tanaka K. Distinct mechanisms of transport of ascorbic acid and dehydroascorbic acid in intestinal epithelial cells (IEC-6). *Res Commun Mol Pathol Pharmacol* 2000; **107**: 219-231 [PMID: [11484876](https://pubmed.ncbi.nlm.nih.gov/11484876/) DOI: [10.1016/j.jnoncrysol.2004.08.128](https://doi.org/10.1016/j.jnoncrysol.2004.08.128)]
  - 46 **Lichtenstein GR**, Cohen LB, Uribarri J. Review article: Bowel preparation for colonoscopy--the importance of adequate hydration. *Aliment Pharmacol Ther* 2007; **26**: 633-641 [PMID: [17697197](https://pubmed.ncbi.nlm.nih.gov/17697197/) DOI: [10.1111/j.1365-2036.2007.03406.x](https://doi.org/10.1111/j.1365-2036.2007.03406.x)]
  - 47 **Ayus JC**, Levine R, Arief AI. Fatal dysnatraemia caused by elective colonoscopy. *BMJ* 2003; **326**: 382-384 [PMID: [12586675](https://pubmed.ncbi.nlm.nih.gov/12586675/) DOI: [10.1136/bmj.326.7385.382](https://doi.org/10.1136/bmj.326.7385.382)]
  - 48 **Markowitz GS**, Stokes MB, Radhakrishnan J, D'Agati VD. Acute phosphate nephropathy following oral sodium phosphate bowel purgative: an underrecognized cause of chronic renal failure. *J Am Soc Nephrol* 2005; **16**: 3389-3396 [PMID: [16192415](https://pubmed.ncbi.nlm.nih.gov/16192415/) DOI: [10.1681/ASN.2005050496](https://doi.org/10.1681/ASN.2005050496)]

**P- Reviewer:** Jha AK, Madalinski M

**S- Editor:** Ma YJ **L- Editor:** A **E- Editor:** Tan WW



## Congenital analbuminemia in a patient affected by hypercholesterolemia: A case report

Patrizia Suppressa, Concetta Carbonara, Francesca Lugani, Monica Campagnoli, Teresa Troiano, Lorenzo Minchiotti, Carlo Sabbà

**ORCID number:** Patrizia Suppressa (0000-0002-3146-9173); Concetta Carbonara (0000-0001-5301-8050); Francesca Lugani (0000-0002-4189-8561); Monica Campagnoli (0000-0002-0983-6854); Teresa Troiano (0000-0002-6836-5816); Lorenzo Minchiotti (0000-0002-7043-482X); Carlo Sabbà (0000-0002-9874-8740).

**Author contributions:** Suppressa P conceived the case report, collected the patient data and performed the follow-up of the patient; Sabbà C helped with insightful discussion; Carbonara C collected the patient data; Troiano T performed the laboratory testing; Lugani F, Campagnoli M, and Minchiotti L performed the mutation analysis of the *ALB*. Minchiotti L contributed also to the drafting of the text. All the authors read and approved the final version of this article.

**Supported by** a Grant of the Italian Ministry of Education, University and Research to the Department of Molecular Medicine of the University of Pavia under the initiative "Dipartimenti di Eccellenza (2018-2022)", and Compagnia di S.Paolo, No. ROL9849.

**Informed consent statement:** The study participants provided informed written consent prior to their treatments and study enrollment.

**Conflict-of-interest statement:** All authors declare no conflict of interest related to this study or its publication.

**Patrizia Suppressa**, Department of Internal Medicine and Rare Disease Centre, University Hospital of Bari, Bari 70124, Italy

**Concetta Carbonara**, Department of Medicine, University of Bari, School of Medicine, Bari 70124, Italy

**Francesca Lugani**, Laboratory of Molecular Nephrology, Istituto Giannini Gaslini, IRCCS, Genova 16148, Italy

**Monica Campagnoli, Lorenzo Minchiotti**, Department of Molecular Medicine, University of Pavia, Pavia 27100, Italy

**Teresa Troiano**, Department of Clinical Pathology, University Hospital of Bari, Bari 70124, Italy

**Carlo Sabbà**, Department of Interdisciplinary Medicine, Geriatric Unit and Rare Disease Center, "Aldo Moro" University, Bari 70125, Italy

**Corresponding author:** Patrizia Suppressa, MD PhD, Doctor, Department of Interdisciplinary Medicine, Geriatric Unit and Rare Disease Center, "A. Moro" University of Bari, Piazza G. Cesare 11, Bari 70124, Italy. [patrizia.suppressa@gmail.com](mailto:patrizia.suppressa@gmail.com)

**Telephone:** +39-80-5592773

**Fax:** +39-80-5478126

### Abstract

#### BACKGROUND

Congenital analbuminemia (CAA) is a very rare disorder. Our data describes the clinical features and laboratory results of a new case established by mutation analysis of the albumin gene in a 39-year-old woman presenting with hypercholesterolemia. Our findings contribute to shed light on the molecular genetics of the disorder and confirm that safe and well tolerated hypocholesterolemic treatment with atorvastatin may be administered in dislipidemic patient with CAA in order to reduce their cardiovascular risk.

#### CASE SUMMARY

Our patient presented with a history of hypercholesterolemia and referred asthenia and heaviness in both legs. She was born from healthy and non-consanguineous parents and her development was normal. She had not familiarity for early cardiovascular disease, and did not report personal history of hypertension, chronic kidney or liver diseases. Clinical laboratories results

**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

**Received:** October 18, 2018

**Peer-review started:** October 18, 2018

**First decision:** November 15, 2018

**Revised:** December 7, 2018

**Accepted:** December 12, 2018

**Article in press:** December 12, 2018

**Published online:** February 26, 2019

showed critically reduced value of albumin whereas other serum proteins were elevated. Main causes of hypoalbuminemia (proteinuria, inflammatory state and insufficient hepatic synthesis) were ruled out by normal procedures and laboratory tests. So the hypothesis of a CAA was tested through mutation analysis of the albumin gene that revealed a homozygous CA deletion in exon 12, at nucleotide positions c1614-1615. This finding brought to the diagnosis of CAA. Currently the patient receives Atorvastatin 20 mg od and undergoes clinical and laboratory follow-up every six months. She never needed albumin infusions.

### CONCLUSION

Our experience shows how treatment with atorvastatin may be safely administered and well tolerated in patients affected by CAA.

**Key words:** Congenital analbuminemia; Hypercholesterolemia; Hypoalbuminemia; Rare disease; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The role of albumin in lipids transport and metabolism makes hypercholesterolemia the most detectable biochemical sign in analbuminemic patients. Despite patients affected by congenital analbuminemia have no severe clinical symptoms in adulthood; they might have high cardiovascular risk, morbidity and perinatal mortality as suggested in our patients' familiar and personal medical history.

**Citation:** Suppressa P, Carbonara C, Lugani F, Campagnoli M, Troiano T, Minchiotti L, Sabbà C. Congenital analbuminemia in a patient affected by hypercholesterolemia: A case report.

*World J Clin Cases* 2019; 7(4): 466-472

**URL:** <https://www.wjnet.com/2307-8960/full/v7/i4/466.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.466>

## INTRODUCTION

Congenital analbuminemia (CAA; Online Mendelian Inheritance in Man database, OMIM # 616000) is a rare autosomal recessive disorder characterized by the complete absence, or severe reduction of circulating serum albumin. Only about ninety cases have been reported worldwide without gender or ethnic predilection, most of which are listed in the Register of analbuminemia cases<sup>[1]</sup>. Although diagnosis is usually made by serum protein electrophoresis, mutation analysis of *ALB* gene is needed for confirmation. No severe clinical phenotype is detected in adult analbuminemic individuals because of the compensatory increase of other serum proteins<sup>[2]</sup>. The most common clinical signs are edema, hypotension and fatigue, while typical biochemical sign is hypercholesterolemia.

In this report we describe the clinical findings of a dislipidemic young woman and the molecular characterisation of the *ALB* gene, which confirmed the diagnosis of CAA.

## CASE PRESENTATION

### Chief complaints

A 39-year-old woman with history of hypercholesterolemia referred asthenia and heaviness in both legs. She had sedentary lifestyle, balanced diet and did not complain intestinal issues.

### Physical examination upon admission

Her weight was 56 kg, height 170 cm, body mass index 19 kg/m<sup>2</sup>, waist circumference 73 cm and her blood pressure was tested at a value of 13/10 KPa.

### Personal and family history

She was born from healthy and non-consanguineous parents and her development was normal. She had not familiarity for early cardiovascular disease, and did not

report personal history of hypertension, chronic kidney or liver diseases.

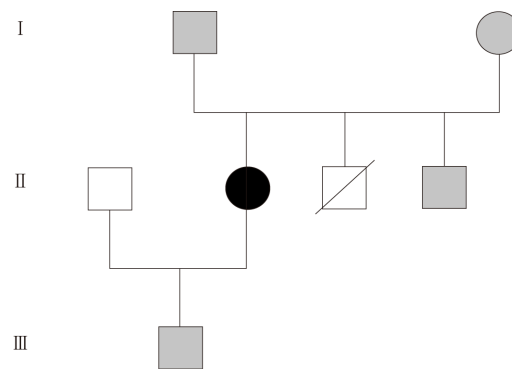
During her life she needed hospitalizations for a miscarriage (28 years old) and a delivery of a healthy male newborn by caesarean section (30 years old). The pedigree of her family is reported in [Figure 1](#) and shows the premature death of one of her brothers for unknown reasons at the age of 1 wk.

### Laboratory examinations

The patient's lipid profile showed a significant elevation of the total (321 mg/dL), low-density lipoprotein (LDL) (161 mg/dL) and high-density lipoprotein (HDL) (118 mg/dL) cholesterol, whereas triglyceride levels were normal. Serum apolipoprotein-A and -B were 291 mg/dL (n.v. 115 mg/dL-210 mg/dL) and 145 mg/dL (n.v. 55 mg/dL-135 mg/dL) respectively.

Since the patient and her relatives had neither clinical history nor physical signs (xanthoma and xanthelasma) of a genetic hypercholesterolemia, we studied other possible causes of her dyslipidemia through further laboratory tests. Blood count, liver and kidney function, coagulation tests, autoimmunity assays, PCR and urine dipstick were normal. Thyroid function tests displayed a subclinical hypothyroidism. Albumin level, detected by nephelometric method, was critically reduced (0.2 g/dL, n.v. 3.4 g/dL-5 g/dL) and total bilirubin was undetectable. Calcium level was 7.5 mg/dL (n.v. 8.5 mg/dL-10.1 mg/dL), total protein 5.8 g/dL (n.v. 6.4 g/dL-8.2 g/dL), pre-albumin 0.46 g/L (n.v. 0.2 g/L-0.4 g/L). Finally, serum protein electrophoresis ([Figure 2](#)) showed the presence of a minimal amount of serum albumin (1.6%, n.v. 55.8%-66.1%), with a simultaneous increase of the other serum protein fractions (alpha-1, alpha-2, beta-1, beta-2 and gamma), including transferrin (607 mg/dL, n.v. 200 mg/dL-360 mg/dL), alpha 1-antitrypsin (2.56 g/L, n.v. 0.9 g/L-2.0 g/L) and complement C4 (0.46 g/L, n.v. 0.10 g/L-0.40 g/L). Those seemed to compensate the lack of albumin<sup>[1]</sup>.

These findings led us to investigate on the patient's hypoalbuminemia. Proteinuria was excluded by normal spot urine albumin/creatinine ratio and total protein/creatinine ratio. Other possible secondary causes of hypoalbuminemia, such as inflammatory state and insufficient hepatic synthesis, were ruled out by normal laboratory tests and abdominal ultrasonography. The latter was negative except for mild hepatic steatosis. All the other examined members of the family (her parents, her brother, and her son) showed albumin level close to the upper limit of the normal range, suggesting that they may be heterozygous for a variation in the *ALB*<sup>[3]</sup>. For the above reasons, we tested the hypothesis of CAA by mutation analysis of the *ALB*<sup>[4]</sup>. After we obtained informed consent, we collected blood samples from all the available members of the family and two unrelated healthy volunteers as a control, and extracted genomic DNA from whole blood. For a rapid identification of variations in the *ALB*, we used a gel-based mutation detection strategy, which we developed and applied to the identification of many other cases of CAA<sup>[2]</sup>. Shortly, we PCR amplified the fourteen genomic fragments of the *ALB* encompassing the fourteen coding exons and their intron-exon junctions, using the specific primer pairs described by Watkins *et al.*<sup>[5]</sup>. The fragments were then examined by heteroduplex and SSCP analysis: The combination of these two techniques usually allowed us to identify the region of *ALB* containing the molecular defect, which was then submitted to direct DNA sequencing<sup>[2]</sup>. In the present case heteroduplex analysis clearly indicated that the only detectable change in both homozygous and heterozygous samples occurred in the 386 bp long region amplified by using PCR primers A23A and A24A encompassing exon 12 and the intron 11-exon 12 and exon 12-intron 13 junctions ([Figure 3A](#)). All the other members of the family are heterozygous, since they show the presence of four bands corresponding to homoduplex and heteroduplex PCR products ([Figure 3A](#), lanes 2, 2', 3, 3', 4, 4' 5, 5'). The homozygous sample ([Figure 3A](#), lanes 1 and 1') revealed only one band but with a different mobility when compared with controls ([Figure 3A](#), lanes 6, 7, 6', and 7'). No variation due to conformation polymorphism could be seen under these electrophoretic conditions (data not shown). The results of the DNA sequence analysis performed on the abnormal fragment showed that our patient is homozygous for a CA deletion near the 3' end of exon 12, at nucleotide positions c. 1614-1615, according to the Human Genome Variation Society rules, *i.e.*, starting from the initiator codon ([Figure 3B](#)). The subsequent frame-shift should give rise to a predicted translation product of 516 amino acid residues instead of the 585 found in the mature protein (p.Leu540Phefs\*2), in which the sequence Cys(538)-Thr-Leu-Ser has been changed to Cys(538)-Thr-Phe-Stop. Unfortunately, we could not perform a search for this truncated variant in the serum of our patient, but no evidence was found so far for the presence of the putative albumin molecule produced in all the cases of CAA studied at the molecular level<sup>[1,6]</sup>. The electropherograms from the parents confirmed that they are both heterozygous for the same mutation (data not shown). This result brought to the



**Figure 1 Family pedigree.** The analbuminemic patient is represented by a black symbol. Grey symbols denote heterozygous subjects. Void symbols indicate individuals not examined in the present study. One of the three siblings of generation II died for unknown reasons in his first week of life.

molecular diagnosis of CAA in our patient.

## FINAL DIAGNOSIS

Based on the biochemical findings and on the mutation analysis of the *ALB* the final diagnosis was CAA and hypercholesterolemia, the latter not of genetic origin.

## TREATMENT

CAA did not require albumin infusions, while hypercholesterolemia was successfully treated with atorvastatin. A low-cholesterol, low-saturated fat diet was prescribed without any significant effect on serum lipid (total cholesterol increased from 321 mg/dL to 334 mg/dL, LDL from 161 mg/dL to 192 mg/dL). Successful and well tolerated treatment with atorvastatin was started at the initial daily dose of 10 mg od, increased to 20 mg od: From baseline total and LDL cholesterol dropped by 23% and 47% respectively, HDL cholesterol increased by 22% while apolipoprotein-B decreased by 25%. Apolipoprotein-A showed no relevant modifications. This observation together with the low blood pressure might explain why, despite the high lipid levels, evident clinical signs of early atherosclerosis have not been observed in the patient<sup>[3]</sup>: No plaques on carotid artery walls were found at ultrasound imaging study.

## OUTCOME AND FOLLOW-UP

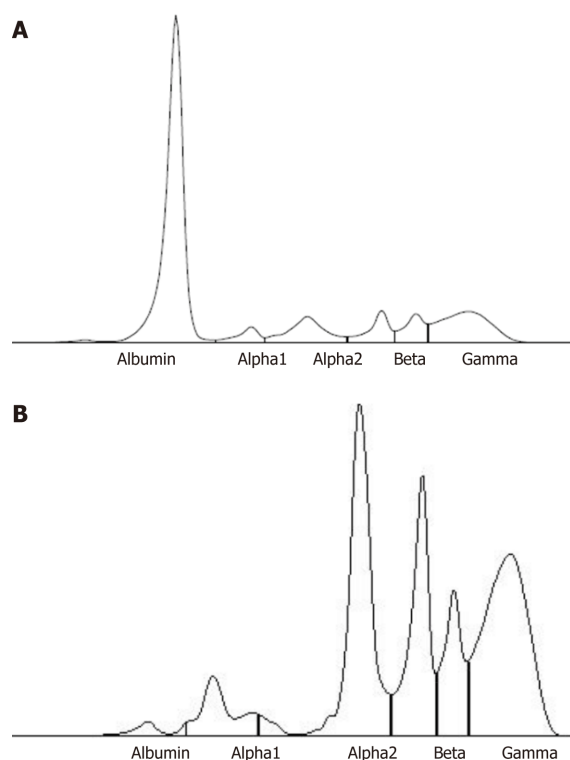
The patient undergoes clinical and laboratory follow-up twice a year. Atorvastatin treatment appears to be successful and well tolerated and so far she never needed albumin infusions.

## DISCUSSION

CAA is a rare autosomal recessive disorder which has an estimated prevalence of less than one in one million<sup>[3]</sup>. The so far reported data on the about 50 cases of CAA studied at the molecular level allowed to identify twenty-five different variations within the *ALB*<sup>[1]</sup>. Among them, the most common are splicing defects, nonsense variants, and frame shift/deletions, as was in the here reported case. Most of the albumin variants are unique and identified only in single individuals or within the same family<sup>[1]</sup>. The variation we identified in our patient was previously found in a young Turkish woman (analbuminemia Safranbolu)<sup>[2]</sup>. The presence of the same defect in unrelated analbuminemic individuals might indicate hypermutable regions in the gene, as it probably is for the relatively more common variants, as analbuminemia Guimarães (c.1289+1G>A), identified in three unrelated families, and especially analbuminemia Kayseri, which accounts for about one-third of the cases characterized at the molecular level<sup>[1]</sup>.

The absence or severe reduction of circulating serum albumin is usually shown by





**Figure 2** Capillary electrophoresis profile of serum proteins in a control (A) and in our patient (B). The latter shows the presence of a minimal amount of serum albumin (1.6%, n.v. 55.8%-66.1%), with a simultaneous increase of the other serum protein fractions: alpha 1 (7%, n.v. 2.9%-4.9%), alpha 2 (29.7%, n.v. 7.1%-11.8%), beta 1 (18.4%, n.v. 4.7%-7.2%), beta 2 (10.7%, n.v. 3.2%-6.5%), gamma (32.6%, n.v. 11.1%-18.8%).

serum protein electrophoresis<sup>[7]</sup>, but the final diagnosis of CAA is based on the mutational analysis of the *ALB*.

The benignancy of clinical manifestations and laboratory tests of analbuminemic patients often lead to a misdiagnosis or to a delayed diagnosis of this rare condition. Despite data of analbuminemic patients' follow-up are not available, early diagnosis of this rare condition may prevent hypercholesterolemia related cardiovascular events<sup>[8]</sup>. Thus, treatment with statins seems to be recommended to reduce long term cardiovascular risk, according to experiences reported in literature<sup>[9,10]</sup>.

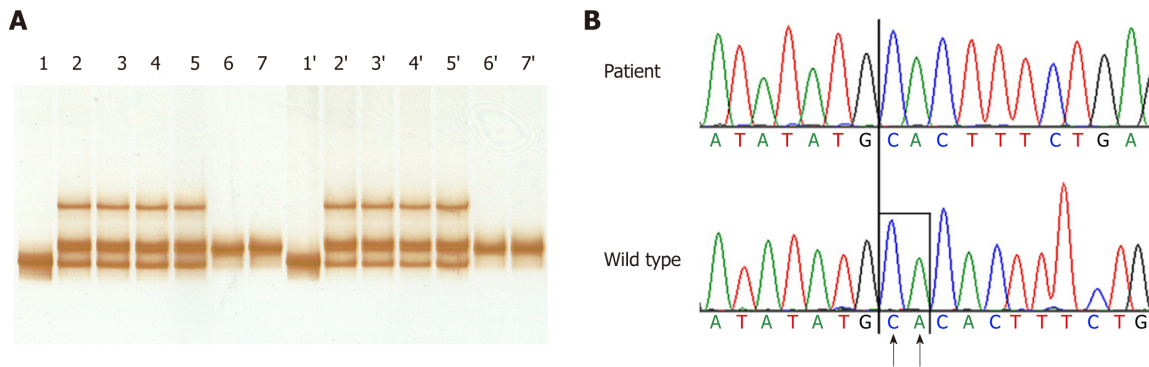
In this case the diagnosis of CAA was not suspected by clinical examination: No edema or lipodystrophy were found. The typical pattern of serum protein electrophoresis and the presence of markedly low albumin level brought to the assumption of the analbuminemia as possible cause of her hypercholesterolemia. Furthermore, the mechanism by which this possible interaction may occur remains equivocal. In vitro data suggest that a key role is played by the increased liver production of other serum proteins such as Apo A1, Apo B, and Apo E and the contextual reduction of the triglycerides-rich lipoprotein clearance. The latter mechanism have been explained through the inhibition of the lipoprotein lipase by free fatty acids not bounded to albumin<sup>[11]</sup>.

In Nagase rats with hereditary analbuminemia increased LDL and HDL cholesterol levels result from HMG-CoA reductase and Hepatic ACAT-2 protein abundance, while LDL-HDL cholesterol liver receptors are normally expressed. Hence, ACAT-2 hepatic protein catalyzes packaging and secretion of apolipoprotein B-containing lipoproteins by the esterification of cholesterol in the liver<sup>[12]</sup>.

On the other hand, normal plasma HDL/total cholesterol ratio in analbuminemia is due to normal activity of converting free cholesterol to cholesterol ester in HDL played by LCAT which seems to be more expressed in female analbuminemic rats.

According to the evidence, CAA should be associated with premature atherosclerosis and cardiovascular events. Even if there is no proved strategy for decreasing cardiovascular risk in analbuminemic patients with hypercholesterolemia, other statin treatment in human analbuminemics are described in literature<sup>[13,14]</sup>.

Treatment with simvastatin 40 mg od in dislipidemic patients with CAA has been administered in two African adults, a 21-year-old caucasian male and a 61-year-old Afro-American male. Serum lipid profile showed a decrease of LDL-cholesterol of 38% and 48% respectively after 20 wk of treatment discontinued for three- to five-fold



**Figure 3 HA and DNA sequence analysis of exon 12 of ALB in the affected family.** A: HA analysis. The DNA encompassing exon 12 and the exon-intron junction from the patient, her mother and father, and two controls were amplified with primers A23A and A24A and the fragments were electrophoresed onto a non-denaturing polyacrylamide gel: lane 1, proband; lane 2, mother; lane 3, father; lane 4, brother, lane 5, son, and lanes 6-7, controls. The same samples were denatured and cooled before loading: lane 1', proband; lane 2', mother; lane 3', father; lane 4', brother, lane 5', son, and lanes 6'-7', controls. Heteroduplexes are evident in the parents, in the brother, and in the son (lanes 2, 3, 4, 5, and 2' 3', 4' and 5'), indicating a heterozygous condition, while an abnormal homoduplex is present in the homozygous proband. No variation due to conformation polymorphism could be seen under these electrophoretic conditions and therefore the result of SSCP analysis are not reported in the figure; B: DNA sequence of the mutated region of exon 12 in the patient. The arrows indicate the two bases that are deleted in the patient: CA at nucleotide positions c. 1614\_1615. The patient is homozygous for this deletion.

increase in creatine kinase<sup>[9]</sup>.

The latter consideration explains why albumin-bound drugs should be carefully administered and monitored during treatment in these patients<sup>[13]</sup>. Long term treatment with atorvastatin was tested in one other Italian patient with CAA: a 38-year-old man received atorvastatin 40 mg od with a decrease of total and LDL cholesterol from baseline by 37.7% and 50.6% respectively. HDL cholesterol increased by 13.4%<sup>[10]</sup>. Treatment was safe and no elevated values of creatine kinase, liver enzymes were detected as in our case.

## CONCLUSION

According to our experience, safe and well tolerated hypocholesterolemic treatment with atorvastatin may be administered in dislipidemic patient with CAA in order to reduce their cardiovascular risk.

Despite analbuminemic patients could be asymptomatic, parents' screening and clinical follow-up is warrant for conditions such as hypercholesterolemia, atherosclerosis, hypercoagulability, osteoporosis, respiratory tract infections, obstetrical complications and pharmacodynamics consequences<sup>[13,14]</sup>. In contrast with the relatively benign presentation of CAA in adult individuals, findings like preterm birth, low birth weight, presence of miscarriages, respiratory distress with frequent hospital admissions, and mild developmental delay have been often described in analbuminemic families<sup>[15]</sup>. Indeed, our patients' familiar and personal medical history seems to confirm perinatal and intrauterine complications, and the crucial role of albumin in the first periods of life.

## REFERENCES

1. Kragh-Hansen U, Minchiotti L, Campagnoli M. The albumin website. Available from: <https://www.albumin.org>
2. Dagnino M, Caridi G, Aydin Z, Ozturk S, Karaali Z, Kazancioglu R, Cefle K, Gursu M, Campagnoli M, Galliano M, Minchiotti L. A novel frameshift deletion in the albumin gene causes analbuminemia in a young Turkish woman. *Clin Chim Acta* 2010; **411**: 1711-1715 [PMID: 20638375 DOI: 10.1016/j.cca.2010.07.009]
3. Peters T. *All About Albumin: Biochemistry, Genetics, and Medical Applications*, 1st ed. San Diego: Academic Press 1995;
4. Minghetti PP, Ruffner DE, Kuang WJ, Dennison OE, Hawkins JW, Beattie WG, Dugaiczky A. Molecular structure of the human albumin gene is revealed by nucleotide sequence within q11-22 of chromosome 4. *J Biol Chem* 1986; **261**: 6747-6757 [PMID: 3009475]
5. Watkins S, Madison J, Galliano M, Minchiotti L, Putnam FW. A nucleotide insertion and frameshift cause analbuminemia in an Italian family. *Proc Natl Acad Sci U S A* 1994; **91**: 2275-2279 [PMID: 8134387 DOI: 10.1073/pnas.91.6.2275]
6. Minchiotti L, Galliano M, Caridi G, Kragh-Hansen U, Peters T. Congenital analbuminaemia: molecular defects and biochemical and clinical aspects. *Biochim Biophys Acta* 2013; **1830**: 5494-5502 [PMID: 23811111]

- 23612153 DOI: [10.1016/j.bbagen.2013.04.019](https://doi.org/10.1016/j.bbagen.2013.04.019)
- 7 **Baldo-Enzi G**, Baiocchi MR, Vigna G, Andrian C, Mosconi C, Fellin R. Analbuminaemia: a natural model of metabolic compensatory systems. *J Inherit Metab Dis* 1987; **10**: 317-329 [PMID: [3126352](https://pubmed.ncbi.nlm.nih.gov/3126352/) DOI: [10.1007/BF01799973](https://doi.org/10.1007/BF01799973)]
- 8 **Demirsoy E**, Sirin G, Ozker E. Coronary artery bypass surgery in a patient with analbuminemia. *Tex Heart Inst J* 2011; **38**: 85-87 [PMID: [21423479](https://pubmed.ncbi.nlm.nih.gov/21423479/)]
- 9 **Burgess LJ**, Marais AD. The use of simvastatin in analbuminaemia. *Cardiovasc Drugs Ther* 2001; **15**: 555-558 [PMID: [11916366](https://pubmed.ncbi.nlm.nih.gov/11916366/) DOI: [10.1023/A:1013780007561](https://doi.org/10.1023/A:1013780007561)]
- 10 **Del Ben M**, Burattin M, Arca M, Ceci F, Violi F, Angelico F. Treatment of severe hypercholesterolemia with atorvastatin in congenital analbuminemia. *Am J Med* 2004; **117**: 803-804 [PMID: [15541334](https://pubmed.ncbi.nlm.nih.gov/15541334/) DOI: [10.1016/j.amjmed.2004.06.039](https://doi.org/10.1016/j.amjmed.2004.06.039)]
- 11 **Maugeais C**, Braschi S, Ouguerram K, Maugeais P, Mahot P, Jacotot B, Darmaun D, Magot T, Krempf M. Lipoprotein kinetics in patients with analbuminemia. Evidence for the role of serum albumin in controlling lipoprotein metabolism. *Arterioscler Thromb Vasc Biol* 1997; **17**: 1369-1375 [PMID: [9261269](https://pubmed.ncbi.nlm.nih.gov/9261269/) DOI: [10.1161/01.ATV.17.7.1369](https://doi.org/10.1161/01.ATV.17.7.1369)]
- 12 **Liang K**, Vaziri ND. HMG-CoA reductase, cholesterol 7alpha-hydroxylase, LCAT, ACAT, LDL receptor, and SRB-I in hereditary analbuminemia. *Kidney Int* 2003; **64**: 192-198 [PMID: [12787409](https://pubmed.ncbi.nlm.nih.gov/12787409/) DOI: [10.1046/j.1523-1755.2003.00041.x](https://doi.org/10.1046/j.1523-1755.2003.00041.x)]
- 13 **Frohlich J**, Pudek MR, Cormode EJ, Sellers EM, Abel JG. Further studies on plasma proteins, lipids, and dye- and drug-binding in a child with analbuminemia. *Clin Chem* 1981; **27**: 1213-1216 [PMID: [7237786](https://pubmed.ncbi.nlm.nih.gov/7237786/)]
- 14 **Koot BG**, Houwen R, Pot DJ, Nauta J. Congenital analbuminaemia: biochemical and clinical implications. A case report and literature review. *Eur J Pediatr* 2004; **163**: 664-670 [PMID: [15300429](https://pubmed.ncbi.nlm.nih.gov/15300429/) DOI: [10.1007/s00431-004-1492-z](https://doi.org/10.1007/s00431-004-1492-z)]
- 15 **Toye JM**, Lemire EG, Baerg KL. Perinatal and childhood morbidity and mortality in congenital analbuminemia. *Paediatr Child Health* 2012; **17**: e20-e23 [PMID: [23730173](https://pubmed.ncbi.nlm.nih.gov/23730173/)]

**P- Reviewer:** Caceres-Loriga FM, Pan SL

**S- Editor:** Wang JL **L- Editor:** A **E- Editor:** Tan WW





## Primary leiomyosarcoma of the thyroid gland with prior malignancy and radiotherapy: A case report and review of literature

Snezana Vujosevic, Djordjije Krnjevic, Milan Bogojevic, Ljiljana Vuckovic, Aleksandar Filipovic, Duško Dunderović, Jelena Sopta

**ORCID number:** Snezana Vujosevic (0000-0003-4990-9365); Djordjije Krnjevic (0000-0003-0597-0770); Milan Bogojevic (0000-0003-1037-1342); Ljiljana Vuckovic (0000-0002-6465-2898); Aleksandar Filipovic (0000-0002-2992-8318); Duško Dunderović (0000-0001-8227-3683); Jelena Sopta (0000-0001-8448-9234).

**Author contributions:** Vujosevic S, Krnjevic D, and Bogojevic M wrote the manuscript and interpreted data; Vuckovic L, Dunderović D, and Sopta J carried out pathological and immunohistological tests on the thyroid tissue; Filipovic A performed the operation and worked with the patient; All authors meet the four criteria from the recommendation of the ICMJE and have read and approved the final manuscript.

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

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016)

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

**Snezana Vujosevic, Djordjije Krnjevic**, Endocrinology, Clinical Center of Montenegro, Podgorica 81000, Montenegro

**Milan Bogojevic**, Internal Medicine Clinic, Clinical Center of Montenegro, Podgorica 81000, Crna Gora, Montenegro

**Ljiljana Vuckovic**, Institute of Pathology, Clinical Center of Montenegro, Podgorica 81000, Montenegro

**Aleksandar Filipovic**, Surgery, Clinical Center of Montenegro, Podgorica 81000, Montenegro

**Duško Dunderović, Jelena Sopta**, Institute of Pathology, University of Belgrade, Belgrade 11000, Serbia

**Corresponding author:** Milan Bogojevic, MD, Doctor, Internal Medicine Clinic, Clinical Center of Montenegro, St. Jelene Savojske 52, Podgorica 81000, Montenegro. [milan.bogojevic@kccg.me](mailto:milan.bogojevic@kccg.me).

**Telephone:** +38-26-9767113

### Abstract

#### BACKGROUND

Leiomyosarcoma (LMS) of the thyroid gland is a rarely presented tumor that offers poor prognosis. To the best of the authors' knowledge, there currently exist only 28 known cases described in the literature (limited to English).

#### CASE SUMMARY

Herein a case is reported of a 60-year-old female patient who had an LMS of the thyroid, which was accompanied by periodic dysphonia and breathing disorder as well as the feeling of pressure in the chest and neck. At the time the disease was diagnosed, no metastases were detected. Prior to the diagnosis, the patient experienced a uterine adenocarcinoma that had been treated by surgical procedure and radiotherapy. For the LMS, a total thyroidectomy was performed, followed by radiotherapy. Since metastases were also discovered in the lungs, sternum, and femur, chemotherapy was administered as well.

Immunohistochemically, the tumor cells in the thyroid indicated positively for alpha smooth muscle actin, calponin, and H-caldesmon, but were negative for CD34, p63, estrogen receptor, progesterone receptor, and Epstein-Barr virus.

#### CONCLUSION

Although the etiology of the LMS is as of yet unknown, prior malignancy and

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

**Received:** November 27, 2018

**Peer-review started:** November 28, 2018

**First decision:** December 15, 2018

**Revised:** January 4, 2019

**Accepted:** January 26, 2019

**Article in press:** January 26, 2019

**Published online:** February 26, 2019

radiation should be considered as risk factors.

**Key words:** Thyroid; Leiomyosarcoma; Smooth muscle tumor; Radiotherapy; Thyroidectomy; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Leiomyosarcoma (LMS) of the thyroid gland is a rare tumor with only 28 cases described in the English literature. Etiology of leiomyosarcoma is still unknown. We present a case of LMS in which the patient had a prior malignancy and radiotherapy along with two similar cases previously described. Therefore, we hypothesize that prior malignancy and radiotherapy is a risk factor. Immunohistochemistry plays a crucial role in the diagnosis, and we provided substantial discussion about differential diagnosis of thyroid tumors, alongside with our microscopic findings that provide practical value for the daily practice of pathology.

**Citation:** Vujosevic S, Krnjec D, Bogojevic M, Vuckovic L, Filipovic A, Dunderovic D, Sopta J. Primary leiomyosarcoma of the thyroid gland with prior malignancy and radiotherapy: A case report and review of literature. *World J Clin Cases* 2019; 7(4): 473-481  
**URL:** <https://www.wjnet.com/2307-8960/full/v7/i4/473.htm>  
**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.473>

## INTRODUCTION

Leiomyosarcoma (LMS) is a malignant tumor derived from or showing evidence of differentiation towards smooth muscle<sup>[1]</sup>. Most commonly, it is found in the pelvic area, gastrointestinal tract, or retroperitoneal area<sup>[2]</sup>. A review of the English literature suggests that LMS is rare, and there have only been 28 such cases described in the thyroid gland. The most common sign is a growing mass in the neck<sup>[3-5]</sup>. There have been only two known cases that had recorded a malignant disease prior to an incidence of an LMS of the thyroid<sup>[15,25]</sup>. While ultrasound, computed tomography scan, and magnetic resonance imaging (MRI) are all useful in diagnosing a thyroid tumor, an immunohistochemical analysis is needed to confirm the diagnosis of a LMS. At the time of this review, an LMS of the thyroid has a poor survivability prognosis<sup>[19]</sup>. Herein, a new case of LMS conjoined with prior endometrial adenocarcinoma is described, which includes a comprehensive review of the literature about thyroid LMS.

## CASE PRESENTATION

### Chief complaints

A 60-year-old woman was admitted to the hospital complaining of pressure in her chest and neck as well as periodic dysphonia and breathing disorder.

### History of present illness

Patient's symptoms started a month ago with worsening in the past 24 h.

### History of past illness

The patient's past medical history included hypertension and a total hysterectomy that revealed an endometrial adenocarcinoma of the uterus. The adenocarcinoma was treated by means of radiotherapy using a micro-selectron application with a vaginal applicator, in which the total dose was 24 Gy in four cycles after surgery. This had been 5 years prior to the diagnosis of LMS.

### Family history

The patient's brother had also had a lung carcinoma.

### Physical examination

Thyroid gland was enlarged, with palpable node in right lobe around 2 cm. The patient's temperature was 36.4°C, heart rate was 80 bpm, respiratory rate was 22



breaths per minute, blood pressure was 145/80 mmHg, and oxygen saturation in room air was 90%.

### **Laboratory testing**

The plasma level of the thyroid stimulating hormone, free thyroxine, free triiodothyronine, calcitonin, and carcinoembryonic antigen were within normal parameters.

### **Imaging examination**

An ultrasound of the neck dating from March 2016 indicated an enlarged thyroid, where the right lobe was 54 mm × 40 mm and its hypoechogenic nodule was 23 mm × 26 mm and calcified on its edges. The left lobe was 55 mm × 25 mm in size in which there were two micro-nodules of 6 mm × 5 mm and 7 mm × 5 mm that had no calcification. No computed tomography or MRI of the thyroid had been done prior to surgical treatment.

### **Further diagnostic**

On July 20, 2016, the patient underwent a total thyroidectomy. There were no local or distant metastases detected, but the patient was found to have multiple enlarged lymph nodes (10 mm on the right and 11 mm on the left edge of the sternocleidomastoid muscle).

**Pathological findings:** Tissue specimens were fixed in a 10% formaldehyde solution, embedded in paraffin, cut into 4 µm thick sections and stained with hematoxylin-eosin. Immunohistochemical staining was carried out according to the avidin-biotin peroxidase complex method.

Immunohistochemical staining with α smooth muscle actin (SMA) (DAKO, Clone 1A4, 1:400), Calponin (Lab Vision, Clone EP798Y, 1:100), H-caldesmon (LabVision, Clone h-CALD, 1:300), CD34 (NOVOCASTRA, Clone L-END/10, 1:100), p53 (NOVOCASTRA, Clone DO-7, 1:50), Ki67 (DAKO, Clone MIB-1, 1:100), estrogen receptor (NOVOCASTRA, Clone 6F11, 1:100), progesterone receptor (NOVOCASTRA, Clone PGR 312, 1:100), and Epstein-Barr virus (EBV) (DAKO, Clone CS.1-4, 1:100) were done manually according to the manufacturer's instructions ([Table 1](#)).

Upon gross examination of the total thyroidectomy specimen, the right lobe and left lobe were measured at 38 mm × 54 mm × 49 mm and 54 mm × 40 mm × 38 mm, respectively. The sectioning of the right lobe indicated a non-encapsulated tumor node, vaguely limited, whitish in color, and firm in its consistency. Its dimensions at a cross section were 23 mm × 20 mm. Histologically, the tumor tissue was composed of atypical spindle cells of irregular fascicular growth, containing large, hyperchromatic nuclei. In a number of tumor cells, the cytoplasm was eosinophilic. While mitoses were common (8-10 per high power field), no necrosis was found. The tumor had invaded the surrounding thyroid parenchyma, but tumor tissue itself was not detected as present in the left lobe of the thyroid gland.

Immunohistochemically, the tumor cells tested positive for alpha SMA, calponin, and H-caldesmon, but were negative for CD34, p53, estrogen receptor, progesterone receptor, and EBV. In approximately 25% of all the tumor cells, the Ki67 proliferative index was positive ([Figure 1](#)).

---

## **FINAL DIAGNOSIS**

---

LMS of the thyroid.

---

## **TREATMENT**

---

The case was presented to the oncological team for soft tissue malignant tumors and to the oncological team for malignant endocrine tumors. It was thereafter decided that the patient should continue treatment under 3D conformal radiotherapy at a total dose of 50 Gy/25 fractions. Although increased therapy in the local tumor region had been planned at a 10 Gy dose divided into 5 fractions, its subsequent level of toxicity (mucositis) prevented it from being carried out.

---

## **OUTCOME AND FOLLOW-UP**

---

Table 1 Markers used

Marker	Clone	Source	Antigen retrieval	Dilutions	Visualization kit manufacturer
α SMA	1A4	DAKO	Microwave oven, citrate, pH 6	1:400	DAKO, EnVision
Calponin	EP798Y	Lab Vision	Microwave oven, citrate, pH 6	1:100	DAKO, EnVision
H-caldesmon	h-CALD	LabVision	Water bath, citrate, pH 6	1:300	DAKO, EnVision
CD34	L-END	NOVOCASTRA	Microwave oven, citrate, pH 6	1:100	DAKO, EnVision
p53	DO-7	NOVOCASTRA	Microwave oven, citrate, pH 6	1:50	DAKO, EnVision
Ki67	MIB-1	DAKO	Microwave oven, DAKO 1700, pH 6	1:100	DAKO, EnVision
ER	6F11	NOVOCASTRA	Microwave oven, citrate, pH 6	1:100	DAKO, EnVision
PR	PGR 312	NOVOCASTRA	Microwave oven, citrate, pH 6	1:100	DAKO, EnVision
EBV	CS.1-4	DAKO	Microwave oven, DAKO 1700, pH 6	1:100	DAKO, EnVision

α SMA: Alpha smooth muscle actin; ER: Estrogen receptor; PR: Progesterone receptor; EBV: Epstein-Barr virus.

Seven months following the surgery (February 2017) as part of her reevaluation, metastatic changes were found in the lungs: Two deposits in the right, measuring 11 mm and 17 mm, and in the left there were three micro-nodular changes that were a maximal size of 6 mm. It was then decided that the patient should begin chemotherapy. The patient was treated with three cycles of Adriamycin + (3, 3-dimethyl-1-triazeno)-imidazole-4-carboxamide after which the metastatic changes were shown to be in regression. However, a pulmonary embolism was also detected as well as a thrombosis of the jugular vein and an infection of the soft tissue of the neck. For this reason, chemotherapy was continued according to the MonoAdria protocol. In August, since osteoblastic activity was detected in the sternum on a scintigraphy, it was decided to initiate palliative radiotherapy and bisphosphonates. In October 2017, a phlebothrombosis of the left popliteal vein was detected. In November 2017, a palliative tracheotomy was performed. As had been suspected, a local metastasis was found. A pathological fracture of the femur in which another metastasis was also detected resulted in an osteosynthesis being performed.

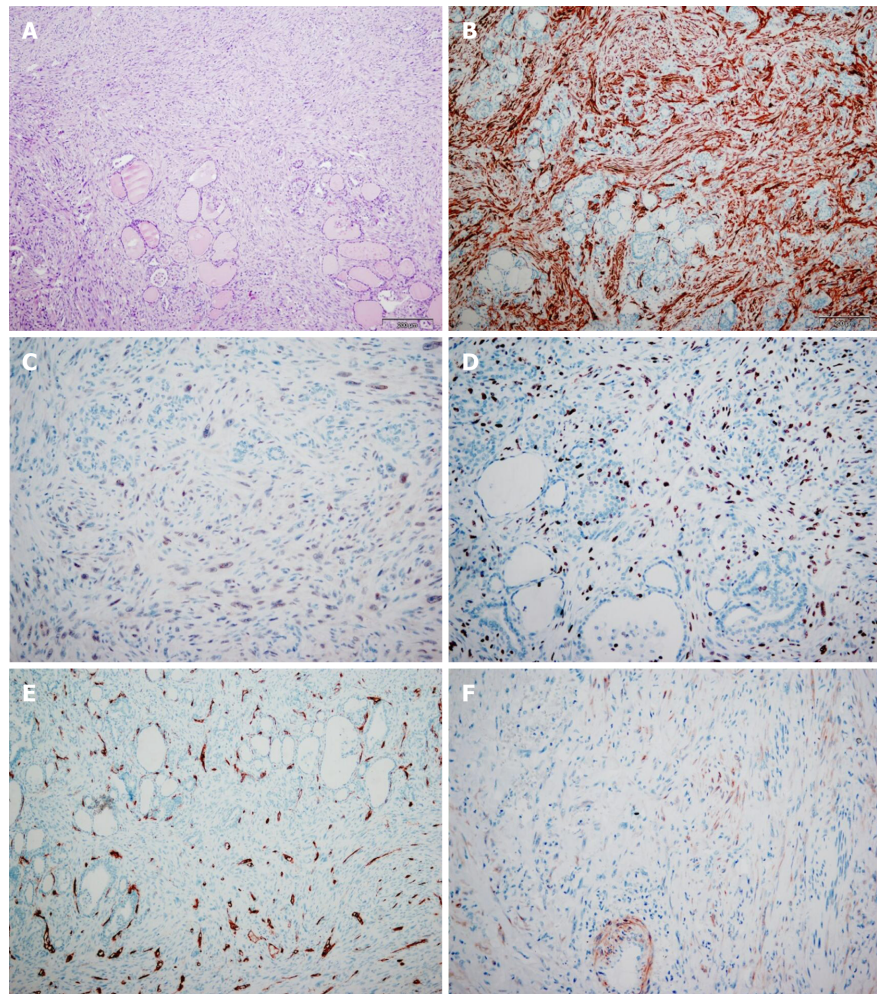
## DISCUSSION

LMS are extremely rare making up only 0.014% of all thyroid tumors<sup>[3]</sup>. The first of such cases were reported in 1969<sup>[4]</sup>. The etiology of LMS is yet unknown, but the current research-based opinion is that it develops from the smooth muscles of the veins in the thyroid gland. A possible association between infection by EBV and LMS has also been investigated, as the youngest reported case was in a 6-year-old child who suffered from congenital immunodeficiency and an EBV infection prior to LMS<sup>[11]</sup>. Two other cases have also been reported of prior malignancy. In the first, the patient had been diagnosed with prostate cancer 3 years prior to LMS, which was thereafter treated by a prostatectomy and an orchiectomy without accompanying radiotherapy<sup>[25]</sup>. The second patient was reported to have had colorectal cancer (29 years prior) as well as breast cancer and then underwent a tumorectomy and radiotherapy 4 years prior to the diagnosis of LMS<sup>[15]</sup>. Given the background of these two cases, the influence of radiation should not be excluded when considering the etiology of LMS.

LMS is found more frequently in females than in males at a ratio of 1.7:1 (Table 2). It is also more often in older patients. The patient age at diagnosis ranges from 32 to 90. Not including the aforementioned case of the 6-year-old child, the median age is 65.

The most common symptom is a growing mass on the neck (23 cases), which is commonly painless<sup>[3-5]</sup>. Symptoms include: Dysphonia<sup>[3,5,22,24,25]</sup>, dysphagia<sup>[3,16,22,23]</sup>, weight loss<sup>[16,17,23]</sup>, breathing disorder<sup>[12,23]</sup>, arm pain<sup>[15,19]</sup>, and skin fistulae<sup>[20]</sup>. Tumor size varies from 1.9 cm to 13 cm (mean: 6.4 cm). At the moment of diagnosis, three total cases recorded lymph-node metastasis. As opposed to anaplastic carcinoma that are more likely to present in lymph node metastasis, LMS often presents in distant metastasis, matching the total of twelve distant metastasis cases recorded here<sup>[3-6]</sup>.

Only two cases had no normal plasma level for thyroid stimulating hormone. The first of which was 0.084 mU/L (normal range, 0.355.5 mU/L) and free thyroxine at 26.79 pmol/L (normal range: 10.4224.32 pmol/L)<sup>[25]</sup>. The second case's levels were elevated at 4.14 IU/mL (normal range: 0.34–3.90 IU/mL)<sup>[13]</sup>. All patients scored within



**Figure 1** The tumor cells tested positive for alpha smooth muscle actin, calponin, H-caldesmon, but were negative for CD34, p53, estrogen receptor, progesterone receptor, and Epstein-Barr virus. In approximately 25% of all the tumor cells, the Ki67 proliferative index was positive. A: Pathological finding of thyroid gland stained (10 ×); B: Positive immunohistochemical staining for α-smooth muscle actin (10 ×); C: Negative immunohistochemical staining for P53 (20 ×); D: Pathological finding of 25% positive Ki67 index in tumor cells (20 ×); E: Negative immunohistochemical staining for CD34 (10 ×); F: Positive immunohistochemical staining for calponin (20 ×).

a normal level of calcitonin.

Albeit radiological examination is useful in discovering and detecting different thyroid tumors, it is of little assistance in defining carcinoma type. Ultrasound can often show a well or ill-defined hypoechoic mass or cystic or calcified nodule<sup>[5,8-10]</sup>. Computed tomography scans most often are able to show a large mass containing necrotic areas that may be accompanied by calcifications well as a local invasion of LMS<sup>[3,9,10,13,15]</sup>. MRI may display an isointense mass on T1-weighted images and an intermediate signal mass on T2-weighted images as fair enhancement on gadolinium-enhanced T1-weighted images<sup>[13]</sup>. Such radiological findings are nonspecific in view of the findings on other thyroid tumors, but the MRI of two cases did manage to indicate as such<sup>[14,15]</sup>. Thyroid scans (scintigraphy) may establish a cold nodule or an enlarged gland in areas of increased and decreased uptake of radioactive iodine<sup>[3,4,8,15]</sup>.

Cut surfaces can differ in color from white to gray, yellowish, brownish-white, or pink. The tumor consistency can vary from firm, hard, or rubbery. Areas often contain necrosis, hemorrhage, calcification, hyalinization, or myxoid changes. The tumor is frequently composed of pleomorphic eosinophilic spindle cells forming fascicles or bundles. This fact complicates it from being distinguished from other undifferentiated carcinoma of the thyroid, such as spindle-cell variants of medullary carcinoma and spindle cell tumors that are thymus-like in differentiation, as well as uncommon primary and metastatic tumors of the thyroid that are predominant in spindle cells. Tumor cells may have normal or hyperchromatic nuclei that may be blunt-ended or cigar shaped that include occasional to frequent mitotic activity. In spite of the need for genetic/molecular studies to distinguish these entities, immunohistochemistry still plays a crucial role in the diagnosis of LMS<sup>[18-21,25]</sup>.

**Table 2 A summary of primary thyroid LMS as reported in the literature (limited to the English language)**

Ref.	Age	Sex	Initial symptoms	Tumor size in cm	ILMN	IDM	Therapy	Outcome
[4]	74	F	Mass ongoing for 4 mo, pain	12	+	+	CT	DWD, 1 mo
[5]	82	M	Mass for 1 mo, dysphonia	5.5	-	-	Lobectomy + ND	DWD 4 mo, RS
[6]	54	F	Mass	?	?	?	Lobectomy	Alive, 15 mo, NED
[7]	?	?	?	?	+	?	?	Alive, 12 mo
[8]	54	F	No symptoms	3.5	?	?	Lobectomy	Alive, 15 mo, NED
[9]	72	F	Mass ongoing for 7 mo	3	-	-	Lobectomy + ND	DWD, 51 mo, MD
[3]	64	F	Mass	7.5	?	+	Uncompleted surgery	DWD, 5 mo, MD
[3]	45	M	Mass ongoing for 1 mo	9	?	+	Lobectomy, CT	Alive, 11 mo, MD
[3]	68	M	Mass, dysphonia	1.9	?	+	Uncompleted surgery	DWD, 18 mo, MD
[3]	83	M	Rapid growing mass, dysphagia	5.5	?	+	Surgery	DWD, 3 mo, MD
[10]	58	F	Mass	5	-	-	Total thyroidectomy + ND	Alive, 25 mo, NED
[11]	6	M	Mass	5	-	+	Tumorectomy	No follow up after 4 mo, MD
[12]	90	F	Breathing disorder and a mass growing for 1 mo	8	?	?	Partial tumorectomy + trachelectomy,	DWD, 2 mo, pneumonia
[13]	66	F	Mass rapidly growing for ? mo	8.5	-	?	Thyroidectomy, laryngectomy	Alive 3 mo, MD, RS
[14]	43	M	Growing mass over 2 mo	6	-	+	Thyroidectomy + ND, CT	DWD 6 mo
[15]	83	F	Mass, pain in the arm	6.7	?	-	Biopsy, palliative therapy	DWD 2 mo, RS
[16]	63	F	Mass, weight loss, dysphagia	7	?	+	Total thyroidectomy	DWD, 5 mo
[17]	65	F	Mass, weight loss, cough	8	-	-	Total thyroidectomy + ND, CT	DWD 4 mo
[18]	-	-	-	-	-	-	-	-
[19]	65	M	Left arm pain	9	-	-	Total thyroidectomy, partial esophagectomy	Alive after 5 yr of follow up
[20]	72	F	Neck mass with accompanying skin fistulae	8.5	-	-	Lobectomy with mass excision	DWD, 2 mo
[21]	64	F	-	-	-	+	Total thyroidectomy	DWD, 3 mo
[22]	56	M	Neck mass dysphonia, dysphagia	3	-	-	Total thyroidectomy + ND	DWD, 8 mo
[23]	39	M	Weight loss, dysphagia	2.5	-	+	Biopsy + RT	DWD 3 mo
[23]	72	F	Mass, breathing disorder	?	+	+	Trachelectomy + biopsy	DWD, 1.5 mo



[25]	65	F	Neck mass dysphonia	8.3	-	+	Total thyroidectomy + lymphadenecto my	?
[25]	83	M	Neck mass, dysphonia	13	-	-	Lobectomy, partial trachelectomy, CT	DWD, 5 mo, MD
[26]	32	F	Neck mass	5	-	-	Lobectomy, RT + CT	?
Our Case	60	F	Mass, dysphonia, breathing disorder	2.5	-	-	Total thyroidectomy, RT + CT	Alive 17 mo, MD, thrombosis

F: Female; M: Male; IDM: Initial distant metastasis; ILNM: Initial lymph nodes metastasis; CT: Chemotherapy; RT: Radiotherapy; ND: Neck dissection; NED: No evidence of disease; DWD: Died with disease; MD: Metastatic disease.

Tumor cells in LMS are immunohistochemically positive for vimentin and SMA and are variable positive for desmin (50%-100%), HHF35, and H-caldesmon. There were three cases that tested negative for desmin. Cytokeratin, thyroglobulin, calcitonin, protein S100, and chromogranins were never positive<sup>[3,5-7]</sup>.

Undifferentiated (anaplastic) thyroid carcinoma might have different microscopic features including spindle cells, which may mimic LMS, fibrosarcoma, or histiocytoma. The clinical presentation of tumor behavior is similar to LMS although anaplastic tumors more often result in metastases in the lymph nodes. Despite the fact that epithelial type cells can often be found in undifferentiated carcinoma, identifying them proves difficult at times. Unlike LMS, the tumor cells in undifferentiated carcinoma frequently test positive for cytokeratins and p53, but epithelial markers can still show negatively in approximately 20% of all cases. Vimentin and desmin are positive in more than 50% while SMA is always negative<sup>[15,20,21,24]</sup>.

The spindle form of medullary carcinoma should always be considered in the differential diagnosis. It scores positive for calcitonin, thyroglobulin, synaptophysin, chromogranin, cytokeratins, CD56, and TTF. Vimentin is a variable positive while SMA is negative<sup>[15,20,21,24]</sup>.

Solitary fibrous tumors are also composed of spindle cells. They often have the clinical presentation of a slow-growing neck tumor. They generally test positive for CD34, BCL2, CD99, and vimentin. Their variable positivity to smooth muscle markers is also noted<sup>[15,20,21,24]</sup>.

Rare primary tumors such as malignant peripheral-nerve sheath tumors, follicular dendritic-cell sarcoma, and histiocytic sarcoma should always be considered in addition to metastatic tumors to the thyroid. An LMS of the uterus is the most common sarcoma metastasizing in the thyroid. Nonetheless, thyroid metastases of any sarcoma or carcinoma that has prominent spindle cells or sarcomatoid features should also be kept in mind when doing a differential diagnosis. A clinico-radiological correlation and application of the panel of antibodies may help in determining the site of the tumor origin.

Of the 25 patients who have known outcomes, 17 passed away. Of these, 15 died within 1 year from their initial diagnosis, one after 18 mo and one after 51 mo. The median survival time was 5 mo<sup>[3-7]</sup>. There is yet to be any consensus on how LMS should be treated. Surgery has been the primary solution. A lobectomy, total thyroidectomy, and neck dissection are available options. Some patients underwent chemotherapy and radiotherapy, but did not yield promising results. Interestingly, none of the three patients that have had long post-treatment survival underwent chemotherapy or radiotherapy. Day *et al*<sup>[14]</sup> reported a patient overexpressing c-kit who was treated with immunotherapy and imatinib-mesylate, but then passed away 3 mo later.

## CONCLUSION

Primary LMS of the thyroid is rare and should be considered when the patient does present with a rapidly growing neck mass. Previous malignancy and radio/chemotherapy should also be taken into consideration as risk factors. The prognosis of the tumor is poor and extensive surgery is often advised.



## ACKNOWLEDGEMENTS

Thanks to pewdiepie for providing good content while writing paper, subscribe to pewdiepie.

## REFERENCES

- 1 **DeLellis RA**, Loyd RV, Heitz PU. Pathology and Genetics of Tumours of Endocrine Organs. World health Organization Classification of Tumours. 3<sup>rd</sup> ed. IARC Press, Lyon. 2004; 49-135
- 2 **Goldblum JR**, Folpe AV, Weiss SW, Enzinger FM. Enzinger and Weiss's soft tissue tumors. 9<sup>th</sup> ed. Mosby Elsevier, Philadelphia Hardcover. 2008; 249-269
- 3 **Thompson LD**, Wenig BM, Adair CF, Shmookler BM, Heffess CS. Primary smooth muscle tumors of the thyroid gland. *Cancer* 1997; **79**: 579-587 [PMID: [9028371](#)]
- 4 **Adachi M**, Wellmann KF, Garcia R. Metastatic leiomyosarcoma in brain and heart. *J Pathol* 1969; **98**: 294-296 [PMID: [5358278](#) DOI: [10.1002/path.1710980411](#)]
- 5 **Kawahara E**, Nakanishi I, Terahata S, Ikegaki S. Leiomyosarcoma of the thyroid gland. A case report with a comparative study of five cases of anaplastic carcinoma. *Cancer* 1988; **62**: 2558-2563 [PMID: [3056606](#)]
- 6 **Kawaguchi Y**, Kanazawa M, Nakayama K, Urazumi K, Takeuchi S, Abe R. A case of leiomyosarcoma of the thyroid gland showing fatal outcome with rapid course. *Nihon Rinsho Gekai Gakkai Zasshi (Jpn J ClinSurg)* 1990; **51**: 1217-1221 [DOI: [10.3919/ringe1963.51.1217](#)]
- 7 **Kaur A**, Jayaram G. Thyroid tumors: cytomorphology of medullary, clinically anaplastic, and miscellaneous thyroid neoplasms. *Diagn Cytopathol* 1990; **6**: 383-389 [PMID: [2292224](#) DOI: [10.1002/dc.2840060603](#)]
- 8 **Chetty R**, Clark SP, Dowling JP. Leiomyosarcoma of the thyroid: immunohistochemical and ultrastructural study. *Pathology* 1993; **25**: 203-205 [PMID: [8367205](#) DOI: [10.3109/00313029309084801](#)]
- 9 **Iida Y**, Katoh R, Yoshioka M, Oyama T, Kawaoi A. Primary leiomyosarcoma of the thyroid gland. *Acta Pathol Jpn* 1993; **43**: 71-75 [PMID: [8465659](#)]
- 10 **Ozaki O**, Sugino K, Mimura T, Ito K, Tamai S, Hosoda Y. Primary leiomyosarcoma of the thyroid gland. *Surg Today* 1997; **27**: 177-180 [PMID: [9018000](#) DOI: [10.1007/BF02385912](#)]
- 11 **Tulbah A**, Al-Dayel F, Fawaz I, Rosai J. Epstein-Barr virus-associated leiomyosarcoma of the thyroid in a child with congenital immunodeficiency: a case report. *Am J Surg Pathol* 1999; **23**: 473-476 [PMID: [10199478](#) DOI: [10.1097/00000478-199904000-00013](#)]
- 12 **Tsugawa K**, Koyanagi N, Nakanishi K, Wada H, Tanoue K, Hashizume M, Sugimachi K. Leiomyosarcoma of the thyroid gland with rapid growth and tracheal obstruction: A partial thyroidectomy and tracheostomy using an ultrasonically activated scalpel can be safely performed with less bleeding. *Eur J Med Res* 1999; **4**: 483-487 [PMID: [10585304](#)]
- 13 **Takayama F**, Takashima S, Matsuba H, Kobayashi S, Ito N, Sone S. MR imaging of primary leiomyosarcoma of the thyroid gland. *Eur J Radiol* 2001; **37**: 36-41 [PMID: [11274837](#) DOI: [10.1016/S0720-048X\(00\)00217-5](#)]
- 14 **Day AS**, Lou PJ, Lin WC, Chou CC. Over-expression of c-kit in a primary leiomyosarcoma of the thyroid gland. *Eur Arch Otorhinolaryngol* 2007; **264**: 705-708 [PMID: [17256123](#)]
- 15 **Just PA**, Guillevin R, Capron F, Le Charpentier M, Le Naour G, Menegaux F, Leenhardt L, Simon JM, Hoang C. An unusual clinical presentation of a rare tumor of the thyroid gland: report on one case of leiomyosarcoma and review of literature. *Ann Diagn Pathol* 2008; **12**: 50-56 [PMID: [18164417](#) DOI: [10.1016/j.anndiagpath.2006.06.006](#)]
- 16 **Mansouri H**, Gaye M, Errihani H, Kettani F, Gueddari BE. Leiomyosarcoma of the thyroid gland. *Acta Otolaryngol* 2008; **128**: 335-336 [PMID: [18274920](#) DOI: [10.1080/00016480500527193](#)]
- 17 **Wang TS**, Ocal IT, Oxley K, Sosa JA. Primary leiomyosarcoma of the thyroid gland. *Thyroid* 2008; **18**: 425-428 [PMID: [18346004](#) DOI: [10.1089/thy.2007.0276](#)]
- 18 **Qin Q**, Liang ZH, Li AH. [Thyroid leiomyosarcoma: report of one cases]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2012; **47**: 75-76 [PMID: [22455785](#)]
- 19 **Mouaqit O**, Belkacem Z, Ifrine L, Mohsine R, Belkouchi A. A rare tumor of the thyroid gland: report on one case of leiomyosarcoma and review of literature. *Updates Surg* 2014; **66**: 165-167 [PMID: [23335096](#) DOI: [10.1007/s13304-013-0196-1](#)]
- 20 **Amal B**, El Fatemi H, Souaf I, Moumna K, Affaf A. A rare primary tumor of the thyroid gland: report a new case of leiomyosarcoma and literature review. *Diagn Pathol* 2013; **8**: 36 [PMID: [23445571](#) DOI: [10.1186/1746-1596-8-36](#)]
- 21 **Tanboon J**, Keskoool P. Leiomyosarcoma: a rare tumor of the thyroid. *Endocr Pathol* 2013; **24**: 136-143 [PMID: [23729187](#) DOI: [10.1007/s12022-013-9251-1](#)]
- 22 **Ege B**, Leventoglu S. Primary leiomyosarcoma of the thyroid. *J Korean Surg Soc* 2013; **85**: 43-46 [PMID: [23833760](#) DOI: [10.4174/jkss.2013.85.1.43](#)]
- 23 **Şahin Mİ**, Vural A, Yüce İ, Çağlı S, Deniz K, Güney E. Thyroid leiomyosarcoma: presentation of two cases and review of the literature. *Braz J Otorhinolaryngol* 2016; **82**: 715-721 [PMID: [27080750](#) DOI: [10.1016/j.bjorl.2015.11.020](#)]
- 24 **Gupta AJ**, Singh M, Rani P, Khurana N, Mishra A. Primary Sarcomas of Thyroid Gland-Series of Three Cases with Brief Review of Spindle Cell Lesions of Thyroid. *J Clin Diagn Res* 2017; **11**: ER01-ER04 [PMID: [28384879](#) DOI: [10.7860/JCDR/2017/22907.9164](#)]
- 25 **Zou ZY**, Ning N, Li SY, Li J, DU XH, Li R. Primary thyroid leiomyosarcoma: A case report and literature review. *Oncol Lett* 2016; **11**: 3982-3986 [PMID: [27313727](#) DOI: [10.3892/ol.2016.4496](#)]
- 26 **Ayadi M**, Gabsi A, Meddeb K, Mokrani A, Yahiaoui Y, Letaief F, Chraiet N, Rais H, Mezlini A. Primary leiomyosarcoma of thyroid gland: the youngest case. *Pan Afr Med J* 2017; **26**: 113 [PMID: [28533836](#) DOI: [10.11604/pamj.2017.26.113.11472](#)]
- 27 **Ordóñez NG**, El-Naggar AK, Hickey RC, Samaan NA. Anaplastic thyroid carcinoma. Immunocytochemical study of 32 cases. *Am J Clin Pathol* 1991; **96**: 15-24 [PMID: [1712540](#) DOI: [10.1093/ajcp/96.1.15](#)]
- 28 **Hurlimann J**, Gardiol D, Scazziga B. Immunohistology of anaplastic thyroid carcinoma. A study of 43 cases. *Histopathology* 1987; **11**: 567-580 [PMID: [2442086](#) DOI: [10.1111/j.1365-2559.1987.tb02667.x](#)]

- 29 **Miettinen M**, Franssila KO. Variable expression of keratins and nearly uniform lack of thyroid transcription factor 1 in thyroid anaplastic carcinoma. *Hum Pathol* 2000; **31**: 1139-1145 [PMID: [11014583](#) DOI: [10.1053/hupa.2000.16667](#)]
- 30 **LiVolsi VA**, Brooks JJ, Arendash-Durand B. Anaplastic thyroid tumors. Immunohistology. *Am J Clin Pathol* 1987; **87**: 434-442 [PMID: [2435145](#)]

**P- Reviewer:** Mogulkoc R, Coskun A

**S- Editor:** Dou Y   **L- Editor:** Filipodia   **E- Editor:** Tan WW



## Endoscopic resection for residual lesion of metastatic gastric cancer: A case report

Kaori Hayashi, Sho Suzuki, Hisatomo Ikehara, Hiroaki Okuno, Akira Irie, Mitsuru Esaki, Chika Kusano, Takuji Gotoda, Mitsuhiko Moriyama

**ORCID number:** Kaori Hayashi (0000-0001-9210-3186); Sho Suzuki (0000-0003-4831-1409); Hisatomo Ikehara (0000-0001-9239-7495); Hiroaki Okuno (0000-0002-3020-7315); Akira Irie (0000-0002-6651-8510); Mitsuru Esaki (0000-0001-7353-2153); Chika Kusano (0000-0002-3789-4787); Takuji Gotoda (0000-0001-6904-6777); Mitsuhiko Moriyama (0000-0002-4617-508X).

**Author contributions:** Hayashi K, Suzuki S, Ikehara H, Okuno H, Irie A, Esaki M, Kusano C, Gotoda T, Moriyama M wrote the paper.

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

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist-2016.

**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

**Kaori Hayashi, Sho Suzuki, Hisatomo Ikehara, Hiroaki Okuno, Akira Irie, Mitsuru Esaki, Chika Kusano, Takuji Gotoda, Mitsuhiko Moriyama,** Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, Tokyo, Chiyoda-ku 1018309, Japan

**Corresponding author:** Sho Suzuki, MD, PhD, Doctor, Research Associate, Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, 1-6 Kanda-Surugadai, Tokyo, Chiyoda-ku 1018309, Japan.

[s.sho.salubriter.mail@gmail.com](mailto:s.sho.salubriter.mail@gmail.com)

**Telephone:** +81-3-32931711

**Fax:** +81-3-32922880

### Abstract

#### BACKGROUND

Chemotherapy is a standard strategy for stage IV gastric cancer patients. However, some cases cannot undergo conversion surgery because of their frailty, even if the patients had response to chemotherapy. For these patients, local tumor progression is a problem. We report here the case of a patient whose residual gastric cancer was resected through endoscopic submucosal dissection (ESD) after concomitant chemotherapy for metastatic gastric cancer.

#### CASE SUMMARY

An 85-year-old male complained of difficulty swallowing, and examination revealed gastric cancer with multiple liver metastases. Although he received concomitant chemotherapy, a residual tumor was observed in the primary lesion while the metastatic lesions disappeared completely. Conversion surgery was considered optional treatment; however, he could not undergo that because of advanced age and comorbidities. Thus, we performed ESD to treat the residual tumor. As a result, we resected the residual lesion completely. The patient has been alive for 29 mo since ESD, without recurrence.

#### CONCLUSION

We achieved local control using ESD, and these findings may provide therapeutic improvements both in local control and patient survival outcomes.

**Key words:** Gastric cancer; Liver metastases; Conversion; Endoscopic submucosal dissection; Chemotherapy; Case report

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

**Received:** October 29, 2018

**Peer-review started:** October 29, 2018

**First decision:** November 27, 2018

**Revised:** December 25, 2018

**Accepted:** January 8, 2019

**Article in press:** January 8, 2019

**Published online:** February 26, 2019

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Some cases cannot undergo conversion surgery because of their frailty, even if the patients had response to chemotherapy. For these patients, local tumor progression is a problem. We resected a residual tumor completely using endoscopic submucosal dissection (ESD) after chemotherapy in an elderly patient who was unable to undergo conversion surgery due to his age and comorbidities. The patient has been alive without recurrence for 29 mo after the ESD. ESD may provide therapeutic improvements in both local control and patient survival outcomes.

**Citation:** Hayashi K, Suzuki S, Ikehara H, Okuno H, Irie A, Esaki M, Kusano C, Gotoda T, Moriyama M. Endoscopic resection for residual lesion of metastatic gastric cancer: A case report. *World J Clin Cases* 2019; 7(4): 482-488

**URL:** <https://www.wjnet.com/2307-8960/full/v7/i4/482.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.482>

## INTRODUCTION

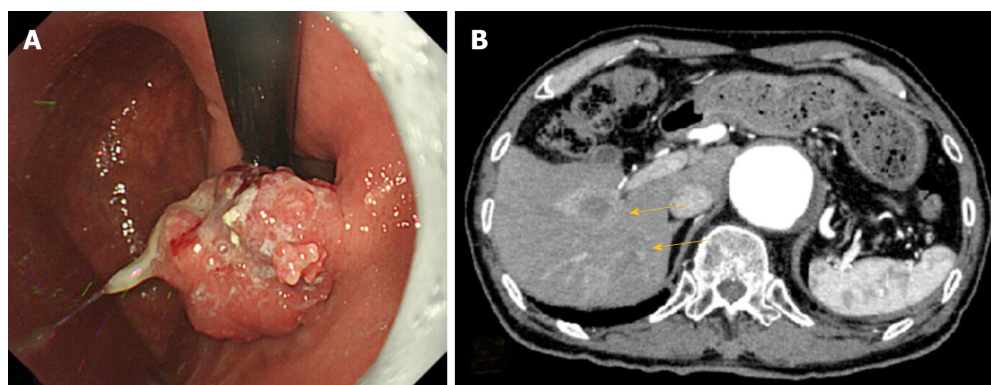
For patients with stage IV gastric cancer, chemotherapy is a standard treatment option. Improvements in chemotherapy regimens have improved patient survival<sup>[1]</sup>; for example, patients' median survival time is longer and the response rate continues to rise<sup>[2,3]</sup>. However, treatment outcomes for metastatic gastric cancer (mGC) remain unsatisfactory. Regardless, the improved efficacy of chemotherapy has led to the ability to perform surgery on tumors that were originally regarded as technically or oncologically unresectable or only marginally resectable. Conversion surgery is one treatment option with the potential to improve survival outcomes<sup>[4]</sup>. Yet, some factors prevent surgical resection, particularly advanced age, low performance status, and comorbidity, even if the patient achieves a complete response from chemotherapy. Many of these patients also experience difficulties with continuing intensive chemotherapy; thus, palliative treatment is often performed. Additionally, gastric stenosis and gastroparesis caused by local recurrence are major problems that decrease a patient's quality of life.

Endoscopic submucosal dissection (ESD) is a common practice used to resect early gastric cancer. It is a minimally intensive procedure that can preserve quality of life. Moreover, the procedure can resect the whole specimen, which allows for correct histopathological assessment. We report herein a case of a patient with mGC that responded to chemotherapy and who underwent a complete resection of the residual tumor using ESD. Twenty-nine months after the ESD, no local recurrence or distant metastases have been found.

## CASE PRESENTATION

An 85-year-old Japanese male complained of difficulty swallowing and visited a hospital. He had a medical history of untreated abdominal aortic aneurysm and pulmonary phthisis. He underwent esophagogastroduodenoscopy to diagnose his symptoms, and a protruding lesion at the gastric cardia was found. Endoscopic biopsy confirmed a moderately differentiated adenocarcinoma. Abdominal computed tomography (CT) showed no evidence of the tumor invading the muscularis propria and serosa, enlarged lymph nodes, or distant metastases. Therefore, this lesion was diagnosed as stage IA gastric cancer (cT1N0M0, 7<sup>th</sup> edition of UICC TNM Classification of Malignant Tumors).

Four months after diagnosis, the patient visited our institution for treatment of this lesion. It took a long time for the patient to visit because he could not decide which treatment to take, with his options being surgery and endoscopic treatment. The tumor (Figure 1A) grew to 20 mm in size, and abdominal CT result showed clear evidence of multiple mass lesions (range: 10-30 mm) in the liver, suggesting metastases of his gastric cancer (Figure 1B); however, there was no evidence of the tumor invading the muscularis propria and serosa or of lymph nodes' swelling. Therefore, the diagnosis was changed to stage IV gastric cancer.



**Figure 1 Objective image findings at the first visit to our institute.** A: EGD findings before treatment. EGD found a protruding lesion, 20 mm in diameter, at the gastric cardia; B: Abdominal CT with contrast before treatment; CT showed multiple mass lesions throughout the liver (arrows). EGD: Esophagogastroduodenoscopy; CT: Computed tomography.

## FINAL DIAGNOSIS

The clinical diagnosis was gastric cancer with liver metastases; stage IV (cT1N0M1).

## TREATMENT

Although the patient was elderly, he was in the Eastern Cooperative Oncology Group performance status of 1 and had adequate organ functions; therefore, chemotherapy was deemed appropriate.

S-1 and oxaliplatin combination therapy (SOX) were administered. S-1 was administered orally (100 mg/d), and oxaliplatin was delivered *via* intravenous infusion (70 mg/m<sup>2</sup> diluted in 500 mL of saline over 120 min). The patient underwent four cycles of SOX, beginning 1 mo after the diagnosis of mGC. Each SOX cycle lasted 3 wk, with S-1 administered daily on days 1 to 14 of each cycle, and oxaliplatin administered only on day 1 of each cycle. After SOX, the primary lesion decreased, although it was confirmed macroscopically, and endoscopic biopsy revealed no atypia. Additionally, CT confirmed no evidence of liver metastases. However, the patient had grade 3 sensory peripheral neuropathy (Common Terminology Criteria for Adverse Events version 4.0; CTCAE4.0); thus, oxaliplatin was discontinued and S-1 was continued for two additional cycles. Thereafter, the primary lesion macroscopically appeared smooth and protruded, and it appeared similar to a submucosal tumor (Figure 2A). The endoscopic biopsy still revealed no atypia. CT results showed no lymph node swelling or distant metastasis (Figure 2B). Therefore, we determined that the patient had had a complete response to the chemotherapy. At that point, the patient's difficulty in swallowing had improved, but he had grade 3 anorexia and sensory peripheral neuropathy (CTCAE4.0); therefore, S-1 was discontinued.

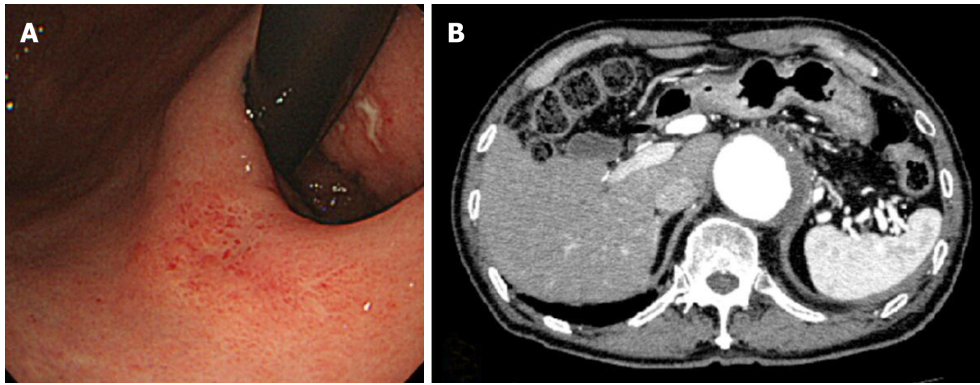
Due to the patient's response to chemotherapy, conversion surgery was considered. The patient's liver metastases had completely disappeared and the primary lesion became unclear macroscopically; however, conversion surgery was difficult because of his advanced age and comorbidities. Although the biopsy specimen at the mucosa of the primary lesion revealed no atypia after chemotherapy, a residual tumor was suspected under the mucosal layer because it looked like there was submucosal tumor. Therefore, ESD was performed for the primary lesion. The lesion was resected with no complications.

## OUTCOME AND FOLLOW-UP

The pathological diagnosis of the specimen was 28 mm × 12 mm, U, type 0-IIc, 1.0 mm × 1.0 mm, well-differentiated tubular adenocarcinoma (tub1), ypT1b (SM), ly0, v0, pHM(-), and pVM(-) in the Japanese Classification of Gastric Cancer (Figure 3A and 3B). After ESD, no recurrence was observed during the 29 mo follow-up period.

## DISCUSSION





**Figure 2 Objective image findings after chemotherapy.** A: EGD finding after chemotherapy. The lesion became unclear. It appeared smooth and protruded, and looked like a submucosal tumor; B: Abdominal CT with contrast after chemotherapy showed multiple lesions had disappeared and no evidence of disease in the liver. EGD: Esophagogastroduodenoscopy; CT: Computed tomography.

To our knowledge, this is the first report describing the efficacy of ESD to prevent local recurrence and help maintain quality of life for a patient with mGC. Effective chemotherapy, followed by complete resection with negative margin using ESD, contributed to a good clinical course.

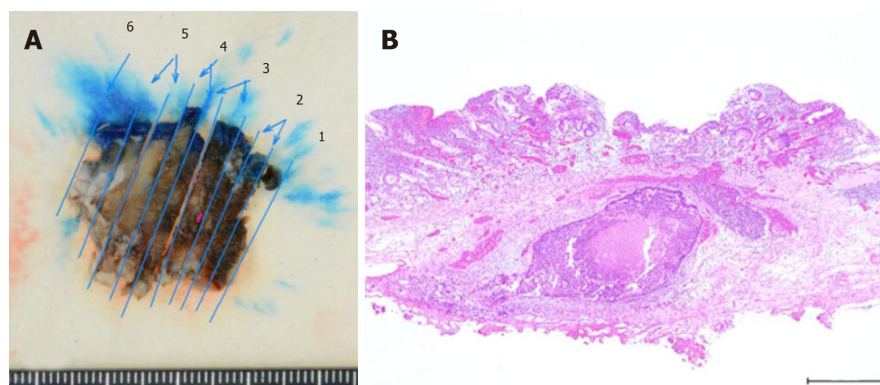
SOX was an effective and safe chemotherapeutic treatment in our patient's case. A previous phase III study (G-SOX study) showed that SOX was not inferior to S-1 plus cisplatin therapy (CS) inefficacy<sup>[5]</sup>. Regarding safety, SOX has shown better tolerability than CS in elderly patients, with a lower frequency of grade 3 or worse adverse events, as well as lower occurrence of increased creatinine levels<sup>[6]</sup>. In clinical practice, SOX is administered more frequently than CS because of its feasibility.

In this case, the patient achieved a complete response for multiple liver masses, and the primary lesion size decreased from SOX, but it was difficult to continue chemotherapy due to adverse events (*i.e.*, peripheral sensory neuropathy and anorexia). Recently, conversion surgery has been proposed and clinically performed following successful chemotherapy. Some studies showed that a microscopically margin-negative (R0) resection for a primary lesion after a good response to chemotherapy improved survival outcomes for mGC patients<sup>[7,8]</sup>. Additionally, cytoreductive or volume-reduction surgery has not shown survival benefits in similar patients<sup>[9,10]</sup>. Sato *et al*<sup>[11]</sup> reported a 5-year overall survival (OS) (median survival time of 47.9 mo) of 48.6% in patients with stage IV gastric cancer who had undergone R0 resection after chemotherapy. Additionally, R0 resection led to a significantly longer OS than did R1 (microscopic residual tumor) and R2 (macroscopic residual tumor) resections. Similarly, in patients with stage IV gastric cancer who had a gastrectomy after chemotherapy, OS was significantly longer from R0 resection only (median OS = 19.2 mo)<sup>[12]</sup>.

Based on newly proposed classification categories for stage IV gastric cancer<sup>[13]</sup>, our case was Category 2. Category 2 is indicative of the absence of macroscopic peritoneal dissemination, with marginally resectable metastatic lesions that are oncologically or technically unresectable. Furthermore, the proposed treatment strategy for Category 2 is conversion surgery, if the patient achieves a satisfactory response to chemotherapy. Therefore, our case was theoretically a good candidate for conversion surgery; however, the patient was elderly and had an untreated large abdominal aortic aneurysm. Moreover, the patient was concerned about decreased performance status due to total gastrectomy.

In cases in which the patient cannot undergo surgery and continue intensive chemotherapy, similar to the case of our patient, most patients shift to less-intensive chemotherapy or supportive care. In the current standard chemotherapy, subsequent chemotherapies are not always less-intensive. In clinical practice, some reports state that less intensive monotherapy was conducted for frail patients<sup>[14,15]</sup>. Nishimura *et al*<sup>[16]</sup> reported that irinotecan monotherapy after refractory response to fluoropyrimidine, platinum and taxanes showed modest activity. However, in these reports, even though most patients' PS were 0-1, their prognoses were poor. For frail patients, if chemotherapy may decrease PS level and worsen prognosis, then supportive care is often chosen as the best alternative. Radiotherapy is one of the therapeutic strategies for these patients to manage localized tumors<sup>[17,18]</sup>. However, in this case, radiotherapy was not conducted due to comorbidity.

Local tumor progression is a problem in many of these patients. Gastric stenosis, obstruction, gastroparesis, bleeding, and pain are common conditions caused by local



**Figure 3 Result of gastric submucosal dissection.** A: Macroscopic findings from a resected specimen; B: Histopathological result of a resected specimen: 28 mm × 12 mm, U, type 0-IIc, 1.0 mm × 1.0 mm, well-differentiated tubular adenocarcinoma (tub1), ypT1b, ly0, v0, pHM(-), and pVM(-).

recurrence or advanced primary lesions that ultimately decrease a patient's quality of life. For these cases, there are some available strategies, such as palliative gastrectomy, palliative gastrojejunostomy, or endoscopic stent. In this case, the patient did not suffer from obstruction at the end of chemotherapy. Although biopsy results both during and after chemotherapy were categorized as Group 1, the primary lesion appeared similar to a submucosal tumor. Considering that a lesion sometimes exists at the submucosa like an inverted polyp<sup>[19]</sup>, a residual tumor was suspected. If the residual tumor had remained and grown, there would have been a risk of obstruction. Therefore, ESD was used to first confirm the residual tumor and then resect the lesion completely. The results of this case suggest that when clinically possible, conversion surgery is a better treatment option, because a tumor may remain even without macroscopic evidence of the disease.

ESD is a less-intensive treatment and common practice to resect early gastric cancer, rather than surgical resection<sup>[20,21]</sup>. ESD enables resection of the whole lesion, thereby allowing a correct histopathological assessment. Furthermore, the procedure is safe and effective in elderly patients. Abe *et al*<sup>[22]</sup> reported that the complication rate (bleeding and perforation rates of 3.2% and 2.8%, respectively) of ESD was similar in elderly (≥ 80 years) and non-elderly patients with early gastric cancer, indicating that ESD was a safe procedure, even for the elderly. Additionally, Watanabe *et al*<sup>[23]</sup> reported no significant differences in adverse events (*i.e.*, rate of perforation, postoperative bleeding, aspiration pneumonia, or stricture) among patients with early gastric cancer, regardless of age. Several studies reported that endoscopic therapies, such as ESD, for locoregional recurrence of esophageal cancer after definitive chemoradiotherapy ensured safety. Furthermore, endoscopic therapy can be locally controlled and may improve both overall and disease-specific survival rates<sup>[24,25]</sup>. In the presented case, we achieved local control from the resection using ESD, which may provide therapeutic improvements in both local control and survival outcomes for the patient.

## CONCLUSION

In conclusion, we presented a rare case of a complete resection with negative margins using ESD for a remnant submucosal lesion after chemotherapy for mGC. The patient has been alive for the past 29 mo after ESD, with no sign of local recurrence.

## REFERENCES

- 1 **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]
- 2 **Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK; ToGA Trial Investigators.** Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]
- 3 **Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M.** S-1 plus

- cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 2008; **9**: 215-221 [PMID: 18282805 DOI: 10.1016/S1470-2045(08)70035-4]
- 4 **Schildberg CW**, Weidinger T, Hohenberger W, Wein A, Langheinrich M, Neurath M, Boxberger F. Metastatic adenocarcinomas of the stomach or esophagogastric junction (UICC stage IV) are not always a palliative situation: a retrospective analysis. *World J Surg* 2014; **38**: 419-425 [PMID: 24146196 DOI: 10.1007/s00268-013-2293-1]
  - 5 **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/mdl472]
  - 6 **Bando H**, Yamada Y, Tanabe S, Nishikawa K, Gotoh M, 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. Efficacy and safety of S-1 and oxaliplatin combination therapy in elderly patients with advanced gastric cancer. *Gastric Cancer* 2016; **19**: 919-926 [PMID: 26474989 DOI: 10.1007/s10120-015-0549-1]
  - 7 **Fukuchi M**, Ishiguro T, Ogata K, Suzuki O, Kumagai Y, Ishibashi K, Ishida H, Kuwano H, Mochiki E. Prognostic Role of Conversion Surgery for Unresectable Gastric Cancer. *Ann Surg Oncol* 2015; **22**: 3618-3624 [PMID: 25663597 DOI: 10.1245/s10434-015-4422-6]
  - 8 **Yamaguchi K**, Yoshida K, Tanahashi T, Takahashi T, Matsuhashi N, Tanaka Y, Tanabe K, Ohdan H. The long-term survival of stage IV gastric cancer patients with conversion therapy. *Gastric Cancer* 2018; **21**: 315-323 [PMID: 28616743 DOI: 10.1007/s10120-017-0738-1]
  - 9 **Fujitani K**, Yang HK, Mizusawa J, Kim YW, Terashima M, Han SU, Iwasaki Y, Hyung WJ, Takagane A, Park DJ, Yoshikawa T, Hahn S, Nakamura K, Park CH, Kurokawa Y, Bang YJ, Park BJ, Sasako M, Tsujinaka T; REGATTA study investigators. Gastrectomy plus chemotherapy versus chemotherapy alone for advanced gastric cancer with a single non-curative factor (REGATTA): a phase 3, randomised controlled trial. *Lancet Oncol* 2016; **17**: 309-318 [PMID: 26822397 DOI: 10.1016/S1470-2045(15)00553-7]
  - 10 **Mahar AL**, Coburn NG, Singh S, Law C, Helyer LK. A systematic review of surgery for non-curative gastric cancer. *Gastric Cancer* 2012; **15** Suppl 1: S125-S137 [PMID: 22033891 DOI: 10.1007/s10120-011-0088-3]
  - 11 **Sato Y**, Ohnuma H, Nobuoka T, Hirakawa M, Sagawa T, Fujikawa K, Takahashi Y, Shinya M, Katsuki S, Takahashi M, Maeda M, Okagawa Y, Naoki U, Kikuch S, Okamoto K, Miyamoto H, Shimada M, Takemasa I, Kato J, Takayama T. Conversion therapy for inoperable advanced gastric cancer patients by docetaxel, cisplatin, and S-1 (DCS) chemotherapy: a multi-institutional retrospective study. *Gastric Cancer* 2017; **20**: 517-526 [PMID: 27553665 DOI: 10.1007/s10120-016-0633-1]
  - 12 **Satoh S**, Okabe H, Teramukai S, Hasegawa S, Ozaki N, Ueda S, Tsuji A, Sakabayashi S, Fukushima M, Sakai Y. Phase II trial of combined treatment consisting of preoperative S-1 plus cisplatin followed by gastrectomy and postoperative S-1 for stage IV gastric cancer. *Gastric Cancer* 2012; **15**: 61-69 [PMID: 21667134 DOI: 10.1007/s10120-011-0066-9]
  - 13 **Yoshida K**, Yamaguchi K, Okumura N, Tanahashi T, Kadera Y. Is conversion therapy possible in stage IV gastric cancer: the proposal of new biological categories of classification. *Gastric Cancer* 2016; **19**: 329-338 [PMID: 26643880 DOI: 10.1007/s10120-015-0575-z]
  - 14 **Shirao K**, Boku N, Yamada Y, Yamaguchi K, Doi T, Goto M, Nasu J, Denda T, Hamamoto Y, Takashima A, Fukuda H, Ohtsu A; Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group. Randomized Phase III study of 5-fluorouracil continuous infusion vs. sequential methotrexate and 5-fluorouracil therapy in far advanced gastric cancer with peritoneal metastasis (JCOG0106). *Jpn J Clin Oncol* 2013; **43**: 972-980 [PMID: 24014884 DOI: 10.1093/jco/hyt114]
  - 15 **Muranaka T**, Yuki S, Komatsu Y, Sawada K, Harada K, Kawamoto Y, Nakatsumi H, Sakamoto N. Efficacy and Safety of Bolus 5-Fluorouracil and L-Leucovorin as Salvage Chemotherapy for Oral Fluoropyrimidine-Resistant Unresectable or Recurrent Gastric Cancer: A Single Center Experience. *J Gastric Cancer* 2016; **16**: 177-181 [PMID: 27752395 DOI: 10.5230/jgc.2016.16.3.177]
  - 16 **Nishimura T**, Iwasa S, Nagashima K, Okita N, Takashima A, Honma Y, Kato K, Hamaguchi T, Yamada Y, Shimada Y, Boku N. Irinotecan monotherapy as third-line treatment for advanced gastric cancer refractory to fluoropyrimidines, platinum, and taxanes. *Gastric Cancer* 2017; **20**: 655-662 [PMID: 27858180 DOI: 10.1007/s10120-016-0670-9]
  - 17 **Hashimoto K**, Mayahara H, Takashima A, Nakajima TE, Kato K, Hamaguchi T, Ito Y, Yamada Y, Kagami Y, Itami J, Shimada Y. Palliative radiation therapy for hemorrhage of unresectable gastric cancer: a single institute experience. *J Cancer Res Clin Oncol* 2009; **135**: 1117-1123 [PMID: 19205735 DOI: 10.1007/s00432-009-0553-0]
  - 18 **Asakura H**, Hashimoto T, Harada H, Mizumoto M, Furutani K, Hasuike N, Matsuoka M, Ono H, Boku N, Nishimura T. Palliative radiotherapy for bleeding from advanced gastric cancer: is a schedule of 30 Gy in 10 fractions adequate? *J Cancer Res Clin Oncol* 2011; **137**: 125-130 [PMID: 20336314 DOI: 10.1007/s00432-010-0866-z]
  - 19 **Odashima M**, Otake M, Nanjo H, Jin M, Horikawa Y, Matsuhashi T, Ohba R, Koizumi S, Kinoshita N, Takahashi T, Shima H, Watanabe S. Hamartomatous inverted polyp successfully treated by endoscopic submucosal dissection. *Intern Med* 2008; **47**: 259-262 [PMID: 18277026]
  - 20 **Isomoto H**, Shikuwa S, Yamaguchi N, Fukuda E, Ikeda K, Nishiyama H, Ohnita K, Mizuta Y, Shiozawa J, Kohno S. Endoscopic submucosal dissection for early gastric cancer: a large-scale feasibility study. *Gut* 2009; **58**: 331-336 [PMID: 19001058 DOI: 10.1136/gut.2008.165381]
  - 21 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229 [PMID: 11156645]
  - 22 **Abe N**, Gotoda T, Hirasawa T, Hoteya S, Ishido K, Ida Y, Imaeda H, Ishii E, Kokawa A, Kusano C, Maehata T, Ono S, Takeuchi H, Sugiyama M, Takahashi S. Multicenter study of the long-term outcomes of endoscopic submucosal dissection for early gastric cancer in patients 80 years of age or older. *Gastric Cancer* 2012; **15**: 70-75 [PMID: 21667133 DOI: 10.1007/s10120-011-0067-8]
  - 23 **Watanabe K**, Hikichi T, Nakamura J, Takagi T, Suzuki R, Sugimoto M, Wagarai Y, Kikuchi H, Konno N, Asama H, Takasumi M, Obara K, Ohira H. Endoscopic submucosal dissection for early gastric cancer in very elderly patients age 85 or older. *Endosc Int Open* 2017; **5**: E17-E24 [PMID: 28191493 DOI: 10.1055/s-0042-122960]

- 24 **Koizumi S**, Jin M, Matsuhashi T, Tawaraya S, Watanabe N, Sawaguchi M, Kanazawa N, Yamada Y, Onochi K, Kimura Y, Ohba R, Kataoka J, Hatakeyama N, Mashima H, Ohnishi H. Salvage endoscopic submucosal dissection for the esophagus-localized recurrence of esophageal squamous cell cancer after definitive chemoradiotherapy. *Gastrointest Endosc* 2014; **79**: 348-353 [PMID: [24125510](#) DOI: [10.1016/j.gie.2013.09.012](#)]
- 25 **Takeuchi M**, Kobayashi M, Hashimoto S, Mizuno K, Kawaguchi G, Sasamoto R, Aoyama H, Aoyagi Y. Salvage endoscopic submucosal dissection in patients with local failure after chemoradiotherapy for esophageal squamous cell carcinoma. *Scand J Gastroenterol* 2013; **48**: 1095-1101 [PMID: [23906093](#) DOI: [10.3109/00365521.2013.822092](#)]

**P- Reviewer:** Fogli L, Zhu YL, Kim JH

**S- Editor:** Dou Y **L- Editor:** Filipodia **E- Editor:** Tan WW



## Peritoneal cavernous hemangiomas: A case report

Li-Yuan Fu, Hong-Yu Chen, Xiao-Li Diao, Zhen-Jun Wang

**ORCID number:** Li-Yuan Fu (0000-0003-1560-6083); Hong-Yu Chen (0000-0002-3660-8523); Xiao-Li Diao (0000-0001-6885-9354); Zhen-Jun Wang (0000-0003-4905-1055).

**Author contributions:** Fu LY and Chen HY collected the patient's clinical data and wrote the paper; Diao XL provided pathological data; Wang ZJ provided guidance.

**Supported by** National High-Tech R and D Program of China (863 Program), No. 2015AA033602; and 1351 Personnel Training Program of Beijing Chao-yang Hospital Affiliated to Capital Medical University, No. CYXZ-2017-09.

**Informed consent statement:**

Consent was obtained from the patient for publication of this report and any accompanying images.

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

**CARE Checklist (2016) statement:**

The guidelines of the CARE Checklist (2016) have been adopted.

**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: <https://creativecommons.org/licenses/by-nc/4.0/>

**Li-Yuan Fu, Hong-Yu Chen, Zhen-Jun Wang,** Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

**Xiao-Li Diao,** Department of Pathology, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China

**Corresponding author:** Zhen-Jun Wang, MD, Doctor, Professor, Surgeon, Department of General Surgery, Beijing Chaoyang Hospital, Capital Medical University, Beijing 100020, China. [drzhenjun@163.com](mailto:drzhenjun@163.com)

**Telephone:** +86-10-85231604

### Abstract

#### BACKGROUND

Cavernous hemangiomas in the liver and spleen has been reported, but it occurs less commonly in the peritoneum. Here we report a case of peritoneal cavernous hemangiomas and share some valuable information about this disease.

#### CASE SUMMARY

A 57-year-old Chinese man had a huge abdominal mass with abdominal distention and a significant reduction of food consumption. An enhanced abdominal and pelvic computed tomography and positron emission tomography-computed tomography revealed multiple cystic masses on the peritoneum, greater omentum, small intestinal mesentery and the surface of the spleen, and a high maximum standardized uptake value of the largest cystic lesion. Exploratory laparotomy was performed, and multiple cystic masses were found on the surface of the peritoneum, greater omentum, mesentery of the small intestine, and surface of the liver and spleen. Dark red bloody cystic fluid was present in the cystic tumor. Pathological examination showed that in the stromal components, the irregular vascular wall was thin. The vessel lumen was interlinked, and the lumen was lined with flat endothelium. According to the intraoperative findings and pathologic results, the patient was diagnosed with peritoneal cavernous hemangiomas.

#### CONCLUSION

The possibility of peritoneal cavernous hemangiomas should be considered when multiple cystic masses are found in the abdominal cavity by preoperative examination.

**Key words:** Peritoneum; Hemangiomas; Cavernous hemangiomas; Cystic lesion; Case report



<http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Received:** September 21, 2018

**Peer-review started:** September 24, 2018

**First decision:** November 1, 2018

**Revised:** December 25, 2018

**Accepted:** December 29, 2018

**Article in press:** December 29, 2018

**Published online:** February 26, 2019

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Cavernous hemangiomatosis in the liver and spleen has been reported, but it rarely occurs diffusely in the abdominal cavity. In 2011, Ribback *et al* reported a case of nodular hemangiomatosis of pleura and peritoneum. We report here a case of peritoneal cavernous hemangiomatosis, along with its pathological type and positron emission tomography-computed tomography findings, for the first time. This case may help us to better understand this disease with regard to clinical manifestations and laboratory examination, imaging and pathologic results.

**Citation:** Fu LY, Chen HY, Diao XL, Wang ZJ. Peritoneal cavernous hemangiomatosis: A case report. *World J Clin Cases* 2019; 7(4): 489-493

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/489.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.489>

## INTRODUCTION

Hemangiomatosis can occur simultaneously in the thoracic and abdominal cavities<sup>[1]</sup>. Peritoneal cavernous hemangiomatosis occurs only in the abdominal cavity. Cystic lesions are widely distributed in the peritoneum, and dark red bloody cystic fluid is found in the lesions. The diagnosis of peritoneal cavernous hemangiomatosis mainly depends on pathological examination. Herein, we report a case of peritoneal cavernous hemangiomatosis and provide some valuable information about this disease.

## CASE PRESENTATION

### Chief complaints

A 57-year-old man was admitted to our hospital for evaluation of a huge abdominal mass causing abdominal distension and a significant reduction of food consumption for nearly 2 mo.

### History of past illness

His past history was unremarkable.

### Personal and family history

His family history was unremarkable.

### Physical examination upon admission

Physical examination revealed a huge, tough mass. The upper boundary of the mass was below the right costal margin. The lower boundary, which was in the pelvic cavity, was hard to touch. The surface of the mass was smooth. The mass was unmovable, and there was no tenderness associated with the mass.

### Laboratory examinations

Routine blood parameters were within the normal range. Tumor marker measurement results were as follows: Carcinoembryonic antigen, 0.00 ng/mL; alpha-fetoprotein, 4.10 ng/mL; CA19-9, 9.80 U/mL; and CA72-4, 1.19 U/mL. An enhanced abdominal and pelvic computed tomography scan revealed a right mid-lower abdominal cystic lesion of 35.6 cm × 14.3 cm × 9.6 cm compressing the intestine and right ureter. Multiple small cystic masses appeared on the peritoneum, greater omentum, small intestinal mesentery, and the surface of the spleen (Figure 1A). Perihepatic effusion was also present. There was a huge mass in the abdominal cavity and the gastrointestinal tract structure was displaced. In order to exclude the extensive metastasis of gastrointestinal tumors, the patient underwent positron emission tomography-computed tomography. The examination revealed a high maximum standardized uptake value (SUVmax) of the largest cystic lesion. The SUVmax of the lesion was 2.7. Mild radioactivity uptake was seen in the multiple cystic lesions on the peritoneum, omentum, small bowel mesentery, and surface of the liver and spleen. Possible preoperative diagnosis was peritoneal metastasis of appendix mucinous tumor.



**Figure 1 Clinical, pathological and immunohistochemical results.** A: The white arrows indicate the abdominal masses; B: The inner cystic wall resected during the operation; C: CD31 staining was positive for the endothelial cells. Magnification  $\times 100$ .

Explorative laparotomy revealed multiple dark red masses on the small intestinal mesentery, peritoneum, and greater omentum, and surface of the liver and spleen. The diameter of the masses ranged from 2 cm to 5 cm. Dark red bloody cystic fluid was seen when the thin wall was incised. A huge soft tumor was found in the right abdominal cavity. The huge cystic lesion was tightly adhered to the small intestine and ascending colon, making it difficult to be separated from the organs. Dark red bloody cystic fluid was present in the mass. An incisional biopsy of the cystic wall and some other smaller cystic lesions was obtained (Figure 1B). Intraoperative frozen section showed that the inner cystic wall was lined with flat endothelium without atypia. However, the high SUVmax and the wide distribution of the masses indicated the possibility of malignancy. Although the sensitivity and specificity of intraoperative frozen pathological examination was very high, we used treatments for malignant tumors considering that the malignant possibility could not be completely excluded after a consultation with the pathologist. We then performed complete aspiration of the seroperitoneum and successively embrocated iodine and carbolic acid on the inner wall of the largest mass. The patient underwent hyperthermic perfusion with 4000 mL normal saline at 43 °C for 1 hr immediately after surgery.

Pathological examination showed that within the stromal components, the irregular vascular wall was thin. The vessel lumen was interlinked, and the lumen was lined with flat endothelium without heterogeneity. Hemorrhage and hemosiderin were present in the stromal components. Immunohistochemical staining of the endothelial cells showed positivity for CD31 and ERG and negativity for D2-40 (Figure 1C). The Ki-67 value was 2%. The diagnosis was peritoneal cavernous hemangiomatosis according to the intraoperative findings and pathologic results.

## FINAL DIAGNOSIS

According to the pathological examination, the final diagnosis was peritoneal cavernous hemangiomatosis.

## TREATMENT

Explorative laparotomy was performed because of multiple abdominal masses causing abdominal distension and the diagnosis was not clear. After surgery, the patient underwent hyperthermic perfusion immediately because the possibility of a malignant disease cannot be completely excluded.

## OUTCOME AND FOLLOW-UP

The patient returned to Shanxi Province after operation and did not go to our hospital for examination. He underwent an abdominal ultrasound examination in a local hospital 2 wk previously. The result showed that multiple cystic masses still existed in the abdominal cavity, but the patient had no discomfort.

## DISCUSSION

Here, we report a case of peritoneal cavernous hemangiomatosis. Cavernous hemangiomatosis can occur in the parenchymal viscera such as liver<sup>[2]</sup> or spleen<sup>[3]</sup>. In 2011, Ribback *et al*<sup>[1]</sup> reported a case of nodular hemangiomatosis of the pleura and peritoneum; however, the advanced pathological type of the hemangiomatosis was not described.

According to the pathology and immunohistochemistry results, the lesion in the present case was composed of vascular components without lymphatic components. Therefore, we excluded the diagnosis of lymphangioma<sup>[4]</sup> and other lymphangiogenic tumors. We also excluded the diagnosis of epithelioid hemangioma since the vascular lumen was lined with flat endothelium without lymphocytes, eosinophils, or the formation of lymphoid follicles, and imaging examination did not show any abnormal soft tissue mass and bone destruction<sup>[5,6]</sup>. Platelet count was not decreased in this patient and no spindle tumor cells or fissured lacuna were found in pathological examination, allowing us to further exclude the diagnosis of kaposiform hemangioendothelioma<sup>[7,8]</sup>. The diagnosis of multicystic mesothelioma was also excluded as the mass contained dark red cystic fluid and the inner cystic wall was lined with flat endothelial cells instead of cubic mesothelial cells<sup>[9]</sup>. Pathological examination showed that the lumen was lined with flat endothelium without heterogeneity, and immunohistochemical examination showed that the Ki-67 value of the endothelial cells was 2%. We excluded the possibility of malignant tumors. The cystic lesions were diffusely located on the surface of the greater omentum, peritoneum, mesentery of the small intestine, and organs including the liver and spleen. No invasion of the intestinal wall or parenchyma of the liver and spleen occurred. Therefore, the final diagnosis was peritoneal cavernous hemangiomatosis.

Although a huge cystic lesion was located in the abdominal cavity, the platelet count and coagulation function tests were within the reference ranges. Thus, Kasabach-Merritt syndrome was excluded<sup>[10]</sup>. We also excluded Maffucci's syndrome because no deformity or activity limitation of the extremities was found<sup>[11]</sup>. Finally, we excluded Klippel-Trenaunay syndrome because the patient had no nevus vascularis, lower extremity deformity, or superficial varicosities<sup>[12]</sup>.

No clear treatments for peritoneal hemangiomatosis have been established. We treated the patient with hyperthermic perfusion because of the malignant clinical manifestation of the tumor. However, the patient developed palpitation after the hyperthermic perfusion. Consequently, we stopped the hyperthermic perfusion treatment. Propranolol can be used to treat gastrointestinal hemangiomatosis<sup>[13]</sup> and hepatic hemangiomatosis<sup>[14]</sup>. We believe that propranolol may be an effective agent to treat the disease such as that described in the present case.

## CONCLUSION

The possibility of peritoneal cavernous hemangiomatosis should be considered when multiple cystic masses are found in the abdominal cavity by preoperative examination.

## REFERENCES

- 1 Ribback S, Thiele A, Rosenberg C, Friesecke S, Neumann V, Tannapfel A, Dombrowski F. Nodular hemangiomatosis of pleura and peritoneum. *Pathol Res Pract* 2011; **207**: 718-721 [PMID: 21978481 DOI: 10.1016/j.prp.2011.08.006]
- 2 Guerra A, Infante A, Rinninella E, Spinelli I, Mazziotti MA, De Gaetano AM, Pompili M, Bonomo L. A peculiar case of diffuse hemangiomatosis of the left hepatic lobe in an asymptomatic adult patient: case report and literature review. *Eur Rev Med Pharmacol Sci* 2017; **21**: 1593-1597 [PMID: 28429345]
- 3 Steininger H, Pfofe D, Marquardt L, Sauer H, Markwat R. Isolated diffuse hemangiomatosis of the spleen: case report and review of literature. *Pathol Res Pract* 2004; **200**: 479-485 [PMID: 15310152 DOI: 10.1016/j.prp.2004.04.004]
- 4 Takeda A, Ito H, Nakamura H. Large Omental Cystic Lymphangioma Masquerading as Mucinous Ovarian Neoplasia in an 8-Year-Old Premenarchal Girl: The Findings from Diagnostic Imaging and Laparoscopic-Assisted Excision. *J Pediatr Adolesc Gynecol* 2017; **30**: 659-662 [PMID: 28629796 DOI: 10.1016/j.jpag.2017.06.003]
- 5 Hejmadi RK, Gey van Pittius D, Stephens M, Chasty R, Braithwaite M. Angiolymphoid hyperplasia with eosinophilia (epithelioid haemangioma) occurring within multiple deep lymph nodes and presenting with weight loss and raised CA-125 levels. *Virchows Arch* 2006; **448**: 366-368 [PMID: 16315021 DOI: 10.1007/s00428-005-0119-8]
- 6 Weissferdt A, Moran CA. Epithelioid hemangioendothelioma of the bone: a review and update. *Adv Anat Pathol* 2014; **21**: 254-259 [PMID: 24911250 DOI: 10.1097/PAP.0000000000000027]
- 7 Putra J, Gupta A. Kaposiform haemangioendothelioma: a review with emphasis on histological

- differential diagnosis. *Pathology* 2017; **49**: 356-362 [PMID: 28438388 DOI: 10.1016/j.pathol.2017.03.001]
- 8 **Cashell J**, Smink GM, Helm K, Xavier F. Kaposiform hemangioendothelioma with Kasabach-Merritt phenomenon in an infant: Successful treatment with prednisolone, vincristine, and addition of sirolimus. *Pediatr Blood Cancer* 2018; **65**: e27305 [PMID: 30070028 DOI: 10.1002/pbc.27305]
  - 9 **Nizri E**, Baratti D, Guaglio M, Sinukumar S, Cabras A, Kusamura S, Deraco M. Multicystic mesothelioma: Operative and long-term outcomes with cytoreductive surgery and hyperthermic intra peritoneal chemotherapy. *Eur J Surg Oncol* 2018; **44**: 1100-1104 [PMID: 29703622 DOI: 10.1016/j.ejso.2018.03.004]
  - 10 **Shimizu Y**, Komura T, Seike T, Omura H, Kumai T, Kagaya T, Ohta H, Kawashima A, Harada K, Kaneko S, Unoura M. A case of an elderly female with diffuse hepatic hemangiomatosis complicated with multiple organic dysfunction and Kasabach-Merritt syndrome. *Clin J Gastroenterol* 2018; **11**: 411-416 [PMID: 29845554 DOI: 10.1007/s12328-018-0871-3]
  - 11 **Fanburg JC**, Meis-Kindblom JM, Rosenberg AE. Multiple enchondromas associated with spindle-cell hemangioendotheliomas. An overlooked variant of Maffucci's syndrome. *Am J Surg Pathol* 1995; **19**: 1029-1038 [PMID: 7661276 DOI: 10.1097/0000478-199509000-00006]
  - 12 **Wang ZK**, Wang FY, Zhu RM, Liu J. Klippel-Trenaunay syndrome with gastrointestinal bleeding, splenic hemangiomas and left inferior vena cava. *World J Gastroenterol* 2010; **16**: 1548-1552 [PMID: 20333801 DOI: 10.3748/wjg.v16.i12.1548]
  - 13 **Akcam M**, Pirgon O, Salman H, Kockar C. Multiple gastrointestinal hemangiomatosis successfully treated with propranolol. *J Pediatr Gastroenterol Nutr* 2015; **60**: e16 [PMID: 23575304 DOI: 10.1097/MPG.0b013e31829530af]
  - 14 **Bosemani T**, Puttgen KB, Huisman TA, Tekes A. Multifocal infantile hepatic hemangiomas--imaging strategy and response to treatment after propranolol and steroids including review of the literature. *Eur J Pediatr* 2012; **171**: 1023-1028 [PMID: 22234480 DOI: 10.1007/s00431-011-1671-7]

**P- Reviewer:** Abdel-Hamid SMM, Chatzizacharias N, Hashimoto R, Kocazeybek B

**S- Editor:** Ji FF **L- Editor:** Filipodia **E- Editor:** Tan WW



## Recurrent acute liver failure associated with novel *SCYL1* mutation: A case report

Jia-Qi Li, Jing-Yu Gong, A S Knisely, Mei-Hong Zhang, Jian-She Wang

**ORCID number:** Jia-Qi Li (0000-0002-3150-3212); Jing-Yu Gong (0000-0002-1149-0979); A S Knisely (0000-0002-9173-539X); Mei-Hong Zhang (0000-0001-9656-7180); Jian-She Wang (0000-0003-0823-586X).

**Author contributions:** Wang JS designed the report and managed the patient; Li JQ was responsible for whole-exome sequencing data analysis and interpretation of sequence variants, validation by Sanger sequencing, and drafting the manuscript; Gong JY and Zhang MH were involved in the acquisition, analysis, and interpretation of clinical data; Knisely AS was responsible for the interpretation of histopathologic data and for manuscript editing; all authors have reviewed the manuscript and approved the final version to be submitted.

**Supported by** the National Natural Science Foundation of China, No. 81570468.

**Informed consent statement:** Informed consent was obtained from the parents of the patient.

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared according to those guidelines.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

**Jia-Qi Li, Jing-Yu Gong, Mei-Hong Zhang,** Department of Pediatrics, Jinshan Hospital of Fudan University, Shanghai 201508, China

**A S Knisely,** Institut für Pathologie, Medizinische Universität Graz, Neue Stiftingtalstraße 6, Graz 8010, Austria

**Jian-She Wang,** The Center for Pediatric Liver Diseases, Children's Hospital of Fudan University, Shanghai 201102, China

**Corresponding author:** Jian-She Wang, PhD, Professor, The Center for Pediatric Liver Diseases, Children's Hospital of Fudan University, 399 Wanyuan Road, Minhang District, Shanghai 201102, China. [jshwang@shmu.edu.cn](mailto:jshwang@shmu.edu.cn)  
**Telephone:** +86-21-64931171  
**Fax:** +86-21-61143167

### Abstract

#### BACKGROUND

Pediatric recurrent acute liver failure (RALF) with recovery between episodes is rare. Causes include autoimmune disease, which may flare and subside; intermittent exposure to toxins, as with ingestions; and metabolic disorders, among them the fever-associated crises ascribed to biallelic mutations in *SCYL1*, with RALF beginning in infancy. *SCYL1* disease manifest with RALF, as known to date, includes central and peripheral neurologic and muscular morbidity (hepatocerebellar neuropathy syndrome). Primary ventilatory and skeletal diseases also have been noted in some reports.

#### CASE SUMMARY

We describe a Han Chinese boy in whom fever-associated RALF began at age 14 mo. Bilateral femoral head abnormalities and mild impairment of neurologic function were first noted aged 8 years 6 mo. Liver biopsy after the third RALF episode (7 years) and during resolution of the fourth RALF episode (8 years 6 mo) found abnormal architecture and hepatic fibrosis, respectively. Whole-exome sequencing revealed homozygosity for the novel frameshift mutation c.92\_93insGGGCCCT, p.(H32Gfs\*20) in *SCYL1* (parental heterozygosity confirmed).

#### CONCLUSION

Our findings expand the mutational and clinical spectrum of *SCYL1* disease. In our patient a substantial neurologic component was lacking and skeletal disease was identified relatively late.



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

**Received:** November 20, 2018

**Peer-review started:** November 20, 2018

**First decision:** December 20, 2018

**Revised:** January 16, 2019

**Accepted:** January 29, 2019

**Article in press:** January 30, 2019

**Published online:** February 26, 2019

**Key words:** SCYL1; Recurrent acute liver failure; Whole-exome sequencing; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** An infant or child with recurrent acute liver failure may have biallelic mutation in SCYL1. A search for causes should include evaluation of this gene even if neurologic or skeletal disease is not appreciated.

**Citation:** Li JQ, Gong JY, Knisely AS, Zhang MH, Wang JS. Recurrent acute liver failure associated with novel SCYL1 mutation: A case report. *World J Clin Cases* 2019; 7(4): 494-499

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/494.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.494>

## INTRODUCTION

An unusual form of acute liver failure (ALF) occurs in children. Starting in infancy, episodes of hepatic insufficiency recur. These episodes may be preceded by fever (and thus be ascribed to antipyretics, in particular paracetamol)<sup>[1-3]</sup>. They may not be restricted to childhood<sup>[4,5]</sup> and may be fatal<sup>[6]</sup>. They are characterized by clinical-laboratory findings that include marked elevations in serum transaminase activity, severe coagulopathy, conjugated hyperbilirubinemia, and normal or only slightly elevated serum gamma-glutamyl transpeptidase activity<sup>[2,4]</sup>. Hepatic crises, with less severe injury, also are seen<sup>[1-5]</sup>. The causes of recurrent ALF (RALF), as this disorder is termed, are incompletely understood, although instances have been ascribed to biallelic mutation in SCYL1 (MIM: 607982)<sup>[7]</sup> or NBAS (MIM: 608025)<sup>[4]</sup>. NBAS disease is multisystem in some patients<sup>[3,6,8]</sup> and manifest only as RALF in others<sup>[2,4]</sup>. The few patients yet reported with SCYL1 disease have exhibited isolated RALF in conjunction with a range of mild to more marked neurological phenotypes, including spinocerebellar ataxia and neurodegeneration<sup>[7,9-11]</sup>. In one, recurrent ventilatory failure was clinically prominent; RALF was not described<sup>[12]</sup>.

We report a novel homozygous mutation in SCYL1 in a Han Chinese boy who had RALF with onset in infancy and in whom, aged 8 years 6 mo, mild neurologic dysfunction and bilateral femoral head abnormalities were identified. Our observations expand the clinical and mutational spectrum of SCYL1 disease.

## CASE PRESENTATION

### Chief complaints

A 7-year-old boy was admitted to our hospital due to recurrent episodes of ALF.

### History of present illness

The patient was well till age 14 mo, when a fever was treated with the non-steroidal anti-inflammatory agent nimesulide. Jaundice developed, with elevated transaminase activity. Injury fell short of frank ALF, constituting instead a "liver crisis". Two episodes of ALF followed at ages of 3 years 6 mo and 6 years 3 mo (Table 1). Clinical, laboratory, and imaging-study findings between episodes were normal. At the age of 7 years, shortly after his third RALF episode, he was referred to our hospital for etiological diagnosis.

### History of past illness

His parents and he denied any history of disease beyond that summarized above.

### Personal and family history

He was born at term (weight 3 kg) by elective cesarean section after an uncomplicated pregnancy. He is the second child of a Han Chinese couple who deny consanguinity. His sibling, a brother, is well.

### Physical examination upon admission

Physical examination on admission found no abnormalities.

**Table 1** Clinical phenotype in three fever-associated acute liver failure episodes

	Age 3 y 6 mo	Age 6 y 3 mo	Age 8 y 5mo	Range
Febrile illness before ALF	Yes	Yes	Yes	
Using antipyretics before ALF	Yes	No	Yes	
Duration	> 4 mo	> 1 mo	> 1 mo	
Max TB (μmol/L)	504.0	305.7	554.0	5.1-17.1
Max DB (μmol/L)	288.0	125.0	264.7	0-6.0
Max ALT (IU/L)	414	1865	881	0-40
Max AST (IU/L)	2050	4900	1648	15-60
Max GGT (IU/L)	117	39	70	7-50
Max PT (s)	24.9	29.5	26.7	11.0-14.5
Hepatomegaly	Yes	Yes	Yes	
Splenomegaly	No	No	Yes	
Additional features	Antipyretic- induced hepatopathy diagnosed	Multi-organ involvement <sup>1</sup>	Multi-organ involvement <sup>2</sup>	

Multi-organ involvement<sup>1</sup>: Acute liver failure, severe acute pancreatitis, bilateral pneumonia, alimentary tract hemorrhage, and septic shock; Multi-organ involvement<sup>2</sup>: Acute liver failure, bronchopneumonia, moderate anemia, ventricular premature beats, hypokalemia, hyponatremia, and bilateral femoral abnormalities. ALF: Acute liver failure; TB: Total bilirubin; DB: Direct bilirubin; ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: gamma-glutamyl transpeptidase; PT: Prothrombin time; Max: Maximum.

### Laboratory examinations

Blood, urine, and stool routine test results, viral serological markers (hepatotropic viruses, Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus 1), biomarkers of toxoplasma infection, of thyroid function, and of hepatobiliary injury, and values for cholesterol, triglycerides, creatine phosphokinase, glucose, ceruloplasmin, blood lactate, blood ammonia, carbonyldiamide, creatinine, uric acid, urine organic acids, alpha-fetoprotein, and immunoglobulin levels were unremarkable. The value of 25-(OH)D<sub>3</sub> was 13.69 ng/mL (normal: 30-100 ng/mL).

### Imaging examinations

Cranial magnetic resonance imaging showed a cyst (90 mm × 30 mm) in the cisterna magna and a relatively small cerebellum. The optic nerves were normal. Abdominal ultrasonography and echocardiography showed no significant abnormalities.

### Other examinations

Findings on ophthalmologic evaluation were unremarkable. Liver biopsy found mildly abnormal architecture, without fibrosis, in a pattern suggesting disordered perfusion; no ultrastructural abnormalities were apparent. The Denver developmental screening test, which covers gross motor, language, fine motor-adaptive, and personal-social skills<sup>[13]</sup>, found a low developmental quotient (< 47, normal > 70) and mental index (63, normal > 70).

### Whole-exome sequencing

With the approval of the ethics committee of the Jinshan Hospital of Fudan University (Jinyi Lunli Keyan-2014-13-01) and after obtaining written informed consent from the parents of the patient, DNA was isolated from peripheral blood samples obtained from the proband, his brother, and his parents. Whole-exome sequencing was conducted as described<sup>[2]</sup> (Genesky Biotechnologies, Shanghai, China). Exomes were captured using a SureSelectXT Human All Exon V6 kit (Agilent Technologies, Foster, CA). Sequencing (250 bp paired-end reads) was performed using the Illumina HiSeq2500 platform following the manufacturer's instructions (Illumina, San Diego, CA). Burrows-Wheeler Aligner software (<http://bio-bwa.sourceforge.net/>) was used for read alignment (hg19; NCBI build 37; February, 2009). Variant calling was performed with Varscan (<http://varscan.sourceforge.net/>) and GATK Haplotype-Caller (<https://software.broadinstitute.org/gatk/best-practices/>). Mean coverage of the exome was 140× with 91% of the exome covered at least 10 times and 80% covered at greater than 20×. The proband's variants were filtered for minor allele frequency < 1% against Thousand Genomes Project (<http://www.1000genomes.org/home>), NHLBI Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), Exome Aggregation Consortium Server (<http://exac.broadinstitute.org/>), and Genesky in-house databases. Variants in ALF-associated genes (Supplementary file 1) assessed by SIFT (<http://sift.jcvi.org/>), Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2/>), or MutationTaster<sup>[14]</sup> (<http://www.mutationtaster.org/>) as pathogenic and present in

accord with inheritance modes were considered of interest. Sanger sequencing was used to confirm variants in the proband and to seek them in his parents and brother.

### Identification of a novel homozygous mutation in exon 1 in SCYL1

The homozygous mutation c.92\_93insGGGCCCT, p.(H32Gfs\*20) in exon 1 in SCYL1 (NM\_020680) was identified in the proband. His brother did not harbor this mutation. Each of their parents was heterozygous for the mutation, consistent with recessive inheritance. The mutation detected by exome sequencing was again identified on Sanger sequencing (Supplementary file 2). No other variant of interest was identified in any ALF-associated gene. The insertion of GGGCCCT between nucleotides 92 and 93 within exon 1 is predicted to result in the substitution of 19 abnormal amino acid residues (His32-Val50), followed by a stop codon at codon 51. MutationTaster predicts that this results in nonsense-mediated mRNA decay<sup>[14]</sup>. The variant is novel; it was not recorded in Exome Aggregation Consortium Server, NHLBI Exome Sequencing Project, Thousand Genomes Project, or Genesky in-house databases.

## FINAL DIAGNOSIS

SCYL1 disease.

## TREATMENT

Supportive care during episodes of ALF or crises followed accepted guidelines and was not specific for any particular form of hepatic dysfunction. They included antibiotics to resist bacterial infection, "liver protectant drugs" (D-glucurono-3,6-lactone, diammonium glycyrrhizinate, polyene phosphatidylcholine, *etc.*), cholagogues and choleretics (ursodeoxycholic acid, cholestyramine, *etc.*), prednisone, and supplementary fat-soluble vitamins A, D, E, and K.

## OUTCOME AND FOLLOW-UP

The patient had his fourth episode of RALF at 8 years 5 mo (Table 1). During resolution of this episode, liver biopsy was conducted again. Alanine transaminase and aspartate aminotransferase values 2 d before liver biopsy were 91 IU/L (expected: 0-40 IU/L) and 476 IU/L (normal: 15-60 IU/L). Ballooning change of hepatocytes, some with cytoplasmic bile pigment, and hepatic fibrosis were observed. A comprehensive skeletal survey by radionuclide bone scan showed bilateral hip joint widening and bilateral femoral head flattening highly suggestive of bilateral femoral head necrosis. The patient failed finger-to-nose testing and Romberg testing, indicating cerebellar ataxia. However, muscle strength, muscular tension, stretch reflexes, deep sensory perception, and motor function were normal. At last evaluation, aged 8 years 7 mo, biomarker values in the proband were normal.

## DISCUSSION

SCYL1 encodes a kinase-like protein in the SCY1-like family<sup>[15,16]</sup>. SCYL1 may function as a scaffolding protein, involved in Golgi-to-endoplasmic reticulum retrograde transport and in Golgi integrity<sup>[17-19]</sup>. SCYL1 also acts in nucleocytoplasmic shuttling of tRNAs<sup>[20]</sup>. Biallelic mutations in SCYL1 were first identified in 2015 as an important cause of a hepatocerebellar neuropathy syndrome (spinocerebellar ataxia, autosomal recessive 21; MIM:616719)<sup>[7]</sup>.

To date, only 5 reports have described 14 patients harboring homozygous or compound heterozygous mutations in SCYL1<sup>[7,9-12]</sup>. Thirteen patients had similar hepatic features<sup>[7,9-11]</sup>; in the 14<sup>th</sup>, features of liver disease were present, but recurrent ventilatory failure dominated clinical illness<sup>[12]</sup>. Neurologic phenotypes varied, including peripheral neuropathy, ataxia, tremor, stuttering, speech-development delay, intellectual disability, microcephaly, and abnormal intracranial magnetic-resonance imaging findings. Six SCYL1-deficient patients had cerebellar atrophy and 2 siblings had optic nerve thinning<sup>[7,10,12]</sup>.

Our patient had a classic hepatic phenotype, with one episode of liver crisis (aged 14 mo) and 3 episodes of acute liver failure (aged 3 years 6 mo, 6 years 3 mo, and 8 years 5 mo). Each episode was preceded by a febrile illness. Hepatobiliary-injury biomarker values recovered completely between episodes. Not all episodes of febrile

illness led to hepatic crisis. Liver biopsy conducted after the third RALF episode (7 years) and during resolution of the fourth RALF episode (8 years 6 mo) revealed slightly abnormal architecture and hepatic fibrosis, respectively.

In our patient, relative cerebellar hypoplasia may be secondary to the cyst in the cisterna magna; vermis atrophy was not identified, and optic nerves were not thinned. Clinical neurologic features are at this writing minimal, with low developmental quotient and mental index, minor intracranial abnormalities, and abnormal coordination tests. These may worsen as the patient ages, or other abnormalities may yet declare themselves. Neurologic abnormalities usually are noted in *SCYL1* disease after liver disease has appeared (Supplementary file 3). To evaluate *SCYL1* in infants or children with RALF thus is indicated even if neurologic disease is inapparent. Long-term follow-up is necessary for best correlation between mutation and phenotypic consequences.

Worth noting is that skeletal abnormalities were observed in 8 of 14 *SCYL1*-deficiency patients. Skeletal phenotypes varied, including short stature, hip dysplasia, coronal cleaving of ribs, scoliosis, lumbar lordosis, and thoracic vertebral abnormalities<sup>[9-11]</sup>. Our patient had bilateral femoral head disease, not reported before in association with *SCYL1* mutation. At least some of the skeletal abnormalities described appear primary rather than resulting from neuromuscular dysfunction, suggesting that such abnormalities may be an important clinical manifestation of *SCYL1* disease. Systemic skeletal examinations thus may be essential for children with *SCYL1* deficiency.

Early identification of the cause of ALF is fundamental for disease-specific management. In our patient, assignment of diagnosis to mutation in a specific gene shifted the context in which we viewed his findings on examination, changing our expectations for the course of what now can be considered as a multisystem disorder. For instance, during the fourth RALF episode, after genetic diagnosis, a comprehensive skeletal survey and more detailed neurologic examination were performed. Our care has become better focused and, with the progress of the disease, may perhaps permit orthopedic, physiatric, and neurologic intervention for directed support. In addition, genetic diagnosis can also help in prenatal screening.

Our patient is the first East Asian described with *SCYL1*-associated RALF. The *SCYL1* mutation c.92\_93insGGGCCCT, p.(H32Gfs\*20), which he harbors in homozygous state, is predicted to result in nonsense-mediated decay of a prematurely terminated RNA transcript. Although we lack direct evidence for lack of *SCYL1* expression in our patient, we thus consider him the 15<sup>th</sup> identified patient with RALF due to *SCYL1* disease. We call attention to his clinical phenotype, with scant neurologic dysfunction identified thus far and relatively late-onset skeletal disease, although our findings are – without life-long follow-up – necessarily incomplete. We suggest that even if neurologic features of disease are few (“atypical” *SCYL1* disease), *SCYL1* defects should be considered as possibly underlying early-onset fever-related RALF. We also note as deserving further study the association between skeletal abnormalities and *SCYL1* disease.

## CONCLUSION

Even if neurologic features of disease are few, *SCYL1* defects should be considered as possibly underlying early-onset fever-related RALF. Systemic skeletal examinations are in order for children with *SCYL1* deficiency.

## ACKNOWLEDGEMENTS

The authors are grateful for the support of the patient’s family and thank referring physicians, nurses, and technical staff.

## REFERENCES

- 1 Calvo PL, Tandoi F, Haak TB, Brunati A, Pinon M, Olio DD, Romagnoli R, Spada M. NBAS mutations cause acute liver failure: when acetaminophen is not a culprit. *Ital J Pediatr* 2017; **43**: 88 [PMID: 28946922 DOI: 10.1186/s13052-017-0406-4]
- 2 Li JQ, Qiu YL, Gong JY, Dou LM, Lu Y, Knisely AS, Zhang MH, Luan WS, Wang JS. Novel NBAS mutations and fever-related recurrent acute liver failure in Chinese children: a retrospective study. *BMC Gastroenterol* 2017; **17**: 77 [PMID: 28629372 DOI: 10.1186/s12876-017-0636-3]
- 3 Stauffer C, Haack TB, Köpke MG, Straub BK, Kölker S, Thiel C, Freisinger P, Baric I, McKiernan PJ, Dikow N, Harting I, Beisse F, Burgard P, Kotzaeridou U, Lenz D, Kühn J, Himbert U, Taylor RW,

- Distelmaier F, Vockley J, Ghaloul-Gonzalez L, Ozolek JA, Zschocke J, Kuster A, Dick A, Das AM, Wieland T, Terrile C, Strom TM, Meitinger T, Prokisch H, Hoffmann GF. Recurrent acute liver failure due to NBAS deficiency: phenotypic spectrum, disease mechanisms, and therapeutic concepts. *J Inher Metab Dis* 2016; **39**: 3-16 [PMID: 26541327 DOI: 10.1007/s10545-015-9896-7]
- 4 **Haack TB**, Staufner C, Köpke MG, Straub BK, Kölker S, Thiel C, Freisinger P, Baric I, McKiernan PJ, Dikow N, Harting I, Beisse F, Burgard P, Kotzaeridou U, Kühr J, Himbert U, Taylor RW, Distelmaier F, Vockley J, Ghaloul-Gonzalez L, Zschocke J, Kremer LS, Graf E, Schwarzmayr T, Bader DM, Gagneur J, Wieland T, Terrile C, Strom TM, Meitinger T, Hoffmann GF, Prokisch H. Biallelic Mutations in NBAS Cause Recurrent Acute Liver Failure with Onset in Infancy. *Am J Hum Genet* 2015; **97**: 163-169 [PMID: 26073778 DOI: 10.1016/j.ajhg.2015.05.009]
- 5 **Casey JP**, McGettigan P, Lynam-Lennon N, McDermott M, Regan R, Conroy J, Bourke B, O'Sullivan J, Crushell E, Lynch S, Ennis S. Identification of a mutation in LARS as a novel cause of infantile hepatopathy. *Mol Genet Metab* 2012; **106**: 351-358 [PMID: 22607940 DOI: 10.1016/j.ymgme.2012.04.017]
- 6 **Capo-Chichi JM**, Mehawej C, Delague V, Caillaud C, Khneisser I, Hamdan FF, Michaud JL, Kibar Z, Mégarbané A. Neuroblastoma Amplified Sequence (NBAS) mutation in recurrent acute liver failure: Confirmatory report in a sibship with very early onset, osteoporosis and developmental delay. *Eur J Med Genet* 2015; **58**: 637-641 [PMID: 26578240 DOI: 10.1016/j.ejmg.2015.11.005]
- 7 **Schmidt WM**, Rutledge SL, Schüle R, Mayerhofer B, Züchner S, Boltshauser E, Bittner RE. Disruptive SCYL1 Mutations Underlie a Syndrome Characterized by Recurrent Episodes of Liver Failure, Peripheral Neuropathy, Cerebellar Atrophy, and Ataxia. *Am J Hum Genet* 2015; **97**: 855-861 [PMID: 26581903 DOI: 10.1016/j.ajhg.2015.10.011]
- 8 **Kortüm F**, Marquardt I, Alawi M, Korenke GC, Spranger S, Meinecke P, Kutsche K. Acute Liver Failure Meets SOPH Syndrome: A Case Report on an Intermediate Phenotype. *Pediatrics* 2017; **139**: e20160550 [PMID: 28031453 DOI: 10.1542/peds.2016-0550]
- 9 **Smith ED**, Radtke K, Rossi M, Shinde DN, Darabi S, El-Khechen D, Powis Z, Helbig K, Waller K, Grange DK, Tang S, Farwell Hagman KD. Classification of Genes: Standardized Clinical Validity Assessment of Gene-Disease Associations Aids Diagnostic Exome Analysis and Reclassifications. *Hum Mutat* 2017; **38**: 600-608 [PMID: 28106320 DOI: 10.1002/humu.23183]
- 10 **Lenz D**, McClean P, Kansu A, Bonnen PE, Ranucci G, Thiel C, Straub BK, Harting I, Alhaddad B, Dimitrov B, Kotzaeridou U, Wenning D, Iorio R, Himes RW, Kuloğlu Z, Blakely EL, Taylor RW, Meitinger T, Kölker S, Prokisch H, Hoffmann GF, Haack TB, Staufner C. SCYL1 variants cause a syndrome with low  $\gamma$ -glutamyl-transferase cholestasis, acute liver failure, and neurodegeneration (CALFAN). *Genet Med* 2018; **20**: 1255-1265 [PMID: 29419818 DOI: 10.1038/gim.2017.260]
- 11 **Shohet A**, Cohen L, Haguel D, Mozer Y, Shomron N, Tzur S, Bazak L, Basel Salmon L, Krause I. Variant in SCYL1 gene causes aberrant splicing in a family with cerebellar ataxia, recurrent episodes of liver failure, and growth retardation. *Eur J Hum Genet* 2019; **27**: 263-268 [PMID: 30258122 DOI: 10.1038/s41431-018-0268-2]
- 12 **Spagnoli C**, Frattini D, Salerno GG, Fusco C. On CALFAN syndrome: report of a patient with a novel variant in SCYL1 gene and recurrent respiratory failure. *Genet Med* 2018 [PMID: 30531813 DOI: 10.1038/s41436-018-0389-6]
- 13 **Frankenburg WK**. The Denver developmental screening test. *Dev Med Child Neurol* 1969; **11**: 260-262 [PMID: 5787726]
- 14 **Schwarz JM**, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; **11**: 361-362 [PMID: 24681721 DOI: 10.1038/nmeth.2890]
- 15 **Kato M**, Yano K, Morotomi-Yano K, Saito H, Miki Y. Identification and characterization of the human protein kinase-like gene NTKL: mitosis-specific centrosomal localization of an alternatively spliced isoform. *Genomics* 2002; **79**: 760-767 [PMID: 12036289 DOI: 10.1006/geno.2002.6774]
- 16 **Liu SC**, Lane WS, Lienhard GE. Cloning and preliminary characterization of a 105 kDa protein with an N-terminal kinase-like domain. *Biochim Biophys Acta* 2000; **1517**: 148-152 [PMID: 11118629 DOI: 10.1016/S0167-4781(00)00234-7]
- 17 **Hamlin JN**, Schroeder LK, Fotouhi M, Dokainish H, Ioannou MS, Girard M, Summerfeldt N, Melançon P, McPherson PS. Scyl1 scaffolds class II Arfs to specific subcomplexes of coatamer through the  $\gamma$ -COP appendage domain. *J Cell Sci* 2014; **127**: 1454-1463 [PMID: 24481816 DOI: 10.1242/jcs.136481]
- 18 **Burman JL**, Hamlin JN, McPherson PS. Scyl1 regulates Golgi morphology. *PLoS One* 2010; **5**: e9537 [PMID: 20209057 DOI: 10.1371/journal.pone.0009537]
- 19 **Burman JL**, Bourbonniere L, Philie J, Stroh T, Dejgaard SY, Presley JF, McPherson PS. Scyl1, mutated in a recessive form of spinocerebellar neurodegeneration, regulates COPI-mediated retrograde traffic. *J Biol Chem* 2008; **283**: 22774-22786 [PMID: 18556652 DOI: 10.1074/jbc.M801869200]
- 20 **Chafe SC**, Mangroo D. Scyl1 facilitates nuclear tRNA export in mammalian cells by acting at the nuclear pore complex. *Mol Biol Cell* 2010; **21**: 2483-2499 [PMID: 20505071 DOI: 10.1091/mbc.E10-03-0176]

**P- Reviewer:** Chatzigeorgiou A, Nacif LS, Zheng YW

**S- Editor:** Wang JL **L- Editor:** Wang TQ **E- Editor:** Tan WW





# Therapeutic plasma exchange and continuous renal replacement therapy for severe hyperthyroidism and multi-organ failure: A case report

Jun-Hui Ba, Ben-Quan Wu, Yan-Hong Wang, Yun-Feng Shi

**ORCID number:** Jun-Hui Ba (0000-0002-8537-2289); Ben-Quan Wu (0000-0003-0476-5118); Yan-Hong Wang (0000-0002-6561-2505); Yun-Feng Shi (0000-0003-0832-2832).

**Author contributions:** Ba JH and Wu BQ designed the report; Wang YH and Shi YF collected the patient's clinical data; Ba JH and Wu BQ analyzed the data and wrote the paper.

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

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**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/>

**Jun-Hui Ba, Ben-Quan Wu, Yan-Hong Wang, Yun-Feng Shi,** Department of Medical Intensive Unit, the Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China

**Corresponding author:** Ben-Quan Wu, MD, Professor, Director, Department of Medical Intensive Unit, the Third Affiliated Hospital of Sun Yat-Sen University, No. 600 Tian He Road, Guangzhou 510630, Guangdong Province, China. [zswbq@163.com](mailto:zswbq@163.com)

**Telephone:** +86-020-85253479

**Fax:** +86-020-85253479

## Abstract

### BACKGROUND

Severe hyperthyroidism is a life-threatening exacerbation of thyrotoxicosis, characterized by high fever and multiorgan failure. The most common medical treatments are administration of antithyroid drugs and radioactive iodine, and thyroidectomy. In some patients, antithyroid therapy is limited due to serious adverse effects or failure to control disease progression. In some extreme cases, such as thyroid storm, conventional therapy alone does not yield effective and rapid improvement before the development of multiorgan failure.

### CASE SUMMARY

This report describes a Chinese patient with severe hyperthyroidism accompanied by multiorgan failure, who was transferred to the medical intensive care unit of our hospital. The patient presented with palpitations, vomiting, diarrhea, and shortness of breath for a week. Laboratory tests showed elevation of thyroid hormones. Hepatic failure occurred with high aminotransferase levels and jaundice. Given her abnormal liver function and medication history, we could not exclude diagnosis of propylthiouracil-induced hepatic failure. Moreover, she also suffered from heart failure. Therapeutic plasma exchange (commonly known as TPE) and continuous renal replacement therapy (commonly known as CRRT) were used as life-saving therapy, which resulted in notable improvement of clinical symptoms and laboratory tests.

### CONCLUSION

Combined TPE and CRRT are safe and effective for patients with hyperthyroidism and multiorgan failure.

**Key words:** Severe hyperthyroidism; Propylthiouracil-induced hepatotoxicity; Multiorgan

<http://creativecommons.org/licenses/by-nc/4.0/>

**Manuscript source:** Unsolicited manuscript

**Received:** November 12, 2018

**Peer-review started:** November 13, 2018

**First decision:** November 27, 2018

**Revised:** December 21, 2018

**Accepted:** December 29, 2018

**Article in press:** December 30, 2018

**Published online:** February 26, 2019

failure; Therapeutic plasma exchange; Continuous renal replacement therapy; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Severe hyperthyroidism accompanied with multiple organ failure has been previously reported but is rare. In this case report, acute liver failure like our patient is a very unusual form of presentation. Considering the patient's medical history, propylthiouracil-induced hepatotoxicity could not be excluded. Therapeutic plasma exchange combined with continuous renal replacement therapy were performed, and successfully stabilized the patient. We suggest that the early application of blood purification technology is feasible in critically patients with severe hyperthyroidism.

**Citation:** Ba JH, Wu BQ, Wang YH, Shi YF. Therapeutic plasma exchange and continuous renal replacement therapy for severe hyperthyroidism and multi-organ failure: A case report. *World J Clin Cases* 2019; 7(4): 500-507

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/500.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.500>

## INTRODUCTION

Hyperthyroidism is characterized by elevated levels of thyroid hormones in the circulation<sup>[1,2]</sup>. The most common causes of hyperthyroidism are Graves' disease, multinodular toxic goiter, and autonomous hyperfunctioning thyroid nodules. Clinical manifestations of thyroid hyperfunction are usually mild or moderate. Severe hyperthyroidism is accompanied by more symptoms, particularly, asthenia followed by nervousness, dyspnea, and loss of weight. Severe hyperthyroidism is rare but life-threatening and can lead to irreversible multiorgan failure and high mortality, especially for older people or patients with cardiovascular disease<sup>[3]</sup>. Thus, a clinically euthyroid state should be achieved as soon as possible.

Traditional medical management has focused on supportive treatment and medication that halt the synthesis, release and peripheral effects of thyroid hormones<sup>[4]</sup>. However, these measures are limited because of adverse effects or failure to relieve the critical condition quickly. As therapeutic plasma exchange (TPE) is able to remove large amounts of serum protein-bound thyroid hormones from the circulation, plasmapheresis has been used as one of the effective alternative treatments since the 1970s<sup>[5]</sup>. Continuous renal replacement therapy (CRRT) can eliminate toxic substances and regulate water-electrolyte and acid-base balance. The combined use of blood purification technology can replace some metabolic functions, thereby effectively improving metabolic disorders.

We report herein a case of severe hyperthyroidism with multiorgan failure, for which antithyroid therapy was contraindicated, and TPE combined with CRRT successfully stabilized the condition of the patient.

## CASE PRESENTATION

### Chief complaints

Chest discomfort, palpitations, anorexia, and bloated legs for a week.

### History of present illness

About 1 year ago, the patient suffered from palpitations and hyperhidrosis. However, she paid no attention to these clinical signs and did not seek medical help. Seven days prior to presentation to a local hospital, she began to experience chest discomfort, palpitations, nausea, vomiting, abdominal pain with diarrhea, lack of energy, anorexia, and bloated legs. Upon admission, the levels of free thyroxine (FT4, > 100 pmol/L, normal range: 11.5-22.7 pmol/L) and free tri-iodothyronine (FT3, 26.33 pmol/L, normal range: 3.5-6.5 pmol/L) were significantly elevated, and the patient was diagnosed with hyperthyroidism. Blood test for liver function showed that alanine aminotransferase (ALT) was 65 U/L (normal range: 3-35 U/L) and aspartate aminotransferase (AST) was 60 U/L (normal range: 13-35 U/L). After taking a small dose of propylthiouracil (PTU, 200 mg), her symptoms worsened and liver function

deteriorated abruptly within 1 d, which was reflected in the level of ALT at 4597 U/L and AST at 7245 U/L (Table 1). Consequently, she was transferred to our hospital.

### **History of past illness**

The patient denied history of hypertension, diabetes mellitus, or exposure to viral hepatitis or tuberculosis. She also denied history of operation, trauma or blood transfusion. She had no known drug or food allergies.

### **Personal and family history**

**Personal history:** (1) Use for drugs: No medications were used daily. (2) Use of alcohol: No drinking. (3) Use of tobacco: No smoking.

**Family history:** (1) Health status or cause of death of parents, siblings and children: All are healthy. (2) Specific diseases related to problems identified in the chief complaint or history of the present illness and/or system review: None. (3) Diseases of family members which may be hereditary or place the patient at risk: No hereditary diseases.

### **Physical examination upon admission**

Temperature: 36.5 °C, Heart rate: 98 beats/min; Respiratory rate: 30 breaths/min; Blood pressure: 110/80 mmHg. Acutely ill-looking, showing agitation and dyspnea. Slightly jaundiced skin and sclerae. Ocular proptosis, bilateral thyroid gland swelling. Rales heard in bilateral lungs. Heart rate of 98 bpm, with normal rhythm. No extra or abnormal heart sound, murmurs or pericardium friction sound. Abdomen flat and soft, marked pitting edema in lower extremities.

### **Laboratory examinations**

**Liver function test results:** ALT 2066 U/L, AST 1229 U/L, total bilirubin 154.8 µmol/L (normal range: 4.0-23.9 µmol/L), direct bilirubin 94 µmol/L (normal range: 0-6.8 µmol/L).

**Thyroid function test results:** FT4 27.56 pmol/L, FT3 5.54 pmol/L, thyroid-stimulating hormone 0.01 µU/mL (normal range: 0.55-4.78 µU/mL). Thyrotrophin receptor antibody: Positive.

**Coagulation test results:** Prothrombin time of 39.3 s (normal range: 11.0-14.5 s), prothrombin activity 17% (normal range: 70%-120%).

**Brain natriuretic peptide:** 2219 pg/mL (normal range: < 100 pg/mL).

**Test for hepatitis virus A, B, C and E, Epstein-Barr virus, cytomegalovirus:** Negative. Immunological tests: Negative.

### **Imaging examinations**

**Ultrasound:** Diffuse enlargement of the thyroid, no cholestasis or extrahepatic obstruction.

**Chest radiography:** Left atrium enlargement (Figure 1).

**Echocardiography:** Left ventricular ejection fraction of 46% with a pericardial and estimated pulmonary arterial systole pressure of 36 mmHg.

## **FINAL DIAGNOSIS**

Burch and Wartofsky score was used to assess the probability of thyrotoxicosis; the patient's Burch and Wartofsky score was 40. According to clinical presentation and laboratory results, the diagnoses of GD, hyperthyroid heart disease, and multiorgan dysfunction was confirmed.

## **TREATMENT**

On the day of admission, we intended to initiate TPE; however, considering the patient's heart failure and severe edema, CRRT was administered with citrate anticoagulation. Continuous venovenous hemodiafiltration was performed and her blood flow rate was maintained at 150-200 mL/min based on target ultrafiltration. Replacement solutions were infused with post-displacement liquid at 1.5 L/h and dialysate liquid at 1.5 L/h. After treatment with CRRT, the vital signs began to

**Table 1** Laboratory results of the patient at a local hospital

Laboratory test	FT3 pmol/L	FT4 pmol/L	TSH $\mu$ U/L	ALT U/L	AST U/L	TB $\mu$ mol/L	DB $\mu$ mol/L
Normal range	3.5-6.5	11.5-22.7	0.55-4.78	3-35	13-35	4.0-23.9	0-6.8
Day 1	26.33	> 100	< 0.005	65	60	34.6	19.1
Day 2	-	55.8	0.072	4597	7254	113.5	70.2

FT3: Free tri-iodothyronine; FT4: Free thyroxine; TSH: Thyroid-stimulating hormone; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TB: Total bilirubin; DB: Direct bilirubin.

stabilize and the agitated mental status was relieved, but the serum bilirubin remained dangerously elevated, which was reflected in the level of total bilirubin at 333.8  $\mu$ mol/L. Therefore, TPE using 2 L fresh frozen plasma was performed in the following 4 d.

Following these procedures, the clinical signs were relieved almost completely. Chest radiography showed moderate improvement of cardiac enlargement (Figure 1). Upon further investigation, PTU-induced hepatic failure could not be excluded, so no antithyroid drugs were administered during this period. Liver biopsy was refused by the patient's parents. On the 5th day, when the laboratory test results were improved (Figures 2 and 3), the patient tried taking methimazole (MMI, 20 mg/d) on the recommendation of an endocrinologist. On the 10th day, her transaminase levels and coagulation parameters were close to normal limits. She was then transferred to the Endocrinology Department for follow-up care.

## OUTCOME AND FOLLOW-UP

The patient was discharged from our hospital after normal liver and thyroid function tests were obtained 1 mo later. Upon regular follow-up in the endocrinology clinic, her thyroid function status was normal and MMI was gradually reduced to 5 mg/d.

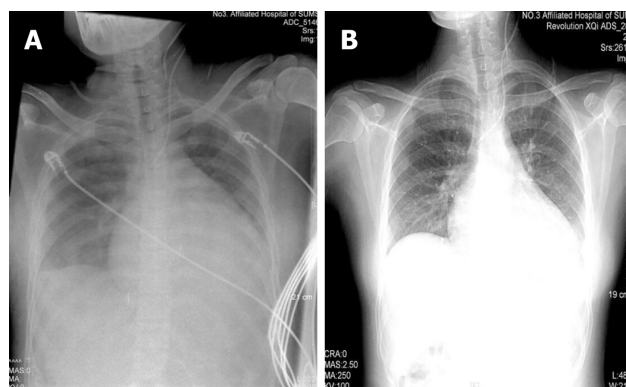
## DISCUSSION

Hyperthyroidism can induce abnormal liver function, especially in the setting of congestive heart failure or thyroid storm, since excessive thyroid hormone levels augment cardiac output without an increase in hepatic blood flow<sup>[6]</sup>, which leads to increased oxygen consumption and decreased liver perfusion that eventually lead to tissue hypoxia<sup>[7,8]</sup>. A previous report cited that mild derangements in liver function tests are common even in patients with subclinical hyperthyroidism but hepatic failure is rare<sup>[9]</sup>.

An unusual feature of our patient was that our patient's transaminases were slightly abnormal at the beginning. On commencing treatment with PTU for 1 d in the local hospital, her liver function deteriorated sharply. PTU was withdrawn and transaminases decreased but remained dangerously elevated. Other causes of hepatic injury, such as viral hepatitis, autoimmune disorders, alcohol consumption, and hepatotoxicity induced by other drugs were excluded. Acute liver failure in this patient was likely to be attributed to hepatocellular injury caused by insufficient oxygen supply as a result of decreased cardiac output. This was compounded by heart failure.

Furthermore, a possible relationship between liver dysfunction and PTU should be considered. However, hepatitis secondary to drug intake is difficult to diagnose. Hanson proposed the criteria to assist with diagnosis of drug-induced hepatitis. Only histological confirmation of hepatocellular injury and drug rechallenge could establish the diagnosis, but it is ethically unusual and only possible practically<sup>[10]</sup>. In our case, the liver biopsy sample was not obtained owing to her parents' refusal.

In fact, transaminases in most patients with thyroid storm are only mildly elevated. An elevation of transaminases with AST levels > 1000 U/L is uncommon<sup>[11]</sup>, and severe acute liver failure, as in the present patient, is an unusual presentation<sup>[12]</sup>. In most reported cases, liver failure occurs a few days or weeks after treatment by PTU<sup>[13]</sup>. Carhill *et al*<sup>[4]</sup> reported a 27-year-old patient with heart failure whose transaminases were elevated significantly after initiation of PTU. The PTU was replaced with MMI, and transaminases then decreased. Both Eisen and Lock reported severe hepatotoxicity after using 300 mg PTU for just 1 d<sup>[14,15]</sup>. PTU-related liver failure can occur at any time over the course of therapy. Unlike MMI, the adverse effects of



**Figure 1 Chest X-ray.** Comparison between the chest X-ray films at admission (left panel) and after treatment (right panel). A: chest X-ray films at admission; B: Chest X-ray films after treatment.

PTU are not dose related. When PTU-related liver failure occurs, the onset is sudden, and the course is rapidly progressive<sup>[16]</sup>.

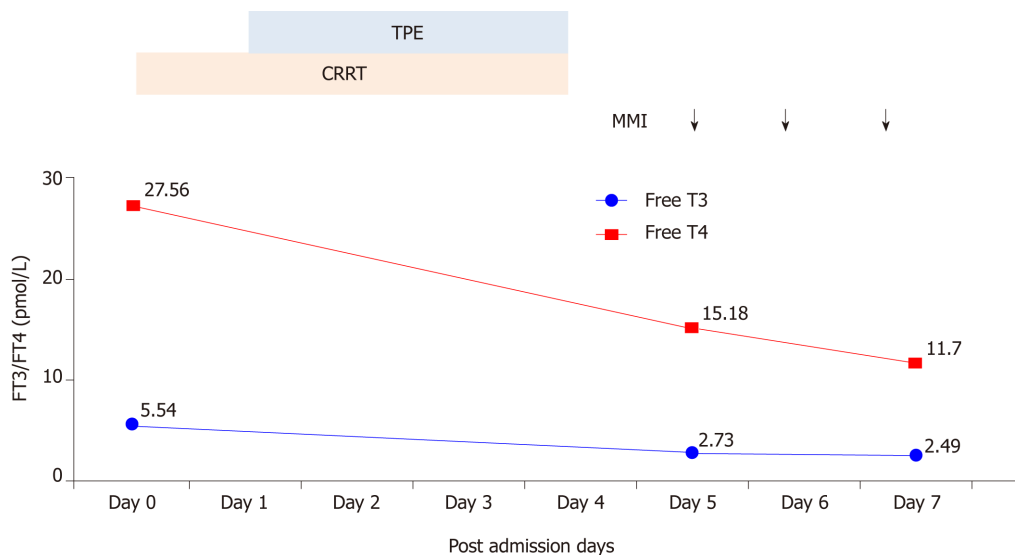
TPE is an extracorporeal blood purification technique designed for rapid removal of harmful plasma constituents or to decrease the concentrations of antibodies and immune complexes<sup>[14,17]</sup>. It is a well-established and effective therapeutic option in many diseases and has been used successfully to treat severe hyperthyroidism in recent years<sup>[18,19]</sup>. The mechanism of this approach is based on the fact that approximately 99% of the circulating thyroid hormones are bound to serum proteins (thyroxine-binding globulin, transthyretin, and albumin), and these protein-bound thyroid hormones can be cleared during TPE. TPE can supplement unbound globulins from fresh frozen plasma to provide new binding sites for free thyroid hormones, which can be removed during the next TPE procedure<sup>[20]</sup>. In addition, TPE provides a particular benefit to patients with coagulopathy of liver disease by supplying coagulation factors. In patients with fulminant hepatic failure, TPE can improve survival of those with sufficient residual hepatic capacity for generation<sup>[15]</sup>. However, TPE alone was not adequately effective in the present patient, which indicated that she needed more urgent therapy.

It is well known that CRRT is conducive to regulation of acid-base balance, electrolytes, and fluid balance to maintain cellular metabolism and homeostasis. Thus, we performed CRRT initially to alleviate symptoms of heart failure and improve life-threatening conditions. CRRT involves continuous dialysis and filtration to facilitate the slower removal of fluids and solutes, which is well tolerated and results in fewer metabolic changes in critically ill patients<sup>[21]</sup>. This approach has advantages in terms of cardiovascular stability, which is suggested as a therapeutic measure in patients with heart failure. Furthermore, it can continually eliminate harmful molecular substances, blood ammonia, and other toxic substances such as lactic acid, which is also beneficial to patients with liver failure.

Our patient responded well to CRRT and TPE. However, approximately 25% of the thyroid hormones are present in the intravascular compartment, and each plasma exchange decreases FT3 and FT4 levels by 30%-50%. The influx of extravascular thyroid hormones into the circulation cannot be ignored<sup>[22]</sup>. Therefore, co-administration of antithyroid drugs is still necessary to maintain a long-term clinical stabilization. Some patients are treated with radioiodine, surgery or MMI after discontinuation of PTU. In recent years, the use of radioiodine in Graves' disease has increased in Asia. In contrast, there is a decreasing trend of radioiodine therapy in the United States and Europe, which may be related to reports of higher rates of radiation-induced malignancies after radioiodine treatment<sup>[23]</sup>. In the present case, PTU was replaced with MMI 20 mg/d orally. Thyroid hormone and transaminases levels were stable. During follow-up of > 2 years, the thyroid and liver function tests were normal.

In conclusion, severe hyperthyroidism with high mortality requires multidisciplinary therapeutic measures in the intensive care setting. TPE combined with CRRT should be considered as a reasonably safe alternative when conventional treatment has failed. Early application of blood purification is feasible in serious clinical situations. Later, conventional medicine can be administered at an appropriate time. This sequential treatment procedure is worth considering in critically ill patients.

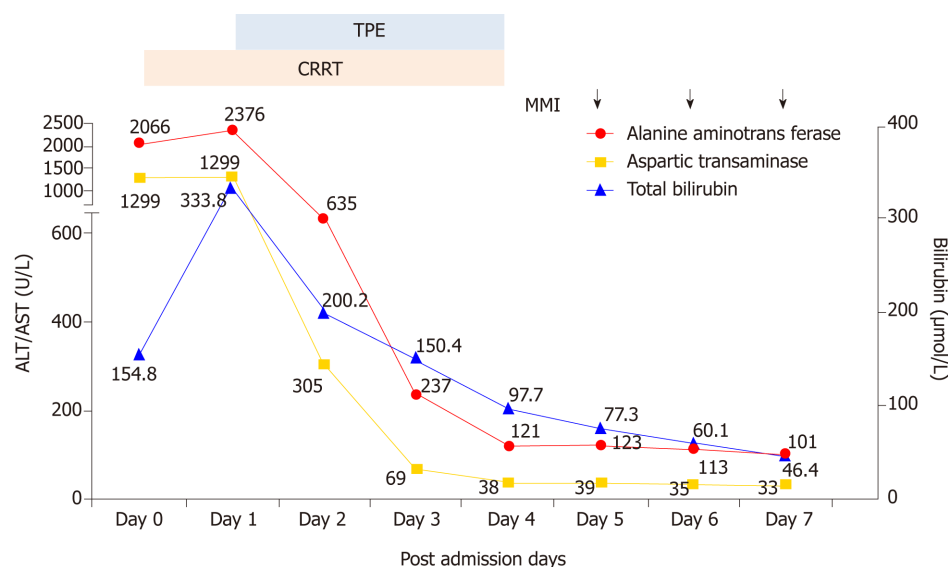




**Figure 2 Changes in FT3 and FT4 during treatment.** The gray and orange shadows showed when TPE and CRRT were carried out. The arrows indicate when MMI was administered. TPE: Therapeutic plasma exchange; CRRT: Continuous renal replacement therapy; MMI: Methimazole; FT3: Free tri-iodothyronine; FT4: Free thyroxine.

## CONCLUSION

If the patient is on antithyroid therapy, and other contributors to liver damage have been excluded, drug-induced hepatotoxicity should be kept in mind. Propylthiouracil-induced liver injury may be very severe, even leading to acute liver failure. It can occur at any time over the course of therapy and is not dose related. TPE combined with CRRT in severe hyperthyroidism patients should be considered as a reasonably safe alternative in serious clinical situations.



**Figure 3** Changes in liver function during treatment. The gray and orange shadows show when TPE and CRRT were carried out. The arrows indicate when MMI was administered. The levels of ALT, AST and TB declined progressively. TPE: Therapeutic plasma exchange; CRRT: Continuous renal replacement therapy; MMI: Methimazole; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TB: Total bilirubin.

## REFERENCES

- 1 Ezer A, Caliskan K, Parlakgumus A, Belli S, Kozanoglu I, Yildirim S. Preoperative Therapeutic Plasma Exchange In Patients With Thyrotoxicosis. *J Clin Apher* 2009; **24**: 111-114 [PMID: 19484727 DOI: 10.1002/Jca.20200]
- 2 Keklik M, Kaynar L, Yilmaz M, Sivgin S, Solmaz M, Pala C, Aribas S, Akyol G, Unluhizarci K, Cetin M, Eser B, Unal A. The Results Of Therapeutic Plasma Exchange In Patients With Severe Hyperthyroidism: A Retrospective Multicenter Study. *Transfus Apher Sci* 2013; **48**: 327-330 [PMID: 23611685 DOI: 10.1016/J.Transci.2013.04.010]
- 3 Iglesias P, DÉVora O, García J, Tajada P, García-ArÉvalo C, DíEz JJ. Severe Hyperthyroidism: Aetiology, Clinical Features And Treatment Outcome. *Clin Endocrinol (Oxf)* 2010; **72**: 551-557 [PMID: 19681915 DOI: 10.1111/J.1365-2265.2009.03682.X]
- 4 Carhill A, Gutierrez A, Lakhia R, Nalini R. Surviving The Storm: Two Cases Of Thyroid Storm Successfully Treated With Plasmapheresis. *BMJ Case Rep* 2012; 2012 [PMID: 23087271 DOI: 10.1136/Bcr-2012-006696]
- 5 Ashkar FS, Katims RB, Smoak WM, Gilson AJ. Thyroid Storm Treatment With Blood Exchange And Plasmapheresis. *JAMA* 1970; **214**: 1275-1279 [PMID: 5536311 DOI: 10.1001/Jama.1970.03180070041007]
- 6 De Campos Mazo DF, De Vasconcelos GB, Pereira MA, De Mello ES, Bacchella T, Carrilho FJ, Cançado EL. Clinical Spectrum And Therapeutic Approach To Hepatocellular Injury In Patients With Hyperthyroidism. *Clin Exp Gastroenterol* 2013; **6**: 9-17 [PMID: 23550044 DOI: 10.2147/CEG.S39358]
- 7 EISEN MJ. Fulminant Hepatitis During Treatment With Propylthiouracil. *N Engl J Med* 1953; **249**: 814-816 [PMID: 13111354 DOI: 10.1056/NEJM195311122492007]
- 8 Barzilay-Yoseph L, Shabun A, Shilo L, Hadary R, Nabrisi D, Kitay-Cohen Y. Thyrotoxic Hepatitis. *Isr Med Assoc J* 2011; **13**: 448-450 [PMID: 21838194]
- 9 Elias RM, Dean DS, Barsness GW. Hepatic Dysfunction In Hospitalized Patients With Acute Thyrotoxicosis: A Decade Of Experience. *ISRN Endocrinol* 2012; **2012**: 325092 [PMID: 23251814 DOI: 10.5402/2012/325092]
- 10 Livadas S, Xyrafis X, Economou F, Boutzios G, Christou M, Zerva A, Karachalios A, Palioura H, Palimeri S, Diamanti-Kandarakis E. Liver Failure Due To Antithyroid Drugs: Report Of A Case And Literature Review. *Endocrine* 2010; **38**: 24-28 [PMID: 20960098 DOI: 10.1007/S12020-010-9348-Y]
- 11 Chong HW, See KC, Phua J. Thyroid Storm With Multiorgan Failure. *Thyroid* 2010; **20**: 333-336 [PMID: 20146655 DOI: 10.1089/Thy.2009.0181]
- 12 Sousa Domínguez A. Severe Acute Liver Failure And Thyrotoxicosis: An Unusual Association. *Rev Esp Enferm Dig* 2015; **107** [PMID: 26176693 DOI: 10.17235/Reed.2015.3607/2014]
- 13 Akmal A, Kung J. Propylthiouracil, And Methimazole, And Carbimazole-Related Hepatotoxicity. *Expert Opin Drug Saf* 2014; **13**: 1397-1406 [PMID: 25156887 DOI: 10.1517/14740338.2014.953796]
- 14 Garla V, Kovvuru K, Ahuja S, Palabindala V, Malhotra B, Abdul Salim S. Severe Hyperthyroidism Complicated By Agranulocytosis Treated With Therapeutic Plasma Exchange: Case Report And Review Of The Literature. *Case Rep Endocrinol* 2018; **2018**: 4135940 [PMID: 29552362 DOI: 10.1155/2018/4135940]
- 15 Aydemir S, Ustundag Y, Bayraktaroglu T, Tekin IO, Peksoy I, Unal AU. Fulminant Hepatic Failure Associated With Propylthiouracil: A Case Report With Treatment Emphasis On The Use Of Plasmapheresis. *J Clin Apher* 2005; **20**: 235-238 [PMID: 16206173 DOI: 10.1002/Jca.20063]
- 16 Bahn RS, Burch HS, Cooper DS, Garber JR, Greenlee CM, Klein IL, Laurberg P, McDougall IR, Rivkees SA, Ross D, Sosa JA, Stan MN. The Role Of Propylthiouracil In The Management Of Graves' Disease In Adults: Report Of A Meeting Jointly Sponsored By The American Thyroid Association And The Food And Drug Administration. *Thyroid* 2009; **19**: 673-674 [PMID: 19583480 DOI: 10.1089/Thy.2009.0169]

- 17 **Jha S**, Waghdhare S, Reddi R, Bhattacharya P. Thyroid Storm Due To Inappropriate Administration Of A Compounded Thyroid Hormone Preparation Successfully Treated With Plasmapheresis. *Thyroid* 2012; **22**: 1283-1286 [PMID: [23067331](#) DOI: [10.1089/Thy.2011.0353](#)]
- 18 **Pasimeni G**, Caroli F, Spriano G, Antonini M, Baldelli R, Appetecchia M. Refractory Thyrotoxicosis Induced By Iodinated Contrast Agents Treated With Therapeutic Plasma Exchange. A Case Report. *J Clin Apher* 2008; **23**: 92-95 [PMID: [18293390](#) DOI: [10.1002/Jca.20161](#)]
- 19 **Muller C**, Perrin P, Faller B, Richter S, Chantrel F. Role Of Plasma Exchange In The Thyroid Storm. *Ther Apher Dial* 2011; **15**: 522-531 [PMID: [22107688](#) DOI: [10.1111/J.1744-9987.2011.01003.X](#)]
- 20 **Min SH**, Phung A, Oh TJ, Han KS, Kim MJ, Kim JM, Lee JH, Park YJ. Therapeutic Plasmapheresis Enabling Radioactive Iodine Treatment In A Patient With Thyrotoxicosis. *J Korean Med Sci* 2015; **30**: 1531-1534 [PMID: [26425054](#) DOI: [10.3346/Jkms.2015.30.10.1531](#)]
- 21 **Park HS**, Kwon SK, Kim YN. Successful Treatment Of Thyroid Storm Presenting As Recurrent Cardiac Arrest And Subsequent Multiorgan Failure By Continuous Renal Replacement Therapy. *Endocrinol Diabetes Metab Case Rep* 2017; 2017 [PMID: [28458893](#) DOI: [10.1530/EDM-16-0115](#)]
- 22 **Kokuho T**, Kuji T, Yasuda G, Umemura S. Thyroid Storm-Induced Multiple Organ Failure Relieved Quickly By Plasma Exchange Therapy. *Ther Apher Dial* 2004; **8**: 347-349 [PMID: [15274688](#) DOI: [10.1111/J.1526-0968.2004.00160.X](#)]
- 23 **Burch HB**, Burman KD, Cooper DS. A 2011 Survey Of Clinical Practice Patterns In The Management Of Graves' Disease. *J Clin Endocrinol Metab* 2012; **97**: 4549-4558 [PMID: [23043191](#) DOI: [10.1210/Jc.2012-2802](#)]

**P- Reviewer:** Sureshkumar K; Akoh JA; Mogulkoc R  
**S- Editor:** Dou Y **L- Editor:** Filipodia **E- Editor:** Tan WW



## Hydrochloric acid enhanced radiofrequency ablation for treatment of large hepatocellular carcinoma in the caudate lobe: Report of three cases

Han-Xia Deng, Jin-Hua Huang, Wan Yee Lau, Fei Ai, Min-Shan Chen, Zhi-Mei Huang, Tian-Qi Zhang, Meng-Xuan Zuo

**ORCID number:** Han-Xia Deng (0000-0002-1250-3444); Jin-Hua Huang (0000-0003-2960-6148); Wan Yee Lau (0000-0002-9802-6537); Fei Ai (0000-0003-2336-6315); Min-Shan Chen (0000-0002-7442-4637); Zhi-Mei Huang (0000-0002-4136-5896); Tian-Qi Zhang (0000-0002-1528-5323); Meng-Xuan Zuo (0000-0003-3589-7316).

**Author contributions:** Huang JH made the study concept and designed the study; Huang JH, Deng HX, and Ai F drafted the manuscript; Lau WY, Chen MS, and Fan YF made critical revision of the manuscript for important intellectual content; Huang ZM and Zhang TQ provided the technical support.

**Supported by** the National Natural Science Foundation of China, No. 81771955.

**Informed consent statement:** Written informed consent was obtained from the patients.

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**Open-Access:** This article is an open-access article which was selected by an in-house editor and

Han-Xia Deng, Jin-Hua Huang, Fei Ai, Zhi-Mei Huang, Tian-Qi Zhang, Meng-Xuan Zuo, Department of Minimally Invasive Interventional Therapy, Cancer Centre of Sun Yat-sen University, Guangzhou 510060, Guangdong Province, China

Han-Xia Deng, Jin-Hua Huang, Fei Ai, Min-Shan Chen, Zhi-Mei Huang, Tian-Qi Zhang, Meng-Xuan Zuo, State Key Laboratory of Oncology in Southern China, Guangzhou 510060, Guangdong Province, China

Wan Yee Lau, Min-Shan Chen, Department of Hepatobiliary Surgery, Cancer Centre of Sun Yat-sen University, Guangzhou 510060, Guangdong Province, China

Wan Yee Lau, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong, China

**Corresponding author:** Jin-Hua Huang, MD, Professor, Department of Minimally Invasive Interventional Radiology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, 651 Dongfeng Road East, Guangzhou 510000, Guangdong Province, China.

[huangjh@sysucc.org.cn](mailto:huangjh@sysucc.org.cn)

**Telephone:** +86-020-87343447

### Abstract

#### BACKGROUND

To report on the use of percutaneous hydrochloric acid (HCl) enhanced radiofrequency ablation (HRFA) for the treatment of large (maximum diameter  $\geq 5$  cm) hepatocellular carcinoma (HCC) in the caudate lobe.

#### CASE SUMMARY

Between August 2013 and June 2016, three patients with a large HCC (maximum diameter: 5.0, 5.7, and 8.1 cm) in the caudate lobe were treated by transarterial chemoembolization followed by computer tomography (CT) guided RFA using a monopolar perfusion RF electrode, which was enhanced by local infusion of 10% HCl at 0.2 mL/min (total volume, 3 to 12 mL). The output power of HRFA reached 100 W, and the average ablation time was 39 min (range, 15 to 60 min). Two patients each underwent one session of HRFA and one patient two sessions. After treatment, CT/magnetic resonance imaging showed that all the three lesions were completely ablated. There was no major complication. Two patients had asymptomatic bile duct dilatation. One patient died of tongue cancer 24 mo

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

**Received:** November 7, 2018

**Peer-review started:** November 12, 2018

**First decision:** November 27, 2018

**Revised:** December 23, 2018

**Accepted:** January 3, 2019

**Article in press:** January 3, 2019

**Published online:** February 26, 2019

after ablation. The remaining two patients were alive and no area of enhancement is detected in the caudate lobe at 28 and 60 mo after ablation, respectively.

## CONCLUSION

Percutaneous CT-guided HRFA is safe and efficacious in treating large HCC in the caudate lobe.

**Key words:** Hydrochloric acid; Radiofrequency ablation; Hydrochloric acid enhanced radiofrequency ablation; Caudate lobe; Large hepatocellular carcinoma; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Caudate lobe hepatocellular carcinoma (HCC) was considered highly technically difficult by surgeons and the outcome of interventional therapies, including transarterial chemoembolization and conventional radiofrequency ablation (RFA), according to previous studies was unsatisfied. Hydrochloric acid enhanced RFA, an innovative technique, can create an ablation zone larger than 5 cm by a single perfusate electrode without major complications, which is promising to treat large caudate lobe HCC patient.

**Citation:** Deng HX, Huang JH, Lau WY, Ai F, Chen MS, Huang ZM, Zhang TQ, Zuo MX. Hydrochloric acid enhanced radiofrequency ablation for treatment of large hepatocellular carcinoma in the caudate lobe: Report of three cases. *World J Clin Cases* 2019; 7(4): 508-515  
**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/508.htm>  
**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.508>

## INTRODUCTION

Hepatocellular carcinoma (HCC) arising from the caudate lobe is rare<sup>[1]</sup>. The caudate lobe is situated deep between the hepatic hilum and the inferior vena cava. Caudate lobectomy is considered to be technically difficult even for small tumors, with high risks of local recurrence and poor overall survival<sup>[2,3]</sup>. For large tumors in the caudate lobe, resection is challenging even in the hands of experienced liver surgeons<sup>[4,5]</sup>. Interventional therapies, including various intravascular and extravascular procedures, have been reported to treat caudate lobe HCC<sup>[6-14]</sup>. However, most of those focused on treating small HCC (maximum diameter < 5 cm). This is a retrospective study on three patients with HCC ≥ 5 cm in the caudate lobe treated by hydrochloric acid (HCl) enhanced radiofrequency ablation (HRFA).

## CASE PRESENTATION

### Case 1

A 61-year-old woman was found to have a caudate lobe lesion (55 mm × 57 mm × 63 mm) on magnetic resonance imaging (MRI). The patient had a history of chronic hepatic B virus (HBV) infection, with Child-Pugh A liver function and a negative serum alpha-fetoprotein (AFP) concentration (0.71 ng/mL). A biopsy confirmed HCC. Transarterial chemoembolization (TACE) using an emulsion of tetrahydropalmatine (THP) 50 mg, lobaplatin 50 mg, and lipiodol 10 mL was performed in August 2013. One month later, computed tomography (CT) showed that the tumor had enlarged (72 mm × 75 mm × 81 mm), and was separated into the superior and inferior parts by a fibrous septum.

Since the tumor enlarged after TACE, the patient was suggested to undergo ablation therapy. Conventional RFA was insufficient to ablate such a huge tumor. Thus, the patient received two sessions of HRFA in September and December 2013, respectively, to ablate the superior and inferior parts of the tumor. HRFA was applied for 60 min in the first and 30 min in the second session. No discomfort during ablation and no complications such as fever, pain, or hemorrhage after HRFA were observed.

One month after the first HRFA, a peripheral hyper-metabolic nodule was detected by PET-CT. Thus, the patient underwent two more sessions of COOL-TIP RFA in December 2013 and March 2014, respectively. After that, the hyper-metabolic lesion



was no longer visible. The last follow-up CT in July 2018 showed that the tumor had decreased to an inactive fibrous tissue mass of about 2 cm in diameter. During the course of treatment and follow-up, there were no major complications. A minor complication was asymptomatic slight dilation (total bilirubin concentration once elevated to 72  $\mu\text{mol/L}$  18 mo after HRFA and returned normal without any treatment) of bile ducts (Figure 1).

### Case 2

A 69-year-old man was found on health checkup to have a caudate lobe tumor, 17 mm  $\times$  25 mm on PET-CT in June 2013. He had a history of chronic HBV infection. The AFP concentration was negative, and he had Child-Pugh A liver function. A biopsy confirmed the diagnosis of HCC. The patient received two sessions of TACE in August and November 2013, respectively. CT performed in March 2014 showed that the lesion had enlarged to 47 mm  $\times$  57 mm, with poor lipiodol deposition. Besides, the patient had a history of tongue cancer and received radiotherapy 4 years ago.

In March 2014, HRFA through an anterior approach was applied for 60 min. There was no acute adverse effect occurring in the peri-ablation and post-ablation periods, and an MRI scan one month later showed no areas of enhancement.

Ten months after HRFA, in January 2015, MRI showed the margin of the lesion to be suspiciously enhanced by contrast. He underwent one session of COOL-TIP RFA. No visible active lesion was detected in the next MRI scan. The last MRI scan in December 2015 found no active lesion in the liver. Twenty-four months after HRFA, the patient died of recurrent tongue cancer (Figure 2).

### Case 3

A 73-year-old man presented with chest and abdomen pain in February 2016. CT showed a mass in the pancreatic neck and a low density lesion, 35  $\times$  50 mm, in the caudate lobe. The patient had a history of chronic HBV infection, Child-Pugh A disease, and a negative serum AFP concentration. The PIVKA level was 20.266 AU/m. An exploratory laparotomy showed a primary tumor in the caudate lobe and that the lesion in pancreatic neck was a spontaneous hematoma. Frozen section analysis of an enlarged portal lymph node showed metastatic HCC. TACE was performed in April 2016, with an emulsion of THP 30 mg, lobaplatin 30 mg, and lipiodol 10 mL. CT after TACE showed good lipiodol deposition.

Three days later, HRFA was applied for 15 min through an anterior approach. No major complications occurred. One month later, the PIVKA concentration dropped to 2.566 AU/mL and no visible contrast enhanced areas on MRI. Follow-up at 28 mo after HRFA showed no signs of relapse or metastasis. (Figure 3)

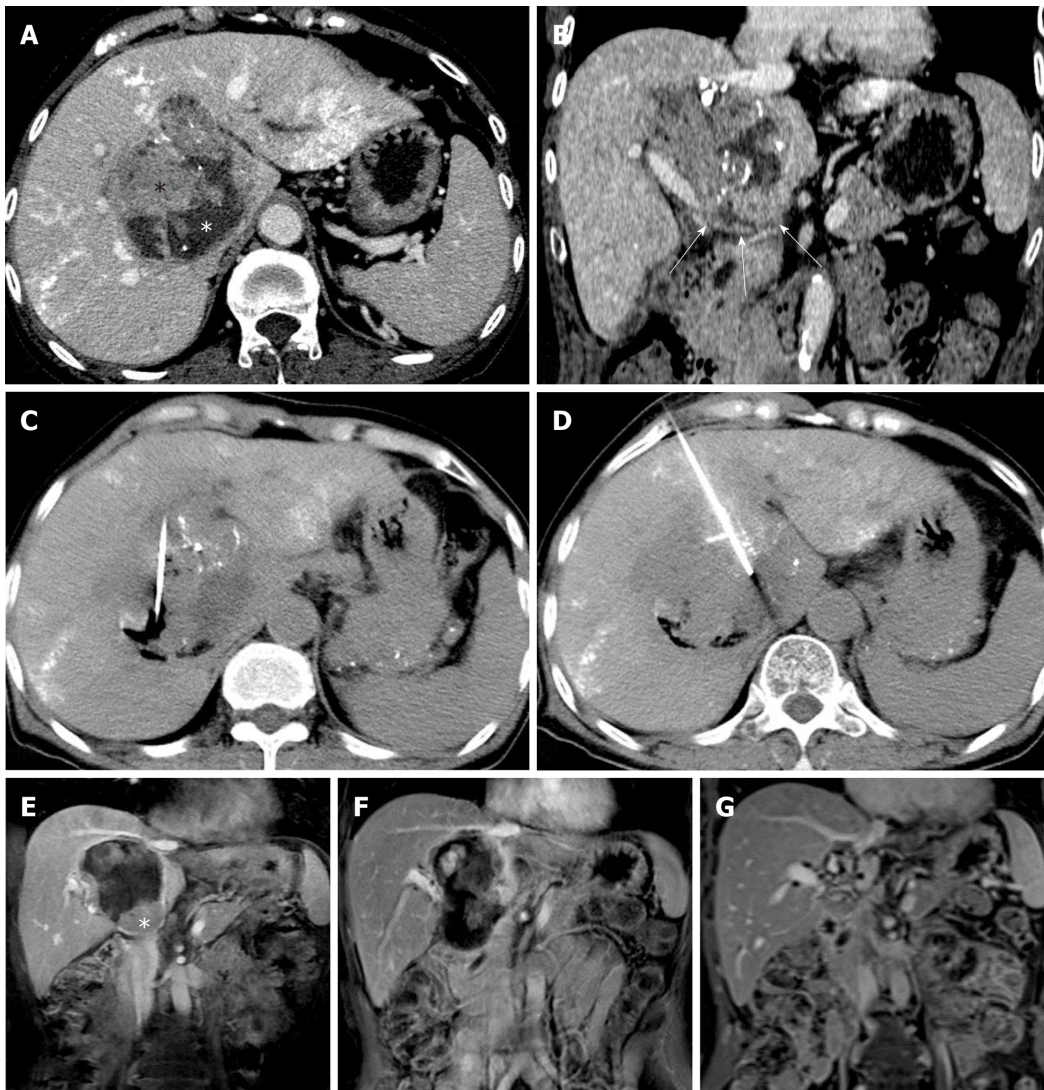
The summary of the three patients is shown in Table 1.

## DISCUSSION

Surgical resection of a caudate lobe tumor is technically challenging as the caudate lobe is situated deep between the hepatic hilum and the inferior vena cava<sup>[1]</sup>. In a report on 12 patients, the median operative time was 568 min, the median intraoperative blood loss was 550 mL, and five patients developed postoperative bile leak with problems in renal function<sup>[4]</sup>. Caudate lobectomy is commonly combined with major or extended hepatectomy with sacrifice of a large amount of non-tumorous liver parenchyma which increases the risk of postoperative liver failure.

Compared to surgery, interventional therapies such as TACE or RFA have a lower risk of treatment morbidities. In 1986, Takayasu *et al*<sup>[6]</sup> reported five patients who underwent transcatheter arterial infusion (TAI) or transcatheter arterial embolization (TAE) for treating advanced-stage caudate lobe HCC. Unfortunately, four of these patients died during a mean of 5.5 mo. With advances in interventional technology and a better understanding of arterial blood supply of caudate lobe HCC<sup>[8,15,16]</sup>, the success rate of selective subsegmental TACE in treating caudate lobe HCC has been greatly improved. However, long-term survival after treatment remains a problem. Kim *et al*<sup>[9]</sup> performed selective TACE to treat 34 patients with caudate lobe HCC with a diameter of less than 3 cm. The 5-year overall survival and progression-free rates were 72% and 21%, respectively. TACE cannot completely block the feeding arteries and gain a complete tumor necrosis, which causes recurrence<sup>[10]</sup>. For a large caudate lobe HCC, the results of TACE are even worse.

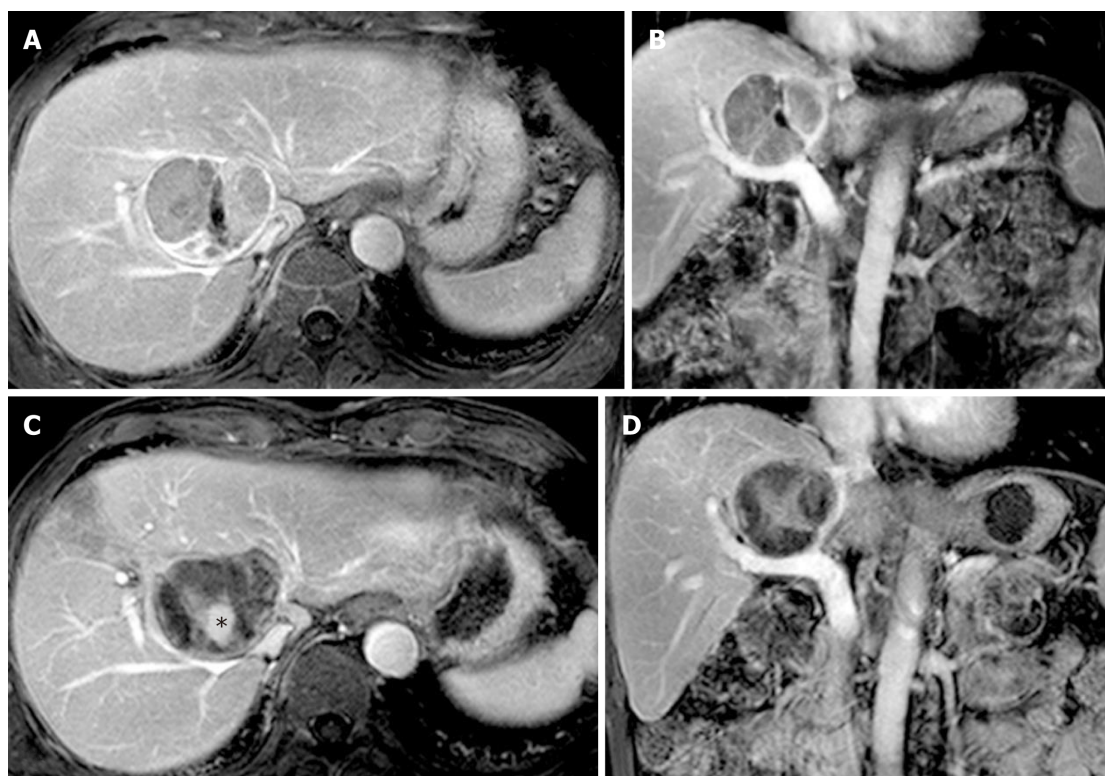
Percutaneous ablation therapies, including percutaneous ethanol injection (PEI), RFA, and MWA, are well-established and widely used treatments for HCC. In 2002, Shibata *et al*<sup>[11]</sup> first introduced PEI with or without TAE to treat 25 patients with caudate lobe HCC (average diameter, 27 mm). Peng *et al*<sup>[13]</sup> reported on 17 patients who underwent RFA treatment for caudate lobe HCC (average diameter, 31 mm).



**Figure 1** Arterial-phase computed tomography images showing a large caudate lobe tumor enhanced after transarterial chemoembolization in a 61-year-old woman with confirmed hepatocellular carcinoma. A: The tumor with poor lipiodol deposition was predominantly composed by isodense tissue (black asterisk), representing the contrast enhanced active part of the tumor and the hypodense necrotic region without enhancement (white asterisk). The hyperdensity in the hepatic parenchyma represents lipiodol deposition after TACE; B: The caudate lobe and porta hepatis were involved (white arrows); C and D: Computed tomography images taken during the ablation show precise placement of the RF electrode into the active regions of the tumor, and the area of destruction can be seen easily; E: The coronal magnetic resonance image (MRI) shows an enhanced inferior residual tumor after the first session of HRFA (white asterisk); F: No active tissue was detected after the second session of HRFA; G: The latest coronal post-contrast T1-weighted image shows focal atrophy and fibrosis formation, resulting in an irregular, non-active region in the caudate lobe about 20 mm in diameter. The bile ducts were slightly dilated on the latest follow-up MRI.

However, most of these studies focused on treating small caudate lobe tumors. Nevertheless, incomplete ablation and recurrence still happened. Nishigaki *et al*<sup>[14]</sup> compared the recurrence rates in patients with caudate lobe HCC or HCC located in other liver segments. They found that the caudate lobe patients had a higher risk of developing tumor recurrence. Caudate lobe HCCs are more difficult to be completely ablated than those in other liver segments due to the restricted approach through which an RFA electrode can be introduced, and the heat sink effect of the inferior vena cava.

In the last decade, several new techniques, such as normal saline perfused radiofrequency ablation (NSRFA) and multi-electrode applications had been developed, aiming to create a large ablative zone<sup>[17]</sup>. Our previous experiments showed that infusing diluted HCl instead of normal saline during RFA could enlarge the diameter of ablation zone from a mean (SD) of 3.52 cm (0.07) to 6.85 cm (0.32) at 30 W-30 min<sup>[18]</sup>. This is because the conductivity of HCl is about three times higher than that of saline, greatly increasing the conductivity around the RF electrode<sup>[19]</sup>. In *in vivo* experiments, HRFA also exhibited a larger ablative zone with favorable safety<sup>[21-22]</sup>. Based on these studies, we have reported performing HRFA on a patient with spontaneously ruptured HCC, which successfully controlled bleeding and achieved complete necrosis after ablation without any complications<sup>[19]</sup>. In a word, HRFA, a



**Figure 2** A well-defined active caudate lobe tumor adjacent to the right hepatic vein in a 69-year-old man with a history of tongue cancer after two sessions of TACE. A and B: The enhanced part was predominant and had poor lipiodol deposition consistent with active tumor tissues; C and D: Post-contrast magnetic resonance imaging (MRI) images after HRFA therapy show a non-enhanced mass with hypointensity. The central irregular hyperintensity (black asterisk, C) was caused by the hemorrhagic content of the necrotic cavity, rather than by contrast enhancement. Relapse was not detected in the most recent MRI examination (data not shown).

technique that can create a large ablation volume by using a monopolar electrode, is promising in treating large caudate lobe HCCs. In the present study, all three patients had unresectable large caudate lobe HCC. One patient underwent two sessions of HRFA and the other two patients underwent one session each. After HRFA and followed complementary COOL-TIP RFA, all the three caudate lobe tumors showed complete necrosis.

Among four sessions of HRFA, three were performed through an anterior approach and the remaining one was through a lateral approach (case 1, the 2<sup>nd</sup> session) in order to protect peripheral vessels and the biliary system. HRFA also avoids repeated punctures because one session of HRFA is sufficient to achieve complete necrosis. Besides, the electrode in HRFA could be placed at the center of the lesion whereas in other RFA techniques, it must reach the tumor margin, which would induce damage to the structure nearby. There was no major complication and asymptomatic bile duct dilatation as a minor complication occurred in patient 1 18 mo after HRFA. It was hypothesized that the non-active lesion, which had shrunk from 7 cm to less than 3 cm, stretched its peripheral liver tissue and induced bile duct dilatation.

## CONCLUSION

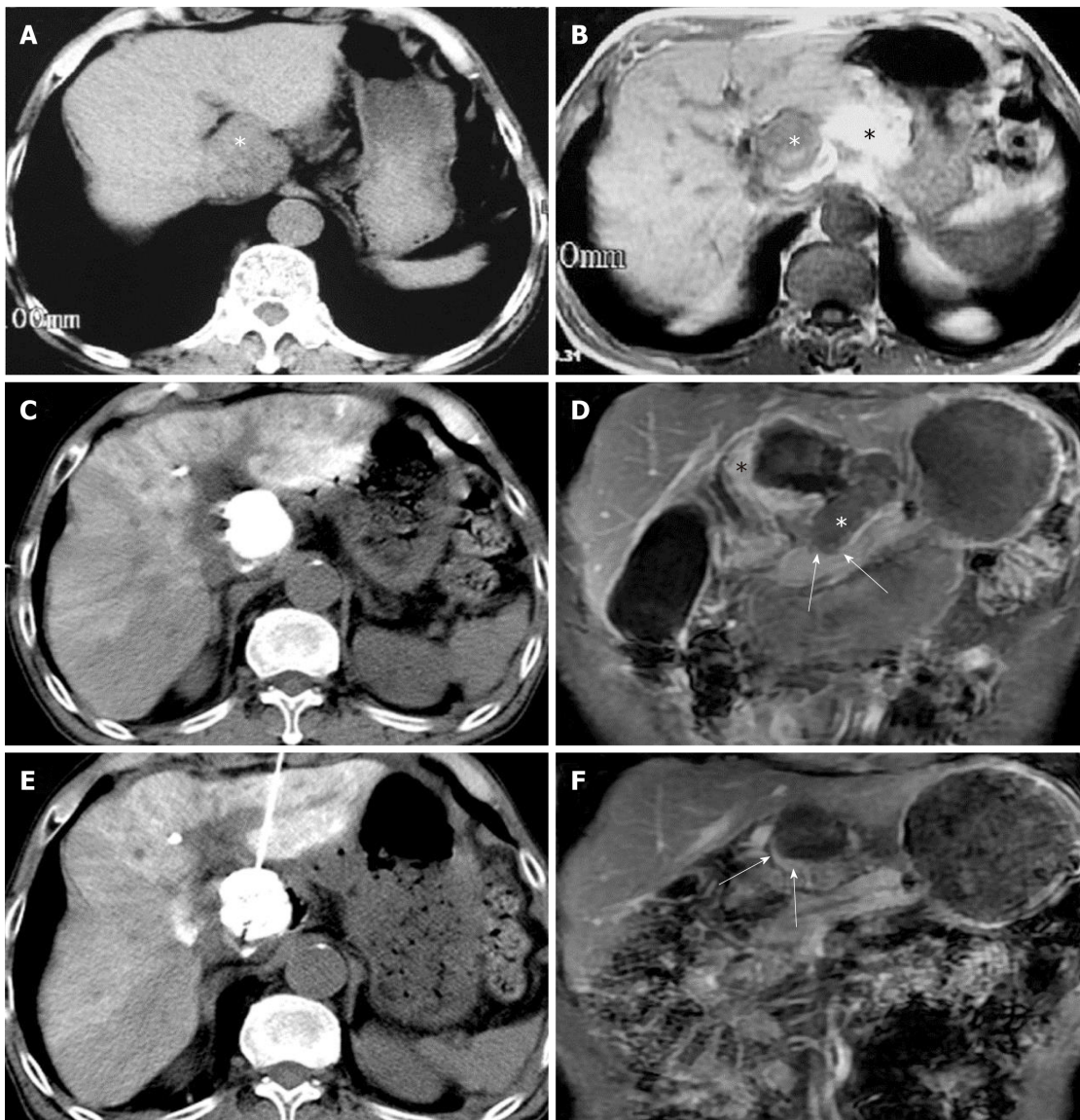
HRFA can be an efficacious and safe choice for patients with a large caudate lobe HCC. However, further research is necessary to determine the appropriate role of HRFA in treating caudate lobe HCCs. Combination of HRFA with TACE or other systemic therapies is expected to further improve the prognosis of patients.



**Table 1** The summary of all three patients

	Case 1	Case 2	Case 3
Sex	Female	Male	Male
Age	61	69	73
Tumor size	75 × 81	47 × 57	35 × 50
Session(s) of HRFA	2	1	1
Complementary RFA	Yes	Yes	None
Survival (mo)	60	48	28
Complications	Asymptomatic bile duct dilatation	None	None
Alive	Alive	Died of tongue cancer recurrence	Alive

HRFA: Hydrochloric acid enhanced radiofrequency ablation.



**Figure 3** Preoperative computed tomography and magnetic resonance imaging images of a 73-year-old male showing a large intrahepatic tumor in the caudate lobe. A and B: A large intrahepatic tumor in the caudate lobe was confirmed as hepatocellular carcinoma by histopathology. A hyperintense mass was noted in the pancreatic neck region on T1-weighted imaging (black asterisk, B). This was shown to be a hematoma on subsequent exploratory laparotomy; C: CT images showing that lipiodol deposition involved almost the entire area of the lesion in the caudate lobe after TACE; D: Peripheral residual tumor medial to the necrotic cavity was demonstrated in a coronal post-contrast T1-weighted image (black asterisk). The non-enhancing soft tissue in the porta hepatis region inferior to the intrahepatic lesion and adjacent to the neck of pancreas (white arrows) is consistent with the shrunken hematoma after exploratory laparotomy (white asterisk). E: CT image showing the needle in the tumor during HRFA; F: A thin enhancing rim-like tissue inferior to the necrotic cavity (white arrows) is evident on the post-contrast T1-weighted image after HRFA therapy. The necrotic region does not show enhancement. No relapse was detected during follow-up MRI examinations. This enhancing

rim probably represents reactive granulation tissues, rather than residual tumor.

## REFERENCES

- 1 **Kumon M.** Anatomy of the caudate lobe with special reference to portal vein and bile duct. *Acta Hepatol Jpn* 1985; **26**: 1193-1199 [DOI: [10.2957/kanzo.26.1193](https://doi.org/10.2957/kanzo.26.1193)]
- 2 **Tanaka S, Shimada M, Shirabe K, Maehara S, Tsujita E, Taketomi A, Maehara Y.** Surgical outcome of patients with hepatocellular carcinoma originating in the caudate lobe. *Am J Surg* 2005; **190**: 451-455 [PMID: [16105535](https://pubmed.ncbi.nlm.nih.gov/16105535/) DOI: [10.1016/j.amjsurg.2004.12.005](https://doi.org/10.1016/j.amjsurg.2004.12.005)]
- 3 **Kumon M.** Anatomical Study of the Caudate Lobe with Special Reference to Portal Venous and Biliary Branches Using Corrosion Liver Casts and Clinical Application. *Liver Cancer* 2017; **6**: 161-170 [PMID: [28275582](https://pubmed.ncbi.nlm.nih.gov/28275582/) DOI: [10.1159/000454682](https://doi.org/10.1159/000454682)]
- 4 **Viganò L, Costa G, Procopio F, Donadon M, Cimino M, Del Fabbro D, Gatti A, Torzilli G.** Parenchyma-Sparing Liver Surgery for Large Segment 1 Tumors: Ultrasound-Guided Lateral and Superior Approaches as Safe Alternatives to Major Hepatectomy. *J Am Coll Surg* 2015; **221**: e65-e73 [PMID: [26272013](https://pubmed.ncbi.nlm.nih.gov/26272013/) DOI: [10.1016/j.jamcollsurg.2015.07.008](https://doi.org/10.1016/j.jamcollsurg.2015.07.008)]
- 5 **Wang ZG, Lau W, Fu SY, Liu H, Pan ZY, Yang Y, Zhang J, Wu MC, Zhou WP.** Anterior hepatic parenchymal transection for complete caudate lobectomy to treat liver cancer situated in or involving the paracaval portion of the caudate lobe. *J Gastrointest Surg* 2015; **19**: 880-886 [PMID: [25759077](https://pubmed.ncbi.nlm.nih.gov/25759077/) DOI: [10.1007/s11605-015-2793-4](https://doi.org/10.1007/s11605-015-2793-4)]
- 6 **Takayasu K, Muramatsu Y, Shima Y, Goto H, Moriyama N, Yamada T, Makuuchi M, Kaneko A, Itabashi M, Shimamura Y.** Clinical and radiologic features of hepatocellular carcinoma originating in the caudate lobe. *Cancer* 1986; **58**: 1557-1562 [PMID: [3017540](https://pubmed.ncbi.nlm.nih.gov/3017540/)]
- 7 **Terayama N, Miyayama S, Tatsu H, Yamamoto T, Toya D, Tanaka N, Mitsui T, Miura S, Fujisawa M, Kifune K, Matsui O, Takashima T.** Subsegmental transcatheter arterial embolization for hepatocellular carcinoma in the caudate lobe. *J Vasc Interv Radiol* 1998; **9**: 501-508 [PMID: [9618113](https://pubmed.ncbi.nlm.nih.gov/9618113/)]
- 8 **Woo S, Kim HC, Chung JW, Jung HS, Hur S, Lee M, Jae HJ.** Chemoembolization of extrahepatic collateral arteries for treatment of hepatocellular carcinoma in the caudate lobe of the liver. *Cardiovasc Interv Radiol* 2015; **38**: 389-396 [PMID: [24934735](https://pubmed.ncbi.nlm.nih.gov/24934735/) DOI: [10.1007/s00270-014-0929-7](https://doi.org/10.1007/s00270-014-0929-7)]
- 9 **Kim HC, Chung JW, Jae HJ, Yoon JH, Lee JH, Kim YJ, Lee HS, Yoon CJ, Park JH.** Caudate lobe hepatocellular carcinoma treated with selective chemoembolization. *Radiology* 2010; **257**: 278-287 [PMID: [20697120](https://pubmed.ncbi.nlm.nih.gov/20697120/) DOI: [10.1148/radiol.10100105](https://doi.org/10.1148/radiol.10100105)]
- 10 **Goldberg SN, Ahmed M.** Minimally invasive image-guided therapies for hepatocellular carcinoma. *J Clin Gastroenterol* 2002; **35**: S115-S129 [PMID: [12394215](https://pubmed.ncbi.nlm.nih.gov/12394215/) DOI: [10.1097/00004836-200211002-00008](https://doi.org/10.1097/00004836-200211002-00008)]
- 11 **Shibata T, Maetani Y, Ametani F, Kubo T, Itoh K, Konishi J.** Efficacy of nonsurgical treatments for hepatocellular carcinoma in the caudate lobe. *Cardiovasc Interv Radiol* 2002; **25**: 186-192 [PMID: [12058213](https://pubmed.ncbi.nlm.nih.gov/12058213/) DOI: [10.1007/s00270-001-0111-x](https://doi.org/10.1007/s00270-001-0111-x)]
- 12 **Yamakado K, Nakatsuka A, Akeboshi M, Takaki H, Takeda K.** Percutaneous radiofrequency ablation for the treatment of liver neoplasms in the caudate lobe left of the vena cava: electrode placement through the left lobe of the liver under CT-fluoroscopic guidance. *Cardiovasc Interv Radiol* 2005; **28**: 638-640 [PMID: [16132396](https://pubmed.ncbi.nlm.nih.gov/16132396/) DOI: [10.1007/s00270-004-0104-7](https://doi.org/10.1007/s00270-004-0104-7)]
- 13 **Peng ZW, Liang HH, Chen MS, Zhang YJ, Li JQ, Zhang YQ, Lau WY.** Percutaneous radiofrequency ablation for the treatment of hepatocellular carcinoma in the caudate lobe. *Eur J Surg Oncol* 2008; **34**: 166-172 [PMID: [17851020](https://pubmed.ncbi.nlm.nih.gov/17851020/) DOI: [10.1016/j.ejso.2007.08.004](https://doi.org/10.1016/j.ejso.2007.08.004)]
- 14 **Nishigaki Y, Tomita E, Hayashi H, Suzuki Y, Iritani S, Kato T, Yamada T.** Efficacy and safety of radiofrequency ablation for hepatocellular carcinoma in the caudate lobe of the liver. *Hepatol Res* 2013; **43**: 467-474 [PMID: [23072582](https://pubmed.ncbi.nlm.nih.gov/23072582/) DOI: [10.1111/j.1872-034X.2012.01095.x](https://doi.org/10.1111/j.1872-034X.2012.01095.x)]
- 15 **Yoon CJ, Chung JW, Cho BH, Jae HJ, Kang SG, Kim HC, Choi YH, Jeon UB, Park JH.** Hepatocellular carcinoma in the caudate lobe of the liver: angiographic analysis of tumor-feeding arteries according to subsegmental location. *J Vasc Interv Radiol* 2008; **19**: 1543-50; quiz 1550 [PMID: [18755606](https://pubmed.ncbi.nlm.nih.gov/18755606/) DOI: [10.1016/j.jvir.2008.07.008](https://doi.org/10.1016/j.jvir.2008.07.008)]
- 16 **Miyayama S, Yamashiro M, Yoshie Y, Nakashima Y, Ikeno H, Orito N, Yoshida M, Matsui O.** Hepatocellular carcinoma in the caudate lobe of the liver: variations of its feeding branches on arteriography. *Jpn J Radiol* 2010; **28**: 555-562 [PMID: [20972854](https://pubmed.ncbi.nlm.nih.gov/20972854/) DOI: [10.1007/s11604-010-0471-8](https://doi.org/10.1007/s11604-010-0471-8)]
- 17 **Kang TW, Rhim H.** Recent Advances in Tumor Ablation for Hepatocellular Carcinoma. *Liver Cancer* 2015; **4**: 176-187 [PMID: [26674766](https://pubmed.ncbi.nlm.nih.gov/26674766/) DOI: [10.1159/000367740](https://doi.org/10.1159/000367740)]
- 18 **Jiang XY, Gu YK, Huang JH, Gao F, Zou RH, Zhang TQ.** Ex Vivo Liver Experiment of Hydrochloric Acid-Infused and Saline-Infused Monopolar Radiofrequency Ablation: Better Outcomes in Temperature, Energy, and Coagulation. *Cardiovasc Interv Radiol* 2016; **39**: 600-605 [PMID: [26486153](https://pubmed.ncbi.nlm.nih.gov/26486153/) DOI: [10.1007/s00270-015-1218-9](https://doi.org/10.1007/s00270-015-1218-9)]
- 19 **Huang JH, Morelli JN, Ai F, Zou RH, Gu YK, Gao F, Zhang TQ, Yao W, Jiang XY, Zhang YY.** Hydrochloric acid-enhanced radiofrequency ablation for treating a large hepatocellular carcinoma with spontaneous rupture: a case report. *Chin J Cancer* 2017; **36**: 1 [PMID: [28061892](https://pubmed.ncbi.nlm.nih.gov/28061892/) DOI: [10.1186/s40880-016-0161-8](https://doi.org/10.1186/s40880-016-0161-8)]
- 20 **Weijian F, Zan L, Suhong H, Hongmei Z, Lei Z, Yanjie Z, Yi C, Ni J.** Destructive effect of percutaneous hydrochloric acid injection therapy for liver cancer--a preliminary experimental and clinical study. *Gan To Kagaku Ryoho* 2006; **33**: 1852-1856 [PMID: [17212126](https://pubmed.ncbi.nlm.nih.gov/17212126/)]
- 21 **Yao W, Gu YK, Wang J, Gao F, Liu WL, Huang JH.** Safety evaluation of a potential ablation agent-hydrochloric acid in the rabbits' model. *Ann Palliat Med* 2014; **3**: 250-262 [PMID: [25841905](https://pubmed.ncbi.nlm.nih.gov/25841905/) DOI: [10.3978/j.issn.2224-5820.2014.02.01](https://doi.org/10.3978/j.issn.2224-5820.2014.02.01)]
- 22 **Zhang TQ, Huang SM, Gu YK, Gao F, Huang ZM, Jiang XY, Liu DX, Huang JH.** Safety and effect on ablation size of hydrochloric acid-perfused radiofrequency ablation in animal livers. *Int J Hyperthermia* 2018; **34**: 925-933 [PMID: [29457524](https://pubmed.ncbi.nlm.nih.gov/29457524/) DOI: [10.1080/02656736.2018.1442588](https://doi.org/10.1080/02656736.2018.1442588)]

**P- Reviewer:** Bramhall S, Jani K, Ekpenyong CE

**S- Editor:** Dou Y **L- Editor:** Wang TQ **E- Editor:** Tan WW





## Long-term follow-up of a patient with venlafaxine-induced diurnal bruxism treated with an occlusal splint: A case report

Jia-Min Chen, Ying Yan

**ORCID number:** Jia-Min Chen (0000-0001-6059-4881); Ying Yan (0000-0002-3134-2463).

**Author contributions:** Yan Y and Chen JM examined the patient and collected the clinical data; Chen JM wrote the paper; Yan Y edited the manuscript and approved the final version.

**Informed consent statement:**

Informed consent was obtained from the patient for publication of this report and any accompanying images and videos.

**Conflict-of-interest statement:** All authors declare no conflict of interest for this article.

**CARE Checklist (2016) statement:**

The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**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

**Jia-Min Chen, Ying Yan**, Department of Prosthodontics, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, Guangzhou 510055, Guangdong Province, China

**Corresponding author:** Ying Yan, MSc, Associate Professor, Chief Doctor, Department of Prosthodontics, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Stomatology, No. 56, West Linyuan Road, Guangzhou 510055, Guangdong Province, China. [yanying2@mail.sysu.edu.cn](mailto:yanying2@mail.sysu.edu.cn)  
**Telephone:** +86-136-6073-2785  
**Fax:** +86-020-83822807

### Abstract

#### BACKGROUND

Bruxism is a jaw-muscle activity characterized by the clenching or grinding of teeth. It can be divided into nocturnal bruxism and diurnal bruxism (DB). DB secondary to antidepressants is rare and refractory. Reports associated with antidepressant-induced DB are mostly anecdotal without long-term follow-up. The effect of drug intervention on antidepressant-induced DB is still contested. We herein report the first case of successful treatment of venlafaxine-induced DB with an occlusal splint.

#### CASE SUMMARY

This case report describes detailed 7-year follow-up of a patient with venlafaxine-induced DB treated with an occlusal splint. The patient who complained about involuntary daytime tooth grinding after taking venlafaxine for a period of 4 mo and was diagnosed with venlafaxine-induced DB. Subsequently, an occlusal splint with modified bilateral buccal-ptyergoid pads was used to treat his tooth grinding and to protect the dental structures from tooth wearing. The patient reported remission of symptoms after several months of treatment. His grinding activity was gradually and stably controlled after 2 years, with an almost complete recovery from DB after 6 years.

#### CONCLUSION

The maxillary buccal-ptyergoid splint can be used as a noninvasive approach to treat venlafaxine-induced DB.

**Key words:** Occlusal splint; Venlafaxine; Diurnal bruxism; Tooth grinding; Movement disorders; Treatment; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Received:** November 14, 2018**Peer-review started:** November 14, 2018**First decision:** December 22, 2018**Revised:** January 8, 2019**Accepted:** January 26, 2019**Article in press:** January 26, 2019**Published online:** February 26, 2019

**Core tip:** Secondary diurnal bruxism (DB) is rare and refractory. The existing literature associated with antidepressant-induced DB mostly consists of anecdotal reports without long-term follow-up. Therapeutic effects of drug intervention are still unclear. This case is the first to describe successful treatment of venlafaxine-induced DB with an occlusal splint.

**Citation:** Chen JM, Yan Y. Long-term follow-up of a patient with venlafaxine-induced diurnal bruxism treated with an occlusal splint: A case report. *World J Clin Cases* 2019; 7(4): 516-524

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/516.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.516>

## INTRODUCTION

Bruxism is a jaw-muscle activity characterized by the clenching or grinding of teeth. Its prevalence is reported to be 8% to 20% among the adult population<sup>[1]</sup>. Bruxism can be divided into nocturnal bruxism and diurnal bruxism (DB) according to the different circadian rhythms. The diurnal form, a kind of parafunction that occurs while awake<sup>[2]</sup>, is always secondary to neurological diseases<sup>[3,4]</sup> and psychotropic drug use<sup>[5-7]</sup> as a manifestation of movement disorders in the oromandibular region.

Furthermore, focal movement disorder mainly manifesting as DB has not been well studied by dentists and there exist few reports of successful treatment. In this paper, we report a patient with venlafaxine-induced DB successfully treated with a maxillary buccal-ptyergoid splint.

## CASE PRESENTATION

### Chief complaints

A 69-year-old married man who suffered from “involuntary daytime tooth grinding” for a period of 2 mo presented to the Department of Prosthodontics of the Sun Yat-sen University Hospital of Stomatology.

### History of present illness

He reported involuntary tooth grinding and jaw tenderness during the day after taking venlafaxine 150 mg/d, quetiapine 100 mg/d, and lorazepam 2.0 mg/d for 4 mo to treat his major depressive disorder. The bruxism occurred during the daytime and subsided when asleep. And in the 2 mo that followed, the patient’s DB became aggravated and he had his mandibular posterior tooth extracted after a crown fracture. Thus, the patient turned to us for help.

### History of past illness

The patient had a history of ischemic stroke decades ago. Antithrombotic agents (clopidogrel/cilostazol) combined with atorvastatin/rosuvastatin and butylphthalide were routinely used to lower the risk of stroke and heart complications. Eight months prior, the patient was admitted to the Psychology Department of the Huifu Xi Branch of Guangdong General Hospital for “insomnia for 20 years and exacerbation in 20 d”. An initial diagnosis of insomnia was made. Routine blood work and biochemical and thyroid function examinations were within the normal range. Head MRI and MRA showed multiple remote ischemic areas at the bilateral frontal lobe and parietal cortex. Sleep monitoring tests indicated no abnormal limb movements or sleep apnea syndrome, but poor sleep quality with an efficiency of only 76.7%. While hospitalized, duloxetine 60 mg/d, lorazepam 2.5 mg/d, and zopiclone 7.5 mg/d were given, but the patient was unable to comply with medication usage instructions. Thus, a combination of physical and psychological therapy was started. After therapy, the patient had improvement in sleep quality and remission of stress and anxiety. A discharge diagnosis of “depressive disorder” was reached and duloxetine 60 mg/d, lorazepam 3.0 mg/d, and quetiapine 100 mg/d were prescribed after the patient left the hospital. Two months later, the patient began to complain about headaches, dizziness, insomnia, feelings of worthlessness, and decreased energy. He was diagnosed with major depressive disorder at the Department of Psychiatry of Guangdong General Hospital. Subsequently, venlafaxine 150 mg/d, lorazepam 2.0

mg/d, and quetiapine 100 mg/d were started for the treatment of depression. After 4 mo of therapy, the patient had a partial remission from depression.

### **Personal and family history**

He had no previous personal or familial history of movement disorder.

### **Physical examination upon admission**

He seemed to be depressive and numb, but he could answer our questions consciously and clearly. Daytime involuntary tooth grinding occurred while resting, but paused when he spoke. Dental examination showed the following findings: (1) No facial asymmetry; (2) No evoked pain or tenderness was noticed when palpating the bilateral temporomandibular joint (TMJ) regions (the anterior wall of external auditory canal and the region anterior to the tragus); (3) Examination of mandibular movement revealed a normal mandibular opening (maximum opening 35 mm) and closing pattern without pain or noise. Mandibular lateral, protrusive, and retruded movements were also within the normal range; (4) Intensified bilateral temporalis and masseter in resting position; and (5) Further intraoral study indicated no stable intercuspal position due to the involuntary tooth grinding. Multiple molar teeth (#45, #17, #27, #37, and #47) were lost. All of the residual teeth of the patient were dramatically worn down (the palatal cusps of #24 and #25 and buccal cusps of #34 and #35 were missing). Cervical wedge-shaped defects could be found in the buccal side of #25, #34, #35, and #44.

### **Imaging examinations**

Panoramic radiograph showed a fresh extraction socket in #45 and suspected root fracture in #26 (Figure 1). Cone-beam computed tomography (CBCT) examination of the TMJ indicated no significant change of bone substance, but a change of the right joint space (Figure 2).

---

## **FINAL DIAGNOSIS**

---

An initial clinical diagnosis of venlafaxine-induced DB was reached.

---

## **TREATMENT**

---

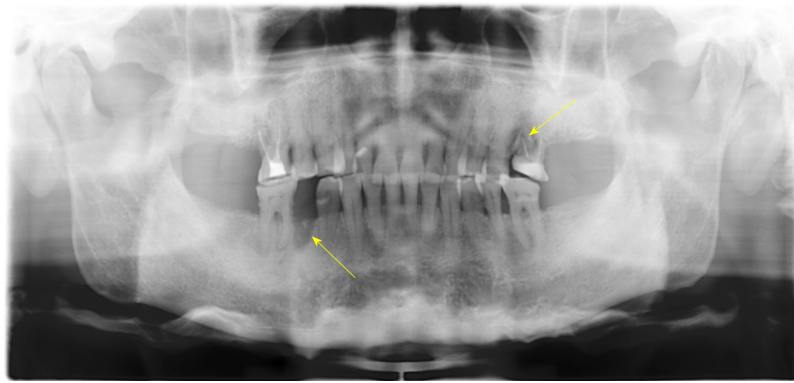
A maxillary buccal-ptyergoid splint was recommended to relieve the symptoms and protect the dental structures from tooth wearing. The maxillary buccal-ptyergoid splint (Patent No. 201620908577.7)<sup>[8]</sup> was designed by Dr. Yan Ying based on the grinding feature of non-centric bruxism. It is composed of the occlusal plate and bilateral buccal-ptyergoid pads. The buccal-ptyergoid pads extending from the distal side of maxillary canine teeth to the mesial side of maxillary second molar teeth cover approximately two-thirds of the buccal side of the mandibular corresponding teeth, leaving a suitable horizontal gap of 1.5 mm. The occlusal plate with convex surface will open the bite 1.5-2.0 mm in the posterior teeth. Constant daytime use of the splint was suggested, with the patient removing it only during mealtimes. The patient then underwent long-term follow-up.

---

## **OUTCOME AND FOLLOW-UP**

---

The patient insisted on the same drug recipe and the final stable medication doses were venlafaxine 150 mg/d, quetiapine 100 mg/d, and lorazepam 2.0 mg/d across 7-year follow-up. All of the results of splint therapy and prognosis were recorded in detail and regular occlusal adjustment was performed on the splint when needed. Jaw tenderness improved dramatically 1 mo after therapy, but deep scratch traces could be found on the bilateral buccal-ptyergoid pads (mainly the right side), indicating the presence of tooth grinding. The patient felt a decrease in the frequency of the grinding activity in the early morning, but an increase after noon 3 mo after the procedure. When he returned back after a period of 4 mo of therapy, cracks in the bilateral buccal-ptyergoid pads and deeper scratch trace could be seen. Thus, a modified maxillary buccal-ptyergoid splint with reinforced wire fused into bilateral buccal-ptyergoid pads was given to the patient (Figure 3). Interestingly, the patient reported marked improvement in the frequency and amplitude of daytime tooth grinding 7 mo postoperatively. However, newly-found bite scars on the splint suggested the occurrence of clenching when awake. At a return visit after 9 mo, clenching subsided



**Figure 1** Panoramic radiograph indicating a fresh extraction socket in #45 and suspected root fracture in #26. Multiple molar teeth (#45, #17, #27, #37, and #47) were lost.

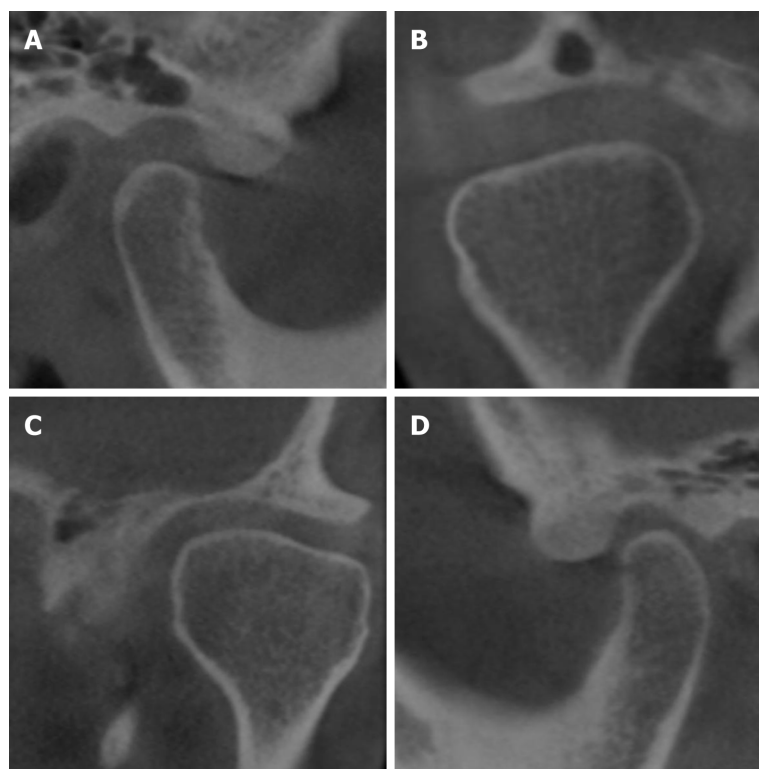
when wearing the splint and gradually recurred after removing it. At 12 mo postoperatively, the patient again complained about a relapse of tooth grinding with scratch traces found mainly on the left buccal-ptyergoid pad. At 24 mo postoperatively, the patient presented with a fracture in tooth #26, possibly due to suspected root fracture and long-term weight overloading. At 30 mo postoperatively, scratch traces on the splint fortunately became less and less noticeable and no new bite scar was noticed. Grinding activity was stably controlled when wearing the splint (Figure 4A and Video 1), but gradually recurred after removing the splint (Figure 4B and Video 2). When he returned for a 6-year follow-up visit, there were no scratch traces but sporadic bite scars on the bilateral posterior region of the splint. Therefore, bilateral buccal-ptyergoid pads of the splint were removed, leaving the appliance acting as a stabilization splint to relieve the clenching of the teeth (Figure 5). Three months later, the patient had almost completely ceased clenching and grinding his teeth when wearing the splint (Figure 6A and Video 3). When the splint was removed, he just had slight relapse of clenching, but could control it consciously (Figure 6B and Video 4). A 7-year follow-up CBCT (Figure 7) indicated no change when compared with the previous images. Registration of the pre-CBCT and post-CBCT was performed to further verify longitudinal changes of the bilateral TMJ (Figure 8).

## DISCUSSION

There exist few reports of successful treatment of secondary DB, a kind of rare focal movement disorder. Research on the management of antidepressant drug-induced DB mainly focused on drug treatments. Unfortunately, the effect of drug intervention is still not clear. Furthermore, frequently switching from one drug to another may cause side effects<sup>[7]</sup>. In our study, we used a maxillary buccal-ptyergoid splint as a noninvasive approach to treat venlafaxine-induced DB. With the improvement of symptoms, patient compliance correspondingly increased, and he was more willing to wear the splint.

Bilateral buccal-ptyergoid pads of the splint to some extent limited the grinding movement when involuntary grinding occurred. The occlusal plate, which opened the bite 1.5-2.0 mm in the posterior teeth, enabled masticatory muscles to adapt to the new mandibular position and gradually established new working memory. In our case, the patient reported marked improvement of the symptoms, but a relapse at 12 mo. A possible reason for the relapse was that masticatory muscles had not yet adapted to the changed mandibular position. A longer period may be required to establish a stable muscle working memory. Hence, at the follow-up of 24 mo postoperatively, daytime grinding was gradually stably controlled, and the patient had almost completely ceased clenching or grinding his teeth by 6 years postoperatively. CBCT examination of the TMJ indicated no change when compared with the former one before treatment, which also implies that long-term use of a maxillary buccal-ptyergoid splint might be harmless to the joint. We hypothesize that the maxillary buccal-ptyergoid splint blocks the abnormal reflex arc of the grinding movement through the mechanisms of neurophysiological and biofeedback effects on masticatory muscles. It may alter the transmission of neurotransmitters in a corresponding brain region for mandibular movement, causing a decrease in the displacement and lateral force of daytime tooth grinding. However, further research should be performed to verify the exact mechanism of the maxillary buccal-ptyergoid





**Figure 2** Cone-beam computed tomography examination of the temporomandibular joint. A: Sagittal projection of the right temporomandibular joint (TMJ) showing increased posterior joint space; B: Coronal projection of the right TMJ showing increased medial and lateral joint space; C: Coronal projection of the left TMJ; D: Sagittal projection of the left TMJ.

splint on venlafaxine-induced DB.

Furthermore, DB can be affected by many factors. It is difficult to diagnose and treat DB due to its complex pathogenesis. Various neurotransmitters have been implicated in DB<sup>[9]</sup>. Experimental studies have demonstrated that the nigrostriatal and mesocortical pathways are two main, but different, dopaminergic pathways involved in motor control. Dopamine (DA) transmitters are believed to play a central role in the appearance of grinding/biting behavior<sup>[10,11]</sup>. There is also evidence for the involvement of other neurotransmitters, such as serotonin (5-HT) and norepinephrine, which are closely related to the regulation of DA in the central nervous system (CNS). In our case, the patient did not report bruxism or any other oral movement disorder until 4 mo after he took venlafaxine at a dose of 150 mg/d. Venlafaxine is a selective serotonin and norepinephrine reuptake inhibitor. According to its pharmacology, venlafaxine has a high affinity to 5-HT when the dosage exceeds 150 mg/d<sup>[12]</sup>. The sequent excess of 5-HT in the synapses leads to an inhibitory effect on the release of DA from the mesocortical tract, thereby leading to bruxism<sup>[13]</sup>.

The patient in our case continued a complicated combination of medications (zopiclone, lorazepam, quetiapine, clopidogrel, cilostazol, atorvastatin, rosuvastatin, and butylphthalide), which made the observation much more confounded. However, none of those medications have been reported to induce DB. Given that there are increasing reports of DB associated with antidepressant drugs (paroxetine<sup>[13]</sup>, sertraline<sup>[14]</sup>, fluvoxamine<sup>[15]</sup>, venlafaxine<sup>[16]</sup>, fluoxetine<sup>[17]</sup>, and atomoxetine<sup>[18]</sup>), our clinical impression was that the patient's DB was secondary to venlafaxine use. The cause-and-effect relationship is supported by the following observations: (1) The patient exhibited no previous history of bruxism or other movement disorder before taking venlafaxine; (2) Duloxetine 60 mg/d was irregularly taken for 2 mo without occurrence of DB; and (3) DB occurred 4 mo after switching from duloxetine 60 mg/d to venlafaxine 150 mg/d.

Existing reports also indicated that DB can be induced by brain injury through the subsequent disruption of normal interplay of neuronal circuits in different brain areas<sup>[19,20]</sup>. DB, or other presenting forms of movement disorders, were found from several days to several years after frontal lobe or thalamic injury<sup>[21-24]</sup>. Chih-Sung Liang<sup>[24]</sup> explained the mechanism by dendritic plasticity and changing patterns in the synaptic activity of injured brain neurons. In our case, head MRI and MRA performed when the patient was admitted to the Huifu Xi Branch of Guangdong General



**Figure 3** Maxillary buccal-ptyergoid splint with reinforced wire fused into the bilateral buccal-ptyergoid pads was positioned in the mouth.

Hospital indicated no progressive damage. As no additional head MRI or MRA was performed after the DB occurred, we are unable to conclude that his bruxism was associated with his cerebral stroke. Of course, it is possible that his cerebral stroke might contribute as a risk factor, making it more difficult for damaged neurons to recover, and previous brain injury history should be noted when treating refractory DB.

Aiming to regulate the dysfunction of neurotransmitters in CNS, drug interventions have been used to treat antidepressant-induced DB. Nevertheless, it is still contested. According to Fitzgerald *et al*<sup>[7]</sup> in a 1995 report, buspirone, a kind of 5-HT receptor agonist, is only effective in only one of the four cases of SSRI-induced DB with a history of psychotropic drugs consumption. Bostwick *et al*<sup>[14]</sup> claimed that buspirone was effective in treating sertraline-induced DB. Pavlovic *et al*<sup>[16]</sup> found that buspirone was effective in venlafaxine-induced DB. It is also reported that tandospirone can result in remission of paroxetine-induced DB<sup>[12]</sup>. However, Miyaoka *et al*<sup>[15]</sup> reported a failure to relieve fluvoxamine-induced DB using tandospirone. The patient's own medical history, medication history, and the complicated etiology of secondary DB may be responsible for these conflicting results. In addition, Fitzgerald *et al*<sup>[7]</sup> also described a severe relapse of psychic symptoms and drug side effects after drug withdrawal or switching to alternatives. Long-term effects of drug intervention on antidepressant-induced DB are still unknown. To conclude, it is a challenge to find specific antidepressants that can avoid the occurrence of drug-induced DB or other movement disorders and remain effective.

This case may be the first to report the successful treatment of venlafaxine-induced DB with an occlusal splint. Reports associated with antidepressant-induced DB are mostly anecdotal without long-term follow-up. Thus, more research, particularly randomized controlled trials, should be carried out to further study the mechanism and treatment of antidepressant-induced DB.

## CONCLUSION

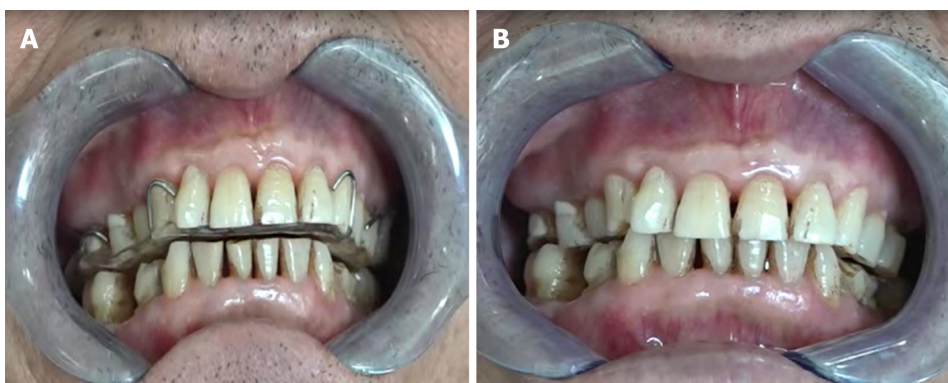
Attention should be paid to the medical and medication history in patients with bruxism. This case report suggests that the maxillary buccal-ptyergoid splint, a noninvasive approach, can be used as the preferred choice when treating drug-induced secondary bruxism before changing the medication regimen.



**Figure 4** Video recordings demonstrating stable control of tooth grinding. A: Wearing the splint, his tooth grinding activity was limited to a relatively smaller range; B: Removing the splint, involuntary tooth grinding gradually re-started.



**Figure 5** Bilateral buccal-ptyergoid pads were removed after daytime tooth grinding symptoms were almost completely controlled.



**Figure 6** Follow-up video recordings demonstrating almost completely ceased tooth grinding and clenching. A: Wearing the splint, his tooth grinding completely ceased with very mild symptoms of clenching; B: Removing the splint, his tooth grinding did not recur. Slight relapse of clenching can be controlled consciously.

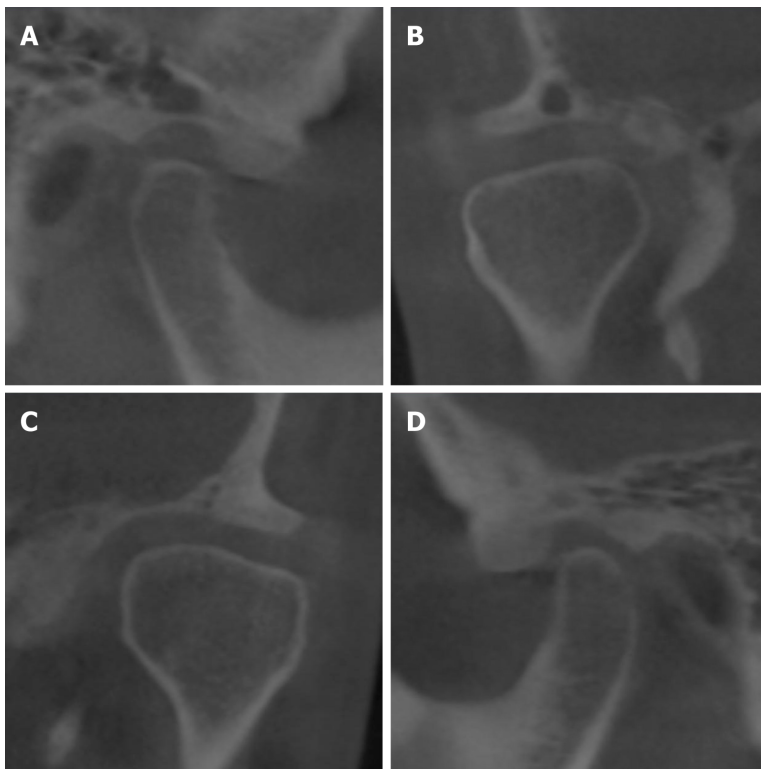


Figure 7 Seven-year follow-up cone-beam computed tomography images indicating no change when compared with the previous images.

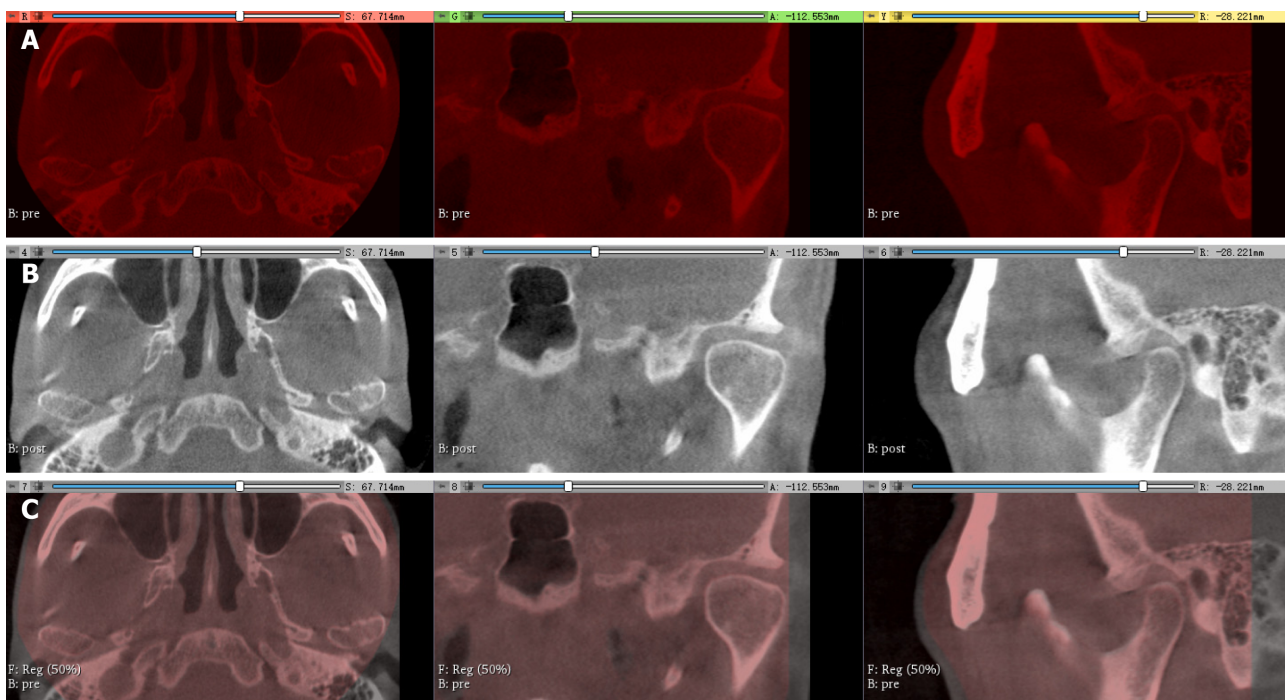


Figure 8 Registration of the pre- and post-cone-beam computed tomography images. A: Cone-beam computed tomography (CBCT) images pre-treatment are labeled red; B: Seven-year follow-up CBCT images post-treatment are labeled gray; C: General registration was performed to superimpose the pre-CBCT and post-CBCT images. Pre-CBCT images served as fixed images with a 50% transparency while post-CBCT images as moving images. A perfect match of the whole images revealed no significant longitudinal changes of the temporomandibular joint.

## REFERENCES

- 1 Lavigne GJ, Khoury S, Abe S, Yamaguchi T, Raphael K. Bruxism physiology and pathology: an overview for clinicians. *J Oral Rehabil* 2008; **35**: 476-494 [PMID: [18557915](#) DOI: [10.1111/j.1365-3113.2008.04001.x](#)]



- 10.1111/j.1365-2842.2008.01881.x]
- 2 **Lobbezoo F**, Ahlberg J, Glaros AG, Kato T, Koyano K, Lavigne GJ, de Leeuw R, Manfredini D, Svensson P, Winocur E. Bruxism defined and graded: an international consensus. *J Oral Rehabil* 2013; **40**: 2-4 [PMID: 23121262 DOI: 10.1111/joor.12011]
- 3 **Watts MW**, Tan EK, Jankovic J. Bruxism and cranial-cervical dystonia: is there a relationship? *Cranio* 1999; **17**: 196-201 [PMID: 10650407 DOI: 10.1080/08869634.1999.11746095]
- 4 **Kwak YT**, Han IW, Lee PH, Yoon JK, Suk SH. Associated conditions and clinical significance of awake bruxism. *Geriatr Gerontol Int* 2009; **9**: 382-390 [PMID: 20002758 DOI: 10.1111/j.1447-0594.2009.00538.x]
- 5 **Mendhekar DN**, Andrade C. Antipsychotic induced bruxism treated with clozapine. *J Neuropsychiatry Clin Neurosci* 2009; **21**: 105-106 [PMID: 19359467 DOI: 10.1176/jnp.2009.21.1.105]
- 6 **Pekkan G**, Kilicoglu A, Algin DI. Treatment of a tardive dyskinesia patient with temporomandibular disorder: a case report. *J Orofac Pain* 2010; **24**: 212-216 [PMID: 20401360]
- 7 **Fitzgerald K**, Healy D. Dystonias and dyskinesias of the jaw associated with the use of SSRIs. *Hum Psychopharm Clin* 1995; **10**: 215-219 [DOI: 10.1002/hup.470100308]
- 8 **Yan Ying**, Wang JH, Feng YF, Jiang LL, Chen JM, Wu MH; inventor; Guangzhou Sanhuan Patent Agency Inc. assignee. Maxillary buccal-ptyergoid splint. Chinese patent CN 201620908577.7. 2017; Apr 10
- 9 **Falisi G**, Rastelli C, Panti F, Maglione H, Quezada Arcega R. Psychotropic drugs and bruxism. *Expert Opin Drug Saf* 2014; **13**: 1319-1326 [PMID: 25195948 DOI: 10.1517/14740338.2014.947262]
- 10 **Mascaro MB**, Bittencourt JC, Casatti CA, Elias CF. Alternative pathways for catecholamine action in oral motor control. *Neurosci Lett* 2005; **386**: 34-39 [PMID: 15978723 DOI: 10.1016/j.neulet.2005.05.062]
- 11 **Gómez FM**, Ortega JE, Horrillo I, Meana JJ. Relationship between non-functional masticatory activity and central dopamine in stressed rats. *J Oral Rehabil* 2010; **37**: 827-833 [PMID: 21039747 DOI: 10.1111/j.1365-2842.2010.02110.x]
- 12 **Stahl SM**, Grady MM, Moret C, Briley M. SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. *CNS Spectr* 2005; **10**: 732-747 [PMID: 16142213 DOI: 10.1017/S1092852900019726]
- 13 **Kishi Y**. Paroxetine-induced bruxism effectively treated with tandospirone. *J Neuropsychiatry Clin Neurosci* 2007; **19**: 90-91 [PMID: 17308240 DOI: 10.1176/jnp.2007.19.1.90]
- 14 **Bostwick JM**, Jaffee MS. Bupirone as an antidote to SSRI-induced bruxism in 4 cases. *J Clin Psychiatry* 1999; **60**: 857-860 [PMID: 10665633 DOI: 10.4088/JCP.v60n1209]
- 15 **Miyaoka T**, Yasukawa R, Mihara T, Shimizu Y, Tsubouchi K, Maeda T, Mizuno S, Uegaki J, Inagaki T, Horiguchi J, Tachibana H. Successful electroconvulsive therapy in major depression with fluvoxamine-induced bruxism. *J ECT* 2003; **19**: 170-172 [PMID: 12972988 DOI: 10.1097/00124509-200309000-00010]
- 16 **Pavlovic ZM**. Bupirone to improve compliance in venlafaxine-induced movement disorder. *Int J Neuropsychopharmacol* 2004; **7**: 523-524 [PMID: 15383159 DOI: 10.1017/S1461145704004638]
- 17 **Oulis P**, Dimitrakopoulos S, Konstantakopoulos G, Tsaltas E, Kollias K. Low-dose aripiprazole in the treatment of SSRI-induced bruxism. *J Neuropsychiatry Clin Neurosci* 2012; **24**: E39 [PMID: 23037677 DOI: 10.1176/appi.neuropsych.11070175]
- 18 **Bahali K**, Yalcin O, Avci A. Atomoxetine-induced wake-time teeth clenching and sleep bruxism in a child patient. *Eur Child Adolesc Psychiatry* 2014; **23**: 1233-1235 [PMID: 25159091 DOI: 10.1007/s00787-014-0607-y]
- 19 **Chen WH**, Lu YC, Lui CC, Liu JS. A proposed mechanism for diurnal/nocturnal bruxism: hypersensitivity of presynaptic dopamine receptors in the frontal lobe. *J Clin Neurosci* 2005; **12**: 161-163 [PMID: 15749418 DOI: 10.1016/j.jocn.2004.07.007]
- 20 **Suri R**, Rodriguez-Porcel F, Donohue K, Jesse E, Lovera L, Dwivedi AK, Espay AJ. Post-stroke Movement Disorders: The Clinical, Neuroanatomic, and Demographic Portrait of 284 Published Cases. *J Stroke Cerebrovasc Dis* 2018; **27**: 2388-2397 [PMID: 29793802 DOI: 10.1016/j.jstrokecerebrovasdis.2018.04.028]
- 21 **Tan EK**, Chan LL, Chang HM. Severe bruxism following basal ganglia infarcts: insights into pathophysiology. *J Neurol Sci* 2004; **217**: 229-232 [PMID: 14706229 DOI: 10.1016/j.jns.2003.10.003]
- 22 **Yi HS**, Kim HS, Seo MR. Trial of oral metoclopramide on diurnal bruxism of brain injury. *Ann Rehabil Med* 2013; **37**: 871-874 [PMID: 24466522 DOI: 10.5535/arm.2013.37.6.871]
- 23 **Scott BL**, Jankovic J. Delayed-onset progressive movement disorders after static brain lesions. *Neurology* 1996; **46**: 68-74 [PMID: 8559423 DOI: 10.1212/WNL.46.1.68]
- 24 **Liang CS**, Chou MK, Yang FW. Delayed-onset diurnal bruxism, psychic akinesia and depression after carbon monoxide poisoning: a case report. *Gen Hosp Psychiatry* 2011; **33**: 82.e9-82.10 [PMID: 21353136 DOI: 10.1016/j.genhosppsych.2010.08.001]

**P- Reviewer:** Teramoto-Matsubara OT, Senol MG

**S- Editor:** Dou Y **L- Editor:** Wang TQ **E- Editor:** Tan WW





## Primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization: A case report

Ke-Lei Zhu, Xiu-Jun Cai

**ORCID number:** Ke-Lei Zhu (0000000164973379); Xiu-Jun Cai (0000000236154680).

**Author contributions:** Zhu KL and Cai XJ solely contributed to this paper.

**Supported by** Yinzhou Young Investigator Award, NO. Yin Ren She 2017-133; and Ministry of Science of Yinzhou District, No. Yin Ke 2017-110.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board of Sir Run Run Shaw Hospital, Hangzhou, China.

**Informed consent statement:** The patient described in the case report gave verbal consent for anonymous description of the case.

**Conflict-of-interest statement:** None of the authors have any conflict of interest to declare.

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**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,

**Ke-Lei Zhu, Xiu-Jun Cai,** Department of General Surgery, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou 310016, Zhejiang Province, China

**Ke-Lei Zhu,** Department of Hepatobiliary Surgery, Yinzhou People's Hospital, Ningbo 315040, Zhejiang Province, China

**Corresponding author:** Xiu-Jun Cai, FRSC, PhD, Professor, Surgeon, Department of General Surgery, Sir Run Run Shaw Hospital, Zhejiang University, No. 3, Qingchun East Road, Hangzhou 310016, Zhejiang Province, China. [cxjzu@zju.edu.cn](mailto:cxjzu@zju.edu.cn)

**Telephone:** +86-571-86006605

### Abstract

#### BACKGROUND

Primary hepatic leiomyosarcoma is rare and reported sporadically, with less than 40 such cases have been reported in the English-language literature. Although it is reported to be associated with acquired immune deficiency syndrome, Epstein-Barr virus infection, Hodgkin's lymphoma, immunosuppression after organ transplantation, and hepatitis C virus-related liver cirrhosis, the precise steps leading to leiomyosarcoma have not been fully identified. Therapeutic strategies include liver wedge resection or lobectomy, chemotherapy, radiotherapy and liver transplantation; however, the prognosis of primary hepatic leiomyosarcoma is dismal.

#### CASE SUMMARY

We describe here the first case of primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization (TACE). The patient was a 68-year-old woman who presented with right upper quadrant pain and weight loss over the past 5 wk before admission. Abdominal computed tomography (commonly known as CT) and ultrasonography showed a mixed echoic mass measuring about 10 cm × 7 cm occupying the right lobe of the liver. Exploratory laparotomy was performed 1 wk after admission. The tumor was unresectable and biopsy was performed. Based on rapid frozen-section and histopathological examination, a final diagnosis of primary hepatic leiomyosarcoma was established. TACE was performed 2 wk later. The postoperative course was uneventful and the patient was discharged on day 7 after the operation. Contrast-enhanced CT showed that the tumor significantly shrunk with satisfactory lipiodol deposition. The patient has been followed up for 82 mo until now, and no progressive enlargement of the tumor or distal metastasis was observed.

#### CONCLUSION

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

**Received:** October 10, 2018

**Peer-review started:** October 10, 2018

**First decision:** November 27, 2018

**Revised:** December 22, 2018

**Accepted:** December 29, 2018

**Article in press:** December 30, 2018

**Published online:** February 26, 2019

TACE is a safe and effective treatment for primary hepatic leiomyosarcoma. The therapeutic effect of TACE combined with surgical resection should be further assessed.

**Key words:** Treatment; Prognosis; Transcatheter arterial chemoembolization; Primary hepatic leiomyosarcoma; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Primary hepatic leiomyosarcoma is rare and reported sporadically. We here describe the first case of primary hepatic leiomyosarcoma successfully treated with transcatheter arterial chemoembolization (commonly known as TACE). The postoperative course was uneventful and the patient was discharged on day 7 after the operation. Contrast-enhanced computed tomography showed that the tumor significantly shrunk with satisfactory lipiodol deposition. The patient has been followed up for 82 mo until now, and no progressive enlargement of the tumor or distal metastasis was observed. This case indicates that TACE is a safe and effective treatment for primary hepatic leiomyosarcoma.

**Citation:** Zhu KL, Cai XJ. Primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization: A case report. *World J Clin Cases* 2019; 7(4): 525-531

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/525.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.525>

## INTRODUCTION

Primary hepatic leiomyosarcoma is rare and only reported sporadically<sup>[1]</sup>, with less than 40 cases having been reported to date. It appears to be associated with acquired immune deficiency syndrome (commonly known as AIDS)<sup>[2]</sup>, Epstein-Barr virus<sup>[3]</sup>, Hodgkin's lymphoma<sup>[4]</sup>, immunosuppression after organ transplantation<sup>[5]</sup>, and hepatitis C virus (commonly known as HCV)-related liver cirrhosis<sup>[6]</sup>, the precise mechanisms leading to leiomyosarcoma have yet been fully identified. Surgical resection is recommended for a curative treatment, while diagnosis of primary hepatic leiomyosarcoma is challenging and often delayed until it reaches a size that results in dismal prognosis.

We herein describe the first case of primary hepatic leiomyosarcoma successfully treated by transcatheter arterial chemoembolization (TACE). The patient has survived for 82 mo with no tumor enlargement and distant metastasis detected at postoperative follow-up.

## CASE PRESENTATION

### Chief complaints

A 68-year-old woman was referred to our hospital on December 2011, due to right upper quadrant pain and a 5-pound weight loss.

### Personal and family history

Other symptoms were not presented and she did not drink alcohol, smoke or have a history of surgery.

### Physical examination upon admission

Physical examination revealed a soft abdomen, no tenderness, no rebound tenderness, and no palpable lymph nodes or abdominal mass.

### Laboratory examinations

The following laboratory data were recorded: hemoglobin of 125 g/L; white cell count of  $5.5 \times 10^9/L$ , with 72.5% neutrophils; platelet count of  $234 \times 10^9/L$ ; alanine aminotransferase of 25 U/L; serum total protein of 75 g/L; albumin of 37.2 g/L; total bilirubin of 11  $\mu\text{mol/L}$ ; direct bilirubin of 9.5  $\mu\text{mol/L}$ ;  $\alpha$ -fetoprotein of 5.23 ng/mL;

and carcinoembryonic antigen of 4.5 ng/L. Serological markers for hepatitis B virus and HCV were negative. The indocyanine green retention rate at 15 min was 2.2%.

### Imaging examinations

Abdominal ultrasonography showed there was a mixed echoic mass measuring around 10 cm × 7 cm in the right hepatic lobe. Abdominal computed tomography (CT) showed a similar finding, that the tumor was inhomogeneous density, with mild delayed enhancement, and had central necrosis (Figure 1A). Gastrointestinal endoscopy and colonoscopy showed negative findings. Chest CT showed no mass over the lung.

Preoperative diagnosis was unconfirmed, exploratory laparotomy was performed 1 wk after admission, and no obvious effusion was found in the abdominal cavity. The entire liver VIII was occupied by a creamy white, firm mass measuring about 10 cm × 7 cm, which protruded into the abdominal cavity and appeared to invade the diaphragm and middle hepatic vein, making the tumor unresectable (Figure 1); thus, a biopsy was performed. Rapid frozen-section biopsy analysis considered the diagnosis of leiomyosarcoma. Histopathological examination showed a hepatic mass that consisted of spindle-shaped cells with mitotic figures and nuclear atypia (Figure 2). Immunohistochemical staining showed that spindle-shaped cells were positive for anti-smooth muscle actin (Figure 3) and desmin (Figure 4) but negative for keratin, S-100, CD34 and CD117. No obvious lesions were observed in the bilateral adnexa and uterus, and no palpable mass was detected in the superficial body.

## FINAL DIAGNOSIS

Taken together, a final diagnosis of primary hepatic leiomyosarcoma was established.

## TREATMENT

TACE was performed 2 wk postoperatively. Superior mesenteric angiography showed no significant abnormality. Common hepatic angiography demonstrated a single mass with hypervascularity, tumor staining and dilated feeding arteries in the anterior segment of the right liver lobe (Figure 1B). A catheter was inserted into the feeding artery of the tumor, and the emulsion containing 50 mg epirubicin and Lipiodol (Lipiodol Ultra-Fluid; Guerbet Japan, Tokyo, Japan) was injected, followed by embolization with gelatin sponge particles (Gelpart; Nihonkayaku, Tokyo, Japan). The postoperative course was uneventful and the patient was discharged on day 7 after the operation. The patient underwent three courses of TACE, and the latest treatment was performed in October 2016. There were no newly emerging lesions observed in the liver (Figure 5B).

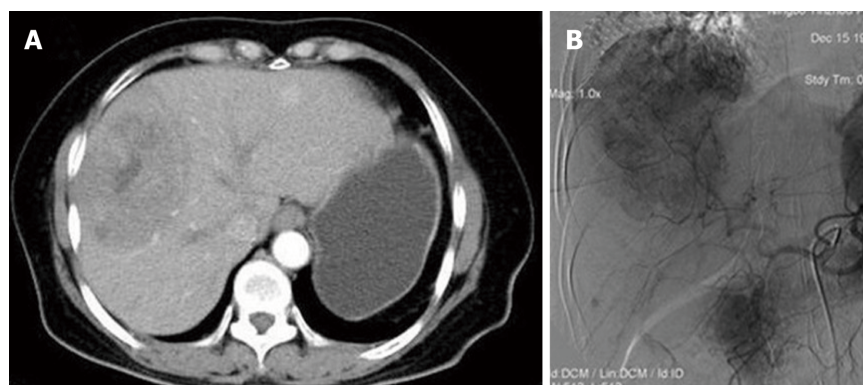
## OUTCOME AND FOLLOW-UP

Routine follow-up was continued in the hepatobiliary clinic. Contrast-enhanced CT in 2018 showed that the tumor had shrunk significantly and was associated with satisfactory lipiodol deposition (Figure 5A). The patient was followed up for 82 mo until now, and no progressive enlargement of the tumor or distal metastasis was observed.

## DISCUSSION

Primary hepatic leiomyosarcoma is extremely rare, and less than 40 such cases have been reported in the English-language literature. Primary hepatic sarcoma comprises only 1%-2% of all malignant hepatic tumors<sup>[7]</sup>, and leiomyosarcoma is particularly rare. Hepatic leiomyosarcoma may arise from intrahepatic vascular structures<sup>[8]</sup>, bile ducts<sup>[9]</sup> or ligamentum teres<sup>[10]</sup>, and is reported to be associated with AIDS<sup>[2]</sup>, Epstein-Barr virus<sup>[3]</sup>, Hodgkin's lymphoma<sup>[3]</sup>, immunosuppression after organ transplantation<sup>[4]</sup>, and HCV-related liver cirrhosis<sup>[5]</sup>. However, the precise steps leading to leiomyosarcoma have not been fully identified.

Leiomyosarcoma has no specific clinical manifestations. It is often asymptomatic until it becomes large and produces nonspecific symptoms, or is unexpectedly found during physical examination. Some patients may have a wide spectrum of symptoms, including abdominal pain, abdominal mass, weight loss, nausea, vomiting, anorexia,



**Figure 1** Imaging features before treatment. A: Computed tomography showed a mass with inhomogeneous density, mild delayed enhancement and central necrosis; B: Hepatic angiography demonstrated a single mass with hypervascular tumor staining and dilated feeding arteries.

abdominal distension, jaundice, and, rarely, acute intra-abdominal bleeding secondary to tumor rupture.

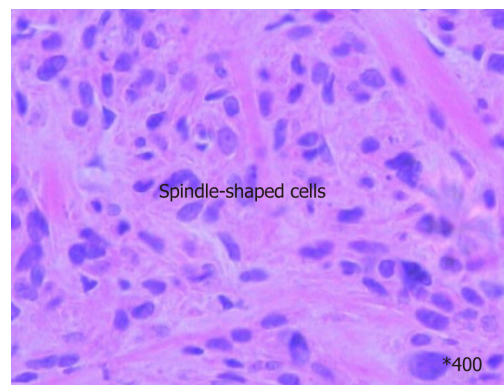
CT findings of primary hepatic leiomyosarcoma have been described as an enlarged, heterogeneous, hypodense mass with peripheral enhancement and evidence of central necrosis, whereas magnetic resonance imaging usually displays the homogeneous or heterogeneous hypointensity tumor on T1-weighted images and hyperintensity on T2-weighted images. Diagnosis of primary hepatic leiomyosarcoma is challenging due to the nonspecific nature of symptoms and lack of serological markers. The clinical manifestations and laboratory and imaging examinations have limited value for diagnosis and differential diagnosis. The definitive diagnosis of leiomyosarcoma mainly depends on pathological and immunohistochemical examinations. The pathological features of leiomyosarcoma include spindle-shaped cells, abundant cytoplasm, nuclear atypia, and presence of mitotic figures. Immunohistochemical staining of tumors is positive for vimentin, desmin and actin, and negative for  $\alpha$ -fetoprotein, CD34, CD117, cytokeratin, and hepatocytes.

Surgical resection is considered to be the only potentially curative treatment. Patients with primary hepatic leiomyosarcoma mostly have no history of hepatitis B and cirrhosis. Therefore, complete resection of the tumor, such as regular hepatic lobectomy, hemihepatectomy and trisegmentectomy, may achieve a satisfactory therapeutic outcome. Matthaei *et al*<sup>[11]</sup> reported three cases with > 10 yr survival after hepatectomy. Liver transplantation for primary hepatic leiomyosarcoma has remained controversial until now. The outcome for liver transplantation has varied among cases. Liang *et al*<sup>[12]</sup> described a patient with primary hepatic leiomyosarcoma who survived for 34 mo after undergoing liver transplantation. In contrast, Saintpaul *et al*<sup>[13]</sup> reported a case with only 15 d survival after surgery. The efficacy of chemotherapy and radiotherapy for primary hepatic leiomyosarcoma has not been confirmed. Adjuvant chemotherapy using doxorubicin and ifosfamide seems to slow progression and help to prolong survival after complete resection<sup>[14]</sup>.

Currently, no effective treatments for unresectable primary hepatic leiomyosarcoma have been reported. Fujita *et al*<sup>[5]</sup> reported a patient with metastatic leiomyosarcoma who just received palliative and conservative therapy and only survived for 3 mo after diagnosis. For our case, the patient underwent three courses of TACE with emulsion of epirubicin and Lipiodol, followed by embolization with gelatin sponge particles. Postoperative contrast-enhanced CT showed significant tumor shrinkage associated with satisfactory lipiodol deposition. Routine follow-up was continued in the hepatobiliary clinic and there were no newly emerging lesions observed in the liver. The case has been followed up for 82 mo until now, and no progressive enlargement of the tumor or distal metastasis has been observed. Primary hepatic leiomyosarcoma is a tumor with rich blood supply, and tumor cells are intolerant to ischemia. The embolic agents injected into the hepatic artery induced ischemic tumor necrosis. This may be the main mechanism underlying the effectiveness of drug-eluting bead TACE (commonly known as DEB-TACE). Otherwise, the epirubicin was used to suppress tumor progression<sup>[15]</sup>.

## CONCLUSION

We have described the first case of primary hepatic leiomyosarcoma successfully



**Figure 2** Histological examination showed a hepatic mass that consisted of spindle-shaped cells with mitotic figures and nuclear atypia.

treated by TACE, and demonstrated that TACE is safe and effective. Further studies are required to assess the therapeutic effect of TACE in combination with surgical resection in primary hepatic leiomyosarcoma.



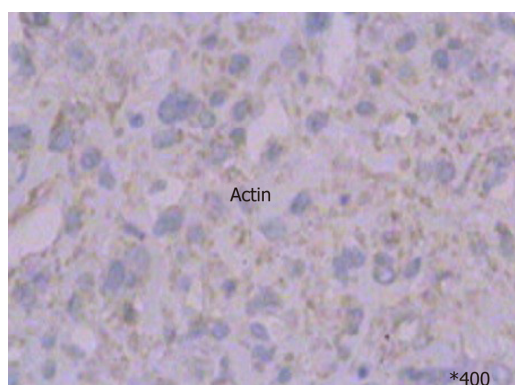


Figure 3 Immunohistochemical staining was positive for anti-smooth muscle actin.

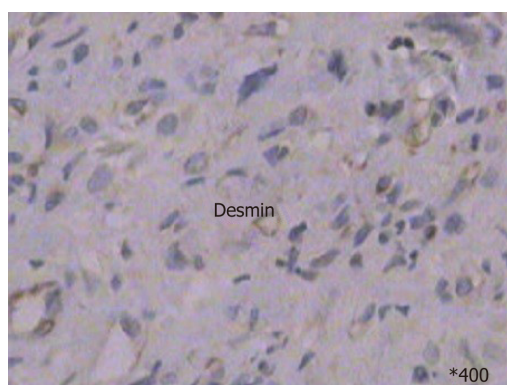


Figure 4 Immunohistochemical staining was positive for desmin.

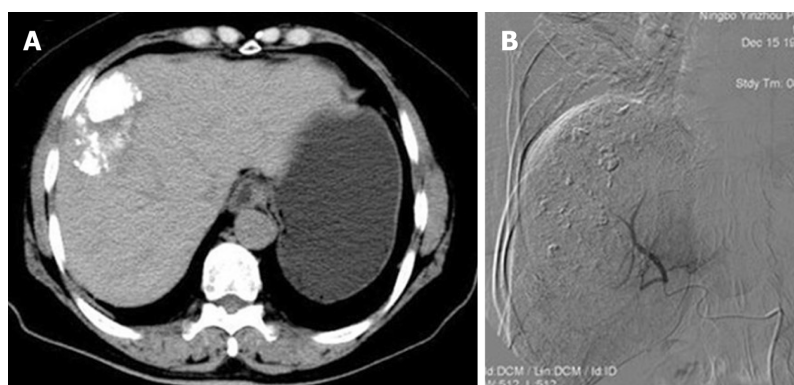


Figure 5 Imaging changes after treatment. A: Computed tomography showed significant tumor shrinkage associated with satisfactory lipiodol deposition; B: No newly emerging lesions and successful embolization of feeding arteries.

## REFERENCES

- 1 **Metta H**, Corti M, Trione N, Masini D, Monestes J, Rizzolo M, Carballido M. Primary hepatic leiomyosarcoma--a rare neoplasm in an adult patient with AIDS: second case report and literature review. *J Gastrointest Cancer* 2014; **45** Suppl 1: 36-39 [PMID: 23921603 DOI: 10.1007/s12029-013-9525-3]
- 2 **Ross JS**, Del Rosario A, Bui HX, Sonbati H, Solis O. Primary hepatic leiomyosarcoma in a child with the acquired immunodeficiency syndrome. *Hum Pathol* 1992; **23**: 69-72 [PMID: 1544673 DOI: 10.1016/0046-8177(92)90014-T]
- 3 **Brichard B**, Smets F, Sokal E, Clapuyt P, Vermeylen C, Cornu G, Rahier J, Otte JB. Unusual evolution of an Epstein-Barr virus-associated leiomyosarcoma occurring after liver transplantation. *Pediatr Transplant* 2001; **5**: 365-369 [PMID: 11560757 DOI: 10.1034/j.1399-3046.2001.00022.x]
- 4 **Giuliente F**, Sarno G, Ardito F, Pierconti F. Primary hepatic leiomyosarcoma in a young man after Hodgkin's disease: diagnostic pitfalls and therapeutic challenge. *Tumori* 2009; **95**: 374-377 [PMID: 19688980 DOI: 10.1177/030089160909500318]

- 5 **Fujita H**, Kiriya M, Kawamura T, Ii T, Takegawa S, Dohba S, Kojima Y, Yoshimura M, Kobayashi A, Ozaki S, Watanabe K. Primary hepatic leiomyosarcoma in a woman after renal transplantation: report of a case. *Surg Today* 2002; **32**: 446-449 [PMID: [12061699](#) DOI: [10.1007/s005950200073](#)]
- 6 **Tsuji M**, Takenaka R, Kashihara T, Hadama T, Terada N, Mori H. Primary hepatic leiomyosarcoma in a patient with hepatitis C virus-related liver cirrhosis. *Pathol Int* 2000; **50**: 41-47 [PMID: [10692176](#) DOI: [10.1046/j.1440-1827.2000.00999.x](#)]
- 7 **Maki HS**, Hubert BC, Sajjad SM, Kirchner JP, Kuehner ME. Primary hepatic leiomyosarcoma. *Arch Surg* 1987; **122**: 1193-1196 [PMID: [3310964](#) DOI: [10.1001/archsurg.1987.01400220103020](#)]
- 8 **Civardi G**, Cavanna L, Iovine E, Buscarini E, Vallisa D, Buscarini L. Diagnostic imaging of primary hepatic leiomyosarcoma: a case report. *Ital J Gastroenterol* 1996; **28**: 98-101 [PMID: [8782003](#)]
- 9 **Holloway H**, Walsh CB, Thomas R, Fielding J. Primary hepatic leiomyosarcoma. *J Clin Gastroenterol* 1996; **23**: 131-133 [PMID: [8877642](#) DOI: [10.1097/00004836-199609000-00014](#)]
- 10 **Yamaguchi J**, Azuma T, Fujioka H, Tanaka K, Furui J, Tomioka T, Kanematsu T. Leiomyosarcoma occurring in the ligamentum teres of the liver: a case report and a review of seven reported cases. *Hepatogastroenterology* 1996; **43**: 1051-1056 [PMID: [8884338](#) DOI: [10.1002/\(SICI\)1097-0347\(199607/08\)43:0.CO;2-V](#)]
- 11 **Matthaei H**, Krieg A, Schmelzle M, Boelke E, Poremba C, Rogiers X, Knoefel WT, Peiper M. Long-term survival after surgery for primary hepatic sarcoma in adults. *Arch Surg* 2009; **144**: 339-44; discussion 344 [PMID: [19380647](#) DOI: [10.1001/archsurg.2009.30](#)]
- 12 **Liang X**, Xiao-Min S, Jiang-Ping X, Jie-Yu Y, Xiao-Jun Z, Zhi-Ren F, Guo-Shan D, Rui-Dong L. Liver transplantation for primary hepatic leiomyosarcoma: a case report and review of the literatures. *Med Oncol* 2010; **27**: 1269-1272 [PMID: [19997990](#) DOI: [10.1007/s12032-009-9372-z](#)]
- 13 **Saint-Paul MC**, Gugenheim J, Hofman P, Arpurt JP, Fabiani P, Michiels JF, Fujita N, Goubeaux B, Loubière R, Delmont J. [Leiomyosarcoma of the liver: a case treated by transplantation]. *Gastroenterol Clin Biol* 1993; **17**: 218-222 [PMID: [8330697](#)]
- 14 **Shivathirthan N**, Kita J, Iso Y, Hachiya H, Kyunghwa P, Sawada T, Kubota K. Primary hepatic leiomyosarcoma: Case report and literature review. *World J Gastrointest Oncol* 2011; **3**: 148-152 [PMID: [22046492](#) DOI: [10.4251/wjgo.v3.i10.148](#)]
- 15 **Pasquali S**, Gronchi A. Neoadjuvant chemotherapy in soft tissue sarcomas: latest evidence and clinical implications. *Ther Adv Med Oncol* 2017; **9**: 415-429 [PMID: [28607580](#) DOI: [10.1177/1758834017705588](#)]

**P- Reviewer:** Farshadpour F, Hann HW, Pallav K, Tajiri K, Vaudo G

**S- Editor:** Yan JP **L- Editor:** Filipodia **E- Editor:** Tan WW



# Anterior cervical corpectomy decompression and fusion for cervical kyphosis in a girl with Ehlers-Danlos syndrome: A case report

Huang Fang, Peng-Fei Liu, Chang Ge, Wen-Zhi Zhang, Xi-Fu Shang, Cai-Liang Shen, Rui He

**ORCID number:** Huang Fang (0000-0001-5931-3886); Peng-Fei Liu (0000-0002-1202-4160); Chang Ge (0000-0002-8765-0991); Wen-Zhi Zhang (0000-0003-1412-3637); Xi-Fu Shang (0000-0002-7311-5103); Cai-Liang Shen (0000-0002-9835-6384); Rui He (0000-0003-0626-1618).

**Author contributions:** He R, Fang H, Liu PF, Ge C, Zhang WZ, Shang XF, and Shen CL examined the patient and collected the clinical data; He R performed the surgery and analyzed the radiologic imaging data; Fang H wrote the paper.

## Informed consent statement:

Informed written consent was obtained from the patient prior to all procedures described in the report as well as for the use of the patient's clinical information and images for published scientific works.

**Conflict-of-interest statement:** All of the authors report no relationships that could be construed as a conflict of interest.

## CARE Checklist (2016) statement:

The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklists (2016).

**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

**Huang Fang, Peng-Fei Liu, Chang Ge, Wen-Zhi Zhang, Xi-Fu Shang, Rui He,** Department of Spinal Surgery, The First Affiliated Hospital of USTC, Hefei 230036, Anhui Province, China

**Cai-Liang Shen,** Department of Spinal Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, Anhui Province, China

**Corresponding author:** Rui He, MD, Chief Doctor, Department of Spinal Surgery, The First Affiliated Hospital of USTC, No. 1, Swan Lake Road, Zhengwu District, Hefei 230000, Anhui Province, China. [herui2005208@sina.com](mailto:herui2005208@sina.com)

**Telephone:** 13966731701

## Abstract

### BACKGROUND

Spinal deformities in Ehlers-Danlos syndrome (EDS; type VI) are generally progressive and severe. Surgical treatment has been described for kyphoscoliosis in the thoracolumbar spine. However, there are few studies describing the consequences of an anterior approach in cervical kyphosis. An anterior approach may not be able to fully decompress the spinal canal and restore the normal curvature of the cervical spine. Therefore, the anterior approach for cervical kyphosis in young children is hard. We describe the first case in an EDS girl with cervical kyphosis who received satisfactory anterior cervical corpectomy decompression and fusion.

### CASE SUMMARY

The chief complaints of a 16-year-old girl with EDS were double upper limb weakness for 7 years and double lower limb walking instability for 2 years. Moreover, the imaging results revealed that the degree of kyphosis from cervical vertebra 2 to 4 accompanying with spinal cord compression was 30°. An anterior cervical corpectomy involving cervical vertebra 3 and a titanium mesh implant were performed with internal fixation. The results at 3 mo after surgery demonstrated that the anterior fusion was solid, and the kyphosis of the cervical spine was corrected. Additionally, the power of all four extremities was significantly improved.

### CONCLUSION

The incidence rate of cervical kyphosis in EDS is rare. The surgical treatment for these patients, especially an anterior approach, is challenging. Therefore, to develop safer and more effective strategies to treat cervical kyphosis in EDS, there is still much work to do.

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

**Received:** November 3, 2018

**Peer-review started:** November 5, 2018

**First decision:** January 5, 2019

**Revised:** January 21, 2019

**Accepted:** January 26, 2019

**Article in press:** January 26, 2019

**Published online:** February 26, 2019

**Key words:** Cervical kyphosis; Ehlers-Danlos syndrome; Anterior cervical corpectomy decompression and fusion; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Ehlers-Danlos syndrome (ESD) is a rare inherited and clinically heterogeneous group of connective tissue disorders. We report a case diagnosed with ESD that was treated by anterior cervical surgery. The clinical outcome is satisfactory.

**Citation:** Fang H, Liu PF, Ge C, Zhang WZ, Shang XF, Shen CL, He R. Anterior cervical corpectomy decompression and fusion for cervical kyphosis in a girl with Ehlers-Danlos syndrome: A case report. *World J Clin Cases* 2019; 7(4): 532-537

**URL:** <https://www.wjgnet.com/2307-8960/full/v7/i4/532.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.532>

## INTRODUCTION

Ehlers-Danlos syndrome (EDS) is an inherited and clinically heterogeneous group of connective tissue disorders<sup>[1,2]</sup>. EDS is characterized by joint laxity and skin fragility<sup>[3]</sup>. Based on the degree of the clinical symptoms, the different biochemical and genetical defects, and the mode of inheritance, six major subtypes of EDS have been recognized since 1998. Among these subtypes, EDS VI (known as the kyphoscoliotic type) is extremely rare. In EDS VI, the kyphoscoliosis is progressive and accompanied with joint hypermobility and abnormal scarring<sup>[4]</sup>. Overall, the incidence rate of EDS is lower than 1 in 100000 live births<sup>[5,6]</sup>. The procollagen-lysine, 2-oxoglutarate 5-dioxygenase (*PLOD1*) gene encodes lysyl hydroxylase (LH1) to catalyze the formation of hydroxylysine in collagen chains<sup>[7,8]</sup>. The kyphoscoliotic type with a deficiency of *PLOD1*/LH1 is of the most interest to a spinal specialist. Among the pediatric population diagnosed with EDS VI, the initial treatment focuses on physical therapy. However, the curve development of scoliosis is rapid. Therefore, once conservative treatment fails or neurologic defects occur because of spinal cord decompression, surgical treatment should be selected<sup>[9,10]</sup>. Many studies have demonstrated surgical treatment for adolescents with thoracolumbar kyphoscoliosis; however, reports involving cervical kyphoscoliosis are few<sup>[11]</sup>. Andrew J. Koberts reported a case where the anterior and posterior approaches were used to treat an infant with EDS type VI with symptomatic cervical kyphoscoliosis<sup>[12]</sup>. Herman and Sonntag reported 20 cases of cervical kyphosis treated by anterior cervical decompression, bone grafting, and anterior cervical plating. The average preoperative kyphosis was 38°, and the postoperative correction was 16°. In addition, 10% of patients had a complete remission of preoperative symptoms, and 55% had improved nerve function<sup>[13]</sup>.

As we all know, because of the severe deformity associated with a significant risk of complications, surgery for these patients is extremely difficult. Moreover, it is difficult to develop a surgical standard of treatment for these diseases, so the individual approach may be advisable. This report concerns a 16-year-old girl with EDS VI with symptomatic cervical kyphosis who underwent anterior cervical corpectomy decompression and fusion (ACCF).

## CASE PRESENTATION

### Chief complaints

The chief complaints of a 16-year-old female with EDS were double upper limb weakness for 7 years and double lower limb walking instability for 2 years.

### History of present illness

The patient developed limb weakness without obvious inducement for 7 years, and did not receive enough attention. She did not receive regular treatment else. And she gradually became unable to walk independently before one month.

### History of past illness

She denied any history of trauma or surgery and did not have hypertension, diabetes,

or cardiovascular and cerebrovascular diseases.

### **Personal and family history**

Her father and brother both had a blue tint to the sclera, joint hypermobility, abnormal scarring, and planovalgus feet.

### **Physical examination upon admission**

This patient was noted to have a blue tint to the sclera (Figure 1A), joint hypermobility (Figure 1B), abnormal scarring (Figure 1C), and planovalgus feet (Figure 1D). The power of main muscles in four extremities was approximately level III-IV.

### **Laboratory examinations**

Routine blood tests, routine urine tests and urinary sediment examination, routine fecal tests and occult blood test, blood biochemistry, immune indexes, and infection indexes were all normal.

### **Imaging examinations**

Imaging results (Figure 2) demonstrated that kyphosis at the C3 level was 30° and the cervical spinal cord was compressed from C2 to C4.

---

## **TREATMENT**

After satisfactory intratracheal anesthesia, the patient was intubated in an appropriate position to avoid excessive activity of the cervical spine. To expose enough field for surgery, a thin pillow was placed behind the shoulder to extend the neck. Through a right-side transverse incision, the cervical spine was exposed carefully. When the gap of C2/3 and C3/4 was certain, a spreader was mounted in the C2 and C4. The intervertebral discs of C3/C4 and C2/C3 were removed to expose the posterior edge of the vertebral body. The operator used rongeur and ultrathin pliers to remove the middle 2/3 of the C3 vertebral body, and the ossified posterior longitudinal ligament was bited. The cartilage surface of C2 and C4 was revealed; the length between C2 and C4 was measured. A titanium mesh was filled with bone from the C3 previously bited and then implanted into the gap between C2 and C4. Because the bone was enough, the iliac crest allograft was not selected. Finally, we used the appropriate titanium nails and plates for the anterior internal fixation. Moreover, during the operation, neuromonitoring was carried out, and no surgical complications were observed. Postoperatively, a satisfactory internal fixation was achieved, and a soft external neck brace was used.

---

## **OUTCOME AND FOLLOW-UP**

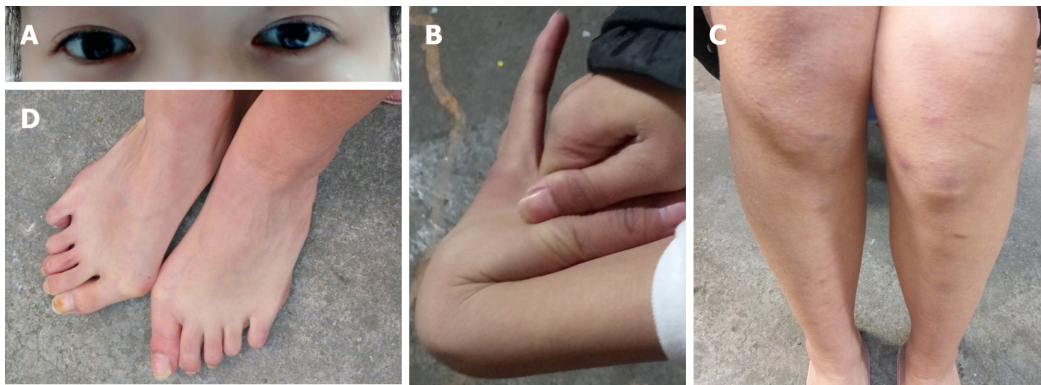
Radiographs 6 wk following anterior cervical corpectomy decompression and fusion showed that the preoperative kyphotic deformity in the cervical spine was significantly corrected (Figure 3). At the 3-mo follow-up, the patient showed significant neurological improvement, as she could walk independently and was able to perform fine movements with her double upper limbs. A computed tomography scan at 5 mo after surgery demonstrated a solid fusion of C2-4 with no displacement of internal hardware anteriorly (Figure 4). Moreover, the normal physiological curvature of the cervical spine was restored.

---

## **DISCUSSION**

Villefranche Nosology demonstrated the major and minor diagnostic standards for the kyphoscoliosis type of EDS in 1998<sup>[4]</sup>. The major standards include joint hypermobility, decrease of muscle tension, and progressive scoliosis. The secondary standards include abnormal scarring and tissue fragility, osteopenia, miceocorneae, and arterial burst. Hypermobility of the cervical spine may lead to craniocervical unbalance and progressive cervical kyphoscoliosis, usually accompanied by motor disfunction, radiculopathy, myelopathy, and sensory disorders<sup>[5]</sup>. Some studies demonstrated that congenital severe hypotonia was always accompanied by a decrease in muscle power. Patients diagnosed with EDS VI may suffer from myopathy<sup>[14]</sup>. Yiş *et al*<sup>[15]</sup> revealed that the patients diagnosed with EDS VI were homozygous for *PLOD1* mutations, leading to either loss of function of LH1 or to its





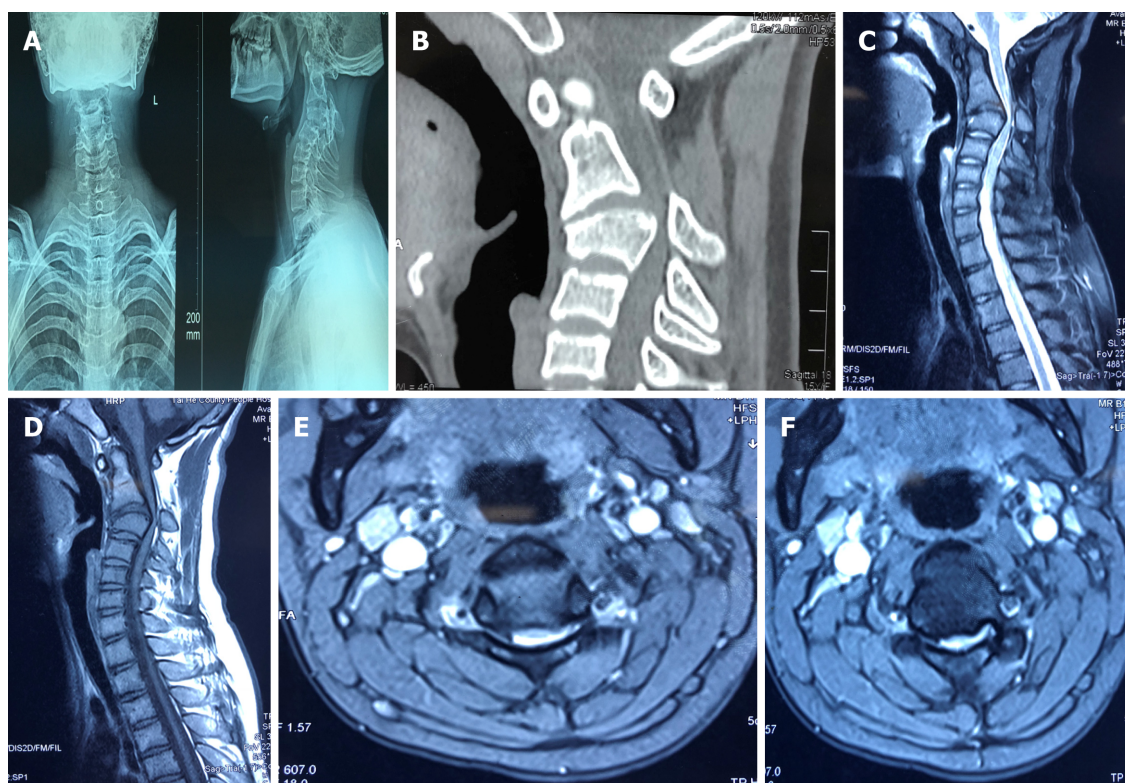
**Figure 1** Clinical features of the girl with Ehlers-Danlos syndrome VI. A: Blue tint to the sclera; B: Joint hypermobility; C: Abnormal scar; D: Planovalgus feet.

deficit. Surgery may be selected when neurological changes or deformity are progressive. However, there is no article describing only anterior surgical management for patients with cervical kyphosis diagnosed with EDS VI.

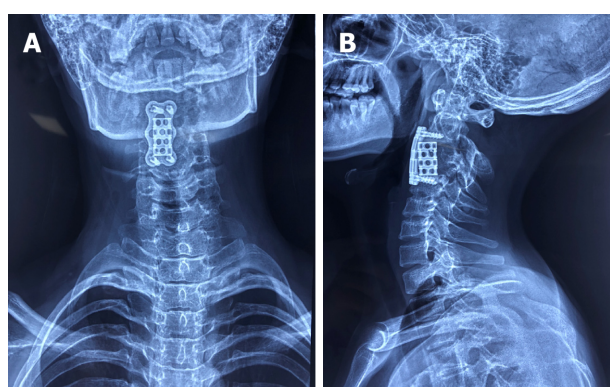
The purpose of surgery is to relieve the compression of the cervical spinal cord, restore the normal curvature of the cervical spine, and stabilize the cervical vertebrae. However, internal fixation in underage girls is challenging due to the anatomy and lack of devices designed specifically for this patient. Moreover, in such limited space, when adequate decompression of the cervical spinal cord and restoration of the physiological curvature are performed, the risk of iatrogenic injury to the spinal cord is high. To prevent screw backout, anterior cervical plates need to be used individually. The autograft and allograft have been found to lead to clinical success, but the first is usually recognized as the gold standard<sup>[16]</sup>. Specifically, the bited bone from C3 is enough for grafting in this case, so we did not use any other materials. The external immobilization is an important process after surgery. Halo fixation may be a good choice, but due to the complications of pin loosening and infection, its use is sometimes limited<sup>[17]</sup>. Due to the reliable internal fixation, in this case, we only used soft fixation for 4-6 wk. Lacking of convincing materials, the long-term effect of internal fixation in pediatric patients is not fully clear. There is still much work to be done. However, many studies have found that few children achieve continued growth after fusion of the subaxial spine<sup>[18]</sup>.

## CONCLUSION

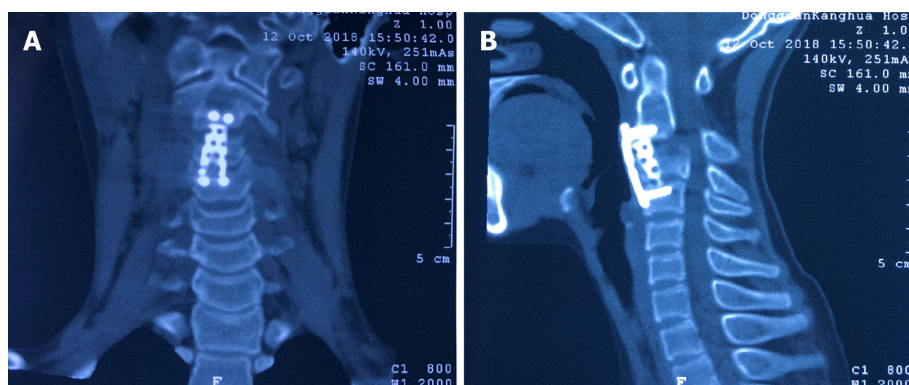
We report the first case of cervical kyphosis in a pediatric patient with EDS VI, who achieved a satisfactory clinical consequence only following ACCF. There is still some work needed to reveal the details about this case. In addition to some biomolecular and genetic experiments, we may need more time to follow her.



**Figure 2** Preoperative imaging of a 16-year-old-female with Ehlers-Danlos syndrome VI. X-ray (A) and computed tomography (B) images demonstrating high cervical kyphosis from C2 to C4. Magnetic resonance imaging (C-F) showing that the cervical ventral spinal cord was compressed by the C3 vertebra.



**Figure 3** Follow-up X-ray images. A: Antero-posterior view radiograph of the cervical spine; B: Lateral view radiograph of the cervical spine. Radiographs acquired 6 wk following anterior cervical corpectomy decompression and fusion showing that the preoperative kyphotic deformity in the cervical spine was significantly corrected.



**Figure 4** Follow-up computed tomography images. Computed tomography acquired 5 mo following surgery with images in the coronal (A) plane and sagittal reconstruction (B) showing maintenance of alignment and evidence of a solid arthrodesis.

## REFERENCES

- 1 **Giunta C**, Superti-Furga A, Spranger S, Cole WG, Steinmann B. Ehlers-Danlos syndrome type VII: clinical features and molecular defects. *J Bone Joint Surg Am* 1999; **81**: 225-238 [PMID: [10073586](#)]
- 2 **Beighton P**, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). *Am J Med Genet* 1998; **77**: 31-37 [PMID: [9557891](#)]
- 3 **Abdalla EM**, Rohrbach M, Bürer C, Kraenzlin M, El-Tayeby H, Elbelbesy MF, Nabil A, Giunta C. Kyphoscoliotic type of Ehlers-Danlos Syndrome (EDS VIA) in six Egyptian patients presenting with a homogeneous clinical phenotype. *Eur J Pediatr* 2015; **174**: 105-112 [PMID: [25277362](#) DOI: [10.1007/s00431-014-2429-9](#)]
- 4 **Giunta C**, Randolph A, Al-Gazali LI, Brunner HG, Kraenzlin ME, Steinmann B. Nevo syndrome is allelic to the kyphoscoliotic type of the Ehlers-Danlos syndrome (EDS VIA). *Am J Med Genet A* 2005; **133A**: 158-164 [PMID: [15666309](#) DOI: [10.1002/ajmg.a.30529](#)]
- 5 **Henderson FC Sr**, Austin C, Benzell E, Bolognese P, Ellenbogen R, Francomano CA, Ireton C, Klinge P, Koby M, Long D, Patel S, Singman EL, Voermans NC. Neurological and spinal manifestations of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet* 2017; **175**: 195-211 [PMID: [28220607](#) DOI: [10.1002/ajmg.c.31549](#)]
- 6 **Porter DA**, Glotzbecker MP, Timothy Hresko M, Hedequist DJ. Deep Surgical Site Infections Following Pediatric Cervical Spine Surgery. *J Pediatr Orthop* 2017; **37**: 553-556 [PMID: [27280897](#) DOI: [10.1097/BPO.0000000000000813](#)]
- 7 **Hyland J**, Ala-Kokko L, Royce P, Steinmann B, Kivirikko KI, Myllylä R. A homozygous stop codon in the lysyl hydroxylase gene in two siblings with Ehlers-Danlos syndrome type VI. *Nat Genet* 1992; **2**: 228-231 [PMID: [1345174](#) DOI: [10.1038/ng1192-228](#)]
- 8 **Kivirikko KI**, Pihlajaniemi T. Collagen hydroxylases and the protein disulfide isomerase subunit of prolyl 4-hydroxylases. *Adv Enzymol Relat Areas Mol Biol* 1998; **72**: 325-398 [PMID: [9559057](#)]
- 9 **McMaster MJ**. Spinal deformity in Ehlers-Danlos syndrome. Five patients treated by spinal fusion. *J Bone Joint Surg Br* 1994; **76**: 773-777 [PMID: [8083268](#)]
- 10 **Vogel LC**, Lubicky JP. Neurologic and vascular complications of scoliosis surgery in patients with Ehlers-Danlos syndrome. A case report. *Spine (Phila Pa 1976)* 1996; **21**: 2508-2514 [PMID: [8923641](#)]
- 11 **Akpınar S**, Gogus A, Talu U, Hamzaoglu A, Dikici F. Surgical management of the spinal deformity in Ehlers-Danlos syndrome type VI. *Eur Spine J* 2003; **12**: 135-140 [PMID: [12709851](#) DOI: [10.1007/s00586-002-0507-6](#)]
- 12 **Kobets AJ**, Komlos D, Houten JK. Congenital cervical kyphosis in an infant with Ehlers-Danlos syndrome. *Childs Nerv Syst* 2018; **34**: 1411-1415 [PMID: [29450629](#) DOI: [10.1007/s00381-018-3750-9](#)]
- 13 **Herman JM**, Sonntag VK. Cervical corpectomy and plate fixation for postlaminectomy kyphosis. *J Neurosurg* 1994; **80**: 963-970 [PMID: [8189276](#) DOI: [10.3171/jns.1994.80.6.0963](#)]
- 14 **Rohrbach M**, Vandersteen A, Yiş U, Serdaroglu G, Ataman E, Chopra M, Garcia S, Jones K, Kariminejad A, Kraenzlin M, Marcelis C, Baumgartner M, Giunta C. Phenotypic variability of the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA): clinical, molecular and biochemical delineation. *Orphanet J Rare Dis* 2011; **6**: 46 [PMID: [21699693](#) DOI: [10.1186/1750-1172-6-46](#)]
- 15 **Yiş U**, Dirik E, Chambaz C, Steinmann B, Giunta C. Differential diagnosis of muscular hypotonia in infants: the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI). *Neuromuscul Disord* 2008; **18**: 210-214 [PMID: [18155911](#) DOI: [10.1016/j.nmd.2007.11.006](#)]
- 16 **Betz RR**, Lavelle WF, Samdani AF. Bone grafting options in children. *Spine (Phila Pa 1976)* 2010; **35**: 1648-1654 [PMID: [20628337](#) DOI: [10.1097/BRS.0b013e3181ce8f4b](#)]
- 17 **Garfin SR**, Roux R, Botte MJ, Centeno R, Woo SL. Skull osteology as it affects halo pin placement in children. *J Pediatr Orthop* 1986; **6**: 434-436 [PMID: [3734066](#)]
- 18 **Anderson RC**, Ragel BT, Mocco J, Bohman LE, Brockmeyer DL. Selection of a rigid internal fixation construct for stabilization at the craniovertebral junction in pediatric patients. *J Neurosurg* 2007; **107**: 36-42 [PMID: [17644919](#) DOI: [10.3171/PED-07/07/036](#)]

P- Reviewer: Lakhdar F, Rodrigues-Lisoni FC

S- Editor: Ma YJ L- Editor: Wang TQ E- Editor: Tan WW





# Rhombencephalitis caused by *Listeria monocytogenes* with hydrocephalus and intracranial hemorrhage: A case report and review of the literature

Jing-Jing Liang, Xiao-Yan He, Hong Ye

**ORCID number:** Jing-Jing Liang (0000-0001-7239-9813); Xiao-Yan He (0000-0002-9446-5292); Hong Ye (0000-0003-4238-7883).

**Author contributions:** Ye H designed the report; He XY collected the patient's clinical data; Liang JJ analyzed the data and wrote the paper.

**Supported by** Young Teacher Foundation of Wuhan University, China, No. 2042017kf0142; and Guidance Fund of Renmin Hospital of Wuhan University, China, No. RMYD2018M19.

**Informed consent statement:**

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

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

**CARE Checklist (2016) statement:**

The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

**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,

**Jing-Jing Liang**, Department of Neurology, Wuhan University, Renmin Hospital, Wuhan 430060, Hubei Province, China

**Xiao-Yan He**, Department of Neurology, Shijiazhuang Second Hospital, Shijiazhuang 050200, Hebei Province, China

**Hong Ye**, Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing 100053, China

**Corresponding author:** Hong Ye, MD, Attending Doctor, Department of Neurology, Xuanwu Hospital, Capital Medical University, No. 45, Changchun Street, Beijing 100053, China.

[chris\\_yehong@126.com](mailto:chris_yehong@126.com)

**Telephone:** +86-10-83198899

**Fax:** +86-10-63131271

## Abstract

### BACKGROUND

*Listeria monocytogenes* (*L. monocytogenes*), a Gram-positive facultatively intracellular bacterium, is the causative agent of human listeriosis. *Listeria* infection is usually found in immunocompromised patients, including elderly people, pregnant women, and newborns, whereas it is rare in healthy people. *L. monocytogenes* may cause meningitis, meningoencephalitis, and some very rare and severe complications, such as hydrocephalus and intracranial hemorrhage, which cause high mortality and morbidity worldwide. Up to now, reports on hydrocephalus and intracranial hemorrhage due to *L. monocytogenes* are few.

### CASE SUMMARY

We herein report a case of rhombencephalitis caused by *L. monocytogenes* in a 29-year-old man. He was admitted to the hospital with a 2-d history of headache and fever. He consumed unpasteurized cooked beef two days before appearance. His medical history included type 2 diabetes mellitus, and contaminated beef intake 2 d before onset. Cerebrospinal fluid analysis revealed Gram-positive rod infection, and blood culture was positive for *L. monocytogenes*. Magnetic resonance imaging findings suggested rhombencephalitis and hydrocephalus. Treatment was started empirically and then modified according to the blood culture results. Repeated CT images were suggestive of intracranial hemorrhage. Although the patient underwent aggressive external ventricular drainage, he died of a continuing deterioration of intracranial conditions.

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

**Received:** October 31, 2018

**Peer-review started:** October 31, 2018

**First decision:** December 9, 2018

**Revised:** December 22, 2018

**Accepted:** January 3, 2019

**Article in press:** January 3, 2019

**Published online:** February 26, 2019

## CONCLUSION

Hydrocephalus, intracranial hemorrhage, and inappropriate antimicrobial treatment are the determinations of unfavorable outcomes.

**Key words:** Rhombencephalitis; *Listeria monocytogenes*; Central nervous system infections; Hydrocephalus; Intracranial hemorrhage; Case report

©The Author(s) 2019. Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** *Listeria monocytogenes* (*L. monocytogenes*) infection occurs predominantly in immunocompromised subjects. Various manifestations of listeriosis have been reported previously, but hydrocephalus and intracranial hemorrhage due to *Listeria* are rare. Hydrocephalus, intracranial hemorrhage, and inappropriate antimicrobial treatment are determinants of unfavorable outcomes. A pertinent literature review might contribute to improving our understanding of the pathogenesis and treatment of this disease.

**Citation:** Liang JJ, He XY, Ye H. Rhombencephalitis caused by *Listeria monocytogenes* with hydrocephalus and intracranial hemorrhage: A case report and review of the literature. *World J Clin Cases* 2019; 7(4): 538-547

**URL:** <https://www.wjnet.com/2307-8960/full/v7/i4/538.htm>

**DOI:** <https://dx.doi.org/10.12998/wjcc.v7.i4.538>

## INTRODUCTION

*Listeria monocytogenes* (*L. monocytogenes*) is one of the very few bacteria that can infect neurons to produce a serious and often fatal disease, with a mortality of 20%-50%<sup>[1-4]</sup>. *L. monocytogenes* infection occurs predominantly in the following populations: elderly people, pregnant women, newborns, and immunodeficient patients; patients with chronic liver disease, malignant hemopathies, and diabetes; patients on chronic hemodialysis; and, less frequently, healthy individuals<sup>[5,6]</sup>. The main routes of transmission are confirmed to be through the consumption of contaminated food and *via* vertical transmission from mother to child<sup>[7]</sup>. Penetration of the intestinal, blood-brain, blood-choroid, and fetoplacental barriers is one of the most important virulence factors of *L. monocytogenes*<sup>[8]</sup>. Therefore, the manifestations of listeriosis are varied, such as gastroenteritis, septicemia, meningitis, and other conditions.

Neurolisteriosis, a central nervous system (CNS) infection caused by *L. monocytogenes*, represents 5%-10% of listeriosis cases and is less common in the world, especially rhombencephalitis<sup>[9-11]</sup>. Hydrocephalus and intracranial hemorrhage are rare complications of listeriosis, occurring in 10%-15% and 3% of neurolisteriosis cases, respectively<sup>[12,13]</sup>. In this paper, we present a young patient with *L. monocytogenes* rhombencephalitis who presented with persistent alteration of consciousness, hydrocephalus, and intracranial hemorrhage. This case is rare due to the occurrence of hydrocephalus and intracranial hemorrhage. Cases published between 1985 and 2018 that are related to *Listeria* hydrocephalus are reviewed in Tables 1 and 2.

## CASE PRESENTATION

### Chief complaints

A 29-year-old Chinese man was admitted to the hospital with a 2-d history of intermittent fevers of up to 39 °C, and forehead headache without nausea.

### History of present illness

Two days prior to onset, he had consumed unpasteurized cooked beef that was stored in the refrigerator for a few days.

### History of past illness

His medical history included type 2 diabetes mellitus, which was poorly controlled, fatty liver, smoking, and drinking.

### Personal and family history



**Table 1** Characteristics of four cases of neonatal listeriosis with hydrocephalus published between 1989 and 2018

Ref.	Gestation/gender	CT/sonography on admission	Time to diagnosis of hydrocephalus	Other complications	Intervention	Outcome
Svare <i>et al</i> <sup>[22]</sup> , 1991	NB, 32 W/M	Not done	6 wk	Epilepsy, intraventricular hemorrhage	VPD	Moderately retarded with reduced muscular tone at 3 mo
Madlinger <i>et al</i> <sup>[25]</sup> , 1998	NB, 34 W/F	Sonography, normal	9 wk	None	VAD, VDP	Recovery
Chan <i>et al</i> <sup>[26]</sup> , 2007	NB, 31 W/M	Not done	10 d	Subtle seizure	VPD	Significant improvement
Laciar <i>et al</i> <sup>[27]</sup> , 2011	NB, 37 W/F	Not done	3 d	None	EVD	NA

NB: Newborn; W: Weeks; M: Male; F: Female; EVD: External ventricular drain; VPD: Ventriculo-peritoneal drain; VAD: Ventriculo-atrial drain; NA: Not available.

He denied a family history of hypertension and stroke.

### **Physical examination upon admission**

The physical examination was unremarkable, except for nuchal rigidity.

### **Laboratory examinations**

The blood laboratory findings showed that glucose, C-reactive protein, and erythrocyte sedimentation rate were high, while white blood cells (WBCs), red blood cells, hemoglobin, urea, creatinine, serum minerals, and autoimmune antibodies were normal. The first lumbar puncture on admission revealed a turbid cerebrospinal fluid (CSF) with 2090 leukocytes/mm<sup>3</sup> (30% neutrophils, 70% monocytes), 233.85 mg/dL protein, 1.4 mmol/L glucose (serum glucose 9 mmol/L), and pressure > 33 cmH<sub>2</sub>O. CSF Gram stain showed Gram-positive rods and was negative for fungi and acid-fast bacilli (Table 3). On the 8<sup>th</sup> day, the blood cultures yielded *L. monocytogenes*, which was susceptible to ampicillin, erythromycin, meropenem, and penicillin but resistant to sulfamethoxazole. CSF and urine cultures were negative. Repeated CSF examination on the 14<sup>th</sup> and 28<sup>th</sup> day showed a greater decrease in WBCs and protein (Table 3).

### **Imaging examinations**

The initial brain CT was unremarkable, and chest CT showed bilateral bronchopneumonia. On the 4<sup>th</sup> day of admission, magnetic resonance imaging (MRI) of the brain showed an abnormally high T2 flow attenuated inversion recovery (FLAIR) signal in the right pons and prominent temporal horns with enlargement of the ventricles (Figure 1). On the 14<sup>th</sup> day, brain CT showed hemorrhage of the right pons and hydrocephalus (bilateral lateral ventricular and the third ventricle hydrocephalus) (Figure 2). The 3<sup>rd</sup> cerebral CT was performed on the day after extraventricular drainage, revealed significant dilatation of fourth ventricle, and no remission in lateral ventricles (Figure 3). The 4<sup>th</sup> brain CT on the 29<sup>th</sup> day showed rehaemorrhage of the lateral ventricle and a larger ventricular system (Figure 4).

## **FINAL DIAGNOSIS**

The patient was finally diagnosed with *Listeria* rhombencephalitis, hydrocephalus, and intracranial hemorrhage.

## **TREATMENT**

Although empiric antibiotic therapy for bacterial meningitis (Ceftriaxone 2 g, every 12 h for 2 d, followed by meropenem 1 g, every 8 h for 2 d) and all other supportive symptomatic treatments were administered after performing blood cultures, the patient developed new symptoms with fever, sinus tachycardia, tachypnea, confusion [Glasgow Coma Scale (GCS) score 12/15], bilateral horizontal nystagmus, bilateral abducens nerve palsy, dysarthria, and weakness of all four limbs. He was transferred to the intensive care unit (ICU) on the 5<sup>th</sup> day. On the 8<sup>th</sup> day, he went into coma (GCS score 5/15), and was intubated and ventilated without autonomous respiration. According to blood cultures, new antibiotic therapy with ampicillin, etimicin and

**Table 2** Characteristics of 18 cases of non-perinatal listeriosis with hydrocephalus published between 1989 and 2018

Ref.	Age/gender	Immune-competent	CT on admission	Time to diagnosis of hydrocephalus	Other complications	Intervention	Outcome
Ulloa-Gutierrez <i>et al</i> <sup>[6]</sup> , 2004	10 Y/M	Yes	Not done	8 d	None	VPD	Recovery
Ulloa-Gutierrez <i>et al</i> <sup>[6]</sup> , 2004	3½ Y/M	Yes	Normal	5 d	None	VPD	Died
Ulloa-Gutierrez <i>et al</i> <sup>[6]</sup> , 2004	6½ Y/M	Yes	Not done	5 d	None	VPD	Died
Kasanmoentalib <i>et al</i> <sup>[12]</sup> , 2010	57 Y/M	Yes	Not done	5 d	Tracheoesophageal fistula	EVD	Severe cognitive slowness
Ito <i>et al</i> <sup>[13]</sup> , 2007	62 Y/M	No	Normal	14 d	Ventriculitis	EVD	Improvement, remained confused and disoriented
McCaffrey <i>et al</i> <sup>[20]</sup> , 2012	57 Y/M	No	Yes, hydrocephalus	1 d	Ventriculitis	EVD	NA
Dhiwakar <i>et al</i> <sup>[21]</sup> , 2007	40 Y/F	No	Not done	2 mo	Seizures, ventriculitis, basal arachnoiditis, cerebellar tonsillar herniation	VPD, VAD	Near-complete recovery
Chan <i>et al</i> <sup>[26]</sup> , 2001	42 Y/M	Yes	Yes, hydrocephalus	4 d	Subdural collection, extensive; cerebritis and ventriculitis	EVD	Died
Lee <i>et al</i> <sup>[28]</sup> , 2010	7 Y/F	Yes	Not done	10 d	None	EVD, VPD	Recovery
Platnaris <i>et al</i> <sup>[29]</sup> , 2009	7 M/M	Yes	Normal	10 d	Seizures	EVD	Normal development having achieved skills according to his age at 22 mo of age
Papandreou <i>et al</i> <sup>[30]</sup> , 2015	3 Y/F	Yes	Normal	8 d	Cerebellar tonsillar herniation, ventriculitis, and AIDP	EVD, VPD	Incomplete recovery
Gaini <i>et al</i> <sup>[31]</sup> , 2015	74 Y/M	Yes	Normal	6 d	Brain abscess	EVD	Severe sequelae, died 1 yr later
Ruggieri <i>et al</i> <sup>[32]</sup> , 2014	27 Y/F	Yes	Yes, hindbrain multifocal lesions	9 d	None	EVD	Only a motor deficit of the right arm remained
Cunha <i>et al</i> <sup>[33]</sup> , 2004	50 Y/M	Yes	Yes, hydrocephalus	1 d	None	No	Died 10 d after admission
Frat <i>et al</i> <sup>[34]</sup> , 2001	72 Y/F	Yes	Normal	12 d	Seizures	VPD	Recovery after 5 mo of rehabilitative care
Raps <i>et al</i> <sup>[35]</sup> , 1989	47 Y/F	No	Not done	Several weeks	Cervical cord compression	EVD, VPD	No significant deficit 6 mo later
Yang <i>et al</i> <sup>[36]</sup> , 2006	42 Y/M	No	Normal	9 d	Seizures	ORI	Recovery
Rana <i>et al</i> <sup>[37]</sup> , 2014	75 Y/M	No	Not done	5 d	None	VPD	Gradual recovery

M: Male; Y: Years; M: Months; F: Female; EVD: External ventricular drain; VPD: Ventriculo-peritoneal drain; VAD: Ventriculo-atrial drain; ORI: Ommaya reservoir implantation; AIDP: Acute inflammatory demyelinating polyneuropathy; NA: Not available.

meropenem was administered. On the 12<sup>th</sup> day, etimicin was discontinued as he became afebrile. We performed an extraventricular drainage to relieve hydrocephalus on the 22<sup>nd</sup> day (Figure 3). On the 29<sup>th</sup> day, because of rehaemorrhagia of the lateral ventricle, his condition rapidly deteriorated (GCS score 3/15), with anisocoria (left pupil 4 mm and right pupil 2 mm).

**Table 3 Cerebrospinal fluid analysis across disease duration**

CSF test	On the 2 <sup>nd</sup> d	On the 14 <sup>th</sup> d	On the 28 <sup>th</sup> d <sup>1</sup>
Color	Turbid	Turbid	Mildly turbid
Pressure(cm H <sub>2</sub> O)	> 33	12.5	NA
Erythrocyte count (/mm <sup>3</sup> )	0	13198	3313
WBC count (/mm <sup>3</sup> )	2090	782	85
WBC distribution (L/N)	70/30	3/97	17/68
Protein (mg/dL)	233.85	441	119
CSF glucose (mmol/L)	1.40	5.42	5.60
Plasma glucose (mmol/L)	9.00	11.05	10.0
Gram stain	Gram-positive rods	Normal	Normal

<sup>1</sup>CSF from brain ventricular draining. WBC: White blood cell; CSF: Cerebrospinal fluid; L: Lymphocytes; N: Neutrophils; NA: No data available.

## OUTCOME AND FOLLOW-UP

The patient died on the 31<sup>st</sup> day. Autopsy could not be performed.

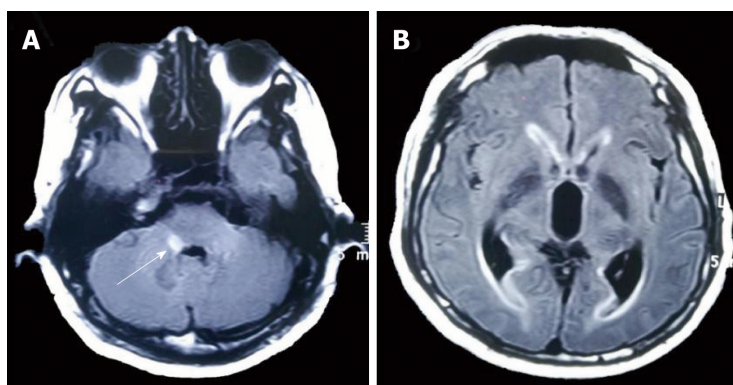
## DISCUSSION

Although *L. monocytogenes* has been reported to be the third most common cause of community-acquired bacterial meningitis, following pneumococcal and meningococcal meningitis in adults, its occurrence is relatively rare, accounting for only 5% of encephalitis cases in metropolitan France<sup>[14]</sup>. *Listeria* has an important impact on public health, with high hospitalization and mortality rates despite antibiotic treatment<sup>[15]</sup>. As listeriosis is not incorporated into the national monitoring system for cases, epidemiological data on *Listeria* are scarce in China<sup>[7,16]</sup>. In a study published in 2013, Feng *et al*<sup>[16]</sup> reviewed 147 cases of listeriosis in China from 1964 to 2010, with neurolisteriosis accounting for 31% of cases. The overall case-fatality rate was 26%, highest among neonatal cases (46%) and lowest among pregnant cases (4%)<sup>[16]</sup>. In a study conducted by Wang *et al*<sup>[7]</sup>, 38 cases of listeriosis, including 5 neonatal, 8 maternal, and 25 nonmaternal cases, were reviewed in China between 1999 and 2011, and the case-fatality rates for neonatal, maternal, and nonmaternal cases were 20%, 0%, and 26%, respectively<sup>[7]</sup>.

CSF and blood cultures are the most specific for diagnosis. Early diagnosis of neurolisteriosis is difficult not only because the presentation of CSF is similar to the manifestations of other bacterial encephalitis and meningitis (pleocytosis, hyperproteinorrachia, and hypoglycorrhachia) but also because approximately 50% of CSF Gram stains are negative<sup>[17]</sup>. Jubelt *et al*<sup>[18]</sup> reported that approximately three-quarters of patients have CSF pleocytosis, with approximately equal percentages of mononuclear and polymorphonuclear cells. In our case, there was an initial predominance of lymphocytic cells, which then turned to mononuclear cell predominance; this change might be related to pathological processes and the application of antibiotics. *Listeria* is usually revealed first on blood cultures, which are positive in 62% of encephalitis cases<sup>[19]</sup>. Therefore, early before antibiotic administration, repeated blood and CSF cultures are necessary and helpful for early and differential diagnoses.

*L. monocytogenes* infection most frequently presents as acute bacterial meningitis, less commonly as meningoencephalitis, and least commonly as rhombencephalitis, accounting for approximately 10% of neurolisteriosis cases<sup>[12,13]</sup>. Although the exact mechanism of rhombencephalitis remains poorly understood, *L. monocytogenes* has a well-known predilection for the brainstem. Karlsson *et al*<sup>[9]</sup> reviewed 120 patients with *Listeria* rhombencephalitis and suggested that *L. monocytogenes* enters the cerebellopontine angle through the trigeminal nerve in a subset of patients, invading the brainstem *via* the sensory trigeminal nuclei. As MRI is superior to CT in detecting subtentorial abnormal lesions, it has become more helpful for diagnosing rhombencephalitis, which has a high signal on T2-FLAIR sequences.

*L. monocytogenes* complications, such as acute hydrocephalus, hemorrhage, brain abscess, spine abscess, cerebritis, and ventriculitis, can develop, and the mortality associated with these complications is significantly high. Hydrocephalus is most common in tuberculous encephalitis but rare in listeriosis, with an approximate 3%



**Figure 1** Axial brain T2-FLAIR magnetic resonance imaging shows a hyperintense lesion of the right pons (A, white arrow), and prominent temporal horns with enlargement of ventricles (B) on the 4<sup>th</sup> d of administration.

incidence of *L. monocytogenes* meningoencephalitis in adults<sup>[13]</sup>. The exact mechanism of hydrocephalus remains unclear. The development of meningitis-associated hydrocephalus may be due to several mechanisms, such as a high level of CSF protein, impaired CSF absorption due to the obliteration of the subarachnoid space by meningeal exudates, and/or blockade of the CSF pathway by leptomeningeal inflammation<sup>[20]</sup>.

Retrospective analysis of hydrocephalus due to listeriosis is scarce at present, and most of the literature consists of case reports. The time to onset of hydrocephalus varies greatly, ranging from 1 d to 9 wk<sup>[20,21]</sup>. Ventricular drainage may not be an effective way to relieve hydrocephalus and improve survival<sup>[12,14]</sup>. A study from the Netherlands reviewed 26 hydrocephalus cases in 577 bacterial meningitis patients (4.5%), including four cases of *L. monocytogenes* (15%), all of whom underwent placement of an external ventricular drain catheter<sup>[14]</sup>. None of these patients improved clinically after catheter placement, and all had poor outcomes for hydrocephalus, with three deaths (75%) and one case of serious sequela (25%), thus indicating that patients with hydrocephalus were at a high risk for unfavorable outcomes and that hydrocephalus was an independent risk factor for death<sup>[14]</sup>. In our case, the patient underwent ventricular drainage, but a continuous improvement in cognitive function was not obvious.

Another rare complication of *Listeria* meningitis is intracranial hemorrhage, which is also one of the determinants of unfavorable outcomes<sup>[2]</sup>. Most reported cases of intracranial hemorrhage occur in infants and young children, while the condition is quite rare in adults. Svarea *et al*<sup>[22]</sup> reported a case of maternal listeriosis resulting in preterm delivery and intraventricular hemorrhage, which was diagnosed by an ultrasound scan. In a prospective study of 860 episodes with bacterial meningitis in the Netherlands, 24 (2.79%) were diagnosed with intracranial hemorrhage, with *S. pneumoniae* accounting for 67% and *L. monocytogenes* accounting for 4%<sup>[2]</sup>. The underlying pathophysiology of intraventricular hemorrhage in *L. monocytogenes* infection is still unknown and may be related to dysregulation of both the coagulation and fibrinolytic pathways and to vascular endothelial cell swelling and activation<sup>[2]</sup>.

An empirical therapy for bacterial meningitis, generally third-generation cephalosporins, is always applied at an early stage when bacterial meningitis is suspected. However, this treatment option does not cover *L. monocytogenes*. Former publications have demonstrated that inappropriate empirical antibiotic therapy leads to unfavorable outcomes<sup>[23]</sup>. Therefore, it is very important to adjust the appropriate antibiotic therapy as soon as possible once *Listeria* is highly suspected or confirmed.

*Listeria* is known to be difficult to treat, not only because *L. monocytogenes* has an intracellular life cycle but also because only a few antibiotics demonstrate activity against *Listeria*<sup>[24]</sup>. Due to the lack of multicenter clinical controlled studies, the optimal antibiotic regimen and duration for neurolisteriosis have not been definitively defined. However, amoxicillin, ampicillin, and penicillin G are generally considered effective regimens in the treatment of listeriosis<sup>[24]</sup>. The addition of aminoglycosides (such as gentamicin) could be considered a treatment regimen for *L. monocytogenes* meningitis, but its use remains controversial due to the occurrence of kidney damage<sup>[24]</sup>. The drugs should be applied at high doses, and the duration of this treatment should be extended to 21 d or longer, until complete eradication, to prevent relapse<sup>[24]</sup>. Furthermore, cotrimoxazole, rifampin, meropenem, linezolid, tetracyclines, and moxifloxacin should also be considered active against *Listeria*<sup>[23]</sup>. In our patient, the combination of ampicillin, etimicin, and meropenem was used for *Listeria*, and it

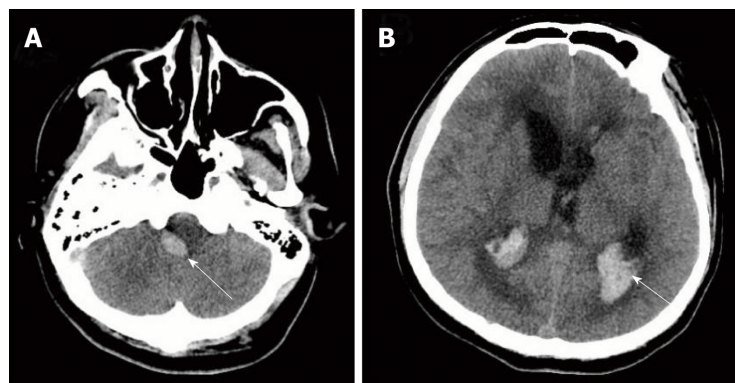


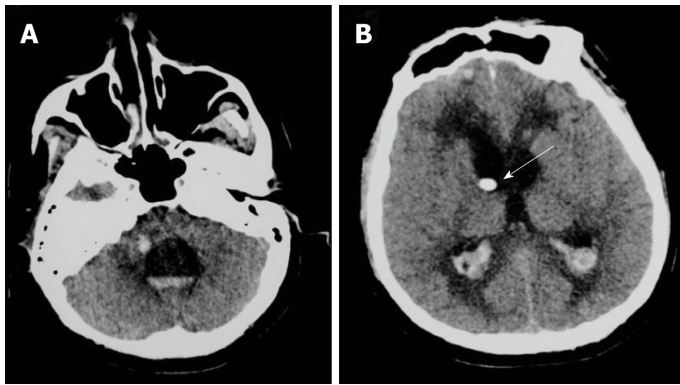
Figure 2 Axial brain computed tomography shows hemorrhage of the right pons (A, white arrow), and gross hydrocephalus and hemorrhage (B, white arrow) on the 14<sup>th</sup> d of administration.

was proven effective by repeated CSF examinations (Table 3).

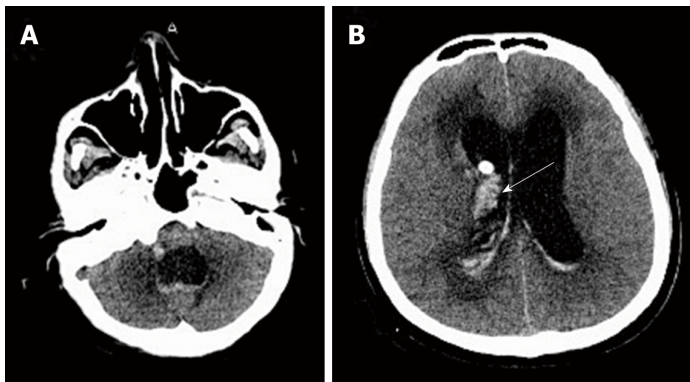
## CONCLUSION

We report a case of acute hydrocephalus and intracranial hemorrhage due to complications from *L. monocytogenes* rhombencephalitis. The pathogenesis of complications has been reviewed. *L. monocytogenes* may be prone to entering the brainstem through the trigeminal nerve; hydrocephalus may be close with a high level of CSF protein and impaired CSF absorption and circulation; the occurrence of intracranial hemorrhage may be related to dysregulation of both the coagulation and fibrinolytic pathways and to vascular endothelial cell swelling and activation. Hydrocephalus, intracranial hemorrhage, and inappropriate antimicrobial treatment are the determinations of unfavorable outcomes.





**Figure 3** Axial brain computed tomography shows no improvement of hydrocephalus in the lateral ventricle on the 22<sup>nd</sup> d of administration (A and B). The ventriculostomy tube is also shown (B, white arrow).



**Figure 4** Axial brain computed tomography shows rehaemorrhage of the lateral ventricle and a larger ventricular system (A and B) on the 29<sup>th</sup> d of administration.

## REFERENCES

- 1 Cossart P. Interactions of the bacterial pathogen *Listeria monocytogenes* with mammalian cells: bacterial factors, cellular ligands, and signaling. *Folia Microbiol (Praha)* 1998; **43**: 291-303 [PMID: [9717257](#) DOI: [10.1007/BF02818615](#)]
- 2 Mook-Kanamori BB, Fritz D, Brouwer MC, van der Ende A, van de Beek D. Intracerebral hemorrhages in adults with community associated bacterial meningitis in adults: should we reconsider anticoagulant therapy? *PLoS One* 2012; **7**: e45271 [PMID: [23028898](#) DOI: [10.1371/journal.pone.0045271](#)]
- 3 Goulenok T, Buzel   R, Duval X, Bruneel F, Stahl JP, Fantin B. Management of adult infectious encephalitis in metropolitan France. *Med Mal Infect* 2017; **47**: 206-220 [PMID: [28336304](#) DOI: [10.1016/j.medmal.2017.01.006](#)]
- 4 Dons L, Jin Y, Kristensson K, Rottenberg ME. Axonal transport of *Listeria monocytogenes* and nerve-cell-induced bacterial killing. *J Neurosci Res* 2007; **85**: 2529-2537 [PMID: [17387705](#) DOI: [10.1002/jnr.21256](#)]
- 5 Ben Shimol S, Einhorn M, Greenberg D. *Listeria* meningitis and ventriculitis in an immunocompetent child: case report and literature review. *Infection* 2012; **40**: 207-211 [PMID: [21877182](#) DOI: [10.1007/s15010-011-0177-6](#)]
- 6 Ulloa-Gutierrez R, Avila-Ag  ero ML, Huertas E. Fulminant *Listeria monocytogenes* meningitis complicated with acute hydrocephalus in healthy children beyond the newborn period. *Pediatr Emerg Care* 2004; **20**: 233-237 [PMID: [15057178](#) DOI: [10.1097/01.pec.0000121243.99242.a9](#)]
- 7 Wang HL, Ghanem KG, Wang P, Yang S, Li TS. Listeriosis at a tertiary care hospital in Beijing, China: high prevalence of nonclustered healthcare-associated cases among adult patients. *Clin Infect Dis* 2013; **56**: 666-676 [PMID: [23175565](#) DOI: [10.1093/cid/cis943](#)]
- 8 Disson O, Lecuit M. Targeting of the central nervous system by *Listeria monocytogenes*. *Virulence* 2012; **3**: 213-221 [PMID: [22460636](#) DOI: [10.4161/viru.19586](#)]
- 9 Karlsson WK, Harboe ZB, Roed C, Monrad JB, Lindelof M, Larsen VA, Kondziella D. Early trigeminal nerve involvement in *Listeria monocytogenes* rhombencephalitis: case series and systematic review. *J Neurol* 2017; **264**: 1875-1884 [PMID: [28730571](#) DOI: [10.1007/s00415-017-8572-2](#)]
- 10 Antal EA, Dietrichs E, L  berg EM, Melby KK, Maehlen J. Brain stem encephalitis in listeriosis. *Scand J Infect Dis* 2005; **37**: 190-194 [PMID: [15849051](#) DOI: [10.1080/00365540410020938](#)]
- 11 Charlier C, Perrodeau   , Leclercq A, Cazenave B, Pilmis B, Henry B, Lopes A, Maury MM, Moura A, Goffinet F, Dieye HB, Thouvenot P, Ungeheuer MN, Tourdjman M, Goulet V, de Valk H, Lortholary O, Ravaud P, Lecuit M; MONALISA study group. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. *Lancet Infect Dis* 2017; **17**: 510-519 [PMID: [28139432](#) DOI: [10.1016/S1473-3099\(16\)30521-7](#)]

- 12 **Kasanmoentalib ES**, Brouwer MC, van der Ende A, van de Beek D. Hydrocephalus in adults with community-acquired bacterial meningitis. *Neurology* 2010; **75**: 918-923 [PMID: [20820003](#) DOI: [10.1212/WNL.0b013e3181f11e10](#)]
- 13 **Ito H**, Kobayashi S, Iino M, Kamei T, Takanashi Y. *Listeria monocytogenes* meningoencephalitis presenting with hydrocephalus and ventriculitis. *Intern Med* 2008; **47**: 323-324 [PMID: [18277040](#) DOI: [10.2169/internalmedicine.47.0509](#)]
- 14 **Pelegriñ I**, Moragas M, Suárez C, Ribera A, Verdager R, Martínez-Yelamos S, Rubio-Borrego F, Ariza J, Viladrich PF, Cabellos C. *Listeria monocytogenes* meningoencephalitis in adults: analysis of factors related to unfavourable outcome. *Infection* 2014; **42**: 817-827 [PMID: [24902522](#) DOI: [10.1007/s15010-014-0636-y](#)]
- 15 **Mailles A**, Lecuit M, Goulet V, Leclercq A, Stahl JP, National Study on Listeriosis Encephalitis Steering Committee. *Listeria monocytogenes* encephalitis in France. *Med Mal Infect* 2011; **41**: 594-601 [PMID: [22036519](#) DOI: [10.1016/j.medmal.2011.07.009](#)]
- 16 **Feng Y**, Wu S, Varma JK, Klena JD, Angulo FJ, Ran L. Systematic review of human listeriosis in China, 1964-2010. *Trop Med Int Health* 2013; **18**: 1248-1256 [PMID: [24016031](#) DOI: [10.1111/tmi.12173](#)]
- 17 **Cunha BA**, Fatehpuria R, Eisenstein LE. *Listeria monocytogenes* encephalitis mimicking Herpes Simplex virus encephalitis: the differential diagnostic importance of cerebrospinal fluid lactic acid levels. *Heart Lung* 2007; **36**: 226-231 [PMID: [17509430](#) DOI: [10.1016/j.hrtlng.2007.01.001](#)]
- 18 **Jubelt B**, Mihai C, Li TM, Veerapaneni P. Rhombencephalitis / brainstem encephalitis. *Curr Neurol Neurosci Rep* 2011; **11**: 543-552 [PMID: [21956758](#) DOI: [10.1007/s11910-011-0228-5](#)]
- 19 **Reynaud L**, Graf M, Gentile I, Cerini R, Ciampi R, Noce S, Borrelli F, Viola C, Gentile F, Briganti F, Borgia G. A rare case of brainstem encephalitis by *Listeria monocytogenes* with isolated mesencephalic localization. Case report and review. *Diagn Microbiol Infect Dis* 2007; **58**: 121-123 [PMID: [17408902](#) DOI: [10.1016/j.diagmicrobio.2006.11.001](#)]
- 20 **McCaffrey LM**, Petelin A, Cunha BA. Systemic lupus erythematosus (SLE) cerebritis versus *Listeria monocytogenes* meningoencephalitis in a patient with systemic lupus erythematosus on chronic corticosteroid therapy: the diagnostic importance of cerebrospinal fluid (CSF) of lactic acid levels. *Heart Lung* 2012; **41**: 394-397 [PMID: [22177759](#) DOI: [10.1016/j.hrtlng.2011.09.002](#)]
- 21 **Dhiwakar M**, Basu S, Ramaswamy R, Mallucci C. Neurolisteriosis causing hydrocephalus, trapped fourth ventricle, hindbrain herniation and syringomyelia. *Br J Neurosurg* 2004; **18**: 367-370 [PMID: [15702836](#) DOI: [10.1080/02688690400005081](#)]
- 22 **Svare J**, Andersen LF, Langhoff-Roos J, Madsen H, Bruun B. Maternal-fetal listeriosis: 2 case reports. *Gynecol Obstet Invest* 1991; **31**: 179-181 [PMID: [2071059](#) DOI: [10.1159/000293148](#)]
- 23 **Hof H**. An update on the medical management of listeriosis. *Expert Opin Pharmacother* 2004; **5**: 1727-1735 [PMID: [15264987](#) DOI: [10.1517/14656566.5.8.1727](#)]
- 24 **van de Beek D**, Cabellos C, Dzupova O, Esposito S, Klein M, Kloek AT, Leib SL, Mourvillier B, Ostergaard C, Pagliano P, Pfister HW, Read RC, Sipahi OR, Brouwer MC; ESCMID Study Group for Infections of the Brain (ESGIB). ESCMID guideline: diagnosis and treatment of acute bacterial meningitis. *Clin Microbiol Infect* 2016; **22** Suppl 3: S37-S62 [PMID: [27062097](#) DOI: [10.1016/j.cmi.2016.01.007](#)]
- 25 **Madlinger AG**, Krauss JK. Intramedullary brain stem cyst and trapped IV ventricle after infection with *Listeria monocytogenes*. *Childs Nerv Syst* 1998; **14**: 747-750 [PMID: [9881629](#) DOI: [10.1007/s003810050309](#)]
- 26 **Chan YC**, Ho KH, Tambyah PA, Lee KH, Ong BK. *Listeria* meningoencephalitis: two cases and a review of the literature. *Ann Acad Med Singapore* 2001; **30**: 659-663 [PMID: [11817300](#) DOI: [10.1097/00000441-200111000-00012](#)]
- 27 **Laciar AL**, Vaca Ruiz ML, Le Monnier A. Neonatal *Listeria*-meningitis in San Luis, Argentina: a three-case report. *Rev Argent Microbiol* 2011; **43**: 45-47 [PMID: [21491067](#) DOI: [10.1590/S0325-75412011000100010](#)]
- 28 **Lee JE**, Cho WK, Nam CH, Jung MH, Kang JH, Suh BK. A case of meningoencephalitis caused by *Listeria monocytogenes* in a healthy child. *Korean J Pediatr* 2010; **53**: 653-656 [PMID: [21189933](#) DOI: [10.3345/kjp.2010.53.5.653](#)]
- 29 **Platnaris A**, Hatzimichael A, Ktenidou-Kartali S, Kontoyiannides K, Kollios K, Anagnostopoulos J, Roilides E. A case of *Listeria* meningoencephalitis complicated by hydrocephalus in an immunocompetent infant. *Eur J Pediatr* 2009; **168**: 343-346 [PMID: [18463893](#) DOI: [10.1007/s00431-008-0739-5](#)]
- 30 **Papandreou A**, Hedra-Fernandez A, Kaliakatsos M, Chong WK, Bhate S. An unusual presentation of paediatric *Listeria* meningitis with selective spinal grey matter involvement and acute demyelinating polyneuropathy. *Eur J Paediatr Neurol* 2016; **20**: 196-199 [PMID: [26371981](#) DOI: [10.1016/j.ejpn.2015.08.004](#)]
- 31 **Gaini S**, Karlsen GH, Nandy A, Madsen H, Christiansen DH, Á Borg S. Culture Negative *Listeria monocytogenes* Meningitis Resulting in Hydrocephalus and Severe Neurological Sequelae in a Previously Healthy Immunocompetent Man with Penicillin Allergy. *Case Rep Neurol Med* 2015; **2015**: 248302 [PMID: [26697245](#) DOI: [10.1155/2015/248302](#)]
- 32 **Ruggieri F**, Cerri M, Beretta L. Infective rhomboencephalitis and inverted Takotsubo: neurogenic-stunned myocardium or myocarditis? *Am J Emerg Med* 2014; **32**: 191.e1-191.e3 [PMID: [24079984](#) DOI: [10.1016/j.ajem.2013.08.047](#)]
- 33 **Cunha BA**, Filozov A, Remé P. *Listeria monocytogenes* encephalitis mimicking West Nile encephalitis. *Heart Lung* 2004; **33**: 61-64 [PMID: [14983142](#) DOI: [10.1016/j.hrtlng.2003.07.001](#)]
- 34 **Frat JP**, Veinstein A, Wager M, Burucoa C, Robert R. Reversible acute hydrocephalus complicating *Listeria monocytogenes* meningitis. *Eur J Clin Microbiol Infect Dis* 2001; **20**: 512-514 [PMID: [11561813](#) DOI: [10.1007/PL00011296](#)]
- 35 **Raps EC**, Gutmann DH, Brorson JR, O'Connor M, Hurtig HI. Symptomatic hydrocephalus and reversible spinal cord compression in *Listeria monocytogenes* meningitis. Case report. *J Neurosurg* 1989; **71**: 620-622 [PMID: [2795184](#) DOI: [10.3171/jns.1989.71.4.0620](#)]
- 36 **Yang CC**, Yeh CH, Tsai TC, Yu WL. Acute symptomatic hydrocephalus in *Listeria monocytogenes* meningitis. *J Microbiol Immunol Infect* 2006; **39**: 255-258 [PMID: [16783458](#)]
- 37 **Rana F**, Shaikh MM, Bowles J. *Listeria* meningitis and resultant symptomatic hydrocephalus complicating infliximab treatment for ulcerative colitis. *JRSM Open* 2014; **5**: 2054270414522223 [PMID: [25057381](#) DOI: [10.1177/2054270414522223](#)]

P- Reviewer: Bhalla AS, Chowdhury FH, Vaudo G

S- Editor: Ji FF L- Editor: Wang TQ E- Editor: Tan WW





Published By Baishideng Publishing Group Inc  
7901 Stoneridge Drive, Suite 501, Pleasanton, CA 94588, USA  
Telephone: +1-925-2238242  
Fax: +1-925-2238243  
E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)  
Help Desk: <https://www.f6publishing.com/helpdesk>  
<https://www.wjgnet.com>

