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## Proteomic insights on the metabolism in inflammatory bowel disease

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### Abstract

Inflammatory bowel diseases (IBD) are chronic and relapsing inflammatory conditions of the gut that include Crohn's disease and ulcerative colitis. The pathogenesis of IBD is not completely unraveled, IBD are multi-factorial diseases with reported alterations in the gut microbiota, activation of different immune cell types, changes in the vascular endothelium, and alterations in the tight junctions' structure of the colonic epithelial cells. Proteomics represents a useful tool to enhance our biological understanding and to discover biomarkers in blood and intestinal specimens. It is expected to provide reproducible and quantitative data that can support clinical assessments and help clinicians in the diagnosis and treatment of IBD. Sometimes a differential diagnosis of Crohn's disease and ulcerative colitis and the prediction of treatment response can be deduced by finding meaningful biomarkers. Although some non-invasive biomarkers have been described, none can be considered as the "gold standard" for IBD diagnosis, disease activity and therapy outcome. For these reason new studies have proposed an "IBD signature", which consists in a panel of biomarkers used to assess IBD. The above described approach characterizes "omics" and in this review we will focus on proteomics.

**Key words:** Proteomics; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Proteins; Biomarkers discovery

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**Core tip:** Patients' heterogeneity is a hallmark for inflammatory bowel diseases (IBD). Some patients present limited bowel involvement and a mild course of the disease, others develop very extensive, aggressive disease and variable response to therapy. In IBD, there is a great need of patient stratification and of new biomarkers as part of a personalized medicine approach to patient care. Biological therapies are more and more widely used for IBD patients, because of their efficacy in patient's refractory to other drugs; still, biological treatments fail in 20%-40% of patients and, to date, no reliable clinical or molecular predictor of response to biological therapeutic strategy has been described. This review aims to collect the "omics" approach for research of serological biomarkers of diagnosis, response to specific biological therapies in the IBD field.

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## INFLAMMATORY BOWEL DISEASE

Ulcerative colitis (UC) and Crohn's disease (CD) are the two main inflammatory bowel diseases (IBD)<sup>[1-4]</sup>. Despite some shared characteristics, they can be distinguished by differences in genetic predisposition, risk factors, and clinical, endoscopic and histological features. CD is characterized by diffuse chronic inflammation throughout the gastrointestinal tract, in a non-continuous manner<sup>[5]</sup>; UC presents with inflammation limited to the colon, spreading continuously from the rectum<sup>[6]</sup>. The pathogenesis of IBD is at present not completely unraveled; however, genetically susceptible individuals seem to have a dysregulated mucosal immune response to the commensal gut flora, which results in bowel inflammation<sup>[7]</sup>. IBD are multi-factorial diseases<sup>[8]</sup> with reported alterations in the gut microbiota<sup>[9-12]</sup>, activation of different immune cell types<sup>[13-15]</sup>, changes in the vascular endothelium<sup>[16,17]</sup>, and alterations in the tight junctions structure of colon epithelial cells<sup>[18-20]</sup>.

Nowadays, the diagnostic and prognostic tools for IBD and the outcome of therapy are largely based on evaluation of clinical symptoms in combination with endoscopy, histology, radiology and non-specific biomarkers from serum or stools<sup>[21]</sup>.

## BIOMARKERS IN INFLAMMATORY BOWEL DISEASE

Inflammation in IBD is characterized by the increased levels of some molecules extensively validated but not all included in the laboratory routine. Some of them are related to the inflammatory acute-phase response, coagulation and fibrinolysis (fibrinogen, plasminogen, complement components), proteinase inhibitors ( $\alpha$ 1-antitrypsin and  $\alpha$ 1-anti-chymotrypsin), transport proteins (haptoglobin and ceruloplasmin) and other serum proteins<sup>[22]</sup> and cytokines<sup>[23]</sup>. Elevated platelet and white blood cell counts may also indicate inflammation but they cannot be considered strictly related to bowel inflammation<sup>[23]</sup>. C-reactive protein (CRP), anti-*Saccharomyces cerevisiae* (ASCA) and anti-neutrophil cytoplasmic antibody are the most widely used indicators. CRP has a short reaction time (6-10 h) and it is useful for the identification of inflammatory disease activity especially in CD, but not in UC<sup>[24]</sup>. CRP has low specificity enabling to differentiate between CD, UC and infectious colitis<sup>[21]</sup>, and also the 25% of IBD patients with demonstrable disease activity have CRP levels above the normal threshold<sup>[22]</sup>. ASCA is an antibody used for the identification of CD patients who are often positive (39%-79% of CD patients, 5%-15% UC patients)<sup>[25,26]</sup>, however a large part of healthy controls is also positive (14%-18%) to this antibody, limiting the diagnostic value of its detection<sup>[27]</sup>. anti-neutrophil cytoplasmic antibodies are antibodies found in immune-mediated pathologies, such as rheumatoid arthritis and Wegener's granulomatosis<sup>[28]</sup>, and have shown a different staining pattern in UC and CD patients<sup>[29-31]</sup>, but as for ASCA 32% of healthy population is also positive to them<sup>[32]</sup>.

Another explored field in the search for IBD biomarkers is the analysis of stool proteins, which can be dysregulated or abnormally present in patients. Stool markers have the advantage of increased specificity for bowel inflammation and reflect any mucosal barrier disruption. Fecal markers can be useful to diagnose CD, where

inflammation is patchy and is possibly missed at endoscopy<sup>[33]</sup>. Fecal calprotectin (FC) accounts for up to 5% of the neutrophil granulocytes' protein content with chemotactic and antimicrobial activities. It is stable in stool for more than a week and can resist to bacterial degradation<sup>[34]</sup>. FC is not a specific marker for IBD, but it correlates with increased disease activity at least in adults<sup>[35]</sup>, but not in pediatric patients where was found with high sensitivity (98%), but only modest specificity (68%)<sup>[36]</sup>. Disease location should also be taken into account when interpreting FC levels. Patients with ileal CD may have ulcers even in the absence of markedly elevated FC levels. Consequently, the cut-off values for ileal CD may differ from those with ileocolic disease<sup>[37,38]</sup>. A study conducted by De Vos *et al*<sup>[39]</sup> has demonstrated that Calprotectin decreased 2 wk after Infliximab administration predicts remission in anti-TNF-naïve patients with UC. The increase of FC can also be a suitable marker for the identification of relapse, given the fact that the levels are increased as early as 6 mo before clinical and endoscopic relapse<sup>[40]</sup>. Lactoferrin is an iron-binding protein expressed by neutrophils during inflammation and represents a defense against infection as part of the innate immune system<sup>[41,42]</sup>. As a biomarker, Lactoferrin can distinguish IBD from Irritable Bowel Syndrome, but not between CD and UC<sup>[27]</sup>.

Although many non-invasive biomarkers have been described, none can be considered as the "gold standard" for IBD diagnosis, disease activity and therapy outcome. A single ideal biomarker is very unlikely to be found. As for other pathologies as pancreatic cancer<sup>[43-46]</sup>, non-small cell lung cancer<sup>[47]</sup> and colorectal cancer<sup>[48]</sup> new studies have proposed the idea of a "Biomarker Signature", which consists in a panel of biomarkers used to assess various pathological conditions and response to therapy<sup>[49]</sup>, and which is applicable also to IBD diagnosis and prognosis. **Table 1** summarizes the biomarkers commonly used for IBD.

## PROTEOMIC APPROACH TO INFLAMMATORY BOWEL DISEASE RESEARCH

Proteomics comprehensively studies the protein composition and abundance in a given cell population and its changes under biological perturbations<sup>[50,51]</sup>. The proteome may be considered the signature of a disease, in fact it is the result of the interactions between the genetic background and environmental factors<sup>[49]</sup>. The novel proteomic technologies now facilitate the analysis of transcriptome variations also in the IBD context and have already provided with new candidate biomarkers<sup>[52]</sup>. They help to investigate the inflammatory response, epithelial barrier function and gut microbiome from different biological samples, *i.e.*, serum/blood, colon samples and feces. The proteomic strategies can be bottom-up and top-down (**Figure 1**). In the bottom-up approach, purified proteins or complex protein mixtures are subjected to proteolytic cleavage and the peptide products are analyzed by mass spectrometry (MS). Conversely, the top-down approach is based either on the analysis of intact proteins followed by the direct measurement of fragment ions by MS or on the isolation of the protein by gel-based separative methods, protein gel elution and MS analysis.

### *Proteomics in the study of IBD pathogenesis*

By LC-MS analysis of colon mucosal biopsies from 10 patients with UC, Bennike *et al*<sup>[53]</sup> identified 5711 quantifiable proteins classified by biological function, sub-cellular location and molecular function. Forty-six proteins demonstrated statistically significant changes in mean abundance between UC biopsies and control biopsies; among those proteins, the one with the largest mean fold abundance change was lactotransferrin, which was 219 times more abundant in the UC group. The relative abundance of lactotransferrin also correlated to the severity of tissue inflammation in the patients with UC, as determined by the colon inflammation grade score based on histology. Good correlation was found between the colon inflammation grade score and the relative abundance of lactotransferrin in the tissue (0.82)<sup>[53]</sup>. Eleven of the 46 proteins identified in the UC biopsies are present in neutrophils and are associated with the formation of neutrophil extra-cellular traps which are released from neutrophils in response to inflammatory stimuli<sup>[54,55]</sup>, and are a sign of chronic inflammation even in the absence of visible inflammation<sup>[54,56,57]</sup>.

Proteomics has also investigated IBD-related immune-cell responses. Riaz *et al*<sup>[58]</sup> compared Th1 and Th17 clones isolated from the intestinal mucosa of CD patients by means of label-free quantitative mass-spectrometry analysis, which led to the identification of a total number of 7401 unique protein groups and demonstrated that 334 proteins were differentially expressed. The largest differences between the two phenotypes were observed in such proteins with cytotoxic function as Granzyme B

**Table 1 Biomarkers in inflammatory bowel disease**

Marker	Setting	Diagnostic accuracy	Ref.
C-Reactive Protein (CRP)	Serum	Higher in CD <i>vs</i> UC 25% IBD patients have levels above normal	Henriksen <i>et al</i> <sup>[24]</sup> , 2008 Vermeire <i>et al</i> <sup>[22]</sup> , 2004
Anti- <i>Saccharomyces cerevisiae</i> Antibodies (ASCA)	Serum	39%-79% CD positive 5%-15% UC positive	Peyrin-Biroulet <i>et al</i> <sup>[25]</sup> , 2015; Reumaux <i>et al</i> <sup>[26]</sup> , 2004
Anti-neutrophil cytoplasmic antibodies (ANCA)	Serum	14%-18% HC positive Different pattern in CD and UC	Bennike <i>et al</i> <sup>[27]</sup> , 2014 Peeters <i>et al</i> <sup>[31]</sup> , 2001; Peyrin-Biroulet <i>et al</i> <sup>[30]</sup> , 2007; Reumaux <i>et al</i> <sup>[29]</sup> , 2003
Calprotectin	Colorectal mucus	32% HC positive Higher in IBD <i>vs</i> HC Higher in UC <i>vs</i> CD	Bernstein <i>et al</i> <sup>[32]</sup> , 2011 Loktionov <i>et al</i> <sup>[79]</sup> , 2016
Calgranulin C (S100A12)		Higher in UC <i>vs</i> CD	
Eosinophil-derived neurotoxin (EDN)		Higher in IBD <i>vs</i> HC Higher in UC <i>vs</i> CD	
Fecal calprotectin (FC)	Stool	It correlates with disease activity in adults	Gisbert <i>et al</i> <sup>[35]</sup> , 2009
Lactoferrin	Stool	It distinguishes IBD from IBS	Bennike <i>et al</i> <sup>[27]</sup> , 2014

CD: Crohn's disease; UC: Ulcerative colitis; HC: Healthy controls; IBS: Irritable bowel syndrome; IBD: Inflammatory bowel disease.

and perforin, which are lower in Th17 cells than in Th1 cells. Other differentially expressed proteins with higher expression in the Th1 clones included several transcription factors with both known and unknown functions in CD4<sup>+</sup> T-cells. The most striking differences at quantitative analysis are about CD4<sup>+</sup> T cells with Th1 phenotype having a much higher degree of cytotoxic features as compared with Th1/Th17 phenotype<sup>[58]</sup>.

As discussed above, the disruption of the intestinal barrier is a typical event in IBD pathogenesis. The intestinal epithelium is the largest surface exposed and coming into contact with the external environment. The intestinal epithelial cells (IECs) are the main component of the physical barrier between the luminal micro-environment and the host and act as the host's first line-of-defense against potential harmful stimulants. They also represent the innate immunity within the gut mucosa<sup>[59]</sup>. Normally, the intestinal epithelium is covered by a single layer of IECs, which are characterized by a fast renewal rate, and act as a protective barrier against luminal antigens, but this barrier can be damaged, thus promoting a state of chronic inflammation due to mucosal immune cell infiltration, as is typically observed in IBD patients<sup>[59]</sup>. The molecular changes in the epithelial layer, extra-cellular matrix and junction proteins in inflamed and non-inflamed intestinal tissue have been only partially addressed to date. In 2012 Poulsen *et al*<sup>[60]</sup> analyzed the proteomic profiles of whole colonic biopsies from UC patients using 2D-gel electrophoresis and MALDI-TOF MS for the identification of differently expressed protein spots. Forty-three proteins were identified differentially expressed between UC inflamed and non-inflamed tissue, including proteins involved in the energy metabolism and in oxidative stress<sup>[60]</sup>. Proteomic studies on isolated IECs obtained from surgical specimens of full-thickness colonic tissues from UC-, CD-affected patients and non-inflamed controls were analyzed by gel-based stable-isotope label technologies (2D-DIGE and ICPL LC-MS/MS) and immunoblot assay to evaluate any proteome changes. Moreover, the results were verified on a group of patients not participating in the discovery phase<sup>[61]</sup>. The differential proteomic approaches have revealed changes in several molecules involved in extracellular matrix, mechano-transduction, metabolic rewiring and autophagy that characterize quiescent UC and quiescent CD epithelial cells and they may help understanding the complex mechanisms associated to IBD. UC patients are characterized by cytoskeletal rearrangement and increased level of specific enzymes that contribute to cell homeostasis, enabling cells to cope with energy requirements and macro-autophagy. CD patients are characterized by metabolic rewiring to sustain the cell metabolism, whereas autophagy and cell renewal are blunted<sup>[61-63]</sup>. **Table 2** provides a summary of the proteins and pathways identified by the proteomic approach as involved in IBD pathogenesis.

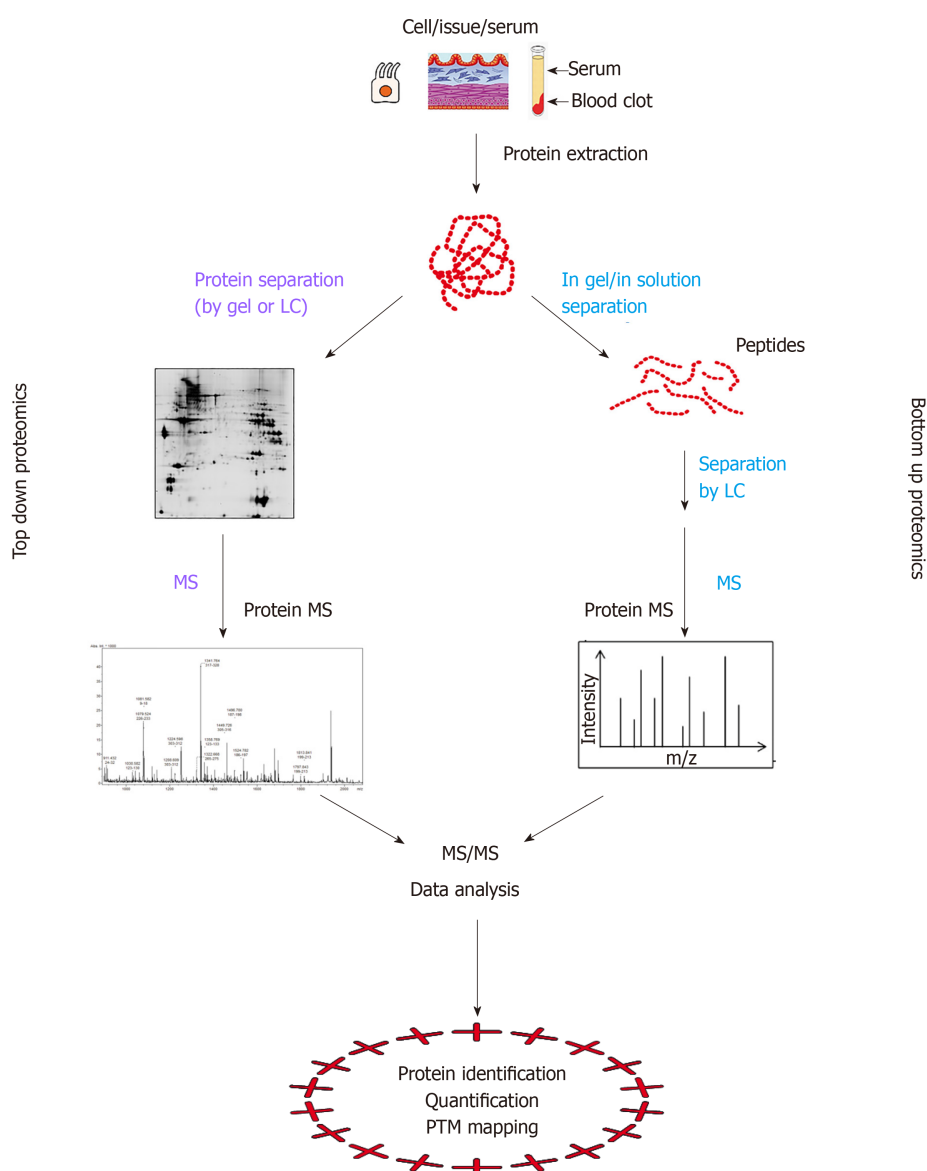


Figure 1 Schematic illustration of the difference between protein-based top-down and peptide-based bottom-up proteomics.

### Proteomics for the identification of novel biomarkers

Another approach is the identification of biomarkers useful for the diagnosis, treatment selection and response monitoring. A recent study focused on diagnosis has identified a serological panel which demonstrates transmural intestinal injury and is able to indicate complications in CD patients with 70% sensitivity and 72.5% specificity<sup>[64]</sup>. The increase of circulating epithelial component proteins may be a sign of transmural intestinal injury and stricturing or fistulizing intestinal complications. The serum biomarkers for the stratification of IBD patients are unable to distinguish between CD and UC<sup>[65]</sup>, while the proteomic profiles of colon biopsies can identify a more precise signature of these diseases<sup>[61,66,67]</sup>. In 2016 Starr *et al*<sup>[68]</sup> established two candidate biomarker panels: A 5-protein panel to discriminate IBD from control patients and a 12-protein panel to distinguish CD from UC patients in children with a new IBD diagnosis.

Proteomics has been applied to the identification of treatment-response biomarkers. The anti-TNF drug called Infliximab is one of the most used drugs in IBD, but the factors predicting the response and the molecular mechanisms that are related to the loss of response or non-responsiveness are not completely known. Meuwis *et al*<sup>[69]</sup> have analyzed sera from responder and non-responder CD patients at baseline and then comparing sera throughout the induction period (week 4 for non-fistulizing and week 10 for fistulizing patients) and have shown that the platelet aggregation Factor 4 (PF4) was higher in non-responders than responders to Infliximab therapy (both before and after treatment). PF4 is considered as an acute-phase reactant because its level increases with general inflammation, as already observed in the plasma of CD

**Table 2** Proteomics in inflammatory bowel disease pathogenesis

Protein	Setting	Diagnostic accuracy	Ref.
Lactotransferrin	UC <i>vs</i> HC biopsies	It correlates to the colon inflammation grade score	Bennike <i>et al</i> <sup>[53]</sup> , 2015
Neutrophil extracellular traps (NETs)		Sign of chronic inflammation	
Granzyme B and Perforin	CD Th1 and Th17 clones from intestinal mucosa	Higher in Th1 <i>vs</i> Th17	Riaz <i>et al</i> <sup>[58]</sup> , 2016
RORC and FOXP3			
Glycerol-3-phosphatedehydrogenase	UC biopsies inflamed <i>vs</i> non-inflamed	Higher in inflamed <i>vs</i> non-inflamed tissue	Poulsen <i>et al</i> <sup>[60]</sup> , 2012
Alphaenolase		Lower in inflamed <i>vs</i> non-inflamed tissue	
Keratins 10, 14, 19	UC intestinal epithelial cells	Higher in QUC <i>vs</i> HC	Moriggi <i>et al</i> <sup>[61]</sup> , 2017
Keratin 8		Lower in QUC <i>vs</i> HC	
Tricarboxylic acid cycle enzymes			
Oxidative phosphorylation enzymes			
Vinculin and $\alpha$ -tubulin			
Keratin 8, 18	CD intestinal epithelial cells	Lower in QCD <i>vs</i> HC	
Heat shock cognate-70 (HSC70)			
Vinculin and $\alpha$ -tubulin		Higher in QCD <i>vs</i> HC	
Fibrinopeptide A (FPA)	CD serum	Higher in CD <i>vs</i> HC	Nanni <i>et al</i> <sup>[62]</sup> , 2009
Complement 3 protein (C3)			
Apolipoprotein A-IV			
Apolipoprotein E		Lower in CD <i>vs</i> HC	
L-lactate dehydrogenase	IBD and HC intestinal epithelial cells	Higher in IBD <i>vs</i> HC; Higher in CD <i>vs</i> UC	Shkoda <i>et al</i> <sup>[63]</sup> , 2007
Carbonyl reductase			
Keratin 19			
Rho-GDI dissociation inhibitor $\alpha$			
Annexin 2	UC intestinal epithelial cells	Higher in UC <i>vs</i> HC	
Programmed cell death protein 8 (PDCD8)			

IBD: Inflammatory bowel disease; CD: Crohn's disease; UC: Ulcerative colitis; QCD: Quiescent Crohn's disease; QUC: Quiescent ulcerative colitis; HC: Healthy controls; CRC: Colorectal carcinoma.

patients<sup>[70-72]</sup>. Gazouli *et al*<sup>[73]</sup> have compared sera before treatment and after IFX induction (week 12) and successfully identified 15 proteins that were differentially accumulated in the sera, most of them modifying the activation of monocytes/macrophages and directly and indirectly regulating the differentiation and activation of CD4+ T-lymphocytes. Also, a recent study by Magnusson *et al*<sup>[74]</sup> reported on the proteomic analysis on biopsies obtained from 6 UC patients (3 responders and 3 non-responders) treated *in vitro* with or without Infliximab and also from 43 UC patients' sera at different time points: Baseline, week 2 and week 14. Those authors have shown that the response in UC patients is associated with reduced monocyte activation 2 wk after therapy initiation, suggesting that the monocytes of these patients are less responsive to inflammatory stimuli when reaching the intestinal mucosa. In therapy responders Infliximab has had influence on Tenascin C, which might be a down-regulator of the two chemokines CCL2 (mcp-1) and CXCL10 (IP-10)<sup>[74]</sup>, which are produced by inflammatory cells and stromal cells, recruit leucocytes, and are induced in inflamed UC mucosa<sup>[75-77]</sup>. Table 3 summarized the potential biomarkers identified by proteomics in IBD.

## CONCLUSION

In the IBD micro-environment a multitude of components interact. No information about a single gene, a single molecule or microbe can exhaustively explain the events that result from such a complex signaling. Also, the wide range of variability between patients' disease features and medical histories makes it difficult to understand how every component of IBD acts and influences other components. On the other hand,

**Table 3** Proteomics in inflammatory bowel disease diagnosis and response to therapy

Proteins	Setting	Diagnostic accuracy	Ref.
Platelet aggregation factor 4 (PF4)	Responder <i>vs</i> non-responder's CD serum	Higher in non-responders	Mewuis <i>et al</i> <sup>[69]</sup> , 2008
Proteins that regulate CD4 <sup>+</sup> T-cell activation	Serum before IFX treatment <i>vs</i> serum after IFX induction period	Higher before treatment	Gazouli <i>et al</i> <sup>[73]</sup> , 2013
Proteins that regulate monocytes/macrophages activation			
Tenascin C	Responder <i>vs</i> non-responder's UC serum	Higher in non-responders	Magnusson <i>et al</i> <sup>[74]</sup> , 2015

CD: Crohn's disease; UC: Ulcerative colitis.

even if the diagnostic gold standard is endoscopy, the introduction of novel molecular biomarkers in clinical practice has always nurtured hopes for new tools that can lead to improvements in diagnostic accuracy. However, the low diagnostic performance of the available markers strongly limits their use in clinical practice. Still, it is reasonable to hypothesize that combining the modification of several biomarkers may identify a sort of fingerprint for IBD with specific disease features.

Indeed, techniques and methodologies that can deal with a very large volume of data and describe a wide picture, rather than focus on single alteration, are likely to represent the necessary step forward in describing and comprehending IBD<sup>[78]</sup>. For all these reasons omics can support the discovery of novel molecular interactions through a better definition of relevant biological pathways and interactions, rather than the analysis of the role of the perturbation of a single element. Omics can lead to the identification of representative patterns of disease which may replace simple biomarkers in clinical practice for the diagnosis, monitoring of IBD and for the personalization of therapies and treatments. Exploiting omic techniques and mastering big data analysis will help researchers to embrace the complexity and overcome the limitations of deciphering inflammatory disorders away from any restricted point of view. **Table 3** provides a summary of the potential biomarkers identified by proteomics in IBD.

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## Basic Study

# Liver stiffness and perfusion changes for hepatic sinusoidal obstruction syndrome in rabbit model

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## Abstract

### BACKGROUND

Hepatic sinusoidal obstruction syndrome (SOS) is caused by damage to hepatic sinusoidal endothelial cells that results in fibrous obliteration of intrahepatic venules and necrosis of hepatocytes. Currently the diagnosis is primarily based on nonspecific clinical features and invasive liver biopsy. Therefore, noninvasive imaging methods are required for the early diagnosis and severity assessment of hepatic SOS.

### AIM

To determine the effectiveness of supersonic shear wave imaging (SSI) and dual energy computed tomography (DECT) for diagnosing hepatic SOS using a rabbit model.

### METHODS

Among nine New Zealand white rabbits (3-4 kg, male), three in control group ingested normal saline for 20 d and six in the SOS group ingested 6-thioguanine (5 mg/kg/d) for 20 d. Liver stiffness was measured using SSI on days 0, 3, 10, and 20. On the same days, liver perfusion was evaluated from virtual monochromatic images of 55 keV and iodine map using DECT. Morphologic changes in the liver were assessed using CT. Final pathology scores were compared between the two groups. Liver stiffness and perfusion parameters were compared according to the groups, days, and pathology scores.

### RESULTS

Final pathology scores were significantly higher in the SOS than the control

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group (median 22 *vs* 2,  $P = 0.024$ ). No gross morphologic changes were seen in livers. Liver stiffness, Hounsfield Unit values, and iodine concentrations were higher in the SOS compared to the control group on days 10 and 20 (all,  $P \leq 0.007$ ). Compared to day 0, liver stiffness and perfusion parameters were higher on day 20 in the SOS group (all,  $P \leq 0.001$ ). Correlation coefficients for liver stiffness ( $r = 0.635$ ), Hounsfield Unit values ( $r = 0.587$ ), and iodine concentration ( $r = 0.611$ ) with final pathology scores were positive without significance (all,  $P > 0.05$ ).

## CONCLUSION

Liver stiffness and perfusion parameters were significantly increased in the livers of a rabbit SOS model. SSI and DECT might aid in early diagnosis of hepatic SOS.

**Key words:** Hepatic sinusoidal obstruction syndrome; Elasticity imaging techniques; Iodine; Computed tomography; Liver; Animals

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**Core tip:** Noninvasive imaging methods are required for the early diagnosis and severity assessment of hepatic sinusoidal obstruction syndrome (SOS). This study showed that liver stiffness on supersonic shear wave imaging was significantly elevated as hepatic SOS progressed in rabbit model. In addition, as hepatic SOS progressed, perfusion parameters measured on dual energy computed tomography were significantly elevated. We suggested that quantitative imaging with supersonic shear wave imaging and dual energy computed tomography could aid in the early diagnosis of hepatic SOS.

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## INTRODUCTION

Hepatic sinusoidal obstruction syndrome (SOS) is a well-known serious complication of hematopoietic stem cell transplantation (HSCT) and is associated with drugs including oxaliplatin-based chemotherapy<sup>[1-4]</sup>. Hepatic SOS, also known as hepatic veno-occlusive disease, is caused by damage to hepatic sinusoidal endothelial cells that results in fibrous obliteration of intrahepatic venules and necrosis of centrilobular hepatocytes<sup>[3,5]</sup>. In pediatric patients, SOS is the most common cause of death after graft-versus-host reaction and infection in patients with HSCT with nonspecific symptoms of painful hepatomegaly, fluid retention, and hyperbilirubinemia<sup>[3,6]</sup>. Liver biopsy is the gold standard for the diagnosis of hepatic SOS, but is limited because of its invasiveness, interobserver variation, and sampling error. Even though conventional computed tomography (CT) or ultrasonography (US) have demonstrated that gross changes in hepatic SOS, such as heterogeneous hypoattenuation and patchy parenchymal enhancement with narrowing of hepatic veins, those imaging findings are nonspecific and present after disease progression while diagnosing hepatic SOS in clinical setting<sup>[7]</sup>. Therefore, new noninvasive imaging biomarker for early diagnosis and severity assessment of hepatic SOS are required as alternatives to liver biopsy and conventional imaging modalities<sup>[8]</sup>.

US elastography is a noninvasive tool for evaluating liver stiffness. In addition to transient elastography (TE) validated for assessment of liver fibrosis<sup>[9,10]</sup>, shear wave elastography (SWE) uses ultrasound waves to quantify the tissue-specific propagation speed of shear waves for guidance of grayscale US images<sup>[11,12]</sup>. SWE gives more reliable results than TE<sup>[13]</sup>. Supersonic shear wave imaging (SSI) is a recently introduced SWE technique characterized by ultrafast image acquisition using multiple push beams and a color map with a wide range. SSI enables real-time analysis with color display, a large field of view and multiple regions of interest, for advantages over acoustic radiation force impulse (ARFI). Although a few studies reported elevated liver stiffness using TE and ARFI in a rat or human SOS<sup>[14-18]</sup> and patients

undergoing HSCT<sup>[19,20]</sup>, no study has evaluated liver stiffness in hepatic SOS using SSI.

Dual energy CT (DECT) uses photon spectra generated by two distinctive tube voltages to obtain additional information about tissue composition based on spectral properties<sup>[21]</sup>. With material-specific information, virtual monochromatic images (VMIs) and concentration map images of materials such as iodine and calcium can be generated by DECT for quantitative diagnosis of liver steatosis and fibrosis<sup>[22-24]</sup>. In hepatic SOS, toxic injury to sinusoidal endothelial cells might cause loss of autoregulation and reduction of blood flow in the sinusoids<sup>[1]</sup>. We postulated that these events could result in different iodine concentrations in livers with SOS, but little has been published on DECT imaging in hepatic SOS. Therefore, the purpose of this study was to know liver stiffness and perfusion changes using SSI and DECT for diagnosis of hepatic SOS using a rabbit model.

## MATERIALS AND METHODS

### *Animal model*

This study was approved by our institution's Animal Experimental Committee and performed according to local animal care guidelines. Nine male New Zealand white rabbits weighting 3-4 kg were used. Three rabbits ingested normal saline *via* a 1 cc syringe for 20 d and were the control group. Six rabbits ingested 6-thioguanine (6-TG, 5 mg/kg/d, four days in a week) for 20 d according to a previous study<sup>[25]</sup> and were the SOS group. Rabbits received standard diets during the experiments. Rabbit conditions were monitored by radiologists and a veterinarian.

### *Laboratory studies*

On days 0, 3, 10, and 20 during medication, laboratory and imaging examinations were performed under sedation. Sedation was accomplished by intramuscular injection of xylazine hydrochloride (1 mg/kg, Rompun; Bayer Korea, Seoul, South Korea) and intravenous injection of alfaxalone (3 mg/kg, Alfaxan®; Careside, Gyeonggi-do, South Korea) with the help of a veterinarian before starting examinations.

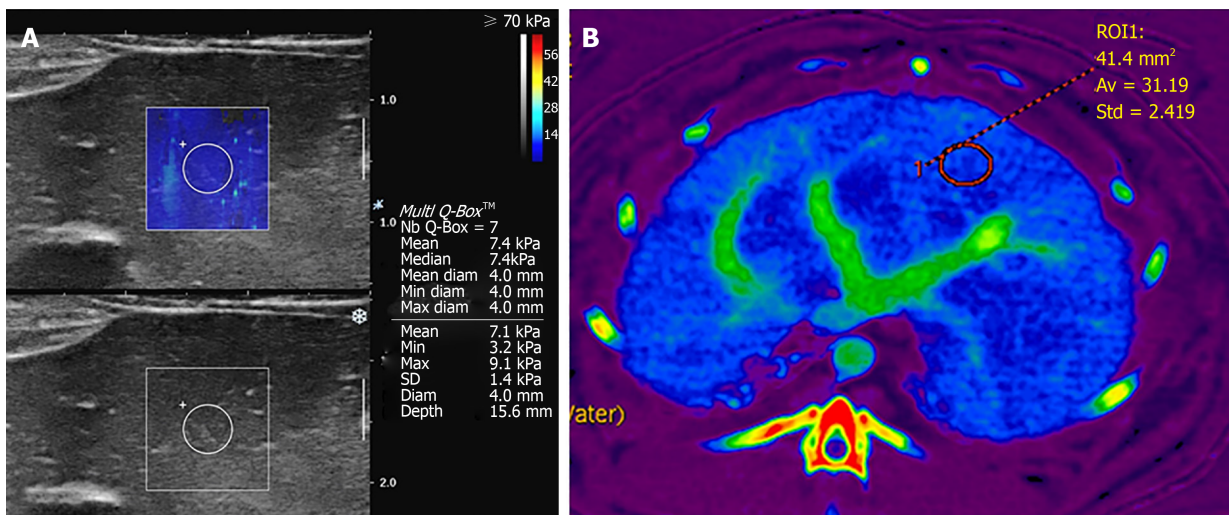
Laboratory tests were performed with samples from ear veins. Tests were for serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (Tbil), and direct bilirubin (Dbil) levels.

### *Imaging studies*

For imaging studies, SWE and DECT were performed on days 0, 3, 10, and 20. Rabbits were in a supine position on the scan table, with forelegs up and tightened with bandages. The upper abdomen was shaved for contact with the US probe. For SWE, SSI (Aixplorer, SuperSonic Imagine, Aix-en-Provence, France; software version of 9.2) was used with a 10-2 MHz linear transducer. An experienced pediatric radiologist performed examinations. Liver stiffness was measured for the liver parenchyma at an epigastric area because in rabbits, the liver left lobe was large and had fewer artifacts from bowel loops than other lobes. Color maps were made for the liver parenchyma avoiding hepatic vessels and bile ducts. A round region of interest (ROI) with diameter 2-4 mm and depth of about 1.5 cm was put on color maps when maps appeared homogeneous during regular free breathing without intubation. Mean value for liver stiffness within the ROI was automatically recorded in kPa units based on Young's modulus. Measurements were repeated seven times as in a previous study<sup>[26]</sup> and mean values were recorded (Figure 1A). All processes were repeated twice and two representative values were used for each rabbit.

CT was performed using DECT with fast kVp switching (80/140 kVp) on a single-source dual-energy 64-channel multidetector CT scanner (GE Discovery CT750 HD scanner, GE Healthcare, Milwaukee, Wisconsin, United States) on the same days. Contrast agent iobitridol (Xenetics 300, Guerbet, Roissy, France) at 2 cc/kg was manually injected into an auricular vein, using the same institutional protocol as for neonates. Scanning parameters were: Helical mode, rapid kVp switching between 80 and 140 kVp, tube current 260 mA, detector collimation 64 mm × 0.625 mm, acquisition mode in axial plane, slice thickness 2.5 mm, pitch 1.375:1, soft convolution kernel, and CT dose index volume 6.48 mGy. Single-phase portal venous phase was acquired at fixed time delay 40 s following contrast material injection according to the institutional CT scan protocol for neonates. Livers were scanned in the craniocaudal direction.

Morphologic liver changes during the study period were assessed using CT. A pediatric radiologist assessed the presence of liver parenchymal as heterogeneous patchy enhancement, gallbladder (GB) wall edema, narrowed or invisible hepatic



**Figure 1** Representative images from supersonic shear wave imaging and dual energy computed tomography of rabbits in a sinusoidal obstruction syndrome group on day 20. A: Mean liver stiffness value for livers in the sinusoidal obstruction syndrome group was 7.4 kPa; B: Iodine concentration in livers of the sinusoidal obstruction syndrome group was 31.19 mg/mL.

veins, hepatomegaly showing inferior liver margin below the right kidney, or presence of ascites using DECT images according to previous studies<sup>[7,27,28]</sup>.

For quantitative assessments, filtered back projection images without iterative reconstruction were used for reconstruction and analysis. VMIs at 55 keV were used for analysis because 70 or 80 kVp images were routinely used for neonate abdominal CT in our hospital. Material density iodine maps were reconstructed. Round ROIs were drawn in liver parenchyma avoiding hepatic vessels using an AW server (GE Healthcare, Milwaukee, Wisconsin, United States; software version 2.0). ROIs were positioned in the same place in the liver parenchyma on VMI 55 keV image and iodine map (Figure 1B). Mean CT attenuations in Hounsfield units (HUs) and iodine concentrations (mg/mL) within ROIs were recorded. ROIs were drawn three times over liver parenchyma and three values were acquired for each parameter.

Radiation dose using mean effective dose by multiplying dose-length product with k (conversion factor, 0.049 used for pediatric patients younger than one year old) of all rabbits were assessed.

### Pathologic scoring of hepatic SOS

On day 21, rabbits were sacrificed and hepatic specimens were obtained. Specimens were fixed in 10% phosphate-buffered formalin (pH 7.1) for 24 h and put in 70% ethanol. After obtaining paraffin blocks, hematoxylin and eosin, Masson's trichrome, and Verhoeff-Van Gieson staining was performed. Final pathology scores for SOS were assessed by an experienced board-certificated pathologist according to a scoring system used previously<sup>[25]</sup>. Scores were assessed as the sum of each subscore in three categories: sinusoidal, central vein, and necro-inflammatory damage. For evaluating sinusoidal damage, sinusoidal dilatation, or congestion were evaluated using subscores from 0 to 3. For evaluation of central vein damage, fraying of endothelial cells and sludge in central veins were assessed. For the evaluation of necro-inflammatory damage, apoptotic bodies, inflammatory cell infiltrates, steatosis, and necrosis were scored separately. All scores were evaluated for 10 areas in lower power fields; the possible range of scores 0 to 240. The pathologist was blind to the final diagnosis of the rabbits.

### Statistical analysis

Statistical analysis was done with SAS (version 9.4, SAS Institute Inc., Cary, NC, United States). Final pathology scores between control and SOS groups were compared using Mann-Whitney U tests. Differences in changing pattern of laboratory results and quantitative imaging parameters including liver stiffness on SSI, HU values on VMI 55 keV images, and iodine concentration (mg/mL) on iodine maps were assessed using linear mixed models. Values were compared between groups for each day and compared between days 0 and 20 for each group in post hoc analysis. Spearman's correlation tests were used to determine correlations between results measured on day 20 and final pathology scores. *P* values less than 0.05 were considered statistically significant.

## RESULTS

During the study period, three of six rabbits in the SOS group died unexpectedly on days 10, 11, and 17. Results for these rabbits until the day of unexpected death were used as much as possible. Pathology results were obtained on the day of death for these rabbits, except the rabbit that died on day 17. The remaining three rabbits in the SOS group all had examinations on days 0, 3, 10, and 20 and pathology results on day 21. Therefore, days 0 and 3 had results from all six rabbits, while day 10 had results from 5 rabbits, and day 20 had results from 3 rabbits in the SOS group. The three rabbits in the control group were alive through day 20.

Final pathology scores were significantly higher in the SOS group ( $n = 5$ , range 20–42, median 22) than the control group ( $n = 3$ , range 2–11, median 2) ( $P = 0.024$ ). According to the categories, the sum of scores for sinusoidal damage was 121 in the SOS group and 15 in the control group. For central vein damage, the score was 3 for the SOS group and 0 for the control group. For necro-inflammatory damage, the score was 9 for the SOS group and 0 for the control group. Liver parenchymal patchy heterogeneous enhancement, GB wall edema, narrowed or invisible hepatic veins, hepatomegaly, or ascites were not presented in CT images for both SOS and control groups during the study period. The mean effective dose of DECT was  $7.9 \pm 1.6$  mSv for all rabbits.

Using linear mixed models, we saw significant differences in changing patterns of liver stiffness, HU values in VMI 55 keV, and iodine concentration between two groups (all,  $P < 0.001$ ) (Figure 2). Laboratory results showed no significant differences between the two groups in patterns over 20 d. Post hoc analyses of laboratory and imaging studies are in Table 1 and 2. Compared to controls, on day 20, AST, ALT, and D.bil values were significantly higher in the SOS group ( $P = 0.004$ , 0.008, 0.013, respectively) (Table 1). Liver stiffness values were significantly higher in the SOS group on day 10 (9.48 kPa *vs* 4.02 kPa,  $P < 0.001$ ) and day 20 (7.71 kPa *vs* 4.67 kPa,  $P = 0.007$ ) compared to the control group (Table 2). HU values for VMI 55 keV were significantly higher in the SOS group on day 3 (167.68 HU *vs* 147.18 HU,  $P = 0.045$ ), day 10 (185.42 HU *vs* 129.05 HU,  $P < 0.001$ ) and day 20 (179.81 HU *vs* 148.95 HU,  $P = 0.006$ ). Iodine concentrations were significantly higher in the SOS group on day 3 (26.74 mg/mL *vs* 20.72 mg/mL,  $P = 0.022$ ), day 10 (31.86 mg/mL *vs* 19.16 mg/mL,  $P < 0.001$ ), and day 20 (35.14 mg/mL *vs* 23.85 mg/mL,  $P < 0.001$ ).

When comparing laboratory results, AST, ALT, and Dbil values were significantly higher on day 20 than on day 0 in the SOS group ( $P = 0.001$ , 0.001, and 0.004, respectively), while there were no significant differences between days 0 and 20 in the control group (Table 3). In quantitative imaging studies (Table 4), liver stiffness values were significantly higher on day 20 than on day 0 in the SOS group (7.71 kPa *vs* 3.89 kPa,  $P < 0.001$ ), but there was no significant difference of stiffness between day 0 and day 20 in the control group (4.67 kPa *vs* 4.35 kPa,  $P = 0.752$ ) (Table 4). HU values for VMI 55 keV were significantly higher on day 20 in the SOS group (179.81 HU *vs* 158.77 HU,  $P = 0.001$ ), with no differences in the control group (147.95 HU *vs* 153.18 HU,  $P = 0.510$ ). Iodine concentrations were also significantly higher on day 20 in the SOS group (35.14 mg/mL *vs* 24.2 mg/mL,  $P < 0.001$ ), with no differences in the control group (23.85 mg/mL *vs* 24.8 mg/mL,  $P = 0.539$ ).

In assessing correlations among final pathology scores and laboratory results (Table 5), correlation coefficients were 0.323 for AST, 0.491 for ALT, 0.156 for Tbil, and 0.261 for Dbil, without statistical significance ( $P > 0.05$ ). In imaging studies, correlation coefficients were 0.635 for liver stiffness, 0.587 for VMI 55 keV, and 0.611 for iodine concentration, even without statistical significance ( $P > 0.05$ ).

## DISCUSSION

In our animal study, hepatic SOS was induced in a rabbit model by orally administered 6-TG. As described by Oancea *et al.*<sup>[25]</sup>, orally administered 6-TG causes hepatic SOS in a dose-dependent manner in a murine model and our findings confirmed early and acute sinusoidal injury in rabbit model by pathology. Our pathology results showed early and acute changes in the SOS group, which was different from the control group. Because rabbits in the SOS group did not show morphologic changes on CT images, these results confirmed the early disease stages of our rabbit model. In this model, non-invasive SSI and DECT examinations had promising results for diagnosing hepatic SOS. Our study was meaningful because changes in quantitative imaging studies with SSI and DECT occurred earlier than morphologic changes in conventional CT.

Our SSI results indicated that hepatic SOS led to elevated liver stiffness. Significant

**Table 1 Comparison of laboratory results between control and sinusoidal obstruction syndrome groups**

Results	Days	Control group	SOS group	Differences	P value
AST (IU/L)	0	32.67 (-55.57, 120.9)	20.5 (-41.89, 82.89)	12.17 (-95.9, 120.23)	0.818
	3	24.67 (-63.57, 112.9)	23 (-39.39, 85.39)	1.67 (-106.4, 109.73)	0.975
	10	20.67 (-67.57, 108.9)	26.4 (-42.5, 95.3)	-5.73 (-117.68, 106.22)	0.917
	20	26.33 (-61.9, 114.57)	221.34 (130.35, 312.33)	-195.01 (-321.75, -68.26)	0.004 <sup>b</sup>
ALT (IU/L)	0	38 (3.97, 72.03)	33.17 (9.11, 57.23)	4.83 (-36.84, 46.51)	0.813
	3	44.67 (10.64, 78.69)	41.83 (17.77, 65.89)	2.83 (-38.84, 44.51)	0.890
	10	33.67 (-0.36, 67.69)	25.41 (-1.18, 51.99)	8.26 (-34.92, 51.44)	0.697
	20	40.67 (6.64, 74.69)	109.35 (74.12, 144.58)	-68.69 (-117.67, -19.71)	0.008 <sup>b</sup>
Tbil (mg/dL)	0	0.27 (-0.18, 0.72)	0.42 (0.1, 0.73)	-0.15 (-0.7, 0.4)	0.579
	3	0.63 (0.18, 1.08)	0.52 (0.2, 0.83)	0.12 (-0.43, 0.67)	0.666
	10	0.9 (0.45, 1.35)	1.18 (0.82, 1.53)	-0.28 (-0.85, 0.29)	0.328
	20	0.53 (0.08, 0.98)	0.96 (0.49, 1.43)	-0.43 (-1.07, 0.22)	0.186
Dbil (mg/dL)	0	0.1 (-0.08, 0.28)	0.12 (-0.01, 0.25)	-0.02 (-0.24, 0.21)	0.879
	3	0.13 (-0.05, 0.32)	0.17 (0.04, 0.3)	-0.03 (-0.26, 0.19)	0.760
	10	0.17 (-0.02, 0.35)	0.26 (0.12, 0.4)	-0.09 (-0.32, 0.14)	0.411
	20	0.13 (-0.05, 0.32)	0.47 (0.28, 0.65)	-0.33 (-0.59, -0.08)	0.013 <sup>a</sup>

Values are estimated mean and 95% confidence interval.

<sup>a</sup> $P < 0.05$ .

<sup>b</sup> $P < 0.01$ . SOS: Sinusoidal obstruction syndrome; AST: Serum aspartate aminotransferase; ALT: Alanine aminotransferase; Tbil: Total bilirubin; Dbil: Direct bilirubin.

stiffness changes measured by SSI were observed starting on day 10, with no obvious morphologic change even with contrast enhanced CT throughout the test days. To the best of our knowledge, this is the first study to investigate the effectiveness of SSI for assessing hepatic SOS. SSI has benefits such as a large area to measure, ultrafast imaging, and low failure rate compared with TE or ARFI<sup>[29]</sup>. Previous studies with TE or ARFI reported an increased liver stiffness in a rat model<sup>[14]</sup> and in pediatric or adult patients following HSCT<sup>[15-18,30]</sup>. A study demonstrated that SWE detects earlier changes than color Doppler study in SOS<sup>[20]</sup>. Increased liver stiffness could be from hepatic venous congestion in the SOS model, although liver inflammation and cholestasis could also affect increased liver stiffness<sup>[31]</sup>. Our study confirmed that SSI could be a method for monitoring disease progression or treatment response to SOS. The SSI values were highest on day 10 and decreased on day 20. Attention is required to interpret decreased SSI value on day 20 because of the relatively small number of animals at day 20. Further clinical research is required to prove the potential utility of SSI in the detection and follow up of hepatic SOS.

Previous studies with conventional CT reported nonspecific morphologic changes such as hepatomegaly, ascites, heterogeneous patchy parenchymal enhancement, narrowed hepatic veins, and GB wall edema for diagnosis of hepatic SOS; these might lack sensitive detection for early changes in hepatic SOS<sup>[28,32]</sup>. Our study demonstrated that quantitative DECT showed increased HU values on VMI 55 keV and increased iodine concentrations in material density maps in the liver parenchyma before gross morphologic changes occurred. These quantitative values also showed increasing trends throughout the study period, which may be promising for follow-up of the severity of hepatic SOS. Low-keV images using VMI could enhance iodine effects<sup>[33]</sup>. This method could aid in the detection of changing patterns of iodine accumulation in the liver parenchyma in hepatic SOS using low-keV images and iodine maps.

Increased HU values and iodine concentrations in our study might be from iodine congestion due to endothelial injury of sinusoids and small intrahepatic venules. A previous study found that on pathology, hepatic SOS showed blood stagnation in dilated sinusoids<sup>[32]</sup>. However, heterogeneous hypoattenuation and patchy liver enhancement were observed in a small number of SOS patients following neoadjuvant chemotherapy or pyrrolizidine alkaloid ingestion<sup>[7,27,34,35]</sup>. These heterogeneous hypoattenuations might be related to delayed blood inflow and decreased flow velocity from damaged sinusoids filled with cell debris, red cells, and extracellular matrix<sup>[34,35]</sup>. Different patterns of enhancement in hepatic SOS could be from different stages and etiologies of SOS according to the studies. Delayed blood flow and decreased flow velocity from hepatic SOS could represent an advanced stage and this could be different from our study. Our study found iodine accumulation in

**Table 2 Comparison of quantitative imaging results between control and sinusoidal obstruction syndrome groups**

Parameters	Days	Control group	SOS group	Differences	P value
Liver stiffness (kPa)	0	4.35 (2.82, 5.88)	3.89 (2.81, 4.97)	0.46 (-1.41, 2.33)	0.623
	3	5.68 (4.16, 7.21)	6.38 (5.3, 7.45)	-0.69 (-2.56, 1.18)	0.458
	10	4.02 (2.49, 5.54)	9.48 (8.29, 10.66)	-5.46 (-7.39, -3.53)	< 0.001 <sup>b</sup>
	20	4.67 (3.14, 6.19)	7.71 (6.17, 9.25)	-3.04 (-5.21, -0.87)	0.007 <sup>b</sup>
VMI 55 keV (HU)	0	153.18 (137.07, 169.29)	158.77 (147.38, 170.16)	-5.59 (-25.33, 14.14)	0.550
	3	147.18 (131.07, 163.29)	167.68 (155.92, 179.44)	-20.5 (-40.44, -0.55)	0.045 <sup>a</sup>
	10	129.05 (112.94, 145.16)	185.42 (173.62, 197.22)	-56.37 (-76.34, -36.4)	< 0.001 <sup>b</sup>
	20	148.95 (132.83, 165.06)	179.81 (166.5, 193.11)	-30.86 (-51.69, -10.03)	0.006 <sup>b</sup>
Iodine concentration (mg/mL)	0	24.8 (20.76, 28.84)	24.2 (21.34, 27.05)	0.61 (-4.34, 5.55)	0.794
	3	20.72 (16.69, 24.76)	26.73 (23.79, 29.67)	-6.01 (-11, -1.02)	0.022 <sup>a</sup>
	10	19.16 (15.12, 23.19)	31.86 (28.91, 34.8)	-12.7 (-17.7, -7.7)	< 0.001 <sup>b</sup>
	20	23.85 (19.81, 27.88)	35.14 (31.84, 38.44)	-11.29 (-16.49, -6.1)	< 0.001 <sup>b</sup>

Values are estimated mean and 95% confidence interval.

<sup>a</sup>*P* < 0.05.

<sup>b</sup>*P* < 0.01. SOS: Sinusoidal obstruction syndrome; VMI: Virtual monochromatic image; HU: Hounsfield unit.

the liver parenchyma in the acute stage of the SOS from blood stagnation. Endothelial injuries and edema of sinusoids could occur in early stage SOS, while hepatic necrosis and deposition of collagen, fibrous tissues could occur in disease progression<sup>[7]</sup>. Therefore, different stages of SOS might have different enhancement patterns. A previous study suggested that the etiology of SOS could cause different enhancement patterns because tumor-related factors also alter liver perfusion and steatosis could affect liver attenuation after receiving chemotherapy<sup>[34]</sup>. Direct quantification of iodine in the liver parenchyma with DECT may be a possible objective method for overcoming the inherent ambiguous nature of attenuation in livers with SOS using conventional CT<sup>[24,36]</sup>. Further studies for assessing DECT results according to stage and etiology of SOS in patients are needed.

This study has several limitations. First, the small number of animals and unexpected death in SOS group in this experimental study was the main reason for the lack of statistical significance in the correlation between final pathology scores and laboratory results or imaging parameters, even though correlation coefficients were about 0.6 for quantitative imaging results. Second, we did not assess hepatic arterial resistance index or flow direction of the portal vein using Doppler US in rabbits. Third, rabbits were assessed until day 20 and advanced disease stages with apparent morphologic changes were not evaluated. However, because this study demonstrated the potential effectiveness of SSI and DECT for hepatic SOS, further large studies with patients and animal models are needed.

In conclusion, changes of quantitative parameters with SSI and DECT could aid the diagnosis of hepatic SOS before morphologic changes were apparent in the liver of a rabbit model. Liver stiffness on SSI, HU values on VMI with low keV, and iodine concentrations on DECT were significantly increased in livers according to SOS progression.

**Table 3 Comparison of laboratory results between days 0 and 20**

Results	Groups	Day 0	Day 20	Difference	P value
AST (IU/L)	Control	32.67 (-55.57, 120.9)	26.33 (-61.9, 114.57)	6.33 (-118.48, 131.15)	0.916
	SOS	20.5 (-41.89, 82.89)	221.34 (130.35, 312.33)	-200.84 (-310.9, -90.78)	0.001 <sup>b</sup>
ALT (IU/L)	Control	38 (3.97, 72.03)	40.67 (6.64, 74.69)	-2.67 (-51.54, 46.21)	0.910
	SOS	33.17 (9.11, 57.23)	109.35 (74.12, 144.58)	-76.19 (-119.14, -33.24)	0.001 <sup>b</sup>
Tbil (mg/dL)	Control	0.27 (-0.18, 0.72)	0.53 (0.08, 0.98)	-0.27 (-0.9, 0.37)	0.387
	SOS	0.42 (0.1, 0.73)	0.96 (0.49, 1.43)	-0.54 (-1.1, 0.02)	0.057
Dbil (mg/dL)	Control	0.1 (-0.08, 0.28)	0.13 (-0.05, 0.32)	-0.03 (-0.29, 0.22)	0.792
	SOS	0.12 (-0.01, 0.25)	0.47 (0.28, 0.65)	-0.35 (-0.57, -0.13)	0.004 <sup>b</sup>

Values are presented as estimated mean and 95% confidence interval.

<sup>b</sup>*P* < 0.01. SOS: sinusoidal obstruction syndrome; AST: Serum aspartate aminotransferase; ALT: Alanine aminotransferase; Tbil: Total bilirubin; Dbil: Direct bilirubin.

**Table 4 Comparison of quantitative imaging results between day 0 and 20**

Parameters	Groups	Day 0	Day 20	Difference	P value
Liver stiffness (kPa)	Control	4.35 (2.82, 5.88)	4.67 (3.14, 6.19)	-0.32 (-2.32, 1.69)	0.752
	SOS	3.89 (2.81, 4.97)	7.71 (6.17, 9.25)	-3.82 (-5.61, -2.02)	< 0.001 <sup>b</sup>
VMI 55 keV (HU)	Control	153.18 (137.07, 169.29)	148.95 (132.83, 165.06)	4.23 (-8.48, 16.95)	0.510
	SOS	158.77 (147.38, 170.16)	179.81 (166.5, 193.11)	-21.04 (-32.7, -9.37)	0.001 <sup>b</sup>
Iodine concentration (mg/mL)	Control	24.8 (20.76, 28.84)	23.85 (19.81, 27.88)	0.95 (-2.12, 4.03)	0.539
	SOS	24.2 (21.34, 27.05)	35.14 (31.84, 38.44)	-10.94 (-13.77, -8.12)	< 0.001 <sup>b</sup>

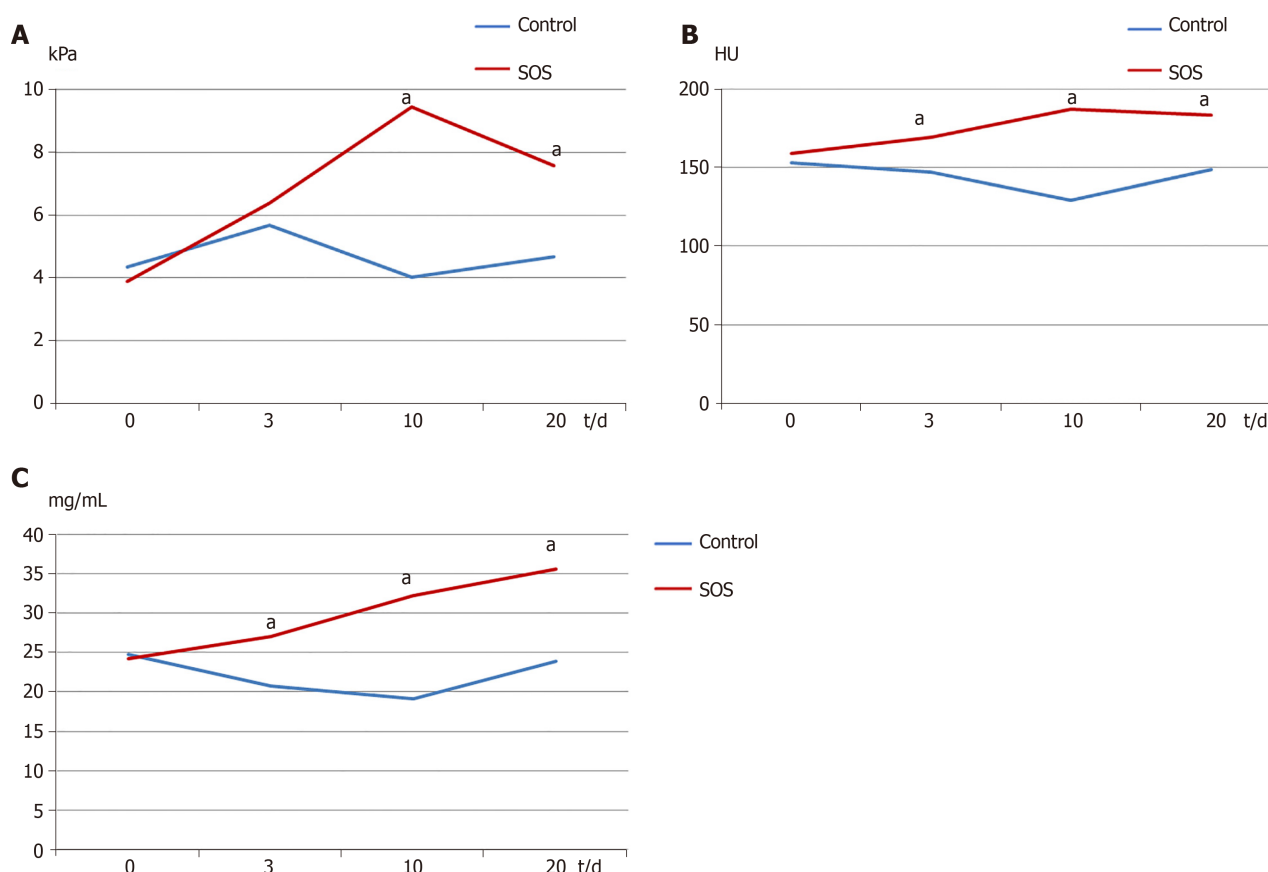
Values are presented as estimated mean and 95% confidence interval.

<sup>b</sup>*P* < 0.01. SOS: Sinusoidal obstruction syndrome; VMI: Virtual monochromatic image; HU: Hounsfield unit.

**Table 5 Assessment of correlation between final pathologic scores and laboratory results and quantitative imaging parameters**

Results	<i>r</i> (95%CI)	P value
AST (IU/L)	0.323 (-0.511, 0.83)	0.453
ALT (IU/L)	0.491 (-0.358, 0.881)	0.230
Tbil (mg/dL)	0.156 (-0.623, 0.771)	0.726
Dbil (mg/dL)	0.261 (-0.557, 0.809)	0.551
Liver stiffness (kPa)	0.635 (-0.171, 0.919)	0.094
VMI 55 keV (HU)	0.587 (-0.241, 0.906)	0.133
Iodine concentration (mg/mL)	0.611 (-0.207, 0.913)	0.112

AST: Serum aspartate aminotransferase; ALT: Alanine aminotransferase; Tbil: Total bilirubin; Dbil: Direct bilirubin; VMI: Virtual monochromatic image; HU: Hounsfield unit; CI: Confidence interval.



**Figure 2 Changing patterns of imaging results.** A: Liver stiffness on supersonic shear wave imaging; B: Hounsfield unit values on virtual monochromatic image of 55 keV; C: Iodine concentration on iodine map of dual energy computed tomography on days 0, 3, 10, and 20 in control (blue) and sinusoidal obstruction syndrome (SOS) (red) groups. All three quantitative values showed significant differences in changing pattern between control and SOS groups for all days ( $P < 0.001$ ). <sup>a</sup>Shows significantly different results between control and SOS groups for each day.

## ARTICLE HIGHLIGHTS

### Research background

Hepatic sinusoidal obstruction syndrome (SOS) is a well-known serious complication of hematopoietic stem cell transplantation and is associated with chemotherapy. Noninvasive imaging methods are required for the early diagnosis and severity assessment of hepatic SOS.

### Research motivation

In pediatric patients, SOS is the most common cause of death in patients with hematopoietic stem cell transplantation with nonspecific symptoms. Currently the diagnosis is primarily based on nonspecific clinical features and invasive liver biopsy. Therefore, noninvasive imaging methods are required for the early diagnosis and severity assessment of hepatic SOS. Supersonic shear wave imaging (SSI) is a recently introduced US elastography technique and is a noninvasive tool for evaluating liver stiffness. Dual energy computed tomography (DECT) could quantify tissue composition or iodine concentration. We postulated that these imaging studies could quantify liver stiffness and perfusion changes in hepatic SOS.

### Research objectives

The purpose of the study was to determine the effectiveness of SSI and DECT for diagnosing hepatic SOS using a rabbit model.

### Research methods

Among nine New Zealand white rabbits, three were in control group and six with 6-thioguanine ingestion were in SOS group. Liver stiffness was measured using SSI and liver perfusion was evaluated from virtual monochromatic images of 55 keV and iodine map using DECT on days 0, 3, 10, and 20. Liver stiffness and perfusion parameters were compared according to the groups, days, and pathology scores.

### Research results

Compared to the control group, final pathology scores were significantly higher in the SOS group, while there were no gross morphologic changes using conventional imaging. Liver stiffness, Hounsfield Unit values, and iodine concentrations were higher in the SOS compared to

the control group as disease progression.

### Research conclusions

This study showed that liver stiffness on SSI and perfusion parameters on DECT were significantly increased according to SOS progression in a rabbit model. We suggested that quantitative imaging with SSI and DECT could aid in the early diagnosis of hepatic SOS.

### Research perspectives

SSI and DECT could be the noninvasive imaging studies for early diagnosis and severity assessment of hepatic SOS. Because this study demonstrated the potential effectiveness of SSI and DECT for hepatic SOS, further large studies with patients are needed.

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## Basic Study

# Role of Tenascin-X in regulating TGF- $\beta$ /Smad signaling pathway in pathogenesis of slow transit constipation

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**Author contributions:** Zhang YC and Chen BX contributed equally to this work; Xie XY and Zhou Y collected the clinical tissues after surgery; Qian Q coordinated the research; Jiang CQ designed and coordinated the research.

### Institutional review board

**statement:** The study was reviewed and approved by the Institutional Review Board of Zhongnan Hospital of Wuhan University.

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## Abstract

### BACKGROUND

Chronic constipation is a gastrointestinal functional disease that seriously harms physical and mental health and impacts the quality of life of patients. Its incidence rate is 2%-27%. Slow transit constipation (STC) is a common type of chronic functional constipation, accounting for 10.3%-45.5% of such cases. Scholars have performed many studies on the pathogenesis of STC. These studies have indicated that the occurrence of STC may be related to multiple factors, such as dysfunction of the enteric nervous system, interstitial cells of Cajal (ICC) damage, and changes in neurotransmitters regulating intestinal peristalsis.

### AIM

To investigate the role of Tenascin-X (TNX) in regulating the TGF- $\beta$ /Smad signaling pathway in the pathogenesis of STC.

### METHODS

This study included an experimental group and a control group. The experimental group included 28 patients with severe colonic STC, and the control group included 18 patients with normal colon tissues. Immunohistochemistry (IHC) was used to detect c-Kit, a specific marker of the ICC. Western blot, immunofluorescence, and IHC were used to detect the localization and expression of TNX and TGF- $\beta$ /Smad.

### RESULTS

IHC showed that the number of ICC with positive c-Kit expression was significantly reduced in the colon of STC patients ( $22.17 \pm 3.28$  vs  $28.69 \pm 3.53$ ,  $P < 0.05$ ) and that the distribution was abnormal. Western blot results showed that c-Kit and Smad7 levels were significantly decreased in the colon of STC patients (c-

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kit:  $0.462 \pm 0.099$  vs  $0.783 \pm 0.178$ ,  $P < 0.01$ ; Smad7:  $0.626 \pm 0.058$  vs  $0.799 \pm 0.03$ ,  $P < 0.01$ ) and that TNX and Smad2/3 levels were higher in the STC group (TNX:  $0.868 \pm 0.028$  vs  $0.482 \pm 0.032$ ,  $P < 0.01$ ). There was no significant difference in TGF- $\beta$  between the two groups ( $0.476 \pm 0.028$  vs  $0.511 \pm 0.044$ ,  $P = 0.272$ ). Pearson correlation analysis showed that the TNX protein exhibited a strong correlation with Smad2/3 and Smad7 ( $P < 0.05$ ,  $|R| > 0.8$ ) and TGF- $\beta$  ( $P < 0.05$ ,  $|R| = 0.7$ ).

## CONCLUSION

The extracellular matrix protein TNX may activate the TGF- $\beta$ /Smad signaling pathway by upregulating the Smad 2/3 signaling protein and thereby induce slight or complete epithelial stromal cell transformation, leading to an abnormal distribution and dysfunction of ICC in the diseased colon, which promotes the occurrence and development of STC.

**Key words:** Slow transit constipation; Tenascin-X; Extracellular matrix glycoproteins; TGF- $\beta$

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**Core tip:** The extracellular matrix protein Tenascin-X may activate the TGF- $\beta$ /Smad signaling pathway by upregulating the Smad2/3 signaling protein and thereby induce slight or complete epithelial stromal cell transformation, leading to an abnormal distribution and dysfunction of interstitial cells of Cajal in the diseased colon, which promotes the occurrence and development of slow transit constipation.

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## INTRODUCTION

Chronic constipation is a gastrointestinal functional disease that seriously harms physical and mental health and impacts the quality of life of patients. Its incidence rate is 2%-27%. Slow transit constipation (STC) is a common type of chronic functional constipation, accounting for 10.3%-45.5% of such cases<sup>[1]</sup>. The clinical features of STC include decreased colonic motility and prolonged colonic transit time. The symptoms of this disease are a decreasing stool frequency, less or no desire to defecate, hard excrement, and abdominal distension. Some patients' symptoms are so stubborn that they cannot be relieved by increasing the dose of laxatives. Scholars have performed many studies on the pathogenesis of STC. These studies have indicated that the occurrence of STC may be related to multiple factors, such as dysfunction of the enteric nervous system, interstitial cells of Cajal (ICC) damage, and changes in neurotransmitters regulating intestinal peristalsis. Among these factors, abnormal distribution and dysfunction of ICC in the colon are particularly important<sup>[2-5]</sup>, which suggests that abnormal changes in ICC may be related to the mechanism of cell transdifferentiation, rather than apoptosis or death of cells<sup>[6]</sup>.

Tenascin-X (TNX) is an important member of the TN family of extracellular matrix glycoproteins, which are involved in the progression of many diseases. Aktar *et al*<sup>[7]</sup> suggested that deficient expression or loss of TNX is highly correlated with Ehlers-Danlos syndrome. Patients with loss of TNX may suffer declining colonic motility as an obvious gastrointestinal symptom. Aktar *et al*<sup>[7]</sup> found that TNX-deficient mice show impaired defecation and neural control of distal colonic motility that can be rescued with a 5-HT4 receptor agonist. Fikree *et al*<sup>[8]</sup> reported that 37% of joint hypermobility syndrome patients experience GI symptoms including increased nausea, abdominal pain, bloating, constipation, and diarrhea compared with non-joint hypermobility syndrome controls<sup>[8]</sup>. The above studies suggest that there is a link between the TNX protein and colonic motility. Our research found that the expression of the TNX protein in the colon tissue of STC patients was notably increased in preliminary experiments; therefore, we inferred that this protein may show a certain

correlation with the occurrence of STC. Over the past few years, research on TNX has gradually shifted toward whether the protein acts as a promoter of a signaling pathway. It was proven that part of the TNX domain activates the TGF- $\beta$  signaling pathway, and the TGF- $\beta$ /Smad signaling pathway was confirmed as an important pattern in cell transition<sup>[9-11]</sup>. Therefore, we further speculated that the TNX protein may induce the transition of ICC through regulation of the TGF- $\beta$ /Smad signaling pathway, causing a decrease in the number of functional ICC changes and further leading to the occurrence of STC.

The aims of this study were to investigate the changes in the expression of TNX, TGF- $\beta$ , Smad2/3, and Smad7 in the colon tissue of STC patients and to explore the influence of the TNX protein and its related signaling pathway on the occurrence of STC. This work will allow the exploration of and speculation about the mechanism of ICC changes at the molecular level.

## MATERIALS AND METHODS

### General information

Patients who were hospitalized in the Colorectal and Anal Surgery Department of Zhongnan Hospital of Wuhan University from January 2015 to October 2018 were selected for this study. The patients were diagnosed with STC according to the Rome III criteria. All patients in the STC group underwent colonic transit tests, defecography, and colonoscopy. The exclusion criteria were as follows: (1) Patients who did not meet the Rome III criteria; (2) Patients with chronic constipation caused by spastic pelvic floor syndrome, rectocele, megacolon, or other organ lesions; (3) Patients with obvious gastric intestinal transit dysfunction; (4) Patients with secondary constipation caused by drugs, endocrine, metabolic, or neurological diseases, renal failure, cirrhosis, severe hypertension, and severe obesity, and those with a body mass index exceeding 30; and (5) Patients with incomplete data that influenced the diagnosis. The control group included patients who experienced regular bowel movements with no history of constipation from whom colon tissue was collected because of abdominal colorectal trauma. All patients were assessed with the Wexner constipation score and the gastrointestinal quality of life index to evaluate their quality of life. The use of the materials was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University and the agreement of the patients.

### Immunohistochemistry and immunofluorescence staining

For immunohistological staining, colon tissue sections were incubated with a 100-fold-diluted TNX antibody (Proteintech, CN, United States) or c-Kit antibody (Abcam, United Kingdom) in blocking solution (2% BSA in PBS) at 4 °C overnight, followed by visualization using a poly-HRP anti-rabbit IgG detection kit.

### Western blot analysis

The instructions for the extraction of cellular proteins for pretreatment were followed. The protein concentration was quantified by the BCA assay (Aspen, CN, United States), and equal amounts of denatured proteins (20  $\mu$ g) were separated *via* 10%-12% SDS-PAGE and subsequently electrotransferred to PVDF membranes. The proteins of interest were detected with specific antibodies against TNX (Proteintech), TGF- $\beta$  (Abcam, United Kingdom), Smad2/3 (CST, United States), and Smad7 (Proteintech). Protein bands were visualized after the binding of the secondary antibody with HRP-conjugated anti-rabbit IgG by using ECL reagents.

### Statistical analysis

Data and statistical analyses were performed using SPSS 17.0, and all data are expressed as the mean  $\pm$  SD. The comparisons of count data were performed by the chi-square test or Fisher's exact test. The data were compared between groups by the *t*-test, and correlation analysis was performed with the Pearson correlation coefficient. A *P* value < 0.05 was regarded as significant.

## RESULTS

### General data of patients

A total of 28 patients with STC and 18 normal controls were collected. Among the 28 STC patients, 7 were male, and 21 were female, with an average age of  $56.86 \pm 13.57$  years. There were 18 subjects in the control group, including 6 males and 12 females; their average age was  $50.00 \pm 12.02$  years. All subjects were examined to determine the

Wexner constipation score to evaluate defecation function and gastrointestinal quality of life index to assess the impact of constipation on their quality of life, as shown in Table 1.

### **Immunohistochemical staining for c-Kit and TNX proteins**

The number of ICC in the colon area of STC patients was significantly decreased compared with that in the control group ( $0.172 \pm 0.013$  vs  $0.256 \pm 0.021$ ,  $P < 0.05$ ), and their distribution was abnormal (Figure 1). TNX was expressed in both cells and the stroma, but the expression of TNX in the STC group was significantly increased compared with that in the control group ( $0.397 \pm 0.023$  vs  $0.226 \pm 0.017$ ,  $P < 0.01$ ) (Figure 2).

### **Western blot analysis of TNX, TGF- $\beta$ , Smad2/3, and Smad7 proteins**

The expression of the TNX and Smad2/3 proteins in the colon tissue of STC patients was significantly higher than that in the control group (TNX:  $0.868 \pm 0.028$  vs  $0.482 \pm 0.032$ ,  $P < 0.01$ ; Smad2/3:  $0.733 \pm 0.045$  vs  $0.405 \pm 0.081$ ,  $P < 0.01$ ). Consistent with this finding, the expression of the Smad7 protein in the colon tissue of STC patients was decreased ( $0.626 \pm 0.058$  vs  $0.799 \pm 0.035$ ,  $P < 0.01$ ), and TGF- $\beta$  expression was not significantly different between the two groups ( $0.476 \pm 0.028$  vs  $0.511 \pm 0.044$ ,  $P = 0.272$ ) (Figure 3).

Pearson correlation analysis showed that the TNX protein presented a strong correlation with Smad2/3 and Smad7 ( $P < 0.05$ ,  $|R| > 0.8$ ) and with TGF- $\beta$  ( $P < 0.05$ ,  $|R| = 0.7$ ).

### **Immunofluorescence staining for TGF- $\beta$ and Smad2/3 proteins**

Laser confocal microscopy showed TGF- $\beta$  expression as red fluorescence and Smad2/3 expression as green fluorescence, and nuclei were indicated by blue fluorescence. Both the STC and normal groups exhibited weak positive expression, which was located in the cytoplasm of stromal cells. The fluorescence intensity and density of Smad2/3 protein expression were significantly higher in the STC group than in the normal group ( $0.029 \pm 0.002$  vs  $0.017 \pm 0.001$ ,  $P < 0.01$ ). There was no significant difference in the fluorescence intensity or density of TGF- $\beta$  between the two groups ( $0.030 \pm 0.005$  vs  $0.033 \pm 0.008$ ,  $P > 0.05$ ) (Figure 4).

## **DISCUSSION**

With additional research, the etiology of STC is becoming clearer. The theory that 5-HT can stimulate colonic smooth muscle cholinergic nerves has been confirmed. Rubina A demonstrated that 5-HT receptor agonists can improve constipation symptoms in TNX knockout mice and suggested a relationship between the TNX protein and gastrointestinal function for the first time. The important role of the relationship between STC and TNX in gastrointestinal functional disease has not been reported previously, so it is worthy of further study.

TGF- $\beta$  belongs to a group of the TGF- $\beta$  superfamily that regulates cell growth, proliferation, and transition through downstream signaling pathways. The precursors of TGF- $\beta$  are cleaved by protease hydrolysis to form disulfide-bonded active TGF- $\beta$  dimers and an inactive binding peptide that binds to a TGF- $\beta$ -binding protein in an inactive form and is stored in the extracellular matrix. Under certain physiological or pathological conditions, active dimers are released from the complex, activate downstream signaling pathways, and regulate cell proliferation and differentiation. The currently recognized downstream pathways include the TGF- $\beta$ /Smad signaling pathway and a Smad protein-independent MAPK pathway. Alcaraz found that the C-terminal fibrinogen-like domain of TNX could promote epithelial-to-mesenchymal transition by activating latent TGF- $\beta$ <sup>[12]</sup>.

In this study, it was found that the expression of the TNX protein in the colon of STC patients was significantly higher than that in the normal group by approximately 1.5-2 times. At the same time, it was found that the expression of Smad2/3 in the STC group was increased significantly, whereas the expression of the Smad7 inhibitor decreased, and these differences were correlated with the change in the TNX expression level. However, there was no significant difference in the change in TGF- $\beta$ . It may be that TNX activates latent TGF- $\beta$  in the extracellular matrix, rather than inducing the synthesis of TGF- $\beta$ .

At this stage, it was found that the expression level of the TNX protein was positively correlated with the TGF- $\beta$ /Smad signaling pathway in the colon tissues of STC patients. The direction of transdifferentiation can be inferred according to the morphological changes in ICC observed by electron microscopy and the patterns of specific expression factors after differentiation, such as  $\alpha$ -SMA in the smooth muscle

Table 1 Comparison of general data between the two groups of patients

Group	Age (yr)	Sex (Male/Female)	Wexner score	GIQLI	Colonic transit test (-/+)
Control	50.00 ± 12.02	6/12	2.5 (0-6)	131.3 (125-138)	-
STC	56.86 ± 13.57	7/21	14.3 (11-18)	95.3 (89-102)	+

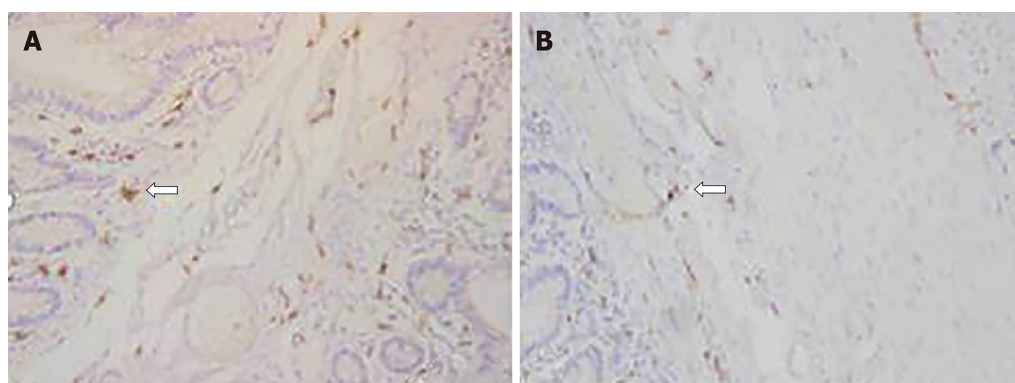
GIQLI: Gastrointestinal quality of life index; STC: Slow transit constipation.

phenotype. Furthermore, ICC transdifferentiation rather than apoptosis or death was verified. Then, based on the cytological animal experiments, the changes in the TGF- $\beta$ /Smad signaling pathway and the occurrence of STC under the two conditions were explored through gene knockout and overexpression of the TNX protein.

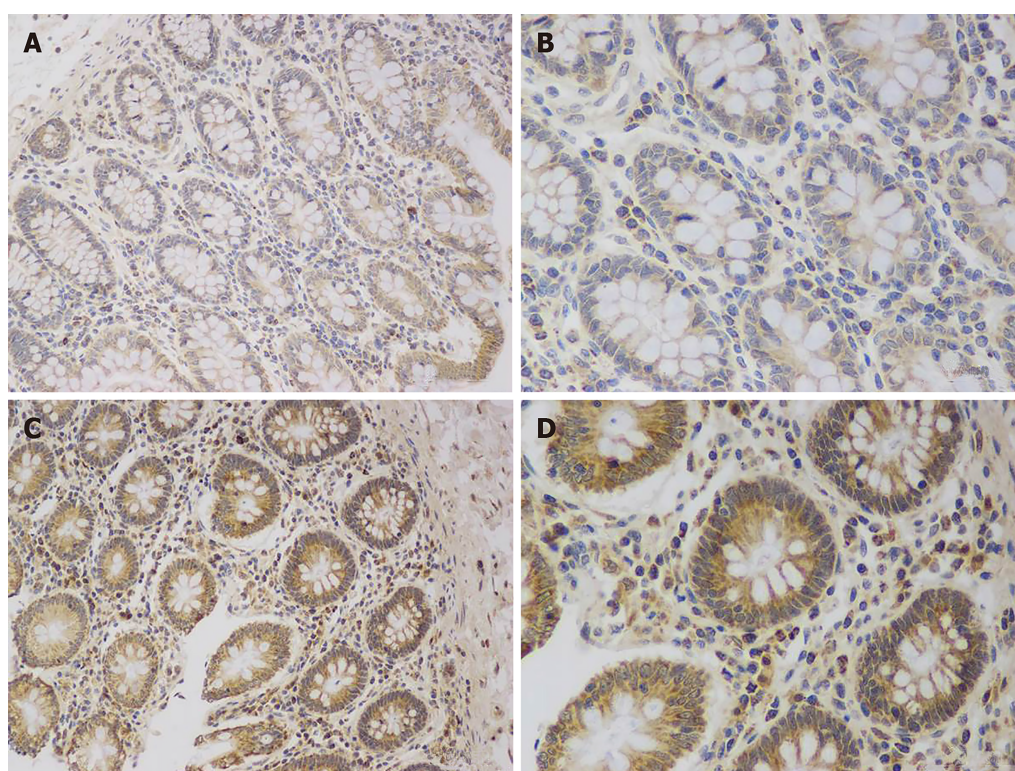
Previous research indicated that Ehlers-Danlos syndrome patients also exhibit colon motility disorders and constipation symptoms, which is contrary to this experimental phenomenon. It is speculated that the changes in ICC are not affected by the TGF- $\beta$ /Smad signaling pathway but are impacted by inhibition of the synthesis and release of cholinergic nerve acetylcholine, by reducing the maximum number of cholinergic receptors bound in the colon, or by reducing the reactivity of colon smooth muscle to Ach, leading to gastrointestinal electromyographic arrhythmia or abnormal changes in other nerves and their transmitters in the enteric nervous system, which destroys the integrity and coordination of the intestinal nervous system and leads to secondary intestinal transmission dysfunction.

This hypothesis is consistent with the observations that the number of AchE-positive neurons in the colonic myenteric plexus is reduced and that Ach expression is decreased in patients with STC<sup>[13]</sup>. Therefore, subsequent detection of cholinergic neurons and neurotransmitters can be performed to investigate whether the degree of change is statistically significant. Furthermore, whether the changes in cholinergic nerves are reversed by interfering with the expression of TNX can be examined through *in vitro* experiments.

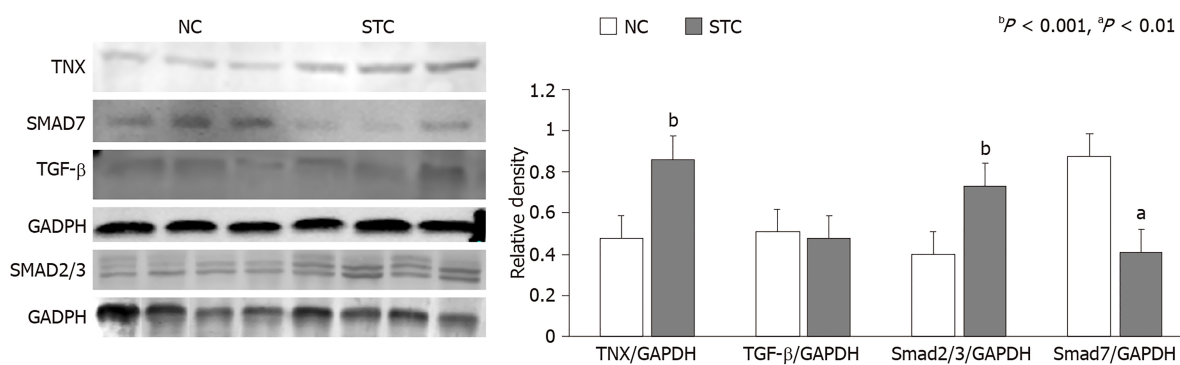
At present, the medical treatment of STC is still accepted by the public, but STC is generally poorly treated by dietary structure adjustment, biofeedback, fecal transplantation, drug ladder treatment, or dependence on laxatives. Therefore, surgical treatment has gradually become mainstream. After continuous improvement, the main surgical procedures applied now include total colectomy and ileorectal anastomosis, subtotal colectomy, colonic exclusion, and ileal pouch anastomosis to reduce the number of bowel movements. Although the surgical treatments for STC tend to be varied, they are clearly associated with major surgical trauma and postoperative complications. Therefore, the in-depth exploration of the pathogenesis of STC and the precise localization of therapy still deserve our attention. This study showed that the expression of the TNX protein is increased in the colon tissue of STC patients, which could affect the transdifferentiation of ICC by activating the TGF- $\beta$ /Smad signaling pathway, upregulating the Smad2/3 excitatory factors, and downregulating the Smad7 inhibitory factor. The transdifferentiation of ICC could be inhibited by interfering with the signaling molecules in this pathway, thereby reducing or reversing the occurrence of STC, providing a new experimental basis for the clinical treatment of STC.



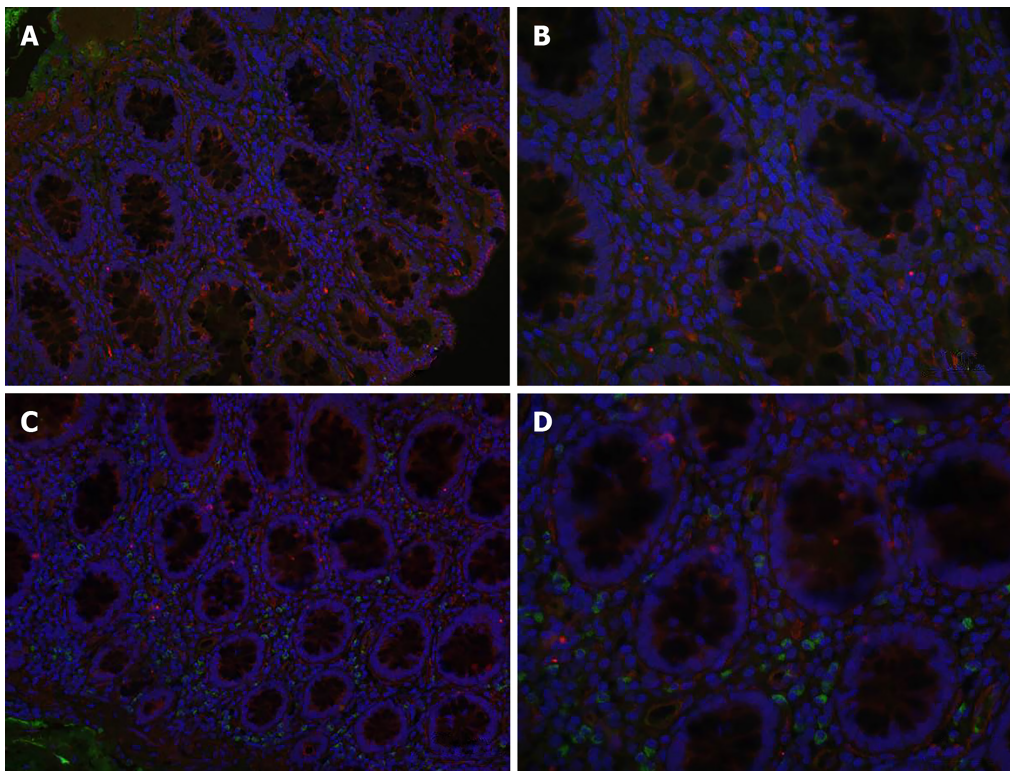
**Figure 1** Immunohistochemical staining for c-Kit and Tenascin-X proteins. A: High expression of c-Kit in the normal colon; B: Low expression of c-Kit in the slow transit constipation colon. The arrows indicate interstitial cells of Cajal showing brown-yellow c-Kit staining, suggesting that the number of interstitial cells of Cajal was reduced in the slow transit constipation group (200 ×).



**Figure 2** Immunohistochemical staining for c-Kit and Tenascin-X proteins. A and B: Low expression of the Tenascin-X protein in the normal group (200 × and 400 ×, respectively); C and D: High expression of the Tenascin-X protein in the slow transit constipation group (200 × and 400 ×, respectively).



**Figure 3** Expression of TNX, TGF-β, Smad2/3, and Smad7 in colon tissue. The change in tenascin-X is consistent with changes in the TGF-β/Smad signaling pathway. NC: Normal group; STC: Slow-transit constipation group. <sup>a</sup> $P < 0.01$ , <sup>b</sup> $P < 0.001$ .



**Figure 4** Expression of TGF- $\beta$  and Smad2/3 in colon tissue. A and B: Normal group (200  $\times$  and 400  $\times$ , respectively); C and D: Slow transit constipation group (200  $\times$  and 400  $\times$ , respectively).

## ARTICLE HIGHLIGHTS

### Research background

Chronic constipation is a gastrointestinal functional disease that seriously harms physical and mental health and impacts the quality of life of patients. Its incidence rate is 2%-27%. Slow transit constipation (STC) is a common type of chronic functional constipation accounting for 10.3%-45.5% of such cases. At present, the most effective treatment for STC is still surgery, but it is more traumatic. We need to further explore the pathogenesis of the disease, through the intervention of gene or protein expression, to alleviate or prevent the occurrence of the disease.

### Research motivation

There is no previous report which has linked constipation to the TGF- $\beta$ /Smad signaling pathway and Tenascin-X (TNX) protein, and it is also the first time that TNX protein has been introduced into clinical research. The pathogenesis of STC is very complex, surgical treatment is effective, but it is traumatic. Therefore, it is necessary to find a new direction of treatment from the pathogenesis.

### Research objectives

The main objective was to explore whether interstitial cells of Cajal (ICC) changes are related to transdifferentiation, and whether TNX protein can cause ICC changes through the TGF- $\beta$ /Smad signaling pathway. At this stage, it was found that the expression level of the TNX protein was positively correlated with the TGF- $\beta$ /Smad signaling pathway in the colon tissues of STC patients. Besides, ICC transdifferentiation rather than apoptosis or death was verified. Next, we need to confirm that the direction of transdifferentiation according to the morphological changes in ICC observed by electron microscopy and the patterns of specific expression factors after differentiation, such as  $\alpha$ -SMA in the smooth muscle phenotype.

### Research methods

We collected surgical specimens of patients and tested them by different laboratory methods including qualitative and quantitative tests, such as colon immunohistochemistry, immunofluorescence staining, and Western blot. The data were compared between groups by the *t*-test, and correlation analysis was performed with the Pearson correlation coefficient.

### Research results

The expression of the TNX protein in the colon of STC patients was significantly higher than that in the normal group by approximately 1.5-2 times. At the same time, it was found that the expression of Smad2/3 in the STC group was increased significantly, whereas the expression of the Smad7 inhibitor decreased, and these differences were correlated with the change in the TNX

expression level. The results may provide a new experimental basis for the clinical treatment of STC.

### Research conclusions

The expression of the TNX protein is increased in the colon tissue of STC patients, which could affect the transdifferentiation of ICC by activating the TGF- $\beta$ /Smad signaling pathway, upregulating the Smad2/3 excitatory factors, and downregulating the Smad7 inhibitory factor. The transdifferentiation of ICC could be inhibited by interfering with the signaling molecules in this pathway, thereby reducing or reversing the occurrence of STC, providing a new experimental basis for the clinical treatment of STC.

### Research perspectives

In the future, based on the cytological animal experiments, the changes in the TGF- $\beta$ /Smad signaling pathway and the occurrence of STC under the two conditions need to be explored through gene knockout and overexpression of the TNX protein. We may be able to regulate the upstream and downstream signaling molecules of this pathway with targeting drugs, so as to alleviate or inhibit the development of STC.

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## Retrospective Cohort Study

# Pre-hepatectomy type IV collagen 7S predicts post-hepatectomy liver failure and recovery

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

### Institutional review board

**statement:** This study was approved by the ethics committee of Keio University Hospital (approval Nos. 20120443 and 20140389).

**Informed consent statement:** All patients involved in this study provided informed consent prior to their study inclusion.

**Conflict-of-interest statement:** All other authors have nothing to disclose.

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## Abstract

### BACKGROUND

Liver resection is an effective treatment for benign and malignant liver tumors. However, a method for preoperative evaluation of hepatic reserve has not yet been established. Previously reported assessments of preoperative hepatic reserve focused only on liver failure in the early postoperative period and did not consider the long-term recovery of hepatic reserve. When determining eligibility for hepatectomy, the underlying pathophysiology needs to be considered to determine if the functional hepatic reserve can withstand both surgery and any postoperative therapy.

### AIM

To identify pre-hepatectomy factors associated with both early postoperative liver failure and long-term postoperative liver function recovery.

### METHODS

This study was a retrospective cohort study. We retrospectively investigated 215 patients who underwent hepatectomy at our hospital between May 2013 and December 2016. Early post-hepatectomy liver failure (PHLF) was defined using the International Study Group of Liver Surgery's definition of PHLF. Long-term

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postoperative recovery of liver function was defined as the time taken for serum total bilirubin and albumin levels to return to levels of  $< 2$  mg/dL and  $> 2.8$  g/dL, respectively, and the time taken for Child-Pugh score to return to Child-Pugh class A.

## RESULTS

Preoperative type IV collagen 7S was identified as a significant independent factor associated with both PHLF and postoperative long-term recovery of liver function. Further analysis revealed that the time taken for the recovery of Child-Pugh scores and serum total bilirubin and albumin levels was significantly shorter in patients with type IV collagen 7S  $\leq 6$  ng/mL than in those with type IV collagen 7S  $> 6$  ng/mL. In additional analyses, similar results were observed in patients without chronic viral hepatitis associated with fibrosis.

## CONCLUSION

Preoperative type IV collagen 7S is a preoperative predictor of PHLF and long-term postoperative liver function recovery. It can also be used in patients without chronic hepatitis virus.

**Key words:** Hepatectomy; Liver failure; Type IV collagen 7S; Liver fibrosis; Postoperative complications; Long-term postoperative liver function recovery

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**Core tip:** In this study, we identified the pre-hepatectomy factor associated with both early postoperative liver failure and long-term postoperative liver function recovery. We found that preoperative type IV collagen 7S is a significant independent factor associated with both post-hepatectomy liver failure and postoperative long-term recovery of liver function. Our analysis revealed that the time required for the recovery of Child-Pugh scores and serum total bilirubin and total bilirubin levels was significantly shorter in patients with type IV collagen 7S  $\leq 6$  ng/mL than in patients with type IV collagen 7S  $> 6$  ng/mL.

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## INTRODUCTION

Liver resection is an effective treatment for benign and malignant liver tumors. Due to advancements in hepatic surgeries and perioperative management, the perioperative mortality in high-volume centers has been reported to be 5% or less<sup>[1-5]</sup>. However, postoperative liver failure remains a severe complication that can result in death.

In addition to preoperatively assessing whether a patient can tolerate hepatectomy, the application of hepatectomy should be determined with considerations on the underlying pathophysiology to decide whether further postoperative therapies will be required to improve the hepatic reserve. Various methods for hepatic reserve evaluation have been reported, including Child-Pugh score<sup>[6]</sup>, indocyanine green retention rate at 15 min (ICG-R15)<sup>[7]</sup>, galactose elimination capacity<sup>[8]</sup>, maximal removal rate of indocyanine green<sup>[9]</sup>, arterial ketone body ratio<sup>[10]</sup>, and technetium-99m galactosyl human serum albumin scintigraphy<sup>[11]</sup>. However, the Child-Pugh score and ICG-R15 may be insufficient for accurately determining the hepatic reserve in patients with arteriovenous shunts or transporter abnormalities. To date, there is no established method for preoperative evaluation of hepatic reserve. Furthermore, existing methods for hepatic reserve assessment primarily focus on postoperative liver failure and do not consider the long-term recovery of liver function. From a therapeutic standpoint, there have been significant advancements in chemotherapy for gastrointestinal cancers in recent years, and the need for postoperative multidisciplinary therapy is currently well recognized. In postoperative therapies,

long-term recovery of liver function is just as important as early liver failure. Therefore, the present study aimed to identify factors that can predict both early postoperative liver failure and long-term postoperative recovery of liver function in patients undergoing hepatectomy.

## MATERIALS AND METHODS

### **Patients**

This study was a retrospective cohort study. Overall, 230 patients who underwent hepatectomy at Keio University Hospital between May 2013 and December 2016 were recruited. Patient data were extracted from the hospital's database. Patients with postoperative follow-up < 1 mo ( $n = 1$ ) and missing data on preoperative liver fibrosis markers ( $n = 14$ ) were excluded, resulting in a sample size of 215 patients. This included 87 patients with hepatocellular carcinoma, 49 with liver metastasis secondary to colon cancer, 24 with hilar cholangiocarcinoma, 20 with intrahepatic cholangiocarcinoma, 22 with liver metastasis due to non-colon cancers, and 13 with other tumors. There were 148 men (68.8%) and 67 women (31.2%), with an overall median age of 68 years (20-88 years). Additional analyses were performed in 158 patients without chronic viral hepatitis. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the ethics committee of our hospital (approval nos. 20120443 and 20140389).

### **Preoperative workup**

The patients underwent preoperative physical examinations and medical history interviews. Serum levels of aspartate aminotransferase, alanine aminotransferase, total bilirubin (TB), albumin (Alb), cholinesterase, total cholesterol, prothrombin time, C-reactive protein, platelet count, ICG-R15, and the fibrosis markers type IV collagen 7S and hyaluronic acid were estimated in each patient. Acoustic radiation force impulse (ARFI) ultrasonography was performed to measure liver stiffness. ARFI was performed 10 times each over the left and right lobes and the mean value was used in the analyses.

### **Surgery**

The inclusion criteria for surgery were as follows. It was assumed that the patients were in a generally good condition to tolerate major laparotomy and that the tumors were within the extent of resections. Hepatic reserve was assessed using biochemical tests and ICG-R15 to determine whether hepatectomy could be tolerated<sup>[12]</sup>. Preoperative computed tomography (Vincent; Fujifilm, Japan) findings were analyzed to calculate the liver volume. The maximum hepatectomy volume was set at 65%.

### **Postoperative workup**

Early postoperative liver failure was diagnosed based on the criteria established by the International Study Group of Liver Surgery (ISGLS)<sup>[13]</sup>. The patients were divided into two groups-the PHLF and non-PHLF groups-based on the presence or absence of early postoperative liver failure, respectively.

Long-term postoperative recovery of liver function was analyzed based on the time to recovery (days) of Child-Pugh class A and serum TB < 2 mg/dL and Alb > 2.8 g/dL. When blood transfusions or blood products were used, recovery was identified based on two consecutive samples without blood transfusions or use of blood products between the samples. The patients were divided into two groups - serum type IV collagen 7S ≤ 6 ng/mL and that with serum type IV collagen 7S > 6 ng/mL-, because type IV collagen 7S ≤ 6 ng/mL is defined as within normal limit in many laboratory companies which measure type IV collagen 7S. The minimum time to recovery was 1 d. For example, if serum TB < 2 mg/dL was observed on postoperative day 3, the time to recovery was 3 d. Even in cases with preoperative serum Alb < 2.8 g/dL, the time to recovery was from the day of surgery to the postoperative day when the levels rose above 2.8 g/dL. All patients were followed up every 1-3 mo after surgery.

### **Histology**

A pathologist evaluated fibrosis of the background liver using the METAVIR score in 91 patients between May 2013 and December 2014<sup>[14]</sup>. We analyzed the pathological data of these patients. Additionally, Elastica van Gieson (EVG) staining of the background liver was performed to determine the areas of fibrosis. For pathological evaluation, 3-μm liver specimen slices were fixed with formalin and stained with EVG. A NanoZoomer HT (Hamamatsu Photonics, Hamamatsu, Japan) was used to obtain an overall tissue image from virtual slides (whole-slide images). The analysis

was performed at 0.46  $\mu\text{m}/\text{pixel}$  in 20 fields of view. Based on this analysis, whole-slide images were placed into five categories-four tissue components (collagen, elastin, nuclei, and cytoplasm) and one non-tissue component-and the amount of each tissue component was measured<sup>[15]</sup>.

### Statistical analysis

Continuous variables are expressed as mean  $\pm$  SD; either the  $\chi^2$  test or Kruskal-Wallis test was used for comparisons. We identified factors associated with significant differences in early postoperative liver failure and long-term postoperative recovery of liver function using multivariate logistic regression and Cox regression. The relationship between collagen and elastin levels and the presence or absence of postoperative early liver failure was examined using the Mann-Whitney *U* test.

We also examined the relationship between collagen and elastin levels and the other parameters. Spearman's correlation coefficient was used for correlations with collagen and elastin levels. Long-term postoperative recovery of liver function was compared between the group of patients with serum type IV collagen 7S  $\leq 6$  ng/mL and that with serum type IV collagen 7S  $> 6$  ng/mL using the Kaplan-Meier method. All statistical analyses were performed using SPSS version 25 (IBM Japan, Tokyo, Japan) with  $P < 0.05$  considered as statistically significant.

Data regarding ICG-R15 and ARFI, which are factors that affect liver function, were missing in 10.2% and 29.8% of cases, respectively. As this harms the reliability and accuracy of the analyses, widens the confidence intervals (CIs), and can result in a bias in the calculation of odds ratios (ORs), we hence compensated for the missing values with multiple imputations using preoperative levels of serum TB, ALB, and cholinesterase and prothrombin time to predict the missing ICG-R15 values and preoperative levels of serum aspartate aminotransferase, type IV collagen 7S, and hyaluronic acid to predict the missing ARFI values. Data were generated for 20 ICG-R15 and ARFI values, which were analyzed separately and combined with the results to calculate the ORs<sup>[16,17]</sup>.

## RESULTS

### Examination of predictive factors for early postoperative liver failure

Table 1 summarizes the patient characteristics, preoperative liver function parameters, and operative procedures. Based on the ISGLS definition, there were 27 cases of early post-hepatectomy liver failure (PHLF) and 188 cases without early PHLF (non-PHLF). Of these, nine were of grade A and 18 were of grade B. There were no intraoperative deaths in this study.

Tables 2 and 3 summarize the results of the analyses of preoperative liver function parameters between the PHLF and non-PHLF groups. In multivariate analysis, preoperative type IV collagen 7S level was a significant independent factor associated with early postoperative liver failure (OR = 1.543; 95%CI: 1.258-1.892;  $P < 0.001$ ).

### Examination of predictive factors for postoperative long-term recovery of liver function

Tables 4, 5, and 6 summarize the results of Cox regression analyses of time to recovery. Preoperative type IV collagen 7S level was a significant independent factor associated with the number of days until recovery to these levels in Cox regression analyses. Figure 1 illustrates the differences in time to recovery. The time to recovery was significantly shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL.

### Examination of cases without chronic viral hepatitis in the background liver

When assessing the presence or absence of fibrosis, there can be bias due to the presence or absence of chronic viral hepatitis, which makes the liver prone to failure. Therefore, an additional analysis of 158 patients without chronic hepatitis virus was performed. Tables 7 and 8 summarize these results. In these analyses, preoperative type IV collagen 7S was a significant factor associated with early postoperative liver failure (OR = 1.423; 95%CI: 1.074-1.886;  $P < 0.001$ ). Figure 2 illustrates the time to recovery in 158 patients without chronic hepatitis virus. For long-term postoperative recovery of liver function as well, the time to recovery was significantly shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL.

### Examination of preoperative jaundice

Similarly, there can be bias due to the presence or absence of preoperative jaundice,

**Table 1** Preoperative clinicopathological, liver function, and operative data of the patients

		Overall (n = 215)	No postoperative liver failure (n = 188)	Postoperative liver failure (n = 27)	P value
Age, yr (median)		68	68	72	0.107
Sex	Men	148	125	23	0.073
	Women	67	63	4	
Child-Pugh score	5	180	162	18	0.01
	6	27	22	5	
	7	7	3	4	
	8	1	1	0	
Liver tumor	Hepatocellular carcinoma	87	75	12	0.030
	Colorectal metastases	49	46	3	
	Hilar cholangiocarcinoma	24	17	7	
	Intrahepatic cholangiocarcinoma	20	16	4	
	Metastatic liver tumor (other than colorectal cancer)	22	21	1	
	Other tumors	13	13	0	
Surgery	Partial resection	112	103	9	0.041
	Sectionectomy	23	22	1	
	Lobectomy (+extended)	73	58	15	
	Three sectionectomy	7	5	2	

which makes the liver prone to failure. Therefore, an additional analysis of 21 patients without preoperative jaundice was performed. **Table 9** summarizes the results of the sub-analysis of 21 patients with preoperative jaundice. Preoperative type IV collagen 7S level was a significant independent factor associated with early postoperative liver failure (OR = 1.540; 95% CI: 1.036-2.288;  $P = 0.033$ ). Further, **Figure 3** illustrates the time to recovery. For long-term postoperative recovery of liver function as well, the time to recovery was significantly shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL. The time to recovery of Alb levels tended to be shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL; however, the difference was not significant.

### Examination of histological factors

A pathologist evaluated fibrosis of the background liver using the METAVIR score in 91 patients between May 2013 and December 2014. METAVIR score was not a significant factor associated with postoperative early liver failure ( $P = 0.801$ ) (**Table 10**). **Figure 4** illustrates the relationships between collagen and elastin levels and early postoperative liver failure. Collagen and elastin levels were no different between patients who experienced early postoperative liver failure and those who did not. Furthermore, when the correlations of collagen and elastin with other data were examined, the preoperative type IV collagen 7S level was correlated with collagen ( $\rho = 0.281$ ,  $P = 0.007$ ) but not with elastin ( $\rho = 0.167$ ,  $P = 0.114$ ). ARFI correlated with both collagen ( $\rho = 0.405$ ,  $P < 0.001$ ) and elastin ( $\rho = 0.316$ ,  $P = 0.002$ ).

## DISCUSSION

The purpose of this study was to analyze predictive factors for early postoperative liver failure and long-term postoperative recovery of liver function. The results indicated that preoperative type IV collagen 7S was a significant independent factor associated with early postoperative liver failure. Additionally, the time to recovery of Child-Pugh classification and serum TB and Alb-which are indicators of long-term postoperative recovery of liver function-was significantly shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL. Similar results were observed irrespective of multiple

**Table 2** Preoperative levels of liver function parameters in patients with and without postoperative liver failure in univariate analysis

Factor	No postoperative liver failure	Postoperative liver failure	Univariate analysis	
	(n = 188)	(n = 27)	OR (95%CI)	P value
Age, yr (median)	68	72		0.095
Sex, male/female	125/63	4/23		0.064
Preoperative biliary drainage	15	7		0.005
Child-Pugh score	5.16 ± 0.45	5.48 ± 0.75	2.505 (1.345-4.665)	0.004
Blood platelet (× 10 <sup>4</sup> /μL)	19.5 ± 7.7	16.7 ± 6.6		0.099
Total bilirubin (mg/dL)	0.80 ± 0.33	0.90 ± 0.39		0.099
PT-INR (INR)	1.00 ± 0.12	1.09 ± 0.13	63.56 (2.533-1595.002)	0.012
Albumin (g/dL)	4.03 ± 0.45	3.64 ± 0.54	0.210 (0.091-0.484)	< 0.001
Aspartate aminotransferase (IU/L)	30.9 ± 23.6	40.6 ± 24.8	1.012 (0.999-1.025)	0.071
Alanine aminotransferase (IU/L)	27.3 ± 20.7	32.6 ± 24.2		0.171
Cholinesterase (IU/L)	284.2 ± 82.1	194.6 ± 69.9	0.982 (0.975-0.990)	< 0.001
Total cholesterol (mg/dL)	188.2 ± 39.2	165.8 ± 37.1	0.984 (0.972-0.996)	0.011
ICG-R15 (%)	10.9 ± 9.01	14.8 ± 11.1	1.034 (0.998-1.071)	0.068
Type IV collagen 7S (ng/mL)	5.50 ± 1.91	8.80 ± 3.06	1.601 (1.340-1.914)	< 0.001
Hyaluronic acid (ng/mL)	88.5 ± 100.6	256.5 ± 216.5	1.005 (1.001-1.009)	0.018
ARFI <sup>TM</sup> (v/s)	1.56 ± 0.57	2.01 ± 0.69	2.785 (1.431-5.422)	0.003
CRP (mg/dL)	0.39 ± 1.01	0.82 ± 1.33	1.319 (0.995-1.747)	0.054
Extent of hepatic resection <sup>[26]</sup>	0.81 ± 0.97	1.37 ± 1.04	1.688 (1.114-2.557)	0.013

PT-INR: Prothrombin time-international normalized ratio; ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

imputations used in the analysis of postoperative liver failure.

Various reports have described existing methods for predicting early postoperative liver failure. Among them, the Child-Pugh classification is widely used worldwide. ICG-R15 is also commonly used in Asia and has been recognized in several other parts of the world<sup>[18]</sup>. However, in the present study, the Child-Pugh classification and ICG-R15 were not sufficient predictors of early postoperative liver failure. Additionally, the Child-Pugh classification, ICG-R15, and other liver function tests only focus on early postoperative liver failure and do not consider the long-term postoperative recovery of liver function.

The present study used the time to recovery of the Child-Pugh classification and serum TB and Alb as indicators of long-term postoperative recovery of liver function. Postoperative recovery of liver function is particularly clinically important when adjuvant therapy is used. In our study, preoperative type IV collagen 7S was a significant independent factor associated with the time to recovery. Furthermore, the time to recovery was significantly shorter in the group with preoperative type IV collagen 7S ≤ 6 ng/mL than in the group with preoperative type IV collagen 7S > 6 ng/mL. This suggests that type IV collagen 7S may also be useful in predicting the long-term postoperative recovery of liver function.

There can be bias due to the presence or absence of chronic viral hepatitis or preoperative jaundice, which makes the liver prone to failure. In additional analyses of patients without chronic viral hepatitis associated with fibrosis, preoperative type IV collagen 7S was also a significant factor associated with early postoperative liver failure and long-term postoperative recovery of liver function. Similarly, in cases of jaundice, type IV collagen 7S was also a significant independent factor associated with early postoperative liver failure and long-term postoperative recovery of liver function. In cases without chronic viral hepatitis associated with fibrosis and cases with jaundice, early postoperative liver failure and postoperative long-term recovery of liver function were also associated with preoperative type IV collagen 7S.

The METAVIR score-which represents histological liver fibrosis-was not significantly associated with early postoperative liver failure. Additionally, we calculated the amount of collagen and elastin fibers in the samples using EVG staining but found no histological differences between patients with early postoperative liver failure and those without (Figure 4).

We evaluated two markers of liver fibrosis-type IV collagen 7S<sup>[19]</sup> and hyaluronic acid<sup>[20]</sup>. In our study, preoperative type IV collagen 7S was a significant independent

**Table 3 Preoperative liver function parameters in patients with and without post-hepatectomy liver failure in multivariate analysis**

Factors	Multivariate analysis	
	OR (95%CI)	P value
Sex, male/female	2.802 (0.813-9.653)	0.0513
ICG-R15 (%)	0.998 (0.951-1.047)	0.466
Type IV collagen 7S (ng/mL)	1.543 (1.258-1.892)	< 0.001
ARFI <sup>TM</sup> (v/s)	1.525 (0.673-3.457)	0.156

ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse.

factor associated with early PHLF and long-term postoperative recovery of liver function. Type IV collagen is a basement membrane protein that is located around the sinusoids in the liver and is an immunohistochemical marker of the basement membrane<sup>[21]</sup>. In a normal liver, sinusoids have no basement membrane; however, as the liver undergoes fibrosis, sinusoidal capillarization occurs and the basement membrane appears. The presence of type IV collagen 7S in the basement membrane is believed to reflect in the form of increased levels in the blood<sup>[22]</sup>.

There are two possible reasons for the association of type IV collagen 7S with early postoperative liver failure and long-term postoperative recovery of liver function. Type IV collagen 7S is affected by factors other than fibrosis. Alternatively, it may reflect a type of fibrosis that does not exhibit histological differences from non-fibrotic tissue. In recent years, type IV collagen 7S has been believed to reflect fibrogenesis—an indicator of collagen production during this process. Therefore, fibrogenesis is different from the histological appearance of fibrosis. Fibrogenesis reflects the current damage to the liver caused by fibrosis and not liver damage that has accumulated over many years, which could be why the histological differences were not observed in the present study. According to Parola *et al.*<sup>[23]</sup>, fibrogenesis is a reaction to liver damage that involves growth of extracellular matrices (ECM) for liver regeneration. Increased ECM in the space of Disse reduces liver function<sup>[24]</sup>. Therefore, fibrogenesis is believed to be a reaction that reduces liver function. The reason why type IV collagen 7S is associated with early postoperative liver failure and postoperative long-term recovery of liver function may be because it reflects fibrosis that does not exhibit histological differences. This suggests that instead of fibrosis—which manifests histologically—fibrogenesis affects the early and long-term postoperative recovery of liver function.

This study has some limitations. Our study was a retrospective analysis at a single center, which limits the validity of the results. Additionally, there is the possibility of bias because only patients that were able to undergo hepatectomy were included. Additionally, a new marker of liver fibrosis-M2BPGI<sup>[25]</sup>—was listed for insurance coverage in Japan in January 2015; however, it was not examined in this study. Going forward, multicenter, prospective studies are required to investigate the application of preoperative serum type IV collagen 7S levels in predicting the postoperative long-term course and early liver failure.

In conclusion, preoperative type IV collagen 7S is a useful predictive factor for early postoperative liver failure and long-term postoperative recovery of liver function. In particular, similar results were obtained in patients without chronic hepatitis virus in the background liver and in those with jaundice. Therefore, preoperative type IV collagen could be useful in predicting early liver failure and long-term postoperative recovery of liver function in different subsets of patients undergoing hepatectomy.

**Table 4** Cox proportional hazards analysis to identify predictors of postoperative recovery of liver function to achieve Child-Pugh class A

Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr (mean)	0.984 (0.972-0.996)	0.007		0.837
Sex, male/female		0.688		0.656
Child-Pugh score	0.577 (0.427-0.780)	< 0.001		0.123
Cholinesterase (IU/L)	1.002 (1.001-1.003)	0.001		0.346
Total cholesterol (mg/dL)	1.004 (1.001-1.007)	0.024		0.932
ICG-R15 (%)	0.980 (0.964-0.997)	0.024		0.756
Type IV collagen 7S (ng/mL)	0.837 (0.784-0.894)	< 0.001	0.779 (0.682-0.890)	< 0.001
Hyaluronic acid (ng/mL)	0.996 (0.994-0.999)	0.003		0.179
ARFI™ (v/s)	0.651 (0.490-0.866)	0.003		0.625
CRP (mg/dL)	0.792 (0.669-0.936)	0.006		0.142
Extent of hepatic resection <sup>[26]</sup>	0.813 (0.707-0.936)	0.004		0.64

ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

**Table 5** Cox proportional hazards analysis to identify predictors of postoperative recovery of liver function to achieve serum total bilirubin < 2 mg/dL

Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr (mean)		0.275		0.53
Sex, male/female		0.127		0.222
Child-Pugh score	0.749 (0.565-0.994)	< 0.001		0.21
PT-INR (INR)	0.195 (0.47-0.803)	0.024		0.155
Cholinesterase (IU/L)	1.002 (1.001-1.004)	0.003		0.106
ICG-R15 (%)		0.165		
Type IV collagen 7S (ng/mL)	0.905 (0.851-0.926)	0.001	0.906 (0.851-0.962)	0.001
Hyaluronic acid (ng/mL)		0.552		
ARFI™ (v/s)		0.056		
CRP (mg/dL)		0.118		
Extent of hepatic resection <sup>[26]</sup>		0.159		

PT-INR: Prothrombin time-international normalized ratio; ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

**Table 6** Cox proportional hazards analysis to identify predictors of postoperative recovery of liver function to achieve serum albumin > 2.8 g/dL

Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr (mean)	0.985 (0.973-0.996)	0.008		0.18
Sex, male/female		0.059		0.457
Child-Pugh score	0.573 (0.424-0.776)	< 0.001		0.611
Cholinesterase (IU/L)	1.002 (1.001-1.003)	0.001		0.39
ICG-R15 (%)	0.979 (0.962-0.996)	0.008		0.983
Type IV collagen 7S (ng/mL)	0.841 (0.787-0.899)	< 0.001	0.759 (0.659-0.875)	< 0.001
Hyaluronic acid (ng/mL)	0.996 (0.994-0.999)	0.004		0.292
ARFI™ (v/s)	0.723 (0.545-0.959)	0.024		0.774
CRP (mg/dL)	0.808 (0.685-0.953)	0.011		0.192
Extent of hepatic resection <sup>[26]</sup>	0.830 (0.722-0.955)	0.009		0.636

ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

**Table 7** Preoperative liver function parameters in post-hepatectomy liver failure and non-post-hepatectomy liver failure patients who did not have hepatitis viruses in univariate analysis

Factors	No postoperative liver failure	Postoperative liver failure	Univariate analysis	
	(n = 136)	(n = 22)	OR (95%CI)	P value
Age, yr (mean)	65.3	70.7		0.09
Sex, male/female	87/49	4/18		0.109
Preoperative biliary drainage	9	4		0.045
Child-Pugh score	5.15 ± 0.39	5.59 ± 0.80	3.694 (1.766-7.729)	0.001
Platelets (× 10 <sup>4</sup> /μL)	21.1 ± 7.7	17.1 ± 7.0		0.991
Total bilirubin (mg/dL)	0.79 ± 0.33	0.91 ± 0.40		0.111
PT-INR (INR)	0.99 ± 0.13	1.1 ± 0.14	123.581 (2.637-5791.435)	0.014
Albumin (g/dL)	4.03 ± 0.44	3.57 ± 0.60	0.157 (0.059-0.414)	< 0.001
Aspartate aminotransferase (IU/L)	30.8 ± 26.6	41.5 ± 25.9		0.121
Alanine aminotransferase (IU/L)	28.0 ± 22.9	33.6 ± 25.5		0.296
Cholinesterase (IU/L)	293.1 ± 85.1	192.2 ± 67.9	0.979 (0.970-0.988)	< 0.001
Total cholesterol (mg/dL)	192.2 ± 42.2	168.4 ± 40.2	0.985 (0.973-0.998)	0.02
ICG-15 clearance rate (%)	10.3 ± 9.11	15.0 ± 11.8	1.034 (0.998-1.081)	0.064
Type IV collagen 7S (ng/mL)	5.36 ± 1.85	9.15 ± 3.13	1.731 (1.383-2.167)	< 0.001
Hyaluronic acid (ng/mL)	78.7 ± 99.2	261.9 ± 207.3	1.005 (1.004-1.011)	< 0.001
ARFI™ (v/s)	1.49 ± 0.53	1.98 ± 0.67	2.785 (1.546-7.887)	0.003
CRP (mg/dL)	0.47 ± 1.14	1.00 ± 1.42		0.056
Extent of hepatic resection <sup>[26]</sup>	0.90 ± 1.0	1.41 ± 1.05	1.638 (1.037-2.587)	0.034

PT-INR: Prothrombin time-international normalized ratio; ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

**Table 8 Preoperative liver function parameters in post-hepatectomy liver failure and non-post-hepatectomy liver failure patients who did not have hepatitis viruses in multivariate analysis**

Factors	Multivariate analysis	
	OR (95%CI)	P value
Cholinesterase (IU/L)	0.987 (0.977-0.998)	0.0083
ICG-R15 (%)	0.959 (0.896-1.026)	0.11
Type IV collagen 7S (ng/mL)	1.423 (1.074-1.886)	0.002
Hyaluronic acid (ng/mL)	1.004 (1.000-1.008)	0.021
ARFI <sup>TM</sup> (v/s)	1.144 (0.406-3.220)	0.6

ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse.

**Table 9 Preoperative liver function parameters in post-hepatectomy liver failure and non-post-hepatectomy liver failure patients who had jaundice in univariate analysis**

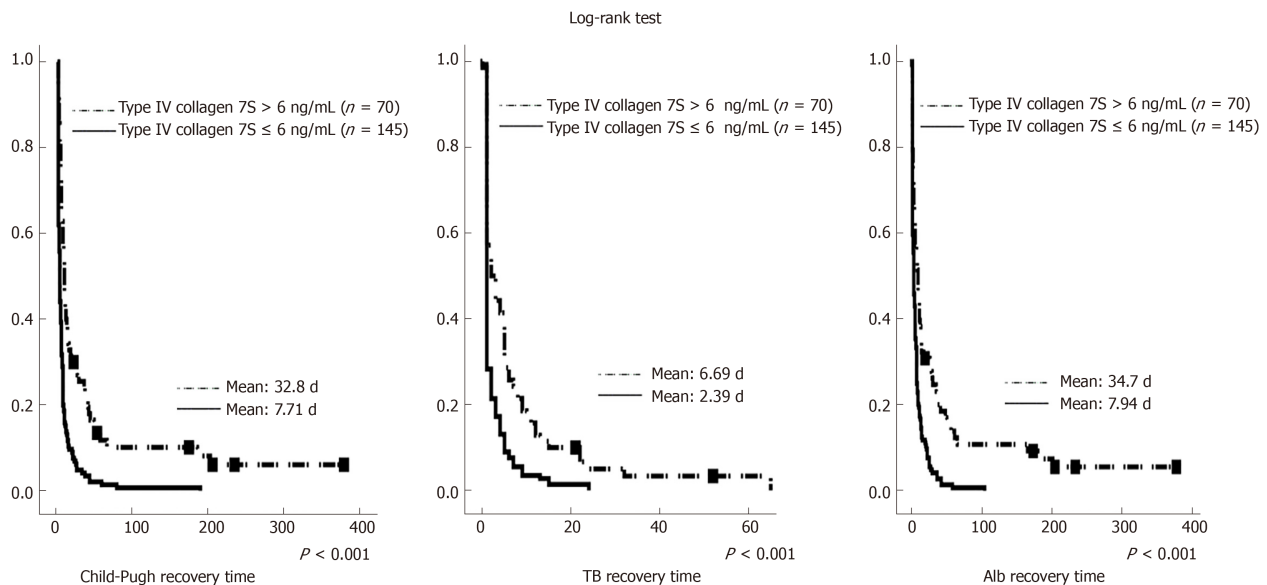
Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr (mean)		0.74		0.817
Sex, male/female		0.105		0.237
Child-Pugh score		0.22		
Cholinesterase (IU/L)	0.979 (0.970-0.988)	0.066		0.157
Total cholesterol (mg/dL)		0.652		
ICG-R15 (%)		0.371		
Type IV collagen 7S (ng/mL)	1.540 (1.036-2.288)	0.033	1.540 (1.036-2.288)	0.033
Hyaluronic acid (ng/mL)		0.187		
ARFI <sup>TM</sup> (v/s)		0.171		
CRP (mg/dL)		0.533		
Extent of hepatic resection <sup>[26]</sup>		0.481		

ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.

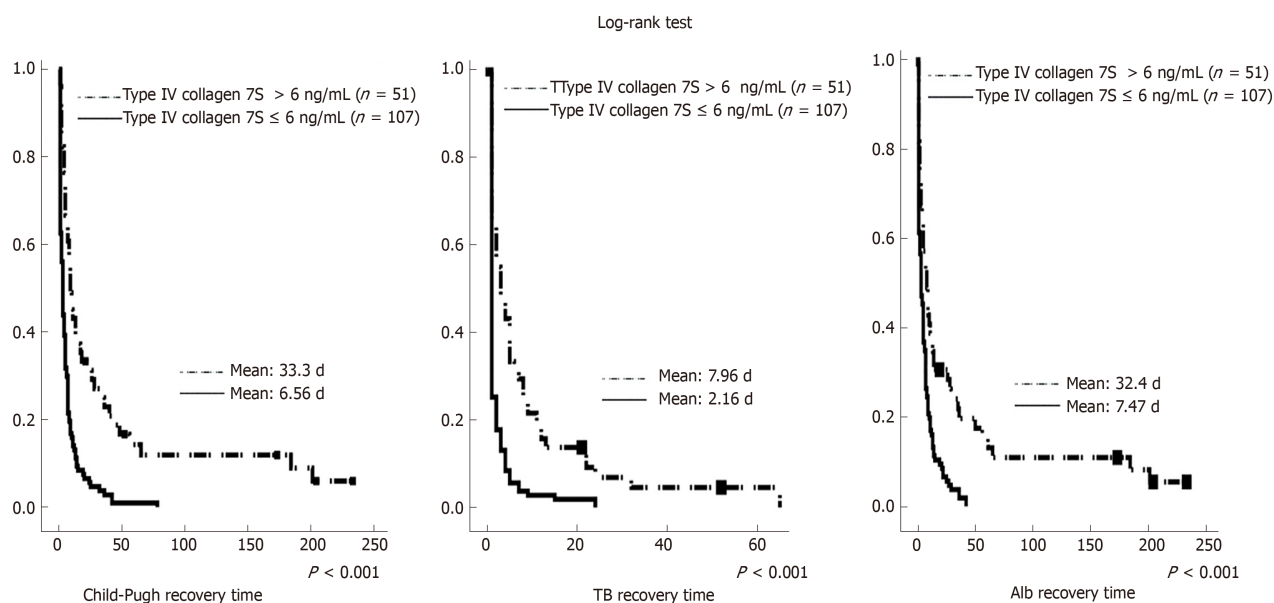
**Table 10** Preoperative liver function parameters in post-hepatectomy liver failure and non-post-hepatectomy liver failure patients in univariate and multivariate analyses for 91 patients

Factors	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Age, yr (mean)		0.083		0.065
Sex, male/female		0.462		0.557
Child-Pugh score		0.347		
Platelets ( $\times 10^4/\mu\text{L}$ )		0.509		
PT-INR (INR)		0.064		
Aspartate aminotransferase (IU/L)		0.087		
Alanine aminotransferase (IU/L)		0.468		
Cholinesterase (IU/L)	0.986 (0.977-0.995)	0.003		0.348
Total cholesterol (mg/dL)		0.128		
ICG-R15 (%)		0.643		
Type IV collagen 7S (ng/mL)	1.514 (0.784-0.894)	< 0.001	2.178 (0.684-0.890)	0.005
Hyaluronic acid (ng/mL)	1.005 (1.001-1.009)	0.018		0.674
ARFI™ (v/s)		0.145		
CRP (mg/dL)		0.495		
METAVIR score		0.801		
Extent of hepatic resection <sup>[26]</sup>	2.296 (0.707-0.936)	0.009		0.86

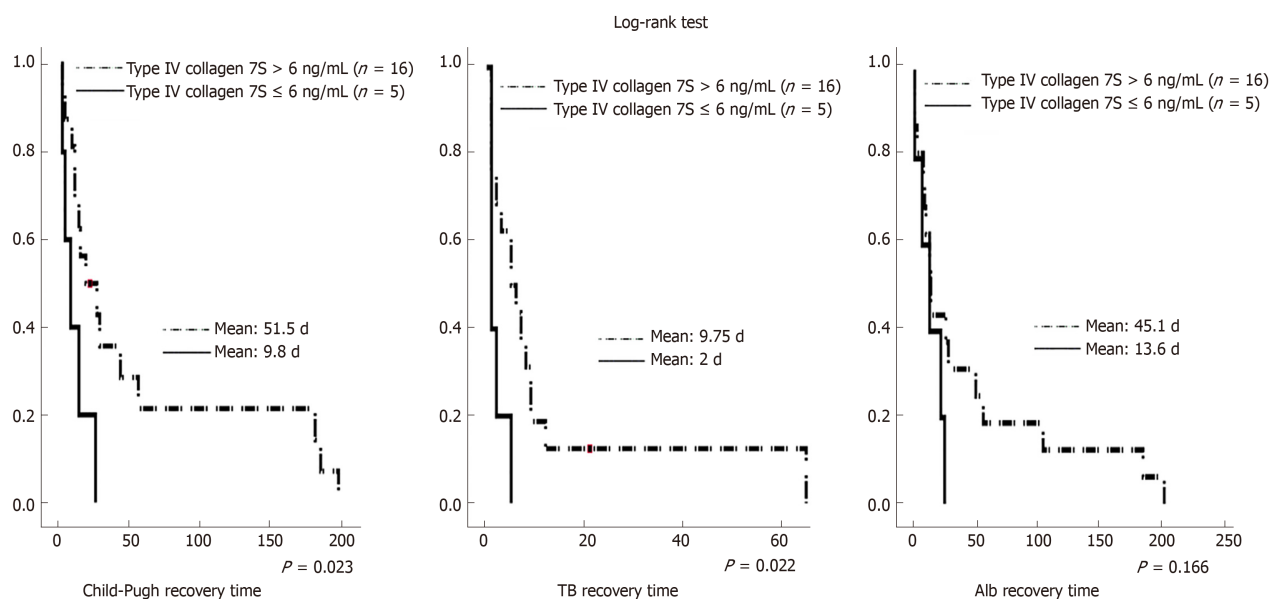
PT-INR: Prothrombin time-international normalized ratio; ICG-R15: Indocyanine green retention rate at 15 min; ARFI: Acoustic radiation force impulse; CRP: C-reactive protein.



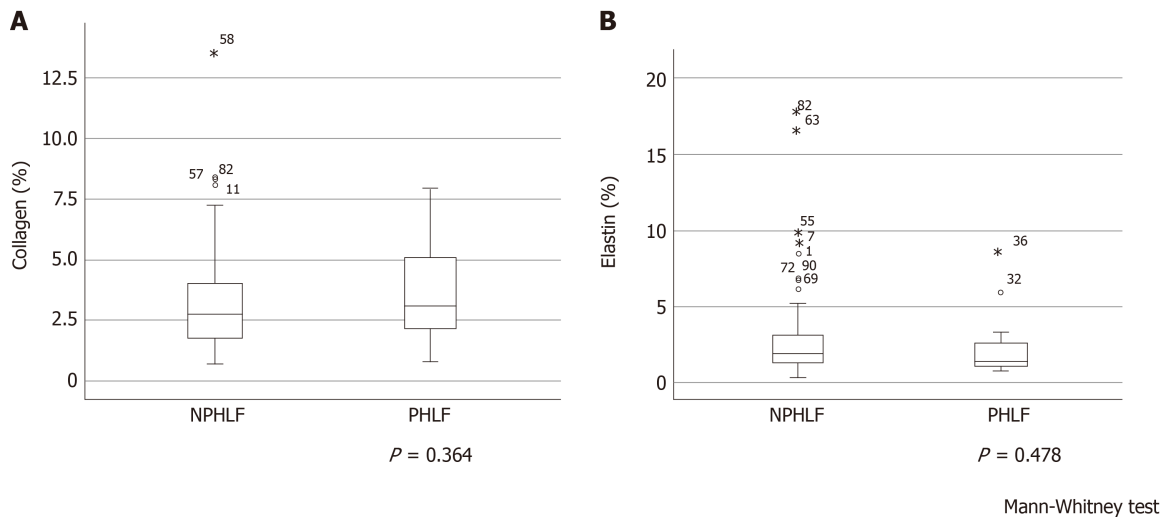
**Figure 1** Postoperative time to recovery of liver function to recovery in this cohort. Time to recovery was defined as return to Child-Pugh class A and return of serum total bilirubin and albumin levels to < 2 mg/dL and > 2.8 mg/dL, respectively. It was compared between patients with serum preoperative type IV collagen 7S > 6 ng/mL ( $n = 70$ ) and those with serum preoperative type IV collagen 7S  $\leq 6$  ng/mL ( $n = 145$ ). The time to recovery was significantly shorter in patients with type IV collagen 7S  $\leq 6$  ng/mL (all,  $P < 0.001$ ; log-rank test). TB: Total bilirubin; Alb: Albumin.



**Figure 2** Postoperative time to recovery of liver function in patients without hepatitis viruses in our study. Time to recovery was defined as return to Child-Pugh class A and return of serum total bilirubin and albumin levels to  $< 2$  mg/dL and  $> 2.8$  mg/dL, respectively. It was compared between patients with serum preoperative type IV collagen 7S  $> 6$  ng/mL ( $n = 51$ ) and those with serum preoperative type IV collagen 7S  $\leq 6$  ng/mL ( $n = 107$ ). The time to recovery was significantly shorter in patients with type IV collagen 7S level  $\leq 6$  ng/mL (all,  $P < 0.001$ ; log-rank test). TB: Total bilirubin; Alb: Albumin.



**Figure 3** Postoperative time to recovery of liver function in patients who had jaundice preoperatively in our study. Time to recovery was defined as return to Child-Pugh class A and return of serum total bilirubin and albumin levels to  $< 2$  mg/dL and  $> 2.8$  mg/dL, respectively. It was compared between patients with serum preoperative type IV collagen 7S  $> 6$  ng/mL ( $n = 16$ ) and those with serum preoperative type IV collagen 7S  $\leq 6$  ng/mL ( $n = 5$ ). For the long-term postoperative recovery of liver function as well, the time to recovery was significantly shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen 7S  $> 6$  ng/mL. The time to recovery of Alb tended to be shorter in the group with preoperative type IV collagen 7S  $\leq 6$  ng/mL than in the group with preoperative type IV collagen  $> 6$  ng/mL; however, the difference was not statistically significant. TB: Total bilirubin; Alb: Albumin.



**Figure 4** Correlation between (A) collagen and (B) elastin concentrations and post-hepatectomy liver failure in this cohort. Circles indicate patients without post-hepatectomy liver failure, whereas squares indicate patients with post-hepatectomy liver failure. PHLF: Post-hepatectomy liver failure; NPHLF: Non-post-hepatectomy liver failure.

## ARTICLE HIGHLIGHTS

### Research background

Liver resection is an effective treatment for benign and malignant liver tumors. However, a method for preoperative evaluation of hepatic reserve has not yet been established. Previously reported assessments of preoperative hepatic reserve focused only on liver failure in the early postoperative period and did not consider the long-term recovery of hepatic reserve.

### Research motivation

When determining eligibility for hepatectomy, the underlying pathophysiology needs to be considered to determine if the functional hepatic reserve can withstand both surgery and any postoperative therapy.

### Research objectives

To identify pre-hepatectomy factors associated with both early postoperative liver failure and long-term postoperative liver function recovery.

### Research methods

This study was a retrospective cohort study. We retrospectively investigated 215 patients who underwent hepatectomy at our hospital between May 2013 and December 2016. Early post-hepatectomy liver failure (PHLF) was defined using the International Study Group of Liver Surgery's definition of PHLF. Long-term postoperative recovery of liver function was defined as the time taken for serum total bilirubin and albumin levels to return to levels of  $< 2$  mg/dL and  $> 2.8$  g/dL, respectively, and the time taken for Child-Pugh score to return to Child-Pugh class A.

### Research results

Preoperative type IV collagen 7S was identified as a significant independent factor associated with both PHLF and postoperative long-term recovery of liver function. Further analysis revealed that the time taken for the recovery of Child-Pugh scores and serum total bilirubin and albumin levels was significantly shorter in patients with type IV collagen 7S  $\leq 6$  ng/mL than in those with type IV collagen 7S  $> 6$  ng/mL. In additional analyses, similar results were observed in patients without chronic viral hepatitis associated with fibrosis.

### Research conclusions

Preoperative type IV collagen 7S is a useful predictive factor for early postoperative liver failure and long-term postoperative recovery of liver function. In particular, similar results were obtained in patients without chronic hepatitis virus in the background liver.

### Research perspectives

Preoperative type IV collagen could be useful in predicting early liver failure and long-term postoperative recovery of liver function in different subsets of patients undergoing hepatectomy in this study. Multicenter and prospective studies are required to investigate the application of preoperative serum type IV collagen 7S levels in predicting the postoperative early liver failure and long-term postoperative recovery of liver function.

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## Retrospective Cohort Study

# Biliary spontaneous dislodgement spiral stent for patients who underwent mechanical lithotripsy

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### Institutional review board

**statement:** The study protocol was reviewed and approved by the Biomedical Research Ethics Committee, West China Hospital, Sichuan University.

### Informed consent statement:

Informed consent was waived by the Ethics Committee.

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## Abstract

### BACKGROUND

The incidence of post-endoscopic retrograde cholangiopancreatography (ERCP) cholangitis (PEC) in patients who underwent mechanical lithotripsy (ML) for large stone removal is high (up to 13.3%). One of the main causes is remaining small fragments or sludge that can impair normal biliary drainage. Endoscopic placement of a nasobiliary tube or a conventional plastic biliary stent has been commonly used under such conditions, but the patient may suffer from significant discomfort after the placement of a nasobiliary tube, while additional endoscopy is required for stent removal. We developed a biliary spontaneous dislodgement spiral stent (BSDSS) to overcome those shortcomings.

### AIM

To evaluate the feasibility, safety, and effectiveness of inserting a BSDSS for patients who underwent ML for large stone removal.

### METHODS

We conducted a single-center, retrospective, cohort study at West China Hospital, Sichuan University. A total of 91 consecutive patients with large biliary stones ( $\geq 10$  mm) in the common bile duct who underwent ML between November 2017 and July 2018 were included. The 49 eligible patients were divided into the BSDSS group and the nasobiliary tube group. Technical success, post-ERCP adverse events (including PEC, post-ERCP pancreatitis, stone recurrence, BSDSS retention, self-extraction and dislocation of the nasobiliary tube), drainage time, and postoperative stay were measured and compared.

### RESULTS

Twenty-one patients in the BSDSS group and 28 patients in the nasobiliary tube group were included in the analyses. The baseline characteristics and clinical

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information were similar in the two groups. Insertions of BSDSS and nasobiliary tube were technically successful in all 49 patients. There was no significant difference in the incidence of overall post-ERCP adverse events between the two groups (4.8% in the BSDSS group *vs* 17.9% in the nasobiliary tube group,  $P = 0.219$ ). The median duration of drainage time (3 d in the BSDSS group *vs* 4 d in the nasobiliary tube group) and length of postoperative stay (4 d in the BSDSS group *vs* 5 d in the nasobiliary tube group) also did not differ ( $P = 0.934$ , and  $P = 0.223$ , respectively).

## CONCLUSION

Endoscopic placement of a BSDSS appears to be feasible, safe and effective for patients who underwent ML for large stone removal.

**Key words:** Cholangitis; Choledocholithiasis; Drainage; Endoscopic nasobiliary drainage; Mechanical lithotripsy; Pancreatitis; Spiral; Stents

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**Core tip:** This retrospective cohort study describes the feasibility, safety, and effectiveness of inserting a biliary spontaneous dislodgement spiral stent (BSDSS) for patients who underwent mechanical lithotripsy for large stone removal. All BSDSSs were inserted successfully and evacuated spontaneously after a median duration of 3 d without additional injuries to the digestive tract. Comparable results of post-endoscopic retrograde cholangiopancreatography adverse events, drainage time, and length of postoperative stay were observed in the BSDSS group ( $n = 21$ ) and the nasobiliary tube group ( $n = 28$ ).

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## INTRODUCTION

Endoscopic retrograde cholangiopancreatography (ERCP) is one of the main methods of removing biliary stones in the common bile duct (CBD)<sup>[1-3]</sup>. Endoscopic sphincterotomy (EST) and endoscopic papillary balloon dilation (EPBD) are the most commonly used modalities for stone removal<sup>[1-3]</sup>, but mechanical lithotripsy (ML) may also be required to remove large biliary stones. ML is the simplest method to fragment large CBD stones, but it carries the risk of remaining debris (including small fragments and sludge), even in patients with successful stone removal according to the judgment of the operating endoscopists. In general, placement of a nasobiliary tube or a conventional plastic biliary stent<sup>[4]</sup> can be performed in this setting, but the applications of both devices have obvious shortcomings. Patients with nasobiliary tubes may suffer from significant discomfort due to the transnasal placement<sup>[5,6]</sup>, which could lead to self-extraction and dislocation of the tube; additionally, bile loss caused by external drainage can lead to electrolyte imbalance<sup>[5,6]</sup>, which is especially risky for patients with arrhythmia. Patients with conventional plastic biliary stents have to undergo another endoscopy for stent removal<sup>[6]</sup>, which incurs additional medical costs. We developed a biliary spontaneous dislodgement spiral stent (BSDSS) to overcome the above shortcomings of nasobiliary tubes and conventional biliary stents<sup>[7,8]</sup>. This retrospective cohort study assessed the feasibility, safety, and effectiveness of the placement of a BSDSS for patients who underwent ML for large stone ( $\geq 10$  mm) removal by comparing the clinical outcomes of BSDSS patients with those of nasobiliary tube patients.

## MATERIALS AND METHODS

### Study design

This single-center, retrospective cohort study was conducted at West China Hospital, Sichuan University, a tertiary hospital. The study protocol was approved by the Biomedical Research Ethics Committee, West China Hospital, Sichuan University.

### Design of the BSDSS

The BSDSS used in this study is made of soft and pliable thermoplastic polyurethane, which is different from the commonly used plastic biliary stent (polytetrafluoroethylene). The main difference in shape between the BSDSS and the conventional plastic biliary stent is the duodenal end (Figure 1). The BSDSS has more than one spiral, whereas the conventional has no or only one spiral (straight type and pigtail type, respectively). There are several side holes in the spirals of the BSDSS, and the diameter of the spirals is 12 mm. Another difference lies in the shape of the flanges in the bile duct end. Unlike the long flanges in the conventional stents, the BSDSSs have two short, thin flanges. The outer diameter of the BSDSS is 7 Fr, and its length is 7 cm.

### Patients

Consecutive patients with large biliary stones ( $\geq 10$  mm) who underwent ML for stone removal between November 2017 and July 2018 were retrospectively collected from our prospectively collected database and the hospital medical records. The exclusion criteria were as follows: (1) Patients with altered anatomy; (2) Patients with percutaneous transhepatic cholangial drainage; (3) Patients with biliary stricture; (4) Patients with intrahepatic stones; and (5) Patients with incomplete stone removal (including failed ML and intolerance to repeated stone extraction).

### ERCP procedures

All ERCP procedures were performed by an experienced endoscopist who underwent > 300 ERCP procedures per year. Patients were administered diazepam, pethidine, and anisodamine for conscious sedation, pain control, and bowel relaxation. ERCP was performed in the prone position using a standard duodenoscope (TJF-260V; Olympus, Tokyo, Japan). After selective cannulation of the CBD, a minimum of 25% omipaque was injected to confirm the number and size of CBD stones. According to the endoscopist's judgment, limited EST (3-5 mm), with or without small EPBD (8-10 mm), was performed to facilitate stone removal, followed by the application of ML. A trapezoid RX wire-guided retrieval basket (Boston Scientific Corporation; Marlborough, MA, USA) was used to fragment the stone, and then a grasping basket (FG-22Q-1; Olympus, Tokyo, Japan) was applied to extract fragments repeatedly.

For patients with nasobiliary tubes, a nasobiliary tube (7 Fr; Micro-Tech (Nanjing) Co., Ltd, Nanjing, China) was inserted into the intrahepatic duct using routine instruments and methods<sup>[5]</sup>. For patients with BSDSSs, a BSDSS [7 Fr  $\times$  7 cm; Micro-Tech (Nanjing) Co., Ltd, Nanjing, China] was advanced into the CBD using a guide wire (Jagwire™; Boston Scientific, Natick, MA, USA) aided by a plastic stent introduction device. The BSDSS was then released under fluoroscopic guidance, leaving the duodenal end with spirals outside the duodenal papilla. The BSDSS location was adjusted by the stent introduction device or endoscopic forceps according to the reference mark. Drainage of bile, small fragments or sludge, were confirmed before withdrawal of the duodenoscope.

### Post-ERCP management

After the procedure, the patients fasted for at least 24 h. Blood tests, including complete blood counts, liver function tests, and pancreatic enzymes, were performed 6-48 h after the procedure. Computed tomography (CT) or magnetic resonance cholangiopancreatography (MRCP) was performed when needed. Daily abdominal radiography was scheduled to determine the BSDSS location until the BSDSS was noted when the patient had a bowel movement. Postoperative cholangiography (for patients with nasobiliary tube and without self-extraction or dislocation of nasobiliary tube) or abdominal ultrasound (for patient with BSDSS, and for patients with nasobiliary tube but with self-extraction or dislocation of nasobiliary tube) was performed to detect residual debris, and additional ERCP was performed when needed. Cholecystectomy was recommended for patients with cystic stones.

Patients were followed *via* clinical visits every 3-6 mo, during which clinical symptoms and laboratory tests including liver function tests were recorded; abdominal ultrasound, CT, or MRCP were performed to identify CBD stone recurrence. Follow-up for each patient was discontinued till CBD stone recurrence or October 2019.



**Figure 1** Biliary spontaneous dislodgement spiral stent and its clinical application in a patient who underwent mechanical lithotripsy for large stone removal. A: the 7-cm × 7-Fr biliary spontaneous dislodgement spiral stent with 12-mm spirals; B and C: the endoscopic and fluoroscopic view of the biliary spontaneous dislodgement spiral stent, respectively, after insertion into the common bile duct.

### Outcome measurements

We evaluated the technical success, post-ERCP adverse events, drainage time, and postoperative stay.

Technical success was defined as the successful insertion of the BSDSS or nasobiliary tube into the bile duct in an appropriate position based on endoscopic and fluoroscopic confirmation.

Post-ERCP adverse events mainly included post-ERCP cholangitis (PEC), post-ERCP pancreatitis (PEP) and CBD stone recurrence. Other events relevant to the BSDSS (BSDSS retention and BSDSS-related injuries to the digestive tract) or nasobiliary tube (self-extraction and dislocation of the nasobiliary tube) were also recorded. PEC was defined as a fever ( $> 38^{\circ}\text{C}$ ), leukocytosis, and evidence of cholestasis<sup>[9]</sup>. PEP was defined as persistent pain associated with a serum amylase (or serum lipase) level  $\geq 3$  times the normal upper limit.<sup>[9]</sup> As a recent study<sup>[10]</sup> revealed that the revised Atlanta criteria<sup>[11]</sup> better reflect the severity of PEP than a previous consensus by Cotton *et al*<sup>[12]</sup>, the revised Atlanta classification was used as a grading standard in this study. CBD stone recurrence was defined as the observation of CBD stones six months or more after ERCP<sup>[13]</sup>.

Because BSDSS dislodgement cannot be precisely detected, the duration from BSDSS placement to evacuation (*i.e.*, evacuation time) was measured as the drainage time for the BSDSS. For patients with nasobiliary tubes, the drainage time was defined as the duration from tube placement to tube extraction.

Postoperative stay was defined as the duration from the ERCP procedure to discharge. For patients who underwent additional ERCP to remove residual debris during the same hospitalization, the postoperative stay was calculated from the initial ERCP to discharge.

### Statistical analysis

SPSS 25.0 was used for statistical analysis. Numerical variables are expressed as the means (standard deviations) or medians (interquartile ranges, IQRs) according to their distribution and were compared using Student's *t* test or Mann-Whitney *U*-test, accordingly. Categorical variables are expressed as the numbers or proportions and were compared using  $\chi^2$  tests or Fisher's exact tests as appropriate. *P* values  $< 0.05$  were considered significant.

## RESULTS

From November 2017 to July 2018, a total of 91 patients with large biliary stones ( $\geq 10$  mm) underwent ML for stone removal (Figure 2), and 49 patients met the criteria for inclusion in this study. Among these 49 patients, 21 underwent endoscopic placement of a BSDSS, while the other 28 patients underwent endoscopic placement of a nasobiliary tube. The baseline characteristics and clinical information in each group were similar (Table 1).

The clinical outcomes in the two groups are shown in Table 2. Insertion of the BSDSS or nasobiliary tube was technically successful with a single attempt in all 49 patients. There was no need to use forceps to adjust the location of the BSDSS. There was no PEC in the two groups, but mild PEP was noted in one patient in the BSDSS group (4.8%, 1/21) and one in the nasobiliary tube group (3.6%, 1/28); which was controlled in both patients with conservative treatment. During a median follow-up of

**Table 1** Baseline characteristics and clinical information of the biliary spontaneous dislodgement spiral stent and nasobiliary groups, *n* (%)

	BSDSS group ( <i>n</i> = 21)	Nasobiliary tube group ( <i>n</i> = 28)	<i>P</i> value
Age, mean ± SD, yr	64 ± 16	67 ± 19	0.572 <sup>5</sup>
Sex, male/female	11/10	13/15	0.680 <sup>6</sup>
Diagnosis			0.951 <sup>7</sup>
Biliary colic	6 (28.6)	10 (35.7)	
Obstructive jaundice	7 (33.3)	8 (28.6)	
Acute cholangitis <sup>1</sup>	6 (28.6)	7 (25.0)	
Acute pancreatitis <sup>1</sup>	2 (9.5)	3 (10.7)	
Comorbidity <sup>2</sup>	10 (47.6)	18 (64.3)	0.243 <sup>6</sup>
Gallbladder status			0.410 <sup>7</sup>
Post cholecystectomy	12 (57.1)	13 (46.4)	
Cholecystectomy after ERCP	2 (9.5)	1 (3.6)	
Gallbladder stones <i>in situ</i>	5 (23.8)	6 (21.4)	
No gallbladder stones	2 (9.5)	8 (28.6)	
Previous EST	2 (9.5)	6 (21.4)	0.438 <sup>7</sup>
Periampullary diverticulum	11 (52.4)	12 (42.9)	0.509 <sup>6</sup>
Maximum CBD diameter, median (IQR), mm	13 (12-16)	15 (13-15)	0.214 <sup>8</sup>
Maximum stone diameter, median (IQR), mm	13 (11-16)	12 (12-15)	0.581 <sup>8</sup>
Minimum stone diameter, median (IQR), mm	10 (9-12)	12 (10-12)	0.761 <sup>8</sup>
Stones number, < 3/≥ 3	14/7	25/3	0.076 <sup>6</sup>
ERCP modalities for stone removal			0.595 <sup>7</sup>
ML <sup>3</sup>	1 (4.8)	4 (14.3)	
EST + ML	3 (14.3)	6 (21.4)	
EPBD + ML <sup>3</sup>	1 (4.8)	2 (7.1)	
EST + EPBD + ML	16 (76.2)	16 (57.1)	
Residual debris <sup>4</sup>	1 (4.8)	5 (17.9)	0.219 <sup>7</sup>

<sup>1</sup>Endoscopic retrograde cholangiopancreatography was performed when acute cholangitis and acute pancreatitis were controlled;

<sup>2</sup>Hypertension, coronary artery disease, diabetes mellitus, chronic obstructive pulmonary disease, liver cirrhosis, neoplastic diseases in other systems;

<sup>3</sup>These patients underwent endoscopic sphincterotomy for stone removal previously;

<sup>4</sup>Residual debris was detected by abdominal ultrasound in the only one patient in the biliary spontaneous dislodgement spiral stent group; Residual debris was detected by postoperative cholangiography in four patients in the nasobiliary tube group, while the remaining one patient was confirmed by abdominal ultrasound owing to dislocation of the nasobiliary tube. All these 6 patients underwent additional endoscopic retrograde cholangiopancreatography for debris removal;

<sup>5</sup>Student's *t* test;

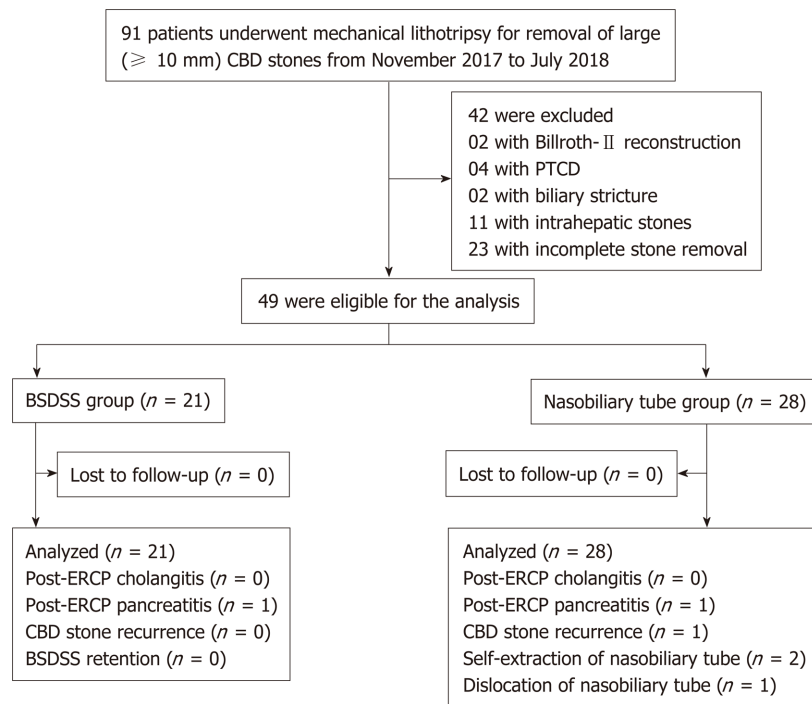
<sup>6</sup> $\chi^2$  test;

<sup>7</sup>Fisher exact test;

<sup>8</sup>Mann-Whitney *U*-test. BSDSS: Biliary spontaneous dislodgement spiral stent; ERCP: Endoscopic retrograde cholangiopancreatography; CBD: Common bile duct; IQR: Interquartile range; EST: Endoscopic sphincterotomy; ML: Mechanical lithotripsy; EPBD: Endoscopic papillary balloon dilation.

18 mo (IQR, 15-21; range, 13-23), CBD stone recurrence was detected in one patient in the nasobiliary tube group (3.6%, 1/28), and additional ERCP was performed to remove the recurrent stone. In addition, all BSDSSs were dislodged and evacuated spontaneously after a median duration of 3 d (IQR, 3-5; range, 2-8), without additional injuries to the digestive tract; most patients (76.2%, 16/21) noticed the dislodged BSDSS when they had a bowel movement. Two patients in the nasobiliary tube (7.1%, 2/28) extracted the tube by themselves on postoperative day 1, due to intolerance of the transnasal placement of the tube; dislocation of the nasobiliary tube was also noted in 1 patient in the nasobiliary tube group (3.6%, 1/28). There were no significant differences in the incidence of overall post-ERCP adverse events (4.8% in the BSDSS group *vs* 17.9% in the nasobiliary tube group, *P* = 0.219).

The median drainage time in the BSDSS group was 3 d and that in the nasobiliary tube group was 4 d, without a significant difference between the groups (*P* = 0.934). The median length of postoperative stay was also similar in the two groups (4 d in the BSDSS group *vs* 5 d in the nasobiliary tube group, *P* = 0.223).



**Figure 2 Study flowchart of patient selection.** CBD: Common bile duct; PTC: Percutaneous transhepatic cholangial drainage; BSDSS: Biliary spontaneous dislodgement spiral stent; ERCP: Endoscopic retrograde cholangiopancreatography.

## DISCUSSION

This retrospective cohort study was conducted to evaluate the feasibility of the placement of a BSDSS for patients who underwent ML for large stone ( $\geq 10$  mm) removal. All inserted BSDSSs were dislodged and evacuated spontaneously without additional injuries to the digestive tract, and the incidence of post-ERCP adverse events in the BSDSS group was low and comparable with that in the nasobiliary tube group. Although the duration from BSDSS placement to evacuation was uncontrollable, similar results in terms of the drainage time and postoperative stay were noted in the two groups. Our findings show the feasibility, safety, and effectiveness of BSDSS for patients who underwent ML for large stone removal.

Apart from EST and EPBD, ML is helpful for removing large stones due to its ability to fragment stones, but the rate of PEC after ML can be high (13.3%, 6/45)<sup>[4]</sup>. Residual small fragments or sludge, as well as injuries to the biliary tract and papillary edema caused by repeated manipulations, may be potential causes. The use of a nasobiliary tube in patients who underwent ML ensures the direct evaluation of the drainage characteristics and facilitates postoperative cholangiography to detect residual debris. However, external drainage from the nasobiliary tube could cause significant discomfort due to the transnasal placement and bile loss. Discomfort in the nostril and throat may lead to self-extraction and dislocation of the nasobiliary tube<sup>[14]</sup>, which was noted in 3 patients with nasobiliary tubes in this study (10.7%, 3/28). Tube kinking, compression ulcers, and aspiration pneumonia can also occur<sup>[5,6,15]</sup>. Although external drainage-induced electrolyte imbalance, such as hypokalemia, is uncommon during short-term biliary drainage (0% in this study), it is risky for patients with arrhythmia once developed. In addition, abdominal ultrasound can be applied to detect residual debris as a substitute for postoperative cholangiography. As shown in Table 1, residual debris was detected by abdominal ultrasound in one patient in the BSDSS group and one patient in the nasobiliary tube group who underwent additional ERCP for debris removal. Given the above, the application of a BSDSS avoids the nasobiliary tube-related medical risks and improves the quality of life of patients.

Compared with conventional plastic biliary stents, the main strength of the BSDSS is its ability to dislodge and evacuate spontaneously after a short period of internal biliary drainage, which was noted in all patients in this study (100%, 21/21). We postulate that the BSDSS is dislodged after papillary edema abates, with the help of bowel movements and/or the passage of high-fiber chyme. In contrast, only 5%-10%

**Table 2** Clinical outcomes of the biliary spontaneous dislodgement spiral stent and nasobiliary tube groups, *n* (%)

	BSDSS group ( <i>n</i> = 21)	Nasobiliary tube group ( <i>n</i> = 28)	<i>P</i> value
Technical success	21 (100)	28 (100)	-
Overall post-ERCP adverse events	1 (4.8)	5 (17.9)	0.219 <sup>4</sup>
Cholangitis	0 (0)	0 (0)	-
Pancreatitis <sup>1</sup>	1 (4.8)	1 (3.6)	1.000 <sup>4</sup>
CBD stone recurrence	0 (0)	1 (3.6)	1.000 <sup>4</sup>
Other events <sup>2</sup>	0 (0)	3 (10.7)	0.250 <sup>4</sup>
Follow-up duration, median (IQR), mo	19 (17-22)	18 (15-21)	0.365 <sup>5</sup>
Drainage time <sup>3</sup> , median (IQR), d	3 (3-5)	4 (2-5)	0.934 <sup>5</sup>
Postoperative stay, median (IQR), d	4 (3-6)	5 (3-7)	0.223 <sup>5</sup>

<sup>1</sup>Pancreatitis was graded as mild in both groups;

<sup>2</sup>There was no biliary spontaneous dislodgement spiral stent (BSDSS) retention in the BSDSS group, while self-extraction (*n* = 2) and dislocation (*n* = 1) of the nasobiliary tube was noted in three patients in the nasobiliary tube group;

<sup>3</sup>Drainage time was defined as the duration from insertion to evacuation in the BSDSS group and the duration from insertion to extraction in the nasobiliary tube group;

<sup>4</sup>Fisher exact test;

<sup>5</sup>Mann-Whitney *U*-test. BSDSS: Biliary spontaneous dislodgement spiral stent; ERCP: Endoscopic retrograde cholangiopancreatography; CBD: Common bile duct; IQR: Interquartile range.

of conventional plastic biliary stents can migrate distally<sup>[16]</sup>; thus, additional endoscopy is frequently required for stent removal. In addition, the BSDSS is soft and has several spirals on the duodenal side, which makes it less likely to lead to stent-related bowel perforation or fistula that have been reported previously in patients with conventional plastic biliary stents<sup>[17-21]</sup>.

The main disadvantage of BSDSS is the lack of control over the timing of dislodgement and evacuation. The length of EST, as well as the size of EPBD, may seriously affect BSDSS dislodgement. For patients who underwent complete EST (and/or large EPBD), the BSDSS may be dislodged within a couple of hours because of the large opening of the papilla; thus, the application of a BSDSS in these patients seems unadvisable. However, as reported by previous studies<sup>[22-24]</sup>, complete EST and large EPBD (12-20 mm) have been regarded to be associated with a higher rate of late adverse events; thus, our routines of performing limited (3-5 mm) EST, small (8-10 mm) EPBD and ML for large stone removal seem reasonable. In addition, we used daily radiography to identify the BSDSS location in this study, but BSDSS dislodgement still could not be detected accurately, and there may be a significant difference in the duration from BSDSS dislodgment to evacuation among patients. Because all BSDSSs were evacuated spontaneously after a median duration of 3 d (IQR, 3-5), daily radiography may not be necessary due to increased radiation exposure. We suggest that single radiography on postoperative day 5 may be preferable for patients with BSDSS if they ignore the evacuated BSDSS when they have a bowel movement. Further methods may be proposed for determining the real-time positioning of BSDSS and to clarify the real drainage time with the BSDSS.

The biodegradable stent reported by Anderloni *et al*<sup>[25]</sup> ensures different degradation times for distinct clinical demands using various polymeric mixtures, making it a promising stent for patients who underwent ML; however, the use of such biodegradable stents should be approached cautiously because partially degraded stents may impair normal drainage and affect the findings of follow-up abdominal imaging.

The present study had several limitations. First, it was a single-center, retrospective study with a small sample size, but consecutive patients who underwent ML for large stone removal were included, which helps reduce the selection bias. Prospective, multicenter, and large-scale studies are needed to further evaluate the role of BSDSSs in such patients. Second, a comparison with the conventional plastic biliary stent was absent. This is mainly due to the rare use of conventional plastic stents in patients who underwent successful stone extraction after ML in our endoscopy center; these patients routinely received nasobiliary tube before the introduction of BSDSS. Third, there was no comparative group without drainage, and thus, the necessity of placing a BSDSS needs to be further investigated. Although the three patients with tube self-extraction or dislocation in the nasobiliary tube group did not develop PEC, considering the reported high incidence of PEC in patients who underwent ML for stone removal (13.3%, 6/45)<sup>[4]</sup>, a comparative study regarding the placement of BSDSS *vs* no BSDSS should be carefully conducted.

In conclusion, endoscopic placement of a BSDSS in patients who underwent ML for large stone removal appears to be feasible, safe and effective.

## ARTICLE HIGHLIGHTS

### Research background

The incidence of post-endoscopic retrograde cholangiopancreatography (ERCP) cholangitis (PEC) in patients who underwent mechanical lithotripsy (ML) for large stone removal is high (up to 13.3%). One of the main causes is remaining small fragments or sludge that can impair normal biliary drainage. Endoscopic placement of a nasobiliary tube or a conventional plastic biliary stent was commonly used under such conditions, but the patient may suffer from significant discomfort after the placement of a nasobiliary tube, while additional endoscopy is required for stent removal.

### Research motivation

We developed a biliary spontaneous dislodgement spiral stent (BSDSS) to overcome nasobiliary tube-related and conventional plastic biliary stent-related shortcomings. The duodenal end of the BSDSS is with several spirals, and its bile duct end has two short and thin flanges. We postulate that the BSDSS is dislodged after papillary edema abates, with the help of bowel movements and/or the passage of high-fiber chyme.

### Research objectives

In this retrospective cohort study, we evaluated the feasibility, safety, and effectiveness of inserting a BSDSS for patients who underwent ML for large stone removal by comparing the clinical outcomes of BSDSS patients with those of nasobiliary tube patients.

### Research methods

From November 2017 to July 2018, a total of 91 consecutive patients underwent ML for large ( $\geq 10$  mm) stone removal. Of these, 49 patients were eligible for this study, and they were divided into the BSDSS group and the nasobiliary tube group. Technical success, post-ERCP adverse events (including PEC, post-ERCP pancreatitis, stone recurrence, BSDSS retention, self-extraction and dislocation of the nasobiliary tube), drainage time, and postoperative stay were measured and compared.

### Research results

Twenty-one patients in the BSDSS group and 28 patients in the nasobiliary tube group were included in the analyses. The baseline characteristics and clinical information were similar in the two groups. Insertions of BSDSS and nasobiliary tube were technically successful in all 49 patients. There was no significant difference in the incidence of overall post-ERCP adverse events between the two groups (4.8% in the BSDSS group *vs* 17.9% in the nasobiliary tube group,  $P = 0.219$ ), as well as the median duration of drainage time (3 d in the BSDSS group *vs* 4 d in the nasobiliary tube group,  $P = 0.934$ ) and the median length of postoperative stay (4 d in the BSDSS group *vs* 5 d in the nasobiliary tube group,  $P = 0.223$ ).

### Research conclusions

Endoscopic placement of a BSDSS appears to be feasible, safe and effective for patients who underwent ML for large stone removal.

### Research perspectives

Multi-center studies with a large sample size are warranted to further confirm the safety and effectiveness of BSDSS. Comparative study regarding the placement of BSDSS *vs* no BSDSS is expected to clarify the necessity of routine application of BSDSS in patients who undergo ML for large stone removal.

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## Retrospective Study

# Modified Child-Pugh grade vs albumin-bilirubin grade for predicting prognosis of hepatocellular carcinoma patients after hepatectomy

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## Abstract

### BACKGROUND

Hepatectomy is the main treatment for patients with hepatocellular carcinoma (HCC) and it has a high possibility for long-term cure potential. But the postoperative mortality and recurrence rates remain high. Since the long-term prognosis of HCC patients is strongly linked to liver function, preoperative assessment of liver function is very important for HCC patients.

### AIM

To compare the predictive power of the modified Child-Pugh (MCP) and albumin-bilirubin (ALBI) grades for the long-term outcome of HCC.

### METHODS

From January 2010 to June 2017, a total of 204 patients with HCC who underwent surgery at the Second Affiliated Hospital of Chongqing Medical University were enrolled in this retrospective study. Multivariate Cox regression analysis was used to determine the independent predictive factors of survival and relapse. The area under the curve (AUC) was used to evaluate the discriminative performance of the MCP grade and ALBI grade to predict the postoperative overall survival (OS) time and recurrence-free survival (RFS) time.

### RESULTS

The median OS and RFS times were 44.0 mo (range: 22.0-74.0 mo) and 22.0 mo (range: 5.0-45.0 mo), respectively. The median OS and RFS times of MCP grades 1, 2, and 3 patients were 60.0, 39.0, and 18.0 mo ( $P < 0.001$ ) and 36.0, 15.0, and 7.0 mo ( $P < 0.001$ ), respectively. The median OS and RFS times of ALBI grades 1, 2, and 3 patients were 56.0, 26.0, and 6.0 mo ( $P < 0.001$ ) and 25.0, 10.0, and 3.0 mo ( $P = 0.003$ ), respectively. Both the MCP and ALBI grades were more accurate than the Child-Pugh grade for predicting long-term prognosis. Further analysis demonstrated that for both predicting OS and RFS, the MCP grade performed

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better than the ALBI grade (AUC: 0.642 *vs* 0.605 for OS; 0.659 *vs* 0.594 for RFS).

## CONCLUSION

The MCP grade is more accurate than the ALBI grade for predicting long-term outcome of patients with HCC.

**Key words:** Modified Child-Pugh grade; Albumin-Bilirubin grade; Hepatocellular carcinoma; Prognosis; Hepatectomy; Child-Pugh

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**Core tip:** We discovered that only a few studies have evaluated the predictive power of prealbumin-involving modified Child-Pugh (MCP) grade for the prognosis of patients with hepatocellular carcinoma (HCC). The objective, recognizable, and simple method, the albumin-bilirubin (ALBI) grade, for predicting the long-term prognosis of HCC patients has been widely proven in the international environment. In this study, receiver operating characteristics curve analysis showed that the MCP grade had higher accuracy than the ALBI grade, and the area under the curve of the MCP grade was larger than that of the ALBI grade. The MCP grade may be the best tool for the selection of HCC treatment strategies.

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## INTRODUCTION

Hepatocellular carcinoma (HCC) is the second most common cancer and the fifth most common cause of cancer-related death in the world<sup>[1]</sup>. Hepatectomy is the main treatment for HCC<sup>[2]</sup> and it has a high possibility for long-term cure potential<sup>[2-4]</sup>. Unfortunately, the postoperative mortality and recurrence rates remain high. It is widely perceived that the prognosis of HCC is related to many clinical factors such as tumor characteristics, vascular invasion, and liver function<sup>[5,6]</sup>. Thus, evaluating liver function is critical for the prognosis of HCC patients.

The Child-Pugh (C-P) grade is widely used as a standard measure of liver function<sup>[7,8]</sup> and prognostic tool<sup>[9,10]</sup>. However, the C-P grading system has some limitations. The two variables of ascites and hepatic encephalopathy are highly subjective, and some indicators such as ascites and albumin are correlated<sup>[11,12]</sup>. Therefore, as an objective, recognizable, and simple method for evaluating HCC liver function, the albumin-bilirubin (ALBI) grade was proposed and has been widely proven in the international environment<sup>[13]</sup>. This score includes only two variables, albumin and total bilirubin, and has a preferable prognostic ability for the survival analysis of HCC patients compared with the C-P score<sup>[12,14]</sup>. Prealbumin (PA) is a new and reliable indicator of liver function. Many studies have confirmed the predictive value of serum PA for liver function<sup>[7,15,16]</sup> and the long-term prognosis of patients with HCC<sup>[16-18]</sup>. It has been reported that the integration of PA into the C-P scoring system to form a modified C-P (MCP) scoring system that includes four factors, PA, albumin, total bilirubin, and prothrombin time, and removes the subjective indicators of ascites and hepatic encephalopathy can improve the accuracy of prognosis prediction in HCC patients<sup>[19]</sup>. However, the relevant data is still not enough. So, based on the fact that both the MCP and ALBI scores are objective, simple, and easy to obtain, we analysed and compared the predictive power of the MCP and ALBI scores for the long-term outcome of hepatectomy patients with HCC. Then, a scoring system with higher accuracy for predicting the overall survival (OS) time and recurrence-free survival (RFS) time of HCC patients can be selected. This will provide a more effective, objective, and simple method to assess the prognosis of HCC patients undergoing hepatectomy and make it easier and more accurate for surgeons to select suitable HCC patients for hepatectomy.

## MATERIALS AND METHODS

### Patients

We included 204 patients with HCC who underwent hepatectomy from January 2010 to June 2017 at the Second Affiliated Hospital of Chongqing Medical University. The inclusion criteria were as follows: (1) Pathology identified as HCC; (2) No treatment prior to hepatic resection and no other malignancies; and (3) Adequate clinical data that could be assessed in the hospital. In the first year after hepatectomy, all of the patients were followed every 3 mo and then every 6 mo in the following years. The diagnosis of tumour recurrence was based on image findings. The last follow-up was in June 2019 or the day the patient died. And during our follow-up, one patient died due to severe postoperative liver failure on the day of the hepatectomy, and two patients were lost to follow-up. This study was registered at [www.chictr.org.cn](http://www.chictr.org.cn) (ChiCTR1900026738) (Supplementary Table 1 contains study data of this study). The study was in line with the ethical guidelines of 1964 Helsinki Declaration and was approved by the review committee of the Second Affiliated Hospital of Chongqing Medical University.

### Data collection

We collected patient data including sex, age, body mass index (BMI), cause of hepatitis, portal hypertension (PH), liver cirrhosis, BCLC (Barcelona Clinic Liver Cancer) stage, and C-P grade. The biochemical indicators were as follows: Serum PA, serum albumin (ALB), total bilirubin (TBIL), prothrombin time (PT), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), international normalised ratio (INR), and platelet count (PLT). The tumor characteristics included the number of tumors, tumor size, portal vein tumor thrombus (PVTT), capsule formation, and differentiation. The intraoperative parameters included surgical bleeding, blood transfusion, type of liver resection, and intraoperative radiotherapy and/or chemotherapy. Based on previous description, the calculation formula of ALBI score was as follows:  $ALBI = \log_{10} \text{bilirubin} \times 0.66 + \text{albumin} \times (-0.085)$ , where the bilirubin is expressed in  $\mu\text{mol/L}$  and the albumin in  $\text{g/L}$ . The ALBI grades were classified as follows:  $\leq -2.60$ , grade 1;  $> -2.60$  to  $\leq -1.39$ , grade 2; and  $\geq -1.39$ , grade 3<sup>[13]</sup>. For the MCP scoring system, the sex difference was considered using receiver operating characteristics (ROC) curve plots to set the optimal cut-off values for male and female PAs, respectively. The PA levels in the male and female patients ranged from 130 mg/L to 190 mg/L and 120 mg/L to 170 mg/L, respectively, similar to a previous report<sup>[19]</sup>. The MCP scoring system is shown in Table 1.

### Statistical analysis

mean  $\pm$  SD or median (quartile range) is used to present the continuous variables. The categorical variables are presented as  $n$  (%) and were compared *via* the  $\chi^2$  test or Fisher's exact test. Kaplan-Meier analysis was used to evaluate the OS and RFS rates and the independent predictive factors for survival and relapse were determined by multivariate Cox regression analysis. The cut-off value was determined *via* the ROC curve. Areas under the ROC curves (AUCs) were used to evaluate the accuracy of the MCP and ALBI scoring systems to predict the postoperative survival time and RFS time in the HCC patients. The difference was considered statistically significant at  $P < 0.05$ . All of the statistical analyses were conducted using SPSS 20.0 software. The statistical methods of this study were reviewed by Jian Gao, who is both the corresponding author and the biomedical statistician of this article.

## RESULTS

### Patient characteristics

A total of 204 patients with HCC who underwent hepatectomy were enrolled in this study, and the median follow-up time was 36 mo. As shown in Table 2, there were 174 (85.3%) males and 30 (14.7%) females with a mean age of  $52.0 \pm 11.4$  years. Most of the patients (85.3%) had a viral hepatitis B background, 120 (58.8%) patients had liver cirrhosis, and most patients (72.5%) have been implanted with radiation particles ( $I^{125}$  or  $I^{131}$ ) or chemotherapy pumps during the surgery. Of the 204 patients, 113 (55.4%) had BCLC stage 0 or A disease, 62 (30.4%) had stage B, and 29 (14.2%) had stage C; and 187 (91.7%) were classified as C-P grade A, 17 (8.3%) were classified as B, and there was no patient classified as grade C. According to the ALBI grade, 136 (66.7%) had grade 1, 66 (32.4%) had grade 2, and only 2 (0.9%) had grade 3. For the MCP grade, 110 (53.9%), 52 (25.5%), and 42 (20.6%) patients were classified as grades 1, 2, and 3, respectively. The median OS and RFS times were 44.0 mo and 22.0 mo,

**Table 1** Modified Child-Pugh scoring system

Item	Score		
	1	2	3
TBIL, $\mu\text{mol/L}$	< 34	34-51	> 51
ALB, g/L	> 35	28-35	< 28
Prothrombin time prolongation, s	< 4	4-6	> 6
PA for males, mg/L	> 190	130-190	< 130
PA for females, mg/L	> 170	120-170	< 120

MCP-1 (score = 4), MCP-2 (score = 5), and MCP-3 (score = 6).

respectively.

### Comparison of the characteristics of three groups in ALBI grade and MCP grade

Statistically significant differences between the three groups in each grade were found for the following variables: In the MCP grade groups for gender ( $P = 0.038$ ), BMI ( $P = 0.008$ ), ALT ( $P = 0.026$ ), C-P grade ( $P < 0.001$ ), BCLC ( $P = 0.01$ ), blood transfusion ( $P = 0.045$ ), and surgical bleeding ( $P = 0.019$ ), in addition to PA, albumin, total bilirubin, and prothrombin time ( $P < 0.001$ ) that are included in this grade; in the ALBI grade groups for INR ( $P = 0.012$ ), C-P grade ( $P < 0.001$ ), BCLC stage ( $P = 0.013$ ), number of tumours ( $P = 0.008$ ), tumour size ( $P = 0.049$ ), blood transfusion ( $P = 0.006$ ), and type of liver resection ( $P = 0.011$ ), in addition to PA, albumin, total bilirubin, and prothrombin time ( $P < 0.001$ ) that are the variables included in this grade (Supplementary Table 1).

### Prognostic factors for OS and RFS

The median OS time and RFS time of the whole cohort were 44.0 mo (range: 22.0-74.0 mo) and 22.0 mo (range: 5.0-45.0 mo), respectively. In order to identify the potential prognostic indicators for the OS and RFS, we included the variables in the univariate analysis with  $P < 0.05$  into the multivariable cox regression analysis (Table 3). As demonstrated by univariate analyses, age, INR, tumor size, surgical bleeding, blood transfusion, hepatectomy type, PVTT, differentiation, C-P grade, BCLC stage, ALBI grade, and MCP grade were significant indicators for OS; except for the indicator of age, the other factors mentioned above were significant indicators for RFS, but liver cirrhosis was not the predictor of OS or RFS. Next, a multivariable regression analysis was conducted to further define the significant independent predictors. As shown in Table 3, age [ $P = 0.002$ , hazard ratio (HR) = 1.853], differentiation ( $P < 0.001$ , HR = 2.230), BCLC stage B ( $P = 0.012$ , HR = 2.635), and MCP grade 2 ( $P = 0.008$ , HR = 1.864) or 3 ( $P = 0.030$ , HR = 2.005) were the independent predictors of OS. For RFS, surgical bleeding ( $P = 0.027$ , HR = 2.246), BCLC stage B ( $P = 0.012$ , HR = 2.635), and MCP grade 2 ( $P = 0.002$ , HR = 1.830) were the independent predictors.

### Discriminatory power of MCP grade and ALBI grade for OS and RFS

The Kaplan-Meier curves of the OS and RFS showed a significant discrimination between the patients in different C-P, MCP, and ALBI grades ( $P < 0.05$ ) (Figure 1). Visual observation of the OS curves indicated that compared with the C-P score, the MCP and ALBI scores had more significant differences in various groups, and the difference in MCP grade displayed a superior over ALBI grade. The median OS times of grades 1, 2, and 3 patients were 60.0, 39.0, and 18.0 mo, and those of ALBI grades 1, 2, and 3 patients were 56.0, 26.0, and 6.0 mo, respectively. Similarly, MCP also demonstrated a greater advantage for predicting RFS than C-P grade and ALBI grade (Figure 2). The median RFS times of MCP grades 1, 2, and 3 patients were 36.0, 15.0, and 7.0 mo, respectively, and those of ALBI grades 1, 2, and 3 patients were 25.0, 10.0, and 3.0 mo, respectively.

The analyses of the ROC curves found that both the MCP and ALBI grades had greater ability to predict the OS and RFS times compared with the C-P grade (Figure 3). For the OS (Figure 3A), the AUC of the MCP grade was 0.642 (95%CI: 0.572-0.707), which was larger than that of the ALBI grade (AUC = 0.605; 95%CI: 0.534-0.673), although the difference was not statistically significant ( $P = 0.263$ ). For the RFS (Figure 3B), we observed the same advantages; the AUC was 0.659 (95%CI: 0.590-0.724) for the MCP grade, and 0.594 (95%CI: 0.524-0.662) for the ALBI grade, and the difference was statistically significant ( $P = 0.038$ ). The differences of the AUCs between the two grades seemed not obvious and the discriminatory power may be a little weak, but

**Table 2** Baseline characteristics of the 204 patients

Variable	All patients (n = 204)
Sex (male/female)	174/30
Age, yr	52.0 ± 11.4
BMI, kg/m <sup>2</sup>	23.2 ± 3.6
Cause of hepatitis (Hepatitis B/C/Others)	174/2/28
PH (yes/no)	48/156
Liver cirrhosis (yes/no)	120/84
P grade (A/B)	187/17
BCLC stage (0 or A/B/C)	113/62/29
PA, mg/L	198.5 (149.3-238.0)
ALB, g/L	41.0 ± 4.7
TBIL, μmol/L	13.3 (9.7-18.3)
PT, s	13.7 (13.0-14.5)
ALT, U/L	36.0 (26.3-56.0)
AFP, μg/L	76.4 (9.02-1082.5)
INR	1.0 (0.98-1.1)
PLT, × 10 <sup>9</sup> /L	135.0 (99.8-181.0)
Number of tumors (1/2/≥3)	161/27/16
Tumor size, cm	4.0 (3.0-6.5)
PVTT (yes/no)	30/174
Capsule formation (yes/no)	27/177
Differentiation (well/poor)	81/123
Surgical bleeding, mL	300 (180-575)
Blood transfusion (yes/no)	68/136
Type of liver resection (minor/major)	159/45
Intraoperative radiotherapy and/or chemotherapy (yes/no)	148/56
ALBI grade (1/2/3)	136/66/2
MCP grade (1/2/3)	110/52/42
Overall survival time, mo	44.0 (22.0-74.0)
Recurrence-free survival time, mo	22.0 (5.0-45.0)

BMI: Body mass index; PH: Portal hypertension; C-P: Child-Pugh; BCLC: Barcelona Clinic Liver Cancer; PVTT: Portal vein tumor thrombus; MCP: Modified Child-Pugh; ALBI: Albumin-bilirubin. Type of liver resection: Minor, ≤ 3 liver segments; major, ≥ 4 liver segments. Intraoperative radiotherapy: Implanted with radiation particles I<sup>125</sup> or I<sup>131</sup>. Intraoperative chemotherapy: Implanted with a chemotherapy pump.

regardless of the OS or RFS, the MCP grade displayed a slight advantage over the ALBI grade.

## DISCUSSION

The long-term outcome of liver cancer patients is closely related to liver function<sup>[5,6]</sup>. The estimation of liver function reserve is key for HCC patients before hepatic resection. The C-P grade is widely used to estimate liver function<sup>[7,8]</sup>. Compared with the C-P grade, the MCP and ALBI grades are more objective, simpler, and easier to obtain<sup>[19,20]</sup>. Based on the same advantages of the two scoring systems mentioned above, and the little data on the MCP grade, we conducted this study to analyse and compare the predictive power of the MCP score and ALBI score for the long-term outcome of HCC patients and to provide clinicians with a more objective, simpler, and more discriminative scoring system.

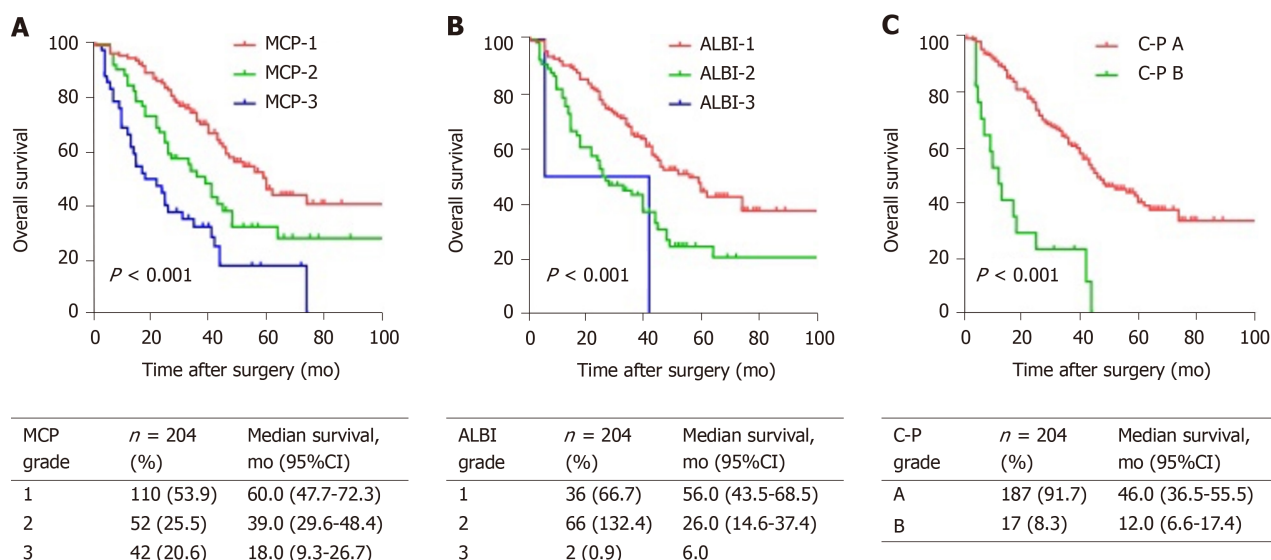
This study demonstrated the advantages of the MCP and ALBI scores in the long-term outcome of HCC patients compared with the C-P score. As shown in **Figure 3**, both the visual inspection and formal statistical analyses demonstrated that the MCP grade had better discrimination in assessing the OS and RFS of HCC patients than the ALBI grade. This study found several strengths of the MCP grading systems compared to the ALBI grading systems. First, PA was included in the MCP classification. Huang *et al*<sup>[7]</sup> demonstrated a close correlation between PA and liver

**Table 3** Univariate and multivariate analyses of prognostic factors for overall survival and recurrence-free survival

Variables	Overall survival		Recurrence-free survival			
	Univariate	Multivariate	Univariate		Multivariate	
	P value	HR (95%CI)	P value	P value	HR (95%CI)	P value
Sex (M/F)	0.595		0.962			
Age (> 50/< 50 yr)	0.020	1.853 (1.243-2.761)	0.002	0.071		
BMI ( $\geq 24$ / $<24$ kg/m <sup>2</sup> )	0.596		0.770			
Cause of hepatitis (HBV/HCV/others)	0.269		0.422			
ALT (> 40/ $\leq 40$ U/L)	0.255		0.069			
AFP ( $\geq 400$ / $< 400$ $\mu$ g/L)	0.111		0.092			
INR ( $\geq 1.3$ / $< 1.3$ )	< 0.001		0.009			
PLT ( $\geq 100$ / $< 100 \times 10^9$ /L)	0.392		0.713			
Number of tumors (single/ $\geq 2$ )	0.243		0.065			
Tumor size ( $\geq 5$ / $< 5$ cm)	< 0.001		< 0.001			
Blood transfusion (yes/no)	0.001		0.003			
Surgical bleeding (> 400/ $\leq 400$ mL)	0.005		0.003		1.437 (1.004-2.058)	0.048
Types of liver resection (minor/major)	< 0.001		< 0.001			
Intraoperative radiotherapy and/or chemotherapy (yes/no)	0.649		0.430			
PVTT (yes/no)	< 0.001		0.005			
Capsule formation (yes/no)	0.408		0.374			
Differentiation (good/poor)	0.004	2.230 (1.452-3.426)	< 0.001	0.029		
PH (yes/no)	0.809		0.542			
Liver cirrhosis (yes/no)	0.944		0.896			
Child-Pugh grade (A/B)	< 0.001		0.001			
BCLC stage (0 or A/B/C)	< 0.001		< 0.001			
0 or A		1			1	
B		2.635 (1.235-5.622)	0.012		2.246 (1.095-4.607)	0.027
ALBI grade (1/2/3)	< 0.001		0.003			
MCP grade	< 0.001		< 0.001			
1		1			1	
2		1.864 (1.174-2.959)	0.008		1.830 (1.246-2.687)	0.002
3		2.005 (1.071-3.753)	0.030			

BMI: Body mass index; PH: Portal hypertension; C-P: Child-Pugh; BCLC: Barcelona Clinic Liver Cancer; PVTT: Portal vein tumor thrombus; ALBI: Albumin-Bilirubin; MCP: Modified Child-Pugh. Type of liver resection: Minor,  $\leq 3$  liver segments; major,  $\geq 4$  liver segments. Intraoperative radiotherapy: Implanted with radiation particles  $I^{125}$  or  $I^{131}$ . Intraoperative chemotherapy: Implanted with a chemotherapy pump.

function in HCC patients undergoing hepatectomy, while Jia *et al*<sup>[16]</sup> proposed that PA can help predict the long-term prognosis of patients after hepatectomy. As Wen *et al*<sup>[19]</sup> reported, the integration of PA into the C-P grading system to form the MCP grading systems can improve the accuracy of postoperative prognosis prediction in HCC patients, and our study is in agreement with this result. Second, the univariate analysis displayed that both the ALBI grade and MCP grade were the predictors of OS or RFS, but the further multivariable regression analysis showed that the independent predictor was MCP grade. Third, the Kaplan-Meier curves showed that the MCP grade had a preferable prognostic discrimination ability (Figures 1 and 2). The median survival time predicted with the MCP grading system was much longer than that with the ALBI grading system, and the median survival time in the same grade could even differ by more than 10 mo (such as grade 2: 39.0 mo *vs* 26.0 mo for OS; grade 1: 36 mo *vs* 25 mo for RFS). Fourth, the MCP grading system had a more favorable patient distribution compared to the ALBI grading system, whose weight tended to be grade 1 (66.7%), and there were only two patients in grade 3. This might have been related to the fact that the liver function of the HCC patients with ALBI grade 3 was too poor to treat by surgery. But the distribution of MCP grades was relatively uniform. The proportions of MCP grades 1, 2, and 3 patients were 53.9%, 25.5%, and 20.6%, respectively. Finally, the ROC curve of the MCP grade had higher

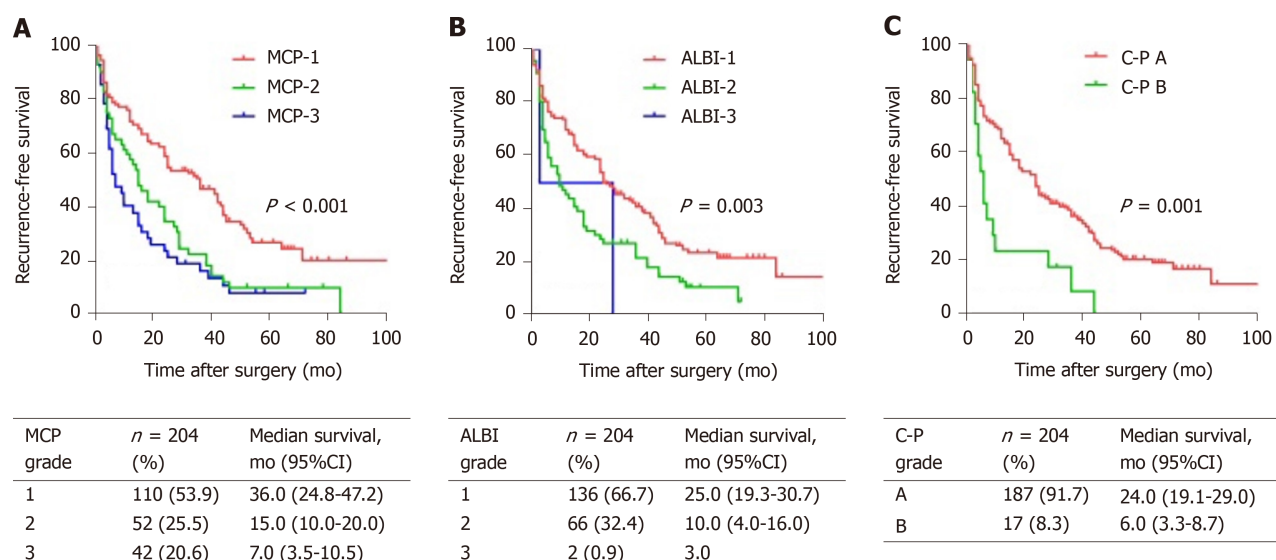


**Figure 1** Kaplan-Meier curves of the overall survival of the 204 hepatocellular carcinoma patients who underwent hepatectomy in the present study. A: Stratified by MCP grade; B: Stratified by ALBI grade; C: Stratified by C-P grade. OS was significantly different among the subgroups stratified by these variables ( $P < 0.05$ ). Associated tables display the median survival for each grade. MCP: Modified Child-Pugh; ALBI: Albumin-bilirubin; C-P: Child-Pugh; CI: Confidence interval.

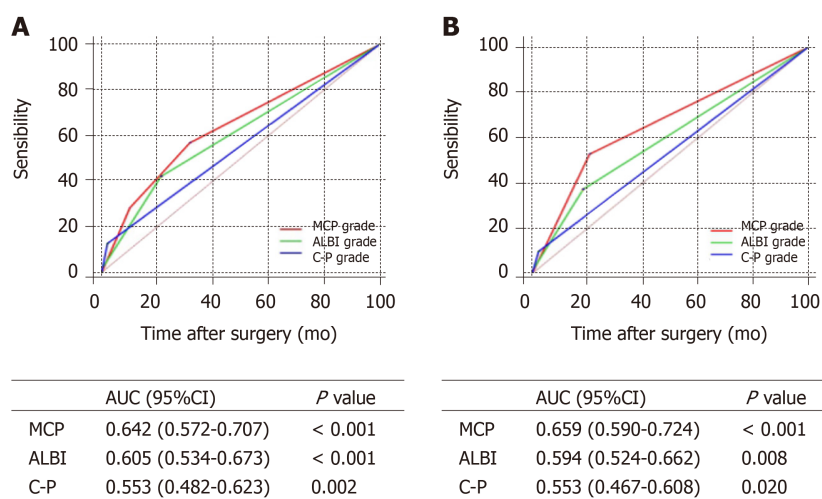
accuracy than the ALBI grade, and the AUC of the MCP grade was larger than the ALBI grade, which further validated that the MCP grade had a better predictive value for the long-term prognosis of HCC patients than the ALBI grade.

There are several limitations to this study. First, the total number of patients was small and all were from one single centre; therefore, larger and multicentre studies are needed to further validate the results. Second, as a retrospective study, it was hard to avoid selection bias. Further prospective studies on the MCP grading system are necessary.

In conclusion, the current study suggests that the MCP grade is more advantageous than the ALBI grade for predicting postoperative long-term outcomes in patients with HCC, and the MCP grade could be conducive to the selection of HCC treatment strategies. This may benefit more HCC patients.



**Figure 2** Kaplan-Meier curves for the recurrence-free survival of the 204 hepatocellular carcinoma patients who underwent hepatectomy in the present study. A: Stratified by MCP grade; B: Stratified by ALBI grade; C: Stratified by C-P grade. RFS was significantly different among the subgroups stratified by these variables ( $P < 0.05$ ). Associated tables display the median survival for each grade. MCP: Modified Child-Pugh; ALBI: Albumin-bilirubin; C-P: Child-Pugh; CI: Confidence interval.



**Figure 3** Comparisons of the area under curve for overall survival and recurrence-free survival predictions among the MCP, ALBI, and C-P grades using receiver operating characteristics curves. A: Overall survival; B: Recurrence-free survival. MCP: Modified Child-Pugh grade; ALBI: Albumin-bilirubin grade; C-P: Child-Pugh grade; AUC: Area under the curve; CI: Confidence interval.

## ARTICLE HIGHLIGHTS

### Research background

Liver resection is the main treatment for hepatocellular carcinoma (HCC) patients and it has a high possibility for long-term cure potential. But the postoperative mortality and recurrence rates remain high. Since the long-term prognosis of HCC patients is strongly linked to liver function, preoperative assessment of liver function is very important.

### Research motivation

The main aim of this study was to compare the predictive power of the modified Child-Pugh (MCP) and albumin-bilirubin (ALBI) grades for the long-term outcome of HCC.

### Research objectives

We can provide a more effective, objective, and simple method to assess the prognosis of HCC patients undergoing hepatectomy and makes it easier and more accurate for surgeons to select suitable HCC patients for hepatectomy.

### Research methods

A total of 204 patients with HCC who underwent surgery were enrolled in this retrospective study, the median follow-up time was 36 mo. Multivariate Cox regression analysis was used to determine the independent predictive factors for survival and relapse. The area under the curve was used to evaluate the discriminative performance of the MCP grade and ALBI grade to predict the postoperative overall survival (OS) time and recurrence-free survival (RFS) time.

### Research results

Both the MCP and ALBI grades were more accurate than the Child-Pugh grade for predicting long-term prognosis. And further analysis demonstrated that for both predicting OS and RFS, the MCP grade performed better than the ALBI grade. This can provide a more effective, objective, and simple tool for the selection of HCC treatment strategies.

### Research conclusions

We found that the new grading system, MCP grade, had predictive value for the long-term prognosis of HCC patients after hepatectomy. Both the MCP and ALBI scoring systems are objective, simple, and discriminative, and the ALBI grade has been widely proven in the international environment for predicting the long-term prognosis of HCC patients. We hypothesized that the MCP grade is superior to the ALBI grade in predicting the prognosis of HCC patients. In this study, we adopted the traditional method to prove the hypothesis, and we found a new phenomenon in which there was no patient in Child-Pugh grade C, and there were few patients in ALBI grade 3, but the patient distribution of MCP grade was relatively uniform. And eventually through the patient distribution, Kaplan-Meier curves and ROC curves of the MCP and ALBI grades and so on, we confirmed the hypothesis that the MCP grade is superior to the ALBI. But in this study, the sample size is not very large, the patients were from one clinical center, and it is a retrospective study, so in the future, we can further confirm the value of MCP in predicting the prognosis of HCC patients through larger samples, multi-center studies and prospective studies. When evaluating liver function of HCC patients before surgery, we should pay more attention to the serum PA levels of patients, and we can choose the MCP grade to assess the prognosis of patients.

### Research perspectives

Larger samples, multi-center studies and prospective studies are needed to further validate the value of the MCP grade for the long-term prognosis of HCC patients. And how to improve the liver function of the HCC patients with a higher MCP grade to further improve the prognosis of HCC patients after surgery remains a question that needs to be answered in future studies.

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## Observational Study

# Benefits of implementing a rapid access clinic in a high-volume inflammatory bowel disease center: Access, resource utilization and outcomes

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## Abstract

### BACKGROUND

Emergency situations in inflammatory bowel diseases (IBD) put significant burden on both the patient and the healthcare system.

### AIM

To prospectively measure Quality-of-Care indicators and resource utilization after the implementation of the new rapid access clinic service (RAC) at a tertiary IBD center.

### METHODS

Patient access, resource utilization and outcome parameters were collected from consecutive patients contacting the RAC between July 2017 and March 2019 in this observational study. For comparing resource utilization and healthcare costs, emergency department (ED) visits of IBD patients with no access to RAC services were evaluated between January 2018 and January 2019. Time to appointment, diagnostic methods, change in medical therapy, unplanned ED visits, hospitalizations and surgical admissions were calculated and compared.

### RESULTS

488 patients (Crohn's disease: 68.4%/ulcerative colitis: 31.6%) contacted the RAC with a valid medical reason. Median time to visit with an IBD specialist following the index contact was 2 d. Patients had objective clinical and laboratory assessment (C-reactive protein and fecal calprotectin in 91% and 73%). Fast-track colonoscopy/sigmoidoscopy was performed in 24.6% of the patients, while

**statement:** We hereby certify that the present study design was approved by The Research Ethics Office (Institutional Review Board) of McGill University. Ethics Committee approval was obtained in accordance to ISO protocol, local legal regulations and McGill University Health Center Research Ethics Board guidelines, prior to initiation of this study.

**Informed consent statement:** Our investigation only included evaluation of clinical data (as approved by the IRB, granting "access to adult health records") and did not required any additional procedures, or influenced health care delivery. As a result, informed consent forms were not required from the subjects for this study.

**Conflict-of-interest statement:** There are no conflicts of interest to report.

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

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

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computed tomography/magnetic resonance imaging in only 8.1%. Medical therapy was changed in 54.4%. ED visits within 30 d following the RAC visit occurred in 8.8% (unplanned ED visit rate: 5.9%). Diagnostic procedures and resource utilization at the ED ( $n = 135$  patients) were substantially different compared to RAC users: Abdominal computed tomography was more frequent (65.7%,  $P < 0.001$ ), coupled with multiple specialist consults, more frequent hospital admission ( $P < 0.001$ ), higher steroid initiation ( $P < 0.001$ ). Average medical cost estimates of diagnostic procedures and services per patient was \$403 CAD *vs* \$1885 CAD comparing all RAC and ED visits.

## CONCLUSION

Implementation of a RAC improved patient care by facilitating easier access to IBD specific medical care, optimized resource utilization and helped avoiding ED visits and subsequent hospitalizations.

**Key words:** Crohn's disease; Ulcerative colitis; Rapid access; Quality-of-care; Emergency department

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**Core tip:** The present study reports a comprehensive analysis of patient access, resource utilization, costs and outcome measures of a newly implemented formal inflammatory bowel diseases (IBD) specific rapid access clinic service compared to usual emergency department visits in IBD patients from a single academic center in North America. Creating a rapid access clinic service for IBD patients is associated with quick patient access, optimized and specific use of diagnostic procedures and services, with similar outcome parameters and lower resource utilization and overall costs compared to regular emergency department visits for IBD patients.

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## INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions which affect the patient's physical health, quality of life and social functioning. This creates an ongoing need for interactions with the healthcare system, as IBD patients are known to have high risk for developing severe disease related complications, as well as drug related adverse events<sup>[1,2]</sup>. As a result, IBD patients are high consumers of acute-care services and the initial point of care for persons having acute health issues related to their IBD are typically the emergency department (ED)<sup>[3]</sup>. Seeking medical care through the ED has been shown to cause a significant socio-economic impact on our health care system due to the substantial burden of resource utilization, especially in chronic conditions, such as IBD<sup>[4]</sup>.

IBD, similar to many other chronic and progressive conditions require continuous follow-up. In the last decade, therapeutic options and tools for disease monitoring have become increasingly complex, which led to a paradigm shift in IBD management. Objective therapeutic targets/endpoints have been defined and more rigorous disease monitoring strategies have been put forward in many expert recommendations, which all require continuous interactions with IBD specialist physicians<sup>[5,6]</sup>. Recently, multiple quality of care indicators have been developed to ensure a standardized and high quality care in IBD management. Among many, patient satisfaction is thought to be an integral part of high quality of care<sup>[7]</sup>. Several data show that "patient access" to treating physician or healthcare services in general is frequently a source of inadequate satisfaction among IBD patients<sup>[8,9]</sup>.

ED services are best reserved for acute, serious and/or life-threatening disease states. Thus optimising and reducing patient load to the ED is an important goal for



global healthcare delivery<sup>[10]</sup>. A large proportion of patients with known chronic conditions could potentially be managed in alternative care settings, more specific to their disease. These specific “rapid access” patient pathways for “rapid” evaluation and management can be established through regular outpatient care providers, which can potentially reduce ED visits, thus saving costs. There are examples for a good performance of rapid access clinics (RAC) in other chronic conditions, *e.g.*, diabetes or cardiology<sup>[11,12]</sup>. Until now, there is no well-defined framework of outpatient RAC in IBD centers across North America. A RAC service can provide quick access and rapid evaluation by an IBD specialist for patients experiencing moderate to severe symptoms in non-emergency situations related to IBD, thus potentially avoiding ED visits. In this study, we aimed to prospectively measure quality of care indicators by assessing patient access, diagnostic procedures, resource utilization and outcome parameters after the implementation of a new, formal RAC service at the McGill University Health Centre (MUHC) tertiary care IBD center. We also aimed to compare the resource utilization and costs of the RAC with regular ED visits of other IBD patients having no access to RAC services.

## MATERIALS AND METHODS

The MUHC IBD center consists of a team of medical professionals including IBD specialized gastroenterologists and fellows, IBD nurses, research fellows who work closely with other consulting professionals to offer a continuous, multi-faceted care to IBD patients<sup>[13]</sup>. The formal RAC service was established within the MUHC IBD center. Treating physicians provided emergency contact information (email/telephone) to all patients, as well as a framework of indications for appropriate consultation to the RAC, known as the Urgent IBD care plan (Supplementary Figure 1). Each email was read and reviewed by an IBD-specialised nurse and/or physician. The patients were offered a RAC visit if the request was deemed appropriate. Consecutive patients from the MUHC IBD Center who contacted the RAC *via* email/telephone or personal visit between July 2017 and April 2019 were prospectively included in this study. Only those above the age of 18 with a known diagnosis of IBD and followed-up at the MUHC IBD center by a member of the gastroenterology service were offered the RAC service. Patients with a recent diagnosis (less than 1 year) or an uncertain diagnosis of IBD were excluded.

Patient and disease related demographics including disease phenotype and severity as well as current and previous medication history was captured upon the RAC visits. Patient access to the RAC in terms of the validity of the request, and time to medical appointment were evaluated. Resource utilization included laboratory inflammatory markers and cultures ordered during the visit, endoscopy and other imaging modalities, requests for consulting services as well as changes in treatment initiated during the visit. We also evaluated outcome parameters such as need for ED visits within a 30 and 90 d period following the RAC visit. These ED visits were categorised based on the fact, whether they were organised by the RAC personnel or initiated by the patient alone and the RAC service was unaware of the event (unplanned ED visits). Hospital admissions or surgery were also registered in the aforementioned period.

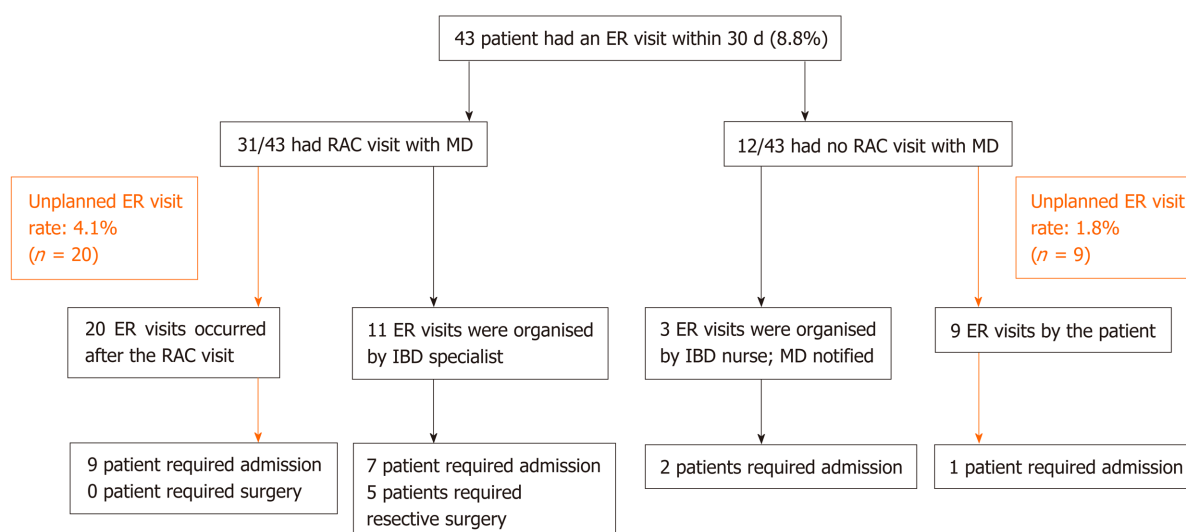
To compare resource utilization and healthcare costs we evaluated consecutive IBD patients who presented to the ED at the MUHC but did not have access to the RAC services. These patients were included in the period between January 2018 and January 2019. Data pertaining to patient access, diagnostic procedures and outcomes similar to the above-mentioned was collected during this period. Comparison of healthcare costs was performed using the non-industry cost estimates for diagnostic tests/procedures and medical services, as reimbursed by the RAMQ in Quebec<sup>[14]</sup>. Average medical cost estimates per patient were calculated for ED visits and RAC visits.

### Statistical analysis

Data was analyzed using SPSS 20.0 software. Descriptive statistics were used to evaluate demographic variables, baseline patient characteristics, frequencies of diagnostic procedures, treatment change and outcome parameters.  $\chi^2$  test was used to calculate differences in frequencies of resource utilization, change in medical therapy or hospitalization events, surgery requirements. Mean (SD) and median (IQR) time to events or length of stay and mean (SD) costs were calculated. A  $P < 0.05$  was regarded as statistically significant.

### Ethical considerations and confidentiality

Ethics Committee approval was obtained in accordance to ISO protocol, local legal



**Figure 1** Emergency department visit rates and patient routes following an initial contact with the rapid access clinic services ( $n = 488$  patients). RAC: Rapid access clinic; ER: Emergency department.

regulations and McGill University Health Center Research Ethics Board guidelines, prior to initiation of this study. All information collected during the course of this study remained confidential to the extent required by law. Data was strictly limited to members of the research team. Authorization to access patient charts was obtained from the Director of Professional Services of the MUHC.

## RESULTS

488 patients [41.3% male, Crohn's disease (CD)/ulcerative colitis (UC) 68.5%/31.5%] who had valid medical reason for contacting the RAC clinic were included during the investigation period (Table 1). For cost- and resource utilization comparison, 135 patients (60.7% male, CD/UC 68.5%/31.5%) who were not followed-up in the MUHC IBD center and were presenting to the ED at the MUHC for symptoms pertaining to a potential IBD flare were included. Detailed patient characteristics data is depicted in Table 1.

Amongst all the email/telephone requests obtained during the study period, 85.8% were deemed appropriate for a rapid appointment as per the Urgent IBD Care plan and consequently, these patients were given appointments for a RAC visit. Amongst these total visits, 333 patients (68.2%) were granted an appointment with an IBD specialist gastroenterologist and 86 patients (17.6%) had a visit with a specialised IBD nurse, where the physician was kept notified of the situation. 69 patients (14.1%) had no visit as their request could be managed *via* email or telephone. The reason for a rapid appointment was potential disease flare in 71.6% of patients presenting for a RAC visit. The median time to a RAC visit with an IBD specialist physician was 2 d (IQR: 0-6 d) following the first point of contact (telephone or email) initiated by the patient.

### Resource utilization and treatment change by the RAC service

RAC visits consisted of fast-track evaluation of disease activity using clinical assessment and laboratory markers. Complete blood count and C-reactive protein (CRP) measurements were performed in 90.9%, while fecal calprotectin (FCAL) in 73%, respectively. Stool culture was ordered in 41.9% of patients and *Clostridium difficile* toxin stool PCR in 43.1%. Colonoscopy and flexible sigmoidoscopy was requested in 17.9% and 6.7% of the patients, while only a minority of patients underwent imaging modalities, including 6.0% abdominal computed tomography (CT) and 2.1% magnetic resonance imaging (MRI). Other specialist consults were ordered in 9.8% of the cases, which included ED visits/consult initiated during a RAC appointment (Table 2). There was no significant difference between resource utilization and outcomes between patients with Crohn's disease and Ulcerative colitis (Supplementary Figure 1).

The study revealed a change in medical therapy in 54.4% of the cases. 21% of the patients experienced initiation or dose adjustment of systemic steroids, and biologics

**Table 1** Characteristics of patients contacting the rapid access clinic vs emergency department

	RAC visits (n = 488)	Emergency department visits (n = 135)
CD/UC (n)	334/154	97/38
Men/Women (%)	41.3/58.7	60.7/39.3
Age (mean $\pm$ SD, yr)	39.3 $\pm$ 14.8	45.2 $\pm$ 18.4
CD localization L1/L2/L3/L4 (%)	25.1/27.9/46.0/1.0	18.4/19.4/62.2/-
CD behavior B1/B2/B3 (%)	66.7/17.6/15.7	38.8/31.6/29.6
CD perianal (%)	22.7	10.2
UC location E1/E2/E3 (%)	8.8/30.4/60.8	-/27.8/72.2
Biological therapy (%)	60.6	42.9
Previous resective surgery (%)	19.8	35.6

RAC: Rapid access clinic; CD: Crohn's disease; UC: Ulcerative colitis.

were started or optimized in 11.9% of cases, respectively (Table 3).

### **Unplanned ED visits and hospital admissions following the RAC visits**

There was an 8.8% ( $n = 43$ ) rate of ED visits within 30 d and 11.1% rate within 90 d following the initial contact with the RAC services. The overall rate of hospital admissions related to IBD within 30 d following the first contact with the RAC was 4.5% ( $n = 22$ ). The overall incidence of unplanned ED visits (not initiated by a physician or IBD nurse) was 5.9% ( $n = 29$ ). Twenty patients presented to the ED following a RAC visit with an IBD specialist and 9 patients were not deemed appropriate for RAC visit with a physician based on complaints. Among patients with unplanned ED visits 10 patients required hospital admission and no patient required surgery. Fourteen patients had ED visits initiated by an IBD specialist or IBD nurse following a RAC visit due to physician concerns during the triage process. Among those, 9 patients required hospital admission and 5 patients underwent surgery. For detailed patient routes see Figure 1.

### **Resource utilization and patient outcomes following ED visits**

Amongst the patients assessed in the ED, 98.5% had at least one CRP value drawn and FCAL was measured in 10.4% of cases, significantly less frequent compared to the RAC visits ( $P < 0.001$ ). 51.1% and 48.9% of patients had a *C. difficile* stool PCR test and stool cultures, the earlier being significantly more frequent compared to the RAC visits ( $P = 0.03$ ). A noteworthy 65.7% of patients underwent abdominal CT imaging, significantly more compared to that during the RAC visits ( $P < 0.001$ ). The frequency of colonoscopy and sigmoidoscopy requested were 26.7% and 14.8%, again significantly more compared to the RAC visits ( $P = 0.005$  and  $P < 0.001$ ). All the patients were assessed by a consultant gastroenterology service. 50.4% were equally seen by internal medicine, 37.8% by colorectal surgery and 9.6% by other consultant services (Table 2).

The overall treatment change rate was similar to that of the RAC cohort, however there was a 42.2% rate of steroid use, significantly more compared to the RAC visits ( $P < 0.001$ ) (Table 3).

Hospital admissions were initiated during an ED visit in 64.4% of the patients, with 5.9% ( $n = 8$ ) of patients requiring surgery. The mean and median length of hospital stay was 8.4 (SD 9.9) and 5 (IQR: 3-10) d with only 16.9% and 13.5% of patient having a 1-2 or 3 d hospital admission. Additional hospitalisations within 30 d following the ED visit occurred in 8.1% ( $n = 11$ ) of the patients.

### **Cost comparison of patient management by the RAC and ED**

We further analyzed medical expenses comparing the average per-patient costs of resources used for patient assessment by the RAC vs the ED. Amongst the 419 patients seen in the RAC, estimated costs per person based on the diagnostic procedures and services utilized was \$403.30 CAD. This is to be compared to ED visits, with an average cost of \$1885.50 CAD per patient, with the cost of emergency visit, interdisciplinary consult and imaging being the most contributive to this total (Figure 2). This estimate do not include the expenses of hospital admissions, although 64.4% of the patients presenting at the ED required at least 1 [median 5 (IQR 3-10)] d of hospital admission. This produced an average additional cost of \$3143 CAD per patient per day related to hospitalisations (the estimated cost of admitting was \$4881 CAD per day). Of note, the average cost related to hospitalizations which incurred

**Table 2 Comparison of resource utilization between rapid access clinic vs emergency department visits**

	RAC resource utilization (%) <sup>1</sup>	ED resource utilization (%) <sup>2</sup>	P value
CRP	90.9	98.5	NS
FCAL	73	10.4	< 0.001
C. diff stool test	43.1	51.1	0.03
Stool Culture	41.9	48.9	0.06
TDM	14.7	0.0	< 0.001
Colonoscopy	17.9	26.7	0.005
Flexible sigmoidoscopy	6.7	14.8	< 0.001
CT abdominal	6.0	65.7	< 0.001
MRI	2.1	2.2	NS
Abdominal ultrasound	11.3	3.7	< 0.001
Gastroenterology consultation	-	100	
Internal medicine consultation	-	50.4	
Colorectal surgery consultation	-	37.8	
Other consults	9.8	9.6	NS

<sup>1</sup>Four hundred and nineteen patients presenting for MD or nurse visit for RAC clinic visit;

<sup>2</sup>One hundred and thirty-five patients presenting for ED visit, with no previous access to RAC services. RAC: Rapid access clinic; ED: Emergency department; CRP: C-reactive protein; FCAL: Fecal calprotectin; TDM: Therapeutic drug monitoring; CT: Computed tomography; MRI: Magnetic resonance imaging.

during all of the RAC visits was \$104.8 CAD per patient per day.

## DISCUSSION

This is the first comprehensive analysis of patient access, resource utilization, costs and outcome measures of a newly implemented formal IBD specific RAC service compared to usual ED visits in IBD patients from a single academic center in North America. The major finding of the present study was that creating a RAC service for IBD patients is associated with quick patient access, optimized and specific use of diagnostic procedures and services, with similar outcome parameters and lower resource utilization compared to regular ED visits for IBD patients.

ED attendance has been reported high for both incident and prevalent cases of IBD. By analyzing trends in ED visits and subsequent hospitalizations in the United States, the frequency of IBD related ED visits increased by 51.8%, from 90846 visits in 2006 to 137946 in 2014 based on the National Emergency Department Sample database<sup>[15]</sup>. For comparison, all-case ED use in this period increased by 14.8%. Inpatient hospitalizations following the ED visits was high, yet showed a decreasing trend for IBD patients (from 64.7% to 52.6%). Of note, the rates of urgent surgery in IBD patients admitted from the ED also decreased from 9.1% of all ED visits in 2006 to 5.6% in 2014. Trends were largely similar for pediatric onset IBD patients, according to a nationwide report on the use of ED resources by children with IBD in the United States. The rate of hospital admission for children was approximately 40% in CD and 60% in UC<sup>[16]</sup>. In a recent population-based study from Manitoba including 300 incident and 3394 prevalent IBD cases, 76% and 49% of patients attended the ED at least once during the study period of 3 years<sup>[3]</sup>. Hospitalization rates were reported lower in this study after presentation to the ED, with only 15% of the patients with known IBD and 44% with a new diagnosis of IBD were being admitted to the hospital. Our results show high rates (64%) for overall hospitalization of IBD patients presenting for regular ED department visits, however the rate of surgical intervention was low, only 5.9%. Direct comparison between these rates is difficult because of different methodology and IBD setting (IBD center *vs* population-based), or other contributing factors (*e.g.*, the availability of IBD specialist gastroenterology consults). Considering all the above, results could suggest that in a significant proportion of cases, IBD care provided in the ED could have been effectively and safely managed in a more cost-optimized outpatient settings, preferably by the attending IBD specialist.

The need for optimized “patient access” and monitoring algorithms for specialized care in acute IBD-related conditions is also expressed by the patients. ED care is associated with a high health care burden (*e.g.*, long waiting hours, assessment and care provided by non-specialized physicians and high costs). Based on a survey from

**Table 3** Inflammatory bowel disease related treatment change based on rapid access clinic/emergency department visit

	RAC visits (n = 488)	Emergency department visits (n = 135)
Treatment change	54.4%	58.5%
Systemic steroid start or dose adjustment	21.0% <sup>b</sup>	42.2% <sup>b</sup>
Biologic start	5.7%	2.2%
Biologic optimization	6.2%	

<sup>b</sup>P < 0.001. RAC: Rapid access clinic.

Manitoba, the majority of persons would be receptive to options other than ED visit when experiencing IBD related symptoms: 77% and 75% of the participants expressed to likely use a phone contact with a specialized IBD nurse, or a gastroenterologist, and 71% would use a walk-in gastroenterology clinic service<sup>[17]</sup>. In a previous study, our group evaluated results from patient satisfaction surveys at the MUHC IBD center, using the “Quality of Care Through the Patient's Eyes - Inflammatory Bowel Disease” questionnaire<sup>[9]</sup>. Results showed that “accessibility” especially in case of acute situation was one of the lowest rated aspects of perceived quality of care. These findings also strengthen the importance of establishing a new route of patient access to IBD specific care.

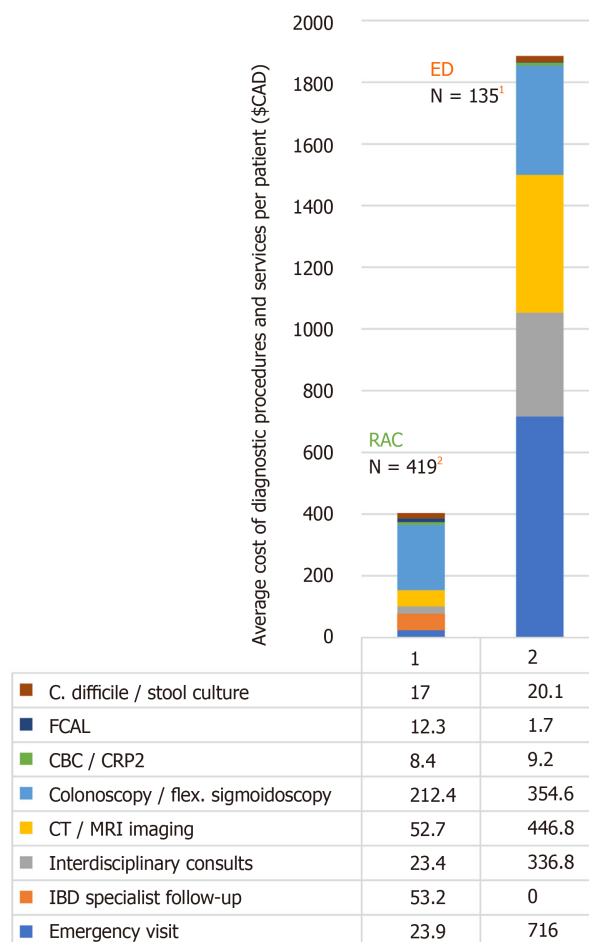
Our study is the first to comparatively evaluate the performance of RAC and ED care pathways, including utilization of diagnostic tests and consulting services. Our results confirm that objective and rapid evaluation was performed at the RAC, including high rates of CRP and FCAL testing (frequently coupled with *Clostridium difficile* and stool cultures), and careful use of fast-track endoscopies in line with a “treat-to-target” framework and objective, timely disease monitoring<sup>[5,6]</sup>. Cross sectional imaging was reserved for suspicion of complicated disease behavior and/or emergency situations, and mainly for CD patients.

In contrast, diagnostic test and service utilization by the ED was significantly different, including a very high utilization of cross-sectional imaging. More than two thirds of the patients underwent CT imaging who presented to the ED, while in the RAC CT was performed in 6% of the IBD patients. The wide use of abdomino-pelvic CT in ED is of concern for radiation exposure and costs. Yarur *et al*<sup>[18]</sup> found in a cross-sectional study that the rate of clinically actionable finding with abdomin-pelvic CT was moderate for CD patients (32.1%) but minimal for UC (12.8%) patients who visited the ED in the United States. For comparison, in the present study, 47.3% of patients underwent CT in the ED even in UC. FCAL test was performed only in 10.4% of the ED visits in the present study. The high frequency of urgent/same day hospitalizations and relatively long in-hospital stay [5 d, (IQR 3-10 d)] by the ED is also an important aspect of excessive resource utilizations.

Of note, 10% of the RAC patients underwent abdominal ultrasound (US) examination as part of their evaluation. US was the preferred method of choice against CT or MRI imaging in patients with the appropriate disease phenotype. Transabdominal US was reported to have comparable overall sensitivity and specificity to MRI and CT imaging in diagnosing ileal CD<sup>[19,20]</sup>. The evolution of US equipment, growing expertise, rapid access and relatively low costs lead to a growing use of intestinal US in the clinical assessment of IBD patients, especially in countries where the use of abdominal ultrasonography has a traditional role in everyday gastroenterology practice, *e.g.*, Germany, Italy<sup>[21]</sup>. A recent study showed that point of care US examinations could play a significant role in guiding therapeutic management in CD, although the proper characterization of disease specific lesions requires training and expertise<sup>[22]</sup>. Another study group reported initial experience of a rapid access US imaging clinic in IBD from the United Kingdom, demonstrating that a combined clinic-radiological approach using fast-track US offers the opportunity for urgent treatment changes and more proper triage of follow up appointment scheduling<sup>[23]</sup>.

Our results also confirm that the therapeutic decisions and optimization of medical therapy were significantly different in the case of RAC *vs* ED visit. Fewer cases of steroid initiation/dose adjustment were performed by IBD specialist physicians in contrast to the ED. In addition, optimization of biologicals and immunosuppressive was more frequent out in RAC settings.

An important outcome parameter is the need for ED visits following a RAC visit, and need for hospital admission and surgery rates within the next 30 or 90 d following the RAC or ED visits. Only 4.1% of patients needed an ED visit following a RAC visit, while the number of those patients who were not granted a RAC visit with



**Figure 2 Average per-patient cost estimates for diagnostic procedures and services in patients presenting to the rapid access clinic vs emergency department (in \$CAD).**<sup>1</sup>One hundred and thirty-five patients presenting for ED visit, with no previous access to RAC services. <sup>2</sup> Four hundred and nineteen patients presenting for a regular ED visit. RAC: Rapid access clinic; ED: Emergency department; CBC: Combined blood count; CRP: C-reactive protein; FCAL: Fecal calprotectin; TDM: Therapeutic drug monitoring; CT: Computed tomography; MRI: Magnetic resonance imaging.

a physician and reported to the ED anyway was even less (1.8%). A significant proportion of the ED visits following the RAC appointment reflected ongoing disease activity. Same day hospitalization rates were much higher following a regular ED visit. Hospital admissions in the next 30 d after a RAC visit was low (4.5%), this was also higher after an ED visit (8.1%). The requirement of urgent surgical interventions was also lower in patients presenting for the RAC [1.3% ( $n = 5$ ) vs 5.9% ( $n = 8$ )].

Finally, ED visits - frequently used by IBD patients - are associated with a known economic burden, thus decreasing ED access offers the potential for cost savings. ED visits contribute to approximately 10% of all ambulatory medical care visits in the United States<sup>[24,25]</sup>. Ballou *et al*<sup>[15]</sup> reported that the frequency of IBD-ED visits increased by 51.8% during an 8 year period (2006-2014) in parallel by a 102.5% rise in the per patient costs and a 207.5% increase in the aggregate national cost of IBD-ED visits, based on the Nationwide Emergency Department Sample database from the United States. The mean total charge of a single ED visit for IBD was \$4342 in 2014<sup>[15]</sup>. To our knowledge, our study is the first in the North American region to compare costs and resource utilization between IBD patients seen in a RAC service and those who presented to ED. Based on the RAMQ reimbursement plan data for procedures and services, and the number of patients included in our observation period we estimated the per-patient costs of one RAC visit at \$403.30 CAD, whereas the per-patient cost incurred during an ED averaged at \$1885.50 CAD. The main cost drivers of the ED visit were emergency service facility fees (\$716 CAD per patient), interdisciplinary consults (\$337 CAD per patient) and cross sectional CT imaging (\$447 CAD per patient). These estimates, however, do not include inpatient costs. Of note, > 60% of patients attending an ED visit had a median 5 night of hospital admission, which adds

\$15715 CAD (\$3143 CAD/d) additional cost per patient. Implementation of a RAC service in IBD would thus alleviate an already saturated acute care pathway and reduce health care costs, as similar outcomes are achievable with an optimized resource utilization.

Nevertheless, the concept of RAC may be less transmissible to community gastroenterology services given differences in IBD patient load and the potential lack of resources, however this strategy was shown clearly beneficial in our high-volume academic tertiary care IBD center. There are other examples of alternative follow-up options/rapid access pathways for IBD patients. A pediatric study by Dykes *et al*<sup>[26]</sup> showed that increasing the availability of IBD specialists and specialized nurses *via* telemedicine (e-visit/e-messaging model) can also decrease the frequency of IBD related ED visits. Another large-scale quality improvement project at a tertiary IBD centre in the UK reported results on the stratification of adult IBD outpatients by risk and disease activity to achieve a more optimal setting for outpatient monitoring. Patients in long-standing remission with a low risk of complications were transferred to a nurse-led telephone clinic monitoring service, with high non-inferior satisfaction rates being reported compared with existing face-to-face clinics. In parallel, the authors reported positive satisfaction results in establishing IBD referral hotlines and RACs, providing a responsive service for patients requiring urgent specialist attention. The median waiting time to a RAC visit was 6.5 d<sup>[27]</sup>.

The strengths of the present study include the single center design in a specialized tertiary care IBD center staffed with specialized IBD clinicians leading to harmonized care and less variation in treatment decisions, and consecutive, prospective patient inclusion with a large cohort size. The study provides a comprehensive and comparative analysis of two acute-care management pathways for IBD patients, including evaluation of patient access, resource utilization, costs and disease outcome parameters. A limitation to our study is that the IBD patient populations attending the RAC and ED services in real-life setting are not fully similar. The two populations had differences in disease characteristics, pointing to the more complex phenotype of the ED cohort. Providing easy access could have potentially boosted patient contact to the RAC clinic, and not all patients would have presented at ED. This is a potential confounder we are not able to fully control for. It may be hypothesized though, that the majority of those patients, who were seen in the RAC clinic would have alternatively presented to the ER (only 14.1% of the requests were deemed inappropriate as triaged by an IBD specialist or nurse). We believe that the differences in resource utilization, treatment decisions, outcomes and costs between the RAC and the ED are straightforward and show a clear benefit of this alternative rapid care pathway for IBD. Thus, the RAC may have accounted for approximately two thirds of potential ED IBD visits in 2018, where RAC and ED visits were monitored prospectively and in parallel. Of note, we could not calculate the exact change in the ED exposure by IBD patients before and after the creation of the RAC, since we did not track ED IBD visit counts before the initiation of the RAC.

In conclusion, results from the present study demonstrate that implementation of a RAC care pathway improved healthcare delivery by facilitating easier access, optimizing resource utilization for patient evaluation and treatment decisions to IBD specific medical care in urgent situations, thus preventing unnecessary ED visits. Patients, who underwent fast-track evaluation by an IBD specialist had low rates of further ED visits and hospital admissions. In addition, RAC pathway was associated with a significant cost reduction compared to ED services.

## ARTICLE HIGHLIGHTS

### Research background

Emergency department (ED) attendance in inflammatory bowel disease (IBD) patients put significant burden on the healthcare system.

### Research motivation

We theorize that a large proportion of IBD patients presenting with urgent IBD specific complaints could potentially be managed in alternative care settings, thus avoiding unnecessary ED visits.

### Research objectives

To report a comprehensive analysis of patient access and resource utilization after the implementation of the new rapid access clinic (RAC) service at a tertiary IBD center, compared to usual ED visits in IBD patients.

### Research methods

Patient access, resource utilization and outcome parameters were collected from consecutive

patients contacting the RAC in a two year period. Comparative analysis of resource utilization and healthcare costs were carried out evaluating ED visits of IBD patients with no access to RAC services.

### Research results

Creating a RAC for IBD patients is associated with quick patient access, optimized and specific use of diagnostic procedures and services, with similar outcome parameters and lower resource utilization and overall costs compared to regular ED visits for IBD patients.

### Research conclusions

Implementation of a RAC facilitated easier access to IBD specific medical care, with optimized resource utilization and helped avoiding potential ED visits and subsequent hospitalisations.

### Research perspectives

A RAC is ideal for providing IBD specific medical care in urgent situations, reducing burden to both the healthcare system and patients.

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## Malignant glomus tumor of the intestinal ileum with multiorgan metastases: A case report and review of literature

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### Abstract

#### BACKGROUND

Glomus tumors (GTs) are rare mesenchymal neoplastic lesions derived from cells of the glomus body. GTs rarely occurs in the visceral organs, where there may be few or no glomus bodies, and the majority of GTs are benign, rarely demonstrating aggressive or malignant behavior and histological features.

#### CASE SUMMARY

We report a patient with malignant GTs of the intestinal ileum with multiorgan metastases who was admitted due to moderate anemia. Capsule endoscopy revealed a bleeding mass in the intestinal ileum, and the patient underwent segmental ileal resection through laparoscopic surgery. The histopathological and immunohistochemical diagnoses were consistent with malignant GT. Long-term follow-up showed that the GT had metastasized to multiple organs such as the colon, brain, and possibly the lung.

#### CONCLUSION

This case was characterized by the highest degree of malignancy and by multiorgan metastases, and it was the first case of intestinal GT uncovered by capsule endoscopy.

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**Core tip:** We report a patient with malignant glomus tumors of the intestinal ileum characterized by the highest degree of malignancy and multiorgan metastases, and it was the first case of intestinal glomus tumor uncovered by capsule endoscopy. We further reviewed the literature on the clinicopathologic features, diagnosis, and treatment of intestinal glomus tumors.

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## INTRODUCTION

Glomus tumors (GTs) are mesenchymal neoplastic lesions derived from cells of the neuromyoarterial glomus or glomus body<sup>[1,2]</sup>. GTs are extremely rare, accounting for approximately 2% of all soft tissue neoplasms, and most often occur in the subungual region of the extremities<sup>[1,3]</sup>. The majority of GTs are benign and rarely demonstrate aggressive or malignant behavior and histological features<sup>[4,5]</sup>. GTs rarely occur in the gastrointestinal tract, where there may be few or no glomus bodies. Among the rarely reported gastrointestinal GTs, the gastric antrum is the most frequent region involved, and GTs that occur in the intestinal tract are extremely rare<sup>[6]</sup>.

Here, we report a patient with malignant GTs of the intestinal ileum with multiorgan metastases and review the literature on the clinicopathologic features, diagnosis, and treatment of intestinal GTs.

## CASE PRESENTATION

### Chief complaints

A 73-year-old woman was admitted with the main complaint of dizziness for 3 mo.

### History of present illness

Patient's dizziness symptoms started 3 mo ago with weakness, which had worsened over the past 1 wk.

### History of past illness

The patient received modified radical mastectomy of the left breast 5 years ago.

### Physical examination

The patient's temperature was 36.5 °C, heart rate was 78 bpm, respiratory rate was 18 breaths per min, and blood pressure was 125/75 mmHg. There were no significant positive signs other than anemic conjunctivae and anemic appearance.

### Laboratory examinations and imaging examinations

Blood routine examination showed that her hemoglobin level was 6.3 g/dL and the fecal occult blood test was positive. Contrast computed tomography (CT), upper gastrointestinal endoscopy, and colonoscopy did not reveal any significant findings. Then the patient underwent a capsule endoscopy examination, which revealed a bleeding mass in the intestinal ileum (Figure 1).

## MULTIDISCIPLINARY EXPERT CONSULTATION

Hong Gao, MD, PhD, Professor and Chief, Department of Colorectal Surgery, Beijing Shijitan Hospital Affiliated to the Capital Medical University.

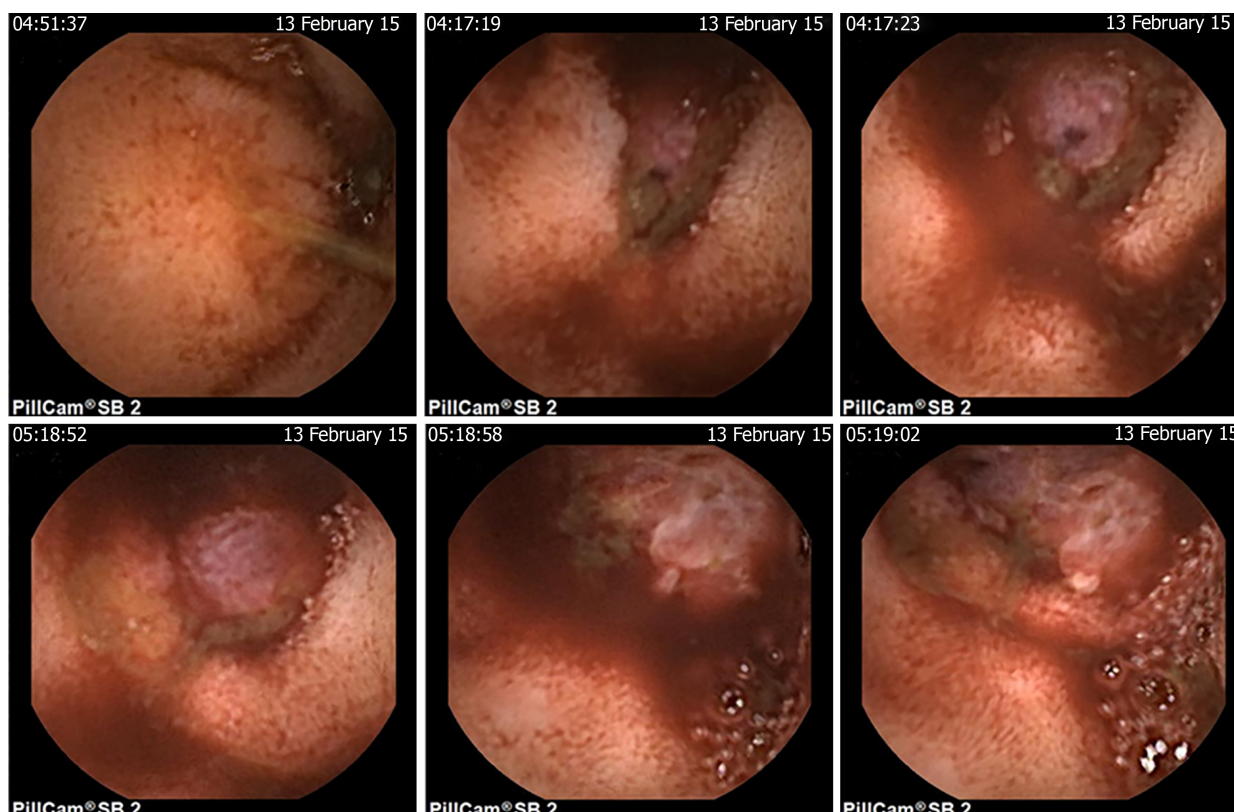


Figure 1 Capsule endoscopic characteristics of the intestinal glomus tumor from different perspectives.

It was recommended that the patient undergo segmental ileum resection through laparoscopic surgery.

## TREATMENT

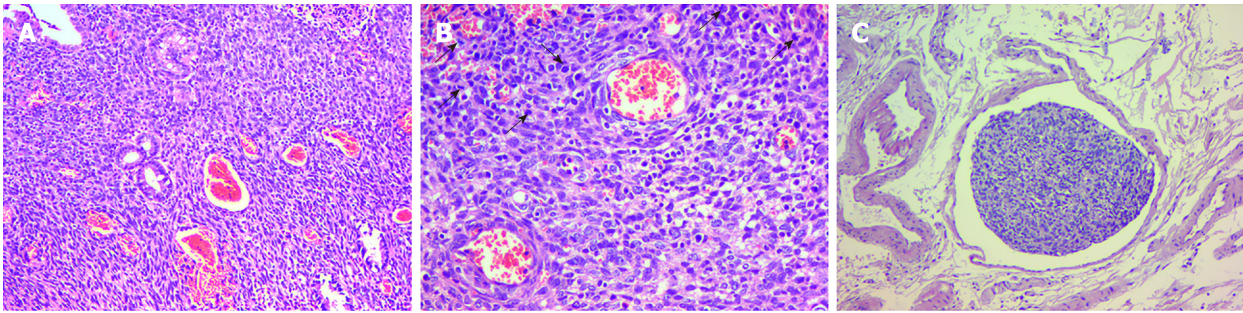
The patient underwent segmental ileum resection through laparoscopic surgery. The tumor measured 2.0 cm × 2.8 cm × 1.2 cm.

## FINAL DIAGNOSIS

The histological examination revealed that the tumor cells were spindle-shaped and surrounded by branched or dilated vessels (Figure 2A), with vascular invasion and focal necrosis, and extended to the muscularis propria. The mitotic activity was ≥ 5/per high-power field (HPF) (× 200) with marked nuclear atypia (Figure 2B and C). Immunohistochemical staining showed that the tumor cells were positive for smooth muscle actin (SMA), vimentin, caldesmon, cluster of differentiation 34 (CD34), and Ki-67 (80%+) and were negative for CD117, desmin, dog-1, s100, leukocyte common antigen and cytokeratin (commonly referred to as CK) (Figure 3). The histopathologic examination and immunohistochemistry results were consistent with a malignant GT.

## OUTCOME AND FOLLOW-UP

At 10 mo after surgery, the patient was re-hospitalized for dizziness and left leg weakness. Cranial magnetic resonance imaging showed the presence of a lesion measuring approximately 2.0 cm in the right frontal lobe that was considered a metastatic tumor. Postoperative pathological examination demonstrated that the lesion had similar histopathological and immunohistochemical features to the primary intestinal GT. Further follow-up showed multiorgan metastases of the GT to the transverse and sigmoid colon (the patient underwent hemicolectomy by laparoscopy), abdominal wall (the patient underwent the resection of the abdominal tumor, enterodialysis, and partial enterectomy by laparotomy), left temporal lobe (the



**Figure 2** Histological characteristics of the malignant glomus tumor in ileum. A: Spindled tumor cells with branched or dilated vessels surrounded [hematoxylin and eosin (H&E) stain, 100 ×]; B: Spindled cells with high mitotic activity and nuclear atypia marked with arrows (H&E stain, 200 ×); and C: Tumor cells with vascular invasion (H&E stain, 100 ×).

patient underwent two tumor resections by craniotomy), and possibly the lung (contrast CT showed a slightly enlarged mass in the inferior lobe of the right lung, and the patient and her family refused further examinations). Eventually, the patient died from multiple organ failure caused by GT metastases. Informed consent was obtained from the patient and her family.

## DISCUSSION

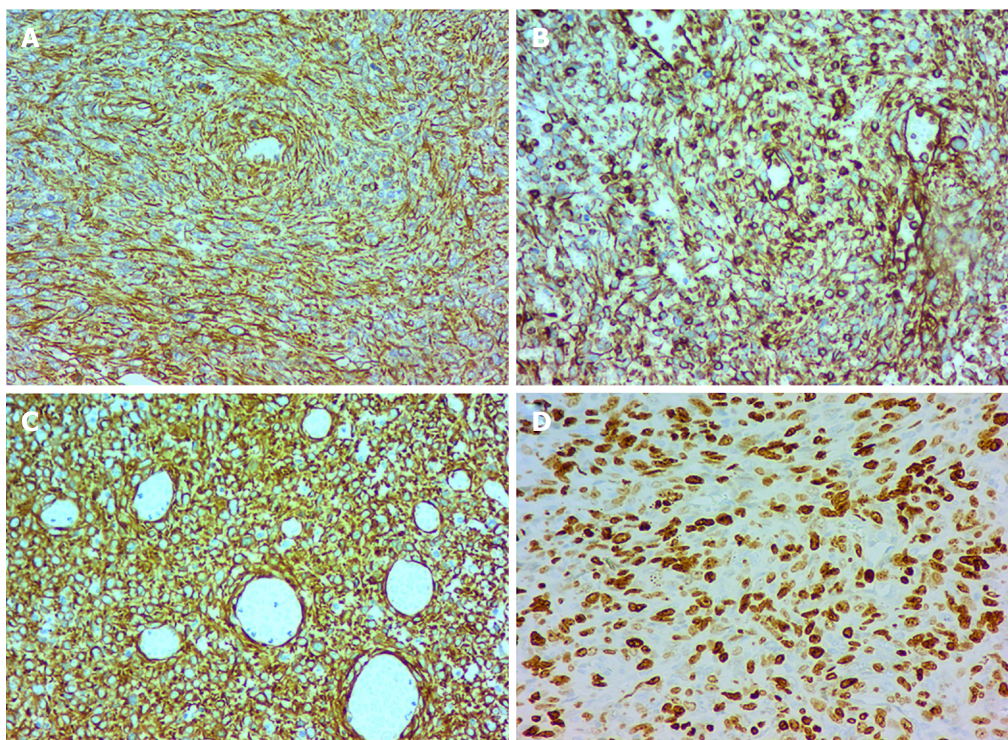
GTs most commonly occur in the dermis or subcutis of the extremities, and the vast majority of GTs are benign; malignant cases account for less than 1% of all GTs<sup>[6,7]</sup>. GTs have been occasionally reported in other locations, including the gastrointestinal tract, where the stomach has been the most frequent site of occurrence. GTs arising from the intestine are extremely rare.

To date, only 20 primary intestinal GTs have been described in the literature, including 9 cases reported by Russian investigators before 1988, for which we could not uncover detailed information<sup>[8-16]</sup>. The clinicopathologic features of the other 11 documented intestinal GTs are summarized in Table 1. The 11 patients ranged from 29-years-old to 82-years-old, and there was a significant male predominance, with 8 males (72.7%), 2 females, and 1 case of unknown sex, while previous data showed a nearly equal sex distribution<sup>[17]</sup>. Intestinal GTs presented with diverse clinical symptoms, the most common of which were melena, vomiting, abdominal pain, and anemic symptoms.

Intestinal GTs can occur in any part of the intestine, and the tumor size ranges from 0.6 cm to 12.8 cm at the longest diameter. The endoscopic appearance of intestinal GTs includes submucosal lesions with either normal mucosa or ulceration. Histologically, intestinal GTs are composed of multiple cellular nodules separated by smooth muscle cells and vascular forms in which numerous dilated blood vessels without GT elements are seen in the tumor periphery. Intestinal GTs can involve mucosa, muscularis, and the whole wall of the intestine, and 54.5% (6/11) of 11 of the previously reported cases involved serosa and even perienteric adipose tissue. Immunohistochemical analyses demonstrated that most intestinal GTs were positive for SMA, caldesmon, calponin, and vimentin and were negative for CD117, desmin, and S-100<sup>[7,17]</sup>.

The diagnosis of malignant GTs should consider the tumor size, infiltrative growth, growth pattern, cellularity, nuclear grade, mitotic activity, atypical mitotic figures, vascular involvement, and necrosis. Folpe *et al*<sup>[7]</sup> studied the features of 52 unusual GTs and proposed the following criteria for the diagnosis of malignant GTs: Tumors with deep locations, more than 2 cm, atypical mitotic figures, moderate to high nuclear grades and a mitotic activity of  $\geq 5/50$  HPFs (400×). World Health Organization classification of soft tissue tumors (2013) recommended that tumors with a deep location and a size of more than 2 cm in the absence of nuclear atypia were classified as glomus tumors of "uncertain malignant potential." According to these criteria, two cases with serosal invasion, large tumor sizes (maximum diameters of 2.5 cm and 12.8 cm), and increased mitotic activity (19/50 HPFs and 4-5/50 HPFs) met the diagnostic criteria for malignant GTs<sup>[5,18]</sup>.

The major differential diagnoses for intestinal GTs were gastrointestinal stromal tumors (GISTs) and gastrointestinal neurogenic tumors. Markku *et al*<sup>[17]</sup> summarized the differences in immunohistochemical findings between gastrointestinal GTs and GISTs. GISTs stained positively for CD117 (100%) and CD34 (69%). In contrast, GTs



**Figure 3** Immunohistochemical staining characteristics of the malignant glomus tumor in ileum. A: Smooth muscle actin; B: Vimentin; C: Caldesmon; and D: Ki-67.

were generally negative for CD117 (100%), and only a few cases were positive for CD34 (20%). Gastrointestinal neurogenic tumors had substantial positive staining for S-100 (paragangliomas and neurilemmomas), CK (carcinoid tumors), and the neuroendocrine markers chromogranin A, neuron-specific enolase, synaptophysin, and CD56 and were negative for SMA and CD117<sup>[1]</sup>.

Complete surgical resection of the tumor is an effective radical treatment for atypical GTs. Markku *et al*<sup>[17]</sup> performed long-term follow-up for 32 atypical gastrointestinal GTs (one intestinal case) after primary surgery and found that one patient died of metastatic disease at 50 mo and that the original tumor had mild atypia and vascular invasion. Malignant GTs were highly invasive, with high rates of recurrence and metastases. Previous studies have shown that 62.5% (10/16) of malignant GTs derived from the trachea, bronchus, or lung were distant metastases, and six patients died during the 60-mo follow-up. Surgical resection is still an effective treatment for malignant GTs, and some patients receive postoperative adjuvant chemotherapy with poor responses to treatment<sup>[19]</sup>. These 11 documented intestinal GT patients that included two malignant cases, underwent laparoscopy or laparotomy, and no recurrence or metastases were reported. Due to the extremely low incidence of intestinal GTs and incomplete clinical information, it is difficult to identify an effective treatment for malignant GTs of the intestine.

Our patient had a malignant intestinal GT with several important and interesting features. (1) This patient had the highest degree of malignancy: Among 11 reported intestinal GTs, 81.8% (9/11) of the cases were benign, and the only two cases that were malignant had increased mitotic activity. Our case exhibited the highest degree of malignancy with extremely high mitotic activity and proliferation capacity (Ki-67, 80% +). (2) This patient had multiorgan metastases: No distant metastases and postoperative recurrence were observed in the two malignant GTs that were previously reported<sup>[5,18]</sup>, while our patient had multiorgan metastases to the transverse colon, sigmoid colon, abdominal wall, left temporal lobe and possibly the lung. This is the first reported case of malignant intestinal GT with multiorgan metastases. And (3) This patient was diagnosed by capsule endoscopy: The tumor occurred in the intestinal ileum, and contrast CT did not show marked enhancement; in addition, upper gastrointestinal endoscopy and colonoscopy could not reach the lesion site. This is the first case of GT identified by capsule endoscopy, and our study added GT to the range of intestinal diseases that can be identified by capsule endoscopy.

**Table 1** Clinicopathological characteristics of documented intestinal glomus tumors

Ref.	Age/sex	Symptoms	Location/size in cm	Invasion	Mitotic activity	Follow up
Abu-Zaid <i>et al</i> <sup>[5]</sup> , 2013	29/female	Constipation vomiting, melena	Ileum 12.8 × 10.2 × 13.1	Serosa	4-5/50 HPFs	6 mo NETR
Tan <i>et al</i> <sup>[18]</sup> , 2015	74/male	Vomiting abdominal pain	Splenic flexure 2.5	Serosa	19/50 HPFs	6 mo NETR
Bennett <i>et al</i> <sup>[20]</sup> , 2015	70/male	Light headedness, melena	Ascending colon 2.3 × 1.6	Muscularis propria	1/50 HPFs	NA
Campana <i>et al</i> <sup>[21]</sup> , 2014	51/male	Melena, orthostasis	Ileum 3.7	Muscularis propria	< 5/50 HPFs	2 yr NETR
Oliphant <i>et al</i> <sup>[22]</sup> , 2007	37/male	Abdominal pain, altered bowel habit	Ascending colon 3.0 × 2.0	Pericolic fat	0/50 HPFs	NA
Barua <i>et al</i> <sup>[23]</sup> , 1988	60/NA	NA	Colon 0.8 × 0.6	Pericolic fat	NA	NA
Miettinen <i>et al</i> <sup>[17]</sup> , 2002	34/female	Appendicitis-like symptoms	Cecum 7.0 × 6.0	NA	1/50 HPFs	NA
Geraghty <i>et al</i> <sup>[24]</sup> , 1991	60/male	Abdominal pain, diarrhea	Ileum 0.6	Serosa	0/50 HPFs	Died <sup>1</sup>
Hamilton <i>et al</i> <sup>[25]</sup> , 1982	82/male	Abdominal pain, anorexia, nausea	Jejunum 1.0 × 1.5	Serosa	NA	6 mo NETR
Knackstedt <i>et al</i> <sup>[26]</sup> , 2007	65/male	Vomiting	Duodenum NA	Submucosa	0/50 HPFs	NA
Tuluc <i>et al</i> <sup>[27]</sup> , 2005	40/male	Rectal bleeding	Colon diminutive	Mucosa	0/50 HPFs	> 1 yr NETR

<sup>1</sup>The patient died 5 d post-operatively from a presumed pulmonary embolus; NA: Not available; NETR: No evidence of tumor recurrence; HPFs: High power fields, 400 ×.

## CONCLUSION

We reported a malignant intestinal GT with the highest degree of malignancy and multiorgan metastases, and this patient was the first GT patient to be diagnosed by capsule endoscopy. Intestinal GTs are extremely rare; most cases are benign, while a few cases demonstrate aggressive or malignant clinical and histological features. The clinical manifestations, imaging and endoscopic features of malignant intestinal GTs lack specificity, and careful histological examinations and immunostaining for appropriate markers are essential for accurate diagnoses. Complete surgical resection is an effective radical treatment for intestinal GTs.

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