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EDITORIAL

Medical imaging for pancreatic diseases: Prediction of severe acute pancreatitis complicated with acute respiratory distress syndrome

Ling-Ji Song, Bo Xiao

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Abstract

In this editorial we comment on the article published in the recent issue of the *World Journal of Gastroenterology* [2022; 28 (19): 2123-2136]. We pay attention to how to construct a simpler and more reliable new clinical predictive model to early identify patients at high risk of acute respiratory distress syndrome (ARDS) associated with severe acute pancreatitis (SAP), and to early predict the severity of organ failure from chest computed tomography (CT) findings in SAP patients. As we all know, SAP has a sudden onset, is a rapidly changing condition, and can be complicated with ARDS and even multiple organ dysfunction syndrome, and its mortality rate has remained high. At present, there are many clinical scoring systems for AP, including the bedside index for severity in AP, acute physiology and chronic health evaluation II, systemic inflammatory response syndrome, Japanese severe score, quick sepsis-related organ failure assessment, *etc.* However, some of these scoring systems are complex and require multiple and difficult clinical parameters for risk stratification. Although the aforementioned biomarkers are readily available, their ability to predict ARDS varies. Accordingly, it is extremely necessary to establish a simple and valuable novel model to predict the development of ARDS in AP. In addition, the extra-pancreatic manifestations of AP patients often involve the chest, among which pleural effusion and pulmonary consolidation are the more common complications. Therefore, by measuring the semi-quantitative indexes of chest CT in AP patients, such as the amount of pleural effusion and the number of lobes involved as pulmonary consolidation, it has important reference value for the early diagnosis of SAP complicated with ARDS and is expected to provide a basis for the early treatment of ARDS.

Key Words: Severe acute pancreatitis; Acute respiratory distress syndrome; Clinical scoring system; Prediction model; Semi-quantitative

Core Tip: Respiratory failure has been confirmed to be the most common type of organ failure in acute pancreatitis (AP) and is closely related to high mortality. Acute respiratory distress syndrome (ARDS) is one of the most common patterns of respiratory failure in AP and is still a little-known disease. Although some studies have shown that it is promising to predict the results of AP-related ARDS, the preventive strategies for ARDS development are still in their infancy. For this reason, we need to establish a simple and valuable new prediction model, combined with chest computed tomography findings, to early identify high-risk patients with severe AP and ARDS and help clinicians take timely intervention measures to prevent disease progression.

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INTRODUCTION

Acute pancreatitis (AP) is a common digestive disease that causes acute abdominal pain. A meta-analysis showed that the annual morbidity and mortality of AP all over the world were 33.74/100000 and 1.60/100000, respectively[1]. According to statistics, AP was the second leading cause of the total hospitalization rate, the largest contributor to the total cost, and the fifth leading cause of in-hospital mortality[2]. Many reports point out that the disease is increasing year by year, which is related to the incidence of biliary disease and the increase of alcoholism[3]. The clinical manifestations of AP vary greatly. Most of the patients who can recover after general treatment are considered to have mild AP, which is called a "self-limited disease"; some patients with transient organ failure (duration < 48 h) are considered to have moderately severe AP[4], with an approximately 20% developing severe AP (SAP). SAP has an aggressive onset with organ failure (duration > 48 h) and presence or absence of pancreatic or peripancreatic tissue necrosis, with a mortality rate of 20%-40%[5].

Acute respiratory distress syndrome (ARDS) is a life-threatening form of respiratory failure. Its main clinical manifestations are shortness of breath and refractory hypoxemia. ARDS is caused by ischemia and hypoxia injury secondary to pulmonary pathological changes under the influence of a variety of intrapulmonary and extrapulmonary factors, resulting in impaired lung function and serious condition of the life of patients[6]. The PaO_2/FiO_2 ratio (oxygenation index) is a component of the assessment of patients with ARDS[7], and refers to the ratio of the partial pressure of arterial oxygen to the concentration of oxygen in the inhaled air, with a normal value of 400-500 mmHg. The Berlin definition of ARDS is the most widely accepted criterion, which defines the specific time from clinical injury to the onset of new respiratory symptoms, specific chest X-ray findings, and the severity of ARDS based on the PaO_2/FiO_2 ratio. ARDS is defined as positive end-expiratory pressure ≥ 5 cm H_2O, the ratio of atrial oxygen partial pressure to inhaled oxygen fraction ≤ 300 mmHg, and bilateral pulmonary infiltrative lesions that cannot be explained completely by fluid overload or heart failure. The Berlin definition also emphasizes that ARDS can be divided into three categories based on the severity of hypoxemia[8]: Mild (PaO_2/FiO_2 200-300), moderate (PaO_2/FiO_2 100-200), and severe (PaO_2/FiO_2 ≤ 100).

SAP is a clinical critical disease with rapid progression, many complications, and high mortality. In addition to causing local disorders, it can also cause damage to other organs, and its serious complications are the main factors leading to poor prognosis[5]. Previous studies have confirmed that the lung is the first damaged target organ in the early induction of systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndrome by SAP, and respiratory failure is the most common organ failure in SAP[9-11]. ARDS is considered to be an important type of respiratory failure with high mortality[12]. It has been reported that 4%-15% of patients with AP have concomitant ARDS[13] , while this percentage may be up to a third in SAP[14]. ARDS is the most dangerous complication of AP, which usually occurs between 2 and 7 d after the onset of pancreatic inflammation. According to the literature data, SAP-related ARDS accounted for 60% of all deaths in SAP patients in the first week of the disease [15]. These data indicate that the more serious the condition of SAP, the worse the progression of the AP-related ARDS, which may further suggest that the severity of SAP is negatively correlated with PaO_2/FiO_2. If the occurrence of ARDS is not predicted early, the patient's lung function will drop sharply, which may even lead to death during the acute reaction period. Thus, there is an urgent need for a simple and accurate new clinical prediction model combined with chest computed tomography (CT) findings of AP patients to diagnose and predict SAP-related ARDS at an early stage. And timely diagnosis and treatment can greatly improve the survival rate of SAP patients.

New prediction model compared with clinical scoring systems

In the recent issue of the *World Journal of Gastroenterology*, Li *et al*[16] published an interesting paper

entitled "Development and external validation of models to predict acute respiratory distress syndrome related to severe acute pancreatitis". This study constructed and validated a new simple and accurate prediction model for SAP-related ARDS. In this multicenter retrospective study, 597 patients diagnosed with AP from four hospitals in different regions of China from 2017 to 2021 were divided into two cohorts: The derivation cohort (n = 407) and the validation cohort (n = 190). Of these, 139 were diagnosed with SAP and 99 were diagnosed with ARDS. Multivariate logistic regression showed that four identical variables of SAP and ARDS were identified as independent risk factors, including heart rate, respiratory rate, serum calcium concentration, and blood urea nitrogen. In the derivation and validation cohorts, the area under the operating characteristic curve (AUC) for predicting SAP was 0.879 and 0.898, respectively, and that value for ARDS was 0.892 and 0.833, respectively. In the derivation cohort for SAP prediction, an AUC value of the new model was significantly better than that of the SIRS (AUC = 0.808) or quick sepsis-related organ failure assessment (qSOFA) (AUC = 0.730); with respect to ARDS prediction, a corresponding AUC value was better than that of the SIRS (AUC = 0.815) or qSOFA (AUC = 0.742). The study developed a novel predictive model for SAP-related ARDS in patients with AP. Furthermore, the results of the new model indicated that patients with AP who exhibited higher respiratory rate, heart rate, and blood urea nitrogen (BUN) concentration, and lower serum calcium concentration on admission might develop SAP and ARDS with a higher risk.

In another study by Ding *et al*[17], 779 AP patients were randomly assigned to the primary cohort (n = 560) and the validation cohort (n = 219), and AP patients in each cohort were further divided into an ARDS group and a non-ARDS group. The heart rate, BUN, and serum calcium in the ARDS group were higher than those in the non-ARDS group, and the heart rate was significantly different between the two groups. Comparing variables in the primary cohort, the heart rate and serum calcium were statistically significantly different between the two groups. Zhang *et al*[18] also divided SAP patients into ARDS and non-ARDS groups, and their results showed statistically significant differences in respiratory rate and heart rate between two groups. Respiratory rate > 30 /min (odds ratio = 2.405) was an independent risk factor for ARDS in patients with SAP. As far as we know, the BUN level has not been used as a direct predictor of ARDS. However, the marker can be used as a predictor of pathogenesis associated with other risk factors, such as AP[6]. BUN not only is selected in the new prediction model of Li *et al*[16], but also is participated in other prediction models of SAP, such as bedside index for severity in AP (BISAP). The results of Dai *et al*[19] showed that the only independent risk factor correlated with 30-d all-cause mortality was BUN level in AP patients. In addition, the validity of BUN as a prognostic marker was further verified using a receiver operating characteristic curve with an AUC of 0.803 for BUN and an optimal cut-off value of 12.01 mmol/L (sensitivity = 0.714, specificity = 0.810). Another study has shown that elevated BUN at admission and within 24 h after admission can predict AP mortality[20]. According to our knowledge, the study of Li *et al*[16] presented the first model to use serum calcium concentration to predict ARDS in SAP. Ye *et al*[21] showed that BISAP and serum calcium were independent predictors of AP severity. The results of the study showed that the model established by the combination of BISAP and serum calcium was remarkably better than that established by BISAP and serum calcium alone. Additionally, the study also found that serum calcium concentration was negatively correlated with the severity of AP, while BISAP was positively correlated with AP severity [21]. It is further suggested that there may be some relationship between serum calcium levels and SAP-related ARDS in patients with AP. A previous study showed that hypocalcemia was an independent risk factor for respiratory failure in SAP[22]. They believed that serum calcium concentration was a valuable tool for evaluating rapidly persistent organ failure in AP patients. Further studies need to be done in this respect.

Because of the high mortality rate of SAP patients, it is necessary to quickly identify patients with a more severe disease state and a higher risk of death at an early stage. For risk stratification, several pancreas-specific or general scoring systems have been established in the past. BISAP is a potential prognostic scoring system for identifying AP patients at high risk of death in hospital, with high specificity[23], but it needs to evaluate pleural exudation. And most hospitalized patients do not undergo the examination within 24 h, so the score cannot be completed[24]. Multi-parameter scores such as the acute physiology and chronic health evaluation II (APACHE II) and BISAP are very important for clinical trials, but the APACHE II score is not specific for AP. In addition, the method is too complex and time-consuming to calculate, and its application in practical clinical work is limited [25]. A clinical study has confirmed that SIRS can be used as an "early warning device" of the severity of AP[26]. At present, the commonly used AP scoring systems, such as the BISAP and the Japanese severe score systems, contain the relevant content of SIRS. The qSOFA score is also rapid and easy to obtain and can be used for rapid evaluation of preclinical patients or emergency patients, but its effect on the prognosis of the disease is limited[27]. Therefore, a simple model with a small number of parameters will be more practical. The novel prediction model reported by Li *et al*[16] involves only four routine parameters for SAP and ARDS prediction. This study confirms that the new model is not inferior to BISAP in predicting SAP-related ARDS, allowing early identification of patients at high risk of SAP and ARDS. It has clinical value for improving the prognosis of AP.

Chest CT findings of AP for early prediction of ARDS

Based on clinical observations, we can evaluate SAP and related ARDS not only from the predictive new model but also from chest CT presentations of AP at the time of initial diagnosis. Up to 55% of AP patients may have abnormal chest CT findings, including pleural effusion, pulmonary atelectasis, pulmonary consolidation, and ARDS-related pulmonary edema[28]. Pulmonary consolidation and pleural effusion are common complications in patients with AP, which are closely related to the severity of AP. The imaging manifestations of pulmonary consolidation are patchy, segmental, and diffuse pulmonary changes, which may contain bronchial inflation signs. It is reported that the incidence of pleural effusion in AP was about 3%-17% in the earlier literature. But recent reports showed that, according to CT scan, the incidence was as high as 46%-50%[29,30]. The chest CT findings of SAP complicated with ARDS mainly include bilateral or left-sided pleural effusion[28,31], and solid changes in the basal segments of lower lobes of both lungs[32] (Figure 1).

Accordingly, we can semi-quantitatively evaluate the pleural effusion volume (PEV) and pulmonary consolidation score (based on the number of involved lobes) in patients with AP. A recent study showed that PEV and pulmonary consolidation lobes can provide early predictions of SAP and organ failure [32]. Peng *et al*[32] reported that PEV was strongly correlated with BISAP score and CT severity index (CTSI) score, but was weakly correlated with the length of hospital stay and APACHE II score. The lung consolidation score was moderately correlated with the BISAP score, CTSI score, and APACHE II score. On the contrary, Yan *et al*[33] showed that there was a strong correlation between PEV and length of hospitalization or APACHE II score. In addition, Peng *et al*[32] described that the accuracy of PEV in predicting SAP was similar to that of the BISAP score, APACHE II score, and CTSI score. As for predicting organ failure, the accuracy of PEV was also similar to that of the BISAP score, APACHE II score, and CTSI score. While Yan *et al*[33] predicted SAP, the accuracy of PEV was distinctly higher than that of the BISAP score and CTSI score, but was significantly lower than that of the APACHE II score. In the prediction of organ failure, the accuracy of PEV was distinctly higher than that of CTSI score, and its accuracy was similar to that from BISAP score or APACHE II score. Peng *et al*[32] also described that the accuracy of the lung consolidation score was similar to that of the BISAP score, APACHE II score, and CTSI score in predicting SAP and organ failure. Although some results of Peng *et al*[32] and Yan *et al*[33] were different, they both confirmed that chest imaging findings of AP patients could indicate the severity of AP and organ failure to some extent.

Previous studies have confirmed that respiratory failure is the most common type of organ failure in AP. Schepers *et al*[11] reported that the proportion of AP patients with respiratory failure was 92% (221/240). It was also found that this was the most common type of organ failure in the early and late stages of AP. Moreover, the distribution of different types of organ failure was different in this study, and the median duration of respiratory failure was 19 d. The mortality rate of respiratory failure was 37%, and the mortality rates of renal failure and circulatory failure was 47% and 40%, respectively. Raghu *et al*[28] claimed that the development of pulmonary consolidation in patients with AP was related to the occurrence of respiratory failure. As mentioned above, the incidence of ARDS was associated with respiratory failure. They also observed that pleural effusion was significantly associated with the severity of AP, the occurrence of respiratory failure, and poor prognosis. To sum up, chest CT findings of AP patients are potentially valuable for early prediction of possible subsequent respiratory failure in our daily clinical work.

Future trends and prospects

The 21st century is the era of big databases. Radiomics is an emerging technology that can extract a large number of parameters from images that are difficult for human eyes to observe and distinguish, and transform image data into high-dimensional and minable data through a variety of algorithms. Therefore, it can carry out a comprehensive quantitative analysis of the heterogeneity of the disease, and assist in clinical diagnosis, treatment, and other work[34]. Lin *et al*[35] first reported a radiomics model based on contrast-enhanced magnetic resonance imaging (MRI) to predict the clinical severity of AP. Their study showed that the prediction accuracy of the portal venous-phase MRI imaging radiomics model reached 85.6% and 81.0% in the training cohort and validation cohort, respectively. These results suggest that the portal venous-phase MRI radiomics model may be more accurate in early predicting the clinical severity of AP, and these findings may have a broad application prospect in the classification of AP severity. We believe that there is also great potential for predicting SAP-related ARDS in the future.

CONCLUSION

In summary, the essence of ARDS in AP is the embodiment of AP complicated with multiple organ dysfunction, with rapid progression and poor prognosis. Clinically, it is necessary to establish a novel predictive model and accurately observe the imaging features of SAP in chest CT. It is conducive to early prediction of the risk of AP patients complicated with ARDS. After that, timely intervention and active treatment of the primary disease will ultimately improve the survival rate of SAP patients.

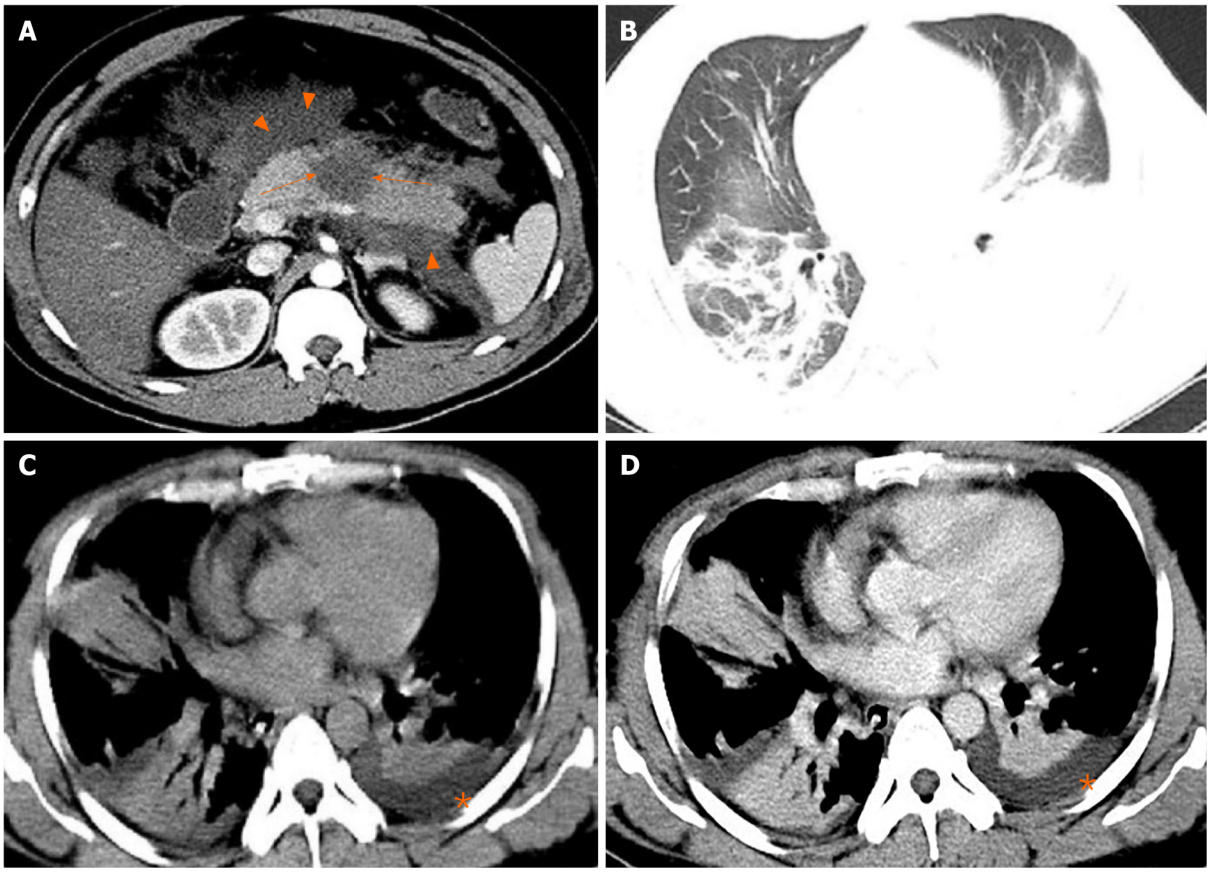

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Figure 1 A 31-year-old man with severe acute pancreatitis complicated with acute respiratory distress syndrome. A: Axial contrast-enhanced computed tomography image in the arterial phase shows flake parenchymal necrosis (arrows) in the region of body of the pancreas, as well as extensive heterogeneous collections (acute necrotic collections) in the peripancreatic and the left pararenal anterior spaces (arrowheads); B: Lung window; C: Mediastinal window; D: Chest axial contrast-enhanced venous phase image. The three images show partial pulmonary consolidation in the middle and lower lobes of the right lung, in which bronchial inflation signs can be seen, and partial consolidation with partial atelectasis in the lower lobe of the left lung caused by external pressure of pleural effusion (asterisks).

FOOTNOTES

Author contributions: Xiao B designed the research study; Song LJ and Xiao B performed the research, analyzed the data, and wrote the manuscript; and all authors have read and approved the final manuscript.

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REVIEW

Role of intestinal flora in primary sclerosing cholangitis and its potential therapeutic value

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Abstract

Primary sclerosing cholangitis (PSC) is an autoimmune disease characterized by chronic cholestasis, a persistent inflammation of the bile ducts that leads to sclerotic occlusion and cholestasis. Gut microbes, consisting of microorganisms colonized in the human gut, play an important role in nutrient intake, metabolic homeostasis, immune regulation, and immune regulation; however, their presence might aid PSC development. Studies have found that gut-liver axis interactions also play an important role in the pathogenesis of PSC. Patients with PSC have considerably reduced intestinal flora diversity and increased abundance of potentially pathogenic bacteria. Dysbiosis of the intestinal flora leads to increased intestinal permeability, homing of intestinal lymphocytes, entry of bacteria and their associated metabolites, such as bile acids, into the liver, stimulation of hepatic immune activation, and promotion of PSC. Currently, PSC effective treatment is lacking. However, a number of studies have recently investigated the targeted modulation of gut microbes for the treatment of various liver diseases (alcoholic liver disease, metabolic fatty liver, cirrhosis, and autoimmune liver disease). In addition, antibiotics, fecal microbiota transplantation, and probiotics have been reported as successful PSC therapies as well as for the treatment of gut dysbiosis, suggesting their effectiveness for PSC treatment. Therefore, this review briefly summarizes the role of intestinal flora in PSC with the aim of providing new insights into PSC treatment.

Key Words: Primary sclerosing cholangitis; Intestinal flora; Antibiotics; Fecal microbiota transplantation; Probiotics; Bile acids

Core Tip: Primary sclerosing cholangitis (PSC) is an autoimmune disease that currently lacks treatment. The intestinal flora comprises microorganisms that colonize the human gut and play essential roles in nutrient intake, metabolic homeostasis, immune regulation, and PSC development. Thus, the intestinal flora may be a potential therapeutic target for PSC, and many recent studies have attempted to regulate it. In this review, we have reviewed the role of the intestinal flora in PSC. We believe that our study makes a significant contribution to the literature because our paper demonstrated the great potential of the gut flora as a therapeutic target for PSC treatment.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is an autoimmune-mediated chronic cholestatic liver disease characterized by progressive bile duct inflammation, leading to intra- and extrahepatic bile duct stenosis and occlusion and cholestatic cirrhosis[1]. Patients with PSC have a greatly increased risk of developing cancers (cholangiocarcinoma, gallbladder cancer, hepatocellular carcinoma, and colorectal cancer); approximately half of patients with PSC develop cancer, ultimately leading to death[2]. Although the etiology of PSC remains unclear, it is generally accepted that interactions between genetics and the environment determine PSC development[3]. Owing to the close anatomical and physiological connection between the intestine and the liver, the intestinal flora is closely related to liver disease[4]. Several studies have suggested that the intestinal flora is involved in PSC development through the gut-liver axis[5,6]. Moreover, patients with PSC have significantly reduced intestinal flora diversity and an increased abundance of potentially pathogenic bacteria[7,8]. Intestinal flora dysbiosis leads to increased intestinal permeability, intestinal lymphocyte homing, entry of bacteria and their associated metabolites [*e.g.* bile acids (BAs)] into the liver, activation of the hepatic immune response, and bile duct inflammation and fibrosis[9].

The incidence of PSC is increasing, but an effective treatment does not exist currently. PSC can eventually progress to cirrhosis or liver failure, but these conditions are still symptomatically treated[10,11]. For patients with end-stage PSC, liver transplantation is the sole option; however, transplantations are not universally available owing to high costs and potential transplant rejection. Furthermore, approximately 30%-50% of patients experience PSC recurrence within 10 years of transplantation[12].

Many studies have reported that the gut flora is a promising therapeutic target for PSC, and that antimicrobial therapy based on gut flora modulation, fecal microbiota transplantation (FMT), and probiotics is an emerging therapeutic options[13,14]. Thus, in this review, we discuss the latest research findings on the role of intestinal flora in PSC and provide important insights into potential microbe-altering interventions.

PSC PATHOPHYSIOLOGY AND THE GUT-LIVER AXIS

PSC is a rare disease with an incidence of 0.91-1.30/100000. The incidence of small bile duct PSC is approximately 0.15/100000, with the highest prevalence in the Nordic countries, reaching an incidence of 16.2/100000[15]. PSC mostly occurs in men aged 40-50 years, with age of diagnosis at 30-40 years and a male to female ratio of approximately 2:1[1]. The pathogenesis of PSC is complex, but it is currently believed that interactions between genetic susceptibility factors and environmental promoters plays a role in the occurrence and development of PSC[16]. Human leukocyte antigen is strongly associated with PSC pathogenesis[17]. However, the risk ratio for first-degree relatives is approximately 11, suggesting that environmental factors play a more critical role in the pathogenesis of PSC[18]. In addition, the geographic distribution of PSC pathogenesis may indicate that the disease is influenced by the environment[19]. Interactions of the gut-liver axis, such as damage to the intestinal mucosal barrier, dysbiosis, and immune interactions, also play a role in the pathogenesis of PSC[1].

The gut-liver axis refers to the bidirectional relationship between the intestine, its microbiota, and the liver, resulting from the interaction of dietary, genetic, and environmental factors[20]. The close association between the intestine and the liver begins during embryonic development, with both organs originating together in the ventral foregut endoderm. From a physiological point of view, the gut-liver

axis is one of the most important links between the intestinal flora and the liver[21]. The liver, which receives approximately 70% of its blood from the portal vein, is a receiver and filter of nutrients and bacterially produced toxins and their metabolites. The secretion of substances such as BAs and antibodies into the intestine acts as a feedback mechanism that affects intestinal homeostasis[22].

Approximately 70% of patients with PSC have concomitant inflammatory bowel disease (IBD) and more commonly ulcerative colitis (UC)[23-25]. Patients with PSC have a reduced risk of death after a colectomy, and after receiving liver transplantation, colectomy significantly reduces the risk of PSC recurrence. This close association between PSC and IBD suggests that intestinal flora may play a key role in the pathogenesis of PSC[26,27] through the gut-liver axis[28].

PATIENTS WITH PSC AND THEIR DYSBIOSIS OF INTESTINAL FIORA

Intestinal flora dysbiosis

Under normal physiological conditions, the human body contains a large and diverse community of intestinal microorganisms, reaching up to 10^{14} organisms that comprise more than 1000 species; this is collectively known as the intestinal flora[29]. A normal intestinal flora is primarily composed of *Firmicutes, Bacteroidetes, Proteobacteria*, and *Actinobacteria*, which promote nutrient digestion and absorption, defend against foreign pathogens, regulate immunity, and participate in metabolic processes[30].

In healthy populations, the intestinal microenvironment is in homeostasis due to the mutual regulation of various flora. Intestinal flora dysbiosis is a disruption of the dynamic balance between intestinal flora when various factors cause disturbances in the human body environment, and changes in the number, species, and ratio of favorable, conditionally pathogenic, and harmful bacteria[31,32]. The manifestations of intestinal flora dysbiosis include intestinal flora translocation (transfer of the original colonizing bacteria from the intestine to the deep mucosa or from the intestine to other sites) and intestinal flora imbalance (decrease in the abundance of the original beneficial intestinal flora and increase in the abundance of pathogenic flora). Dysbiosis of the intestinal flora leads to impairment of the intestinal barrier, increased endotoxemia, and a breakdown of the liver's immune tolerance to the intestinal flora and its metabolites, which in turn causes a strong immune response in the liver[33].

The intestinal flora of patients with PSC

It was found that patients with PSC have a marked dysbiosis of the intestinal flora compared with the healthy population. Rossen *et al*[34] performed the first 16S rRNA analysis of the microbiota of the intestinal mucosa in the ileocecal region of patients with PSC and found that, at the genus level, the relative abundance of uncultured *Clostridium II* was notably lower in patients with PSC compared with patients with UC and non-inflammatory controls. In addition, the mucosal adherent microbiota at the level of the ileocecal region in patients with PSC showed considerably reduced diversity and abundance. Torres *et al*[8], using bacterial 16S rRNA next-generation sequencing, reported that patients with PCS had similar overall microbiome characteristics at different locations in the gut, exhibiting enrichment in *Blautia* and *Barnesiellaceae spp*. A more in-depth taxon analysis at the operational taxonomic unit (OTU) level revealed the most significant changes in *Clostridiales*, with 3 decreases and 66 OTU enrichments. Sabino's study found that species richness (defined as the number of different OTUs observed in the samples) was reduced in patients with PSC compared with healthy controls, that *Enterococcus, Clostridium, Lactobacillus,* and *Streptococcus* were enriched, and that an operational taxonomic unit belonging to the *Enterococcus* genus is positively correlated with the levels of alkaline phosphatase (ALP) levels (a marker of disease severity)[35]. In addition, PSC has its own unique gut microbial profile that is not associated with IBD co-morbidity. Subsequently, a study by Kummen *et al*[36] also confirmed the unique gut microbiota of PSC independent of the receipt of antibiotics and ursodeoxycholic acid (UDCA) treatment, with a marked reduction in bacterial diversity in patients with PSC. Furthermore, Quraishi *et al*[37] explored the intestinal mucosal flora of PSC-IBD patients, further complementing the study by Kummen *et al*[36] *Escherichia, Lachnospiraceae,* and *Megasphera* were markedly increased, whereas *Prevotella* and *Roseburia* (butyrate producers) were decreased in abundance in PSC-IBD patients compared with controls. They hypothesized that intestinal microecological dysregulation in patients with PSC may prompt dysregulation associated with mucosal immunity by modulating abnormal homing of intestinal-specific lymphocytes and intestinal permeability. This is consistent with the hypothesis of Kummen *et al*[36], Rühlemann *et al*[38,39] also showed that PSC itself drives the observed changes in fecal microbiota and that the findings of Kummen *et al*[36] regarding microbiota as a diagnostic marker are promising.

In the last two years, studies on the PSC gut flora have gained more interest. Quraishi *et al*[40] tried to unravel the PSC disease mechanism by integrating mucosal transcriptomics, immunophenotyping, and mucosal microbial analysis; their study reported that PSC-IBD patients had considerably higher abundance of *Bacteroides fragilis, Roseburia spp., Shewanella sp.,* and *Clostridium ramosum* species, which was associated with changes in the BA metabolic pathway. In addition, the amine oxidase-expressing bacterium *Sphingomonas sp*. is upregulated in PSC-IBD. Amine oxidase is associated with abnormal

homing of intestinal lymphocytes to the liver[41]. The upper gastrointestinal tract and bile ducts of patients with PSC are equally affected by microbial ecological dysbiosis. Liwinski *et al*[42] showed that the biliary microbiome of patients with PSC exhibited the most extensive changes, including reduced biodiversity and expansion of pathogenic bacteria, with the marked increase of *Enterococcus spp.* directly causing epithelial barrier damage and mucosal inflammation. Lapidot *et al*[43] found that microbial alterations in PSC were consistent in saliva and gut, with 27 bacterial species present in both the salivary and gut microbiomes, including *Clostridium perfringens XlVa, Veillonella, Lachnospiraceae, Streptococcus,* and *Blautia*. Of these, *Lactobacillus, Ruminococcus gnavus,* and *Streptococcus salivarius* were extensively enriched. The study by Lemoinne *et al*[44] confirmed previous findings of altered bacterial microbiota composition in patients with PSC, such as *Faecalibacterium* and *Ruminococcus* in reduced proportions, and reported for the first time the occurrence of fungal ecological dysbiosis in patients with PSC. PSC-associated fungal ecological dysbiosis is characterized by increased biodiversity (alpha diversity) and altered composition compared with in healthy subjects or patients with IBD, including a marked increase in the abundance of *Exophiala spp*. In some patients. Kummen *et al*[45] provided a detailed functional analysis of microbial genes encoding enzymes and metabolic pathways by using metagenomic shotgun sequencing. *Clostridium spp.* increased in the intestinal flora of patients with PSC, while *Eubacterium spp.* and *Ruminococcus obeum* decreased. Targeted metabolomics revealed reduced concentrations of vitamin B6 and branched-chain amino acids in PSC. Microbial metabolism of essential nutrients and circulating metabolites associated with the disease process were considerably altered in patients with PSC compared with in healthy individuals, suggesting that microbial function may be related to the disease process in PSC.

Most of these studies used 16S gene sequencing to examine the microbiota in the intestinal mucosa and feces of patients with PSC. Although these studies came from all over the world, some of them had relatively small sample sizes, and dietary and lifestyle habits varied between the samples of each study, possibly affecting the final gut floral composition of patients with PSC and limiting the generalization of the results. However, these studies also yielded some common findings that reveal to some extent the gut microbiota characteristics of patients with PSC[46]. Patients with PSC suffer intestinal dysbiosis, which has its own unique biological characteristics, as evidenced by a decrease in gut bacterial α-diversity (average species diversity of the ecosystem) and marked changes in β-diversity (spatial variation in species composition), a decrease in specialized anaerobic bacteria, and an increase in the abundance of potentially pathogenic bacteria (Table 1)[7,35-39,42-44,47]. Among which, *Veillonella, Enterococcus, Streptococcus, Clostridium,* and *Lactobacillus spp.* were markedly elevated[36,38,42-44]. An increase in *Veillonella* species, a potential pathogen in humans, can serve as a biomarker of the severity of certain diseases, such as autoimmune liver disease and cirrhosis[48,49].

INTESTINAL FLORA AND PSC-MECHANISTIC INSIGHTS

The leaky gut hypothesis was first proposed by Bjarnason *et al*[50] in 1984, providing theoretical support for the involvement of the intestinal flora in the development of PSC. Normal intestinal flora plays the role of maintaining the balance of the intestinal environment and preventing pathogenic bacteria and toxins from entering the blood circulation[51]. The germ-free (GF) multidrug resistance 2 knockout (Mdr2-/-) mice is a well-studied PSC model that shows a lack of microbial regulation, which is direct evidence that intestinal flora has a key role in PSC development[52]. Intestinal flora dysbiosis damages the intestinal barrier in patients with PSC, allowing bacteria and enteric-derived endotoxins to enter the liver *via* the portal vein, triggering an immune response[53]. Simultaneously, when liver function is impaired, Kupffer cells cannot inactivate endotoxins as efficiently, impairing bile excretion. Furthermore, this increases intestinal permeability, intestinal lymphocyte nesting, and the entry of bacteria and their metabolites [*i.e.* pathogen-associated molecular patterns (PAMPs)] into the liver, impairs normal BA metabolism, and promotes bile duct inflammation and fibrosis (Figure 1)[54,55].

Intestinal flora dysbiosis activates liver immunity

Intestinal flora dysbiosis damages the intestinal barrier: Lapidot *et al*[43] found that in patients with PSC, a decrease in the relative abundance of commensal bacteria in the intestinal flora, including *Bacteroides thetaiotaomicron* and *Faecalibacterium prausnitzii*, and bacterial diversity led to decreased short-chain fatty acids (SCFAs) with anti-inflammatory effects, such as acetate and butyric acid. This decrease caused intestinal barrier dysfunction and lack of antimicrobial peptides, exacerbating the leaky gut syndrome. When intestinal flora dysregulation occurs in patients with PSC, PAMPs in the gut bind to Toll-like receptors (TLRs) and NOD-like receptors (NLRPs) on the surface of dendritic cells. This event activates the cytoplasmic downstream nuclear transcription factor κB (NF-κB), causing the production and secretion of inflammatory cytokines and chemokines. Disruption of intestinal epithelium tight junctions and the normal intestinal barrier leads to increased intestinal permeability[31,56]. Furthermore, *Enterococcus faecalis* (*E. faecalis*), which increased the most in the intestinal flora of patients with PSC, produces gelatinase, which damages the intestinal epithelium and causes impaired intestinal barrier function[35]. Nakamoto *et al*[57] also found that increased *Klebsiella pneumoniae* during PSC

Table 1 Changes in the intestinal flora of patients with primary sclerosing cholangitis

Methods	Increased	Decreased	Ref.
16S RNA gene sequencing: ileum, colon, and rectal samples	*Actinobacillus*, *Bifidobacterium*, *Fusobacterium*, *Haemophilus*, *Roseburia*	*Bacteroides*	[135]
qPCR: fecal samples	*Enterobacteriaceae*	*Bifidobacterium*, *Lactobacillus*	[136]
16S RNA gene sequencing: fecal samples	*Clostridium*, *Lactobacillus*, *Ruminococcus gnavus*, *Veillonella*	*Coprococcus*, *Faecalibacterium*, *Phascolarctobacterium*, *Ruminococcus*	[137]
16S RNA gene sequencing: duodenal fluid, saliva, duodenal mucosa, and bile samples	Duodenal mucosa biopsy: *Escherichia coli*, *Veillonella dispar*; Bile fluid: *Enterococcus*, *Neisseria*, *Proteobacteria*, *Staphylococcus*, *Veillonella dispar*		[42]
Metagenomic shotgun sequencing	*Clostridiales*	*Eubacterium*, *Ruminococcus obeum*	[45]
16S RNA gene sequencing: fecal and saliva samples	*Bacteroides fragilis*, *Blautia*, *Clostridium spp. Enterococcus*, *Enterobacteriaceae*, *Lactobacillus*, *Ruminococcus gnavus*, *Streptococcus salivarius Veillonella dispar*	*Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii*	[43]
Sigmoid mucosal biopsies and 16S RNA gene sequencing	*Haemophilus parainfluenzae*, *Pseudomonas*, *Streptococcus*	*Lachnospiraceae*	[40]
16S RNA gene sequencing: fecal samples	*Veillonella*	*Blautia*, *Faecalibacterium*, *Lachnoclostridium*, *Ruminococcus*	[44]
16S RNA gene sequencing: fecal samples	*Enterococcus*, *Lactobacillus*, *Streptococcus*, *Veillonella*	*Clostridium cluster IV*, *Coprococcus*, *Desulfovibrio*, *Faecalibacterium*, *Holdemanella*	[38]
16S RNA gene sequencing: fecal samples	*Veillonella*	*Coprococcus*, *Desulfovibrio*, *Phascolarctobacterium*, *Succinivibrio*	[36]
16S RNA gene sequencing: fecal samples	*Clostridium*, *Enterococcus*, *Haemophilus*, *Rothia*, *Streptococcus*, *Veillonella*	*Adlercreutzia equolifaciens*, *Coprococcus catus*, *Faecalibacterium prausnitzii*, *Prevotella copri*, *Ruminococcus gnavus*	[47]
Colonic mucosal biopsies and 16S RNA gene sequencing	*Escherichia*, *Lachnospiraceae*, *Megasphaera*	*Bacteroides*, *Prevotella*, *Roseburia*	[37]
16S RNA gene sequencing: fecal samples	*Veillonella*		[39]
16S RNA gene sequencing: fecal samples	*Enterococcus*, *Streptococcus*, *Veillonella*		[7]
16S RNA gene sequencing: fecal samples	*Bacteroidetes*, *Enterococcus*, *Fusobacterium*, *Lactobacillus*, *Morganella*, *Streptococcus*, *Veillonella*	*Anaerostipes*	[35]
Colonic mucosal biopsies and 16S RNA gene sequencing: ileal samples	*Barnesiellaceae*, *Blautia*		[8]
Mucosal biopsy of the ileocecal region and 16S RNA gene sequencing	*Clostridium clusters IVand XIVa*, *Akkermansia sp.* (*Verrucomicrobia*), *Uncultured Clostridiales II*		[34]

forms pores by disrupting the intestinal epithelium, leading to increased intestinal permeability, thus prompting other bacteria (*e.g. Proteus mirabilis* and *Enterococcus gallinarum*) to cross the intestinal barrier. In turn, a Th17 cell-mediated inflammatory response initiates in the liver. Finally, Manfredo *et al*[58] demonstrated that *Enterococcus gallinarum* could reach several organs, such as the mesentery, mesenteric lymph nodes, liver, and spleen, after crossing the damaged intestinal epithelium, causing autoimmune diseases such as PSC.

In addition, PSC recurrence in patients who had undergone liver transplantation was associated with specific intestinal flora changes before transplantation. The rate of PSC recurrence was decreased in patients with a higher abundance of *Shigella spp*. in the intestinal flora before transplantation, suggesting that *Shigella spp*. may reduce bacterial translocation and endotoxemia by improving the intestinal mucus layer function and repairing the intestinal barrier[59].

Intestinal bacterial translocation induces liver inflammation: Secondary bacterial overgrowth in the small intestine of rats, achieved by using a blind jejunal loop, led to the translocation of intestinal flora and its metabolite. Consequently, the intestines exhibited characteristic pathological changes of PSC, such as irregular dilatation and bead-like changes in the intra- and extrahepatic bile ducts[60]. Furthermore, Tedesco *et al*[61] found elevated serum interleukin (IL)-17 levels in PSC mice; enriched *Lactobacillus gasseri*, peribiliary collagen deposition, and periportal fibrosis; and increased numbers of IL-17A+ and γδTCR+ cells in mouse liver tissues, which are characteristic inflammatory responses.

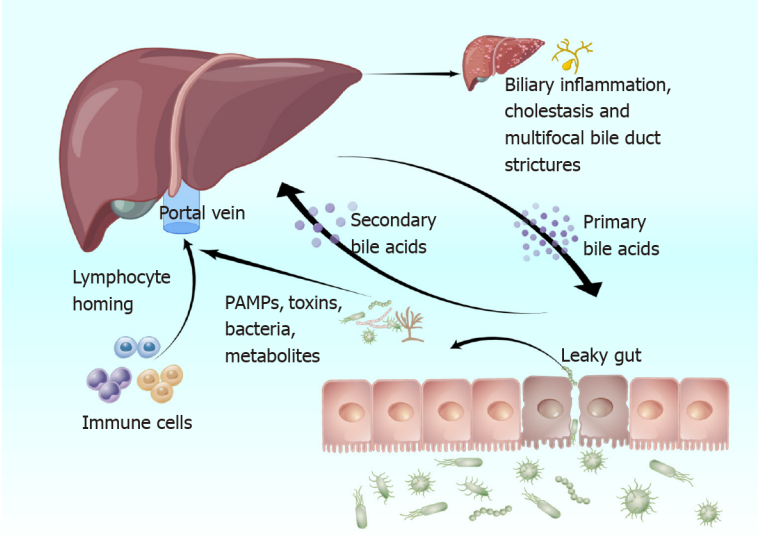

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Figure 1 The effect of intestinal flora dysbiosis in patients with primary sclerosing cholangitis. Intestinal flora dysbiosis causes increased intestinal permeability, intestinal lymphocyte homing, and entry of bacteria and their metabolites (*i.e.* pathogen-associated molecular patterns) into the liver. It also impairs normal bile acid metabolism and promotes bile duct inflammation and fibrosis. PAMPs: Pathogen-associated molecular patterns. By Figdraw, www.figdraw.com.

Additionally, Liao *et al*[62] used Mdr2-/- mice to investigate the role of intestinal flora in PSC, reporting that Mdr2-/- mice had intestinal flora dysbiosis. This caused the NLRP3-mediated innate immune response in the liver, amplified by intestinal barrier failure and enhanced bacterial translocation. Finally, Dhillon *et al*[63] compared the serum soluble cluster of differentiation 14 (sCD14) and lipopolysaccharide-binding protein (LBP) levels of patients with PSC and healthy controls, finding that patients with PSC had elevated levels of sCD14 and LBP. The sCD14 and LBP bind to lipopolysaccharides (typical bacterial translocation markers in humans) in response to significant intestinal flora translocation in patients with PSC[64].

The liver contains many immune cells, including Kupffer cells, natural killer (NK) cells, NK T cells, T cells, and B cells, and is a vital immune organ. In healthy individuals, only a few translocated bacterial products make it to the liver. The liver immune system tolerates these bacterial products to avoid harmful reactions, known as hepatic immune tolerance[65]. The intestinal flora dysbiosis in PSC impairs the intestinal barrier function, allowing bacteria and their products to enter the liver continuously. Thus, the hepatic immune tolerance breaks, inducing local inflammation and immune responses by activating TLR-based pattern recognition receptors on hepatic immune cells. Gram-positive bacteria mainly mediate TLR2 activation, endotoxins mediate TLR4 activation, bacterial flagella mediate TLR5 activation, and unmethylated CpG DNA mediates TLR9 activation[66]. TLR activation promotes a downstream inflammatory cascade that activates the MyD88-mediated NF-κB pathway to induce liver fibrosis[67]. Simultaneously, inflammatory cytokines and chemokines [*e.g.* IL-6 and tumor necrosis factor-α (TNF-α)] are overexpressed, inflammatory cells infiltrate, and oxidative stress and endoplasmic reticulum stress occur in the bile duct epithelium. Eventually, bile duct sclerosis and occlusion, cholestasis, and bile duct fibrosis develop[54,68].

Intestinal lymphocyte homing exacerbates liver inflammation

Up to 70% of patients with PSC also develop IBD, suggesting a correlation between the intestine and the liver in patients with PSC and IBD. The discovery of reciprocal transport pathways of lymphocytes to target tissues, as well as the expression of gut-specific adhesion molecules and chemokines in the liver, suggest the homing of intestinal lymphocytes as a contributing factor to PSC pathogenesis[69,70]. Endothelial cells in the hepatic sinusoids of patients with PSC overexpress mucosal vascular addressin cell adhesion molecule 1 (an endothelial adhesion molecule) and C-C motif chemokine ligand 25 (a chemokine), which bind to α4β7 integrin and C-C motif chemokine receptor expressed by intestinal mucosal lymphocytes. This event prompts the recruitment of lymphocytes of an intestinal origin into the liver, which then recognizes the corresponding antigen and triggers an autoimmune response, causing liver injury[71,72]. Trivedi *et al*[41] suggested that this mechanism is associated with hepatic vascular adhesion protein-1 (VAP-1) overexpression. Increased *Veillonella* in the gut of patients with PSC results in primary amine metabolism, which participates in VAP-1 synthesis (as a VAP-1 substrate). Furthermore, hepatic interstitial cells express VAP-1, which recruits intestine-derived T cells to the liver, promoting liver inflammation and fibrosis[73]. Moro-Sibilot *et al*[74] found that elevated levels of sVAP-

1 were associated with poor disease outcomes in PSC. High sVAP-1 Levels correlate with the expression of mucosal addressin cell adhesion molecule 1 in the liver, which contributes to the homing of intestinally activated T cells to the hepatobiliary tract[75]. Meanwhile, sVAP-1 triggers oxidative stress in hepatocytes and aggravates liver injury[76]. B cells in the liver are also derived from intestine-associated lymphoid tissue. B cells are activated by intestinal bacteria and enter the liver, producing antibacterial molecules, such as immunoglobin A, that aggravate liver damage.

Intestinal flora affects PSC through BAs metabolism

It has been established that several intestinal bacterial genera produce BA hydrolases, such as *Lactobacillus, Clostridium, Enterococcus,* and *Bifidobacterium*. Normal microbial metabolism increases BA diversity as well as hydrophobicity, which facilitates BA excretion[77,78]. Intestinal flora plays a key role in the pathogenesis of PSC by mediating BA biosynthesis and farnesol X receptor (FXR) signaling. FXR regulates BA synthesis through a negative feedback loop thereby affecting the intestinal flora[79]. BAs can directly damage intestinal bacterial cell membranes and indirectly affect the intestinal flora composition by binding to FXR and enhancing the action of antimicrobial peptides. Intestinal flora can also alter BA metabolism by affecting the ab initio synthesis of BAs and enterohepatic circulation[80]. Liwinski *et al*[42] found that patients with PSC had increased taurolithocholic acid concentrations in their bile, which causes inflammation; the levels were closely related to the abundance of *Enterococcus.* BA hydrolase expression, which catalyzes the conversion of primary BAs to secondary BAs, is highest when the human intestinal flora contains *E. faecalis*. Thus, a significant increase in *E. faecalis* in the bile of patients with PSC may affect BA metabolism and cause excessive accumulation of secondary BAs in the body, exacerbating PSC[7,42,81]. Tabibian *et al*[82] found that Mdr2-/- mice produced similar biochemical and histological features of PSC (confirmed by liver pathology and hydroxyproline assays) compared to conventionally reared Mdr2-/- mice; these mice were deficient in secondary BAs due to lack of intestinal flora. Further studies showed that GF-Mdr2-/- mice and antibiotic-induced specific pathogen-free Mdr2-/- mice showed imbalance in BA homeostasis, increased BA reuptake, and accelerated accumulation of harmful BAs in the liver due to dysregulation of intestinal microecology [83].

A recent study showed that *Prevotella copri* in the human gut regulates BA metabolism and transport pathways through gut microbiota interactions, especially the FXR signaling pathway, significantly improving chlorosis and liver fibrosis in 3,5-diethoxy-carbonyl-1,4-dihydropyridine-induced PSC mice [84]. Another study showed that intestinal flora attenuates liver damage by promoting UDCA production. The mechanism of UDCA, which has antioxidant, immunomodulatory, hepatocyte-protective, and membrane-maintaining functions, includes re-establishing the intestinal flora, and is widely used to treat PSC[85]. Lee *et al*[86] found that *Ruminococcus gnavus N53* and *Collinsella aerofaciens* in normal human intestinal flora catalyze the conversion of goose deoxycholic acid to UDCA by expressing the 7β-hydroxysteroid dehydrogenase gene, which increases UDCA acid, thereby reducing liver damage in pathological conditions.

TARGETED INTESTINAL FLORA MODULATION FOR PSC TREATMENT

There are no clear and effective options for treating PSC. Pharmacological and endoscopic treatments exist; however, these treatments primarily target the symptoms, and the only effective treatment for end-stage PSC is liver transplantation[16]. In recent years, the incidence of PSC has increased, but intestinal flora research has also expanded, resulting in antimicrobial therapy based on intestinal flora modulation and FMT as potential PSC treatment options[87]. Studies have found that antibiotics, probiotics, and FMT improve intestinal flora disorders, thereby treating PSC (Table 2)[88,89].

Antibiotics

Studies have shown that patients with PSC treated with vancomycin had significant reductions in their serum ALP and bilirubin levels and Mayo PSC risk scores (MRSs) and significant improvements in clinical symptoms, such as fatigue and pruritus[90,91]. An open-label prospective therapeutic clinical trial study showed that oral vancomycin was well tolerated in patients with PSC, with peripheral blood γ-gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT) concentrations, white blood cell counts, and neutrophil counts returning to normal from pre-treatment elevated levels within 3 mo of oral administration. Cholangiography, histological, and liver stiffness assessment at the end of follow-up showed improved results, and the trial also showed that that peripheral blood levels of CD4 + CD25hiCD127 Lo and CD4 + FoxP3 + regulatory T cells were also elevated in PSC-IBD patients treated with oral vancomycin[92,93]. Furthermore, Britto *et al*[94] found fewer potentially pathogenic bacteria, such as *Fusobacterium, Haemophilus,* and *Neisseria*, in the intestinal flora of patients with PSC after oral vancomycin treatment. A significant recovery in flora diversity was also observed, suggesting that vancomycin treatment indirectly leads to a secondary increase in bacterial diversity by prompting the intestinal flora to suppress mucosal inflammation. The efficacy of vancomycin for PSC may be related to its selectivity for *Clostridium perfringens*[95]. Shah *et al*[96] reported that vancomycin has a relatively

Table 2 Intestinal flora regulation in primary sclerosing cholangitis treatment

Treatment			Results	Ref.
Oral Antibiotics	Vancomycin	125 mg four times daily for 90 d	Fecal calprotectin and serum GGT levels returned to normal	[94]
		125 mg four times daily for 12 wk	Significantly decreased ALP, MRS, ESR, and GGT levels. Fatigue, pruritus, diarrhea, and anorexia significantly improved	[91]
		50 mg/kg/d for 30 to 118 mo	Decreased ALT, AST, GGT, and ESR levels. Jaundice improved. The overall rate of positive serum autoantibodies decreased after 3.5 mo	[138]
	Vancomycin and metronidazole	Vancomycin: 125 mg or 250 mg four times daily for 12 wk; Metronidazole: 250 mg or 500 mg three times daily for 12 wk	Decreased ALP and MRS levels. The decrease in ALP level was more pronounced following vancomycin administration	[90]
	Metronidazole with ursodeoxycholic acid	Taken together for 36 mo	Significantly decreased ALP and MRS levels	[98]
	Azithromycin	500 mg three days per week for 6 wk	Decreased ALP and TBIL levels and cholestasis-related symptoms. The urine was turned dark in color again	[139]
	Rifaximin	550 mg twice daily for 12 wk	Decreased GGT and CRP levels; Improved pruritus symptoms	[95]
	Minocycline	100 mg twice daily for one year	Significantly decreased ALP and MRS levels	[100]
Fecal microbiota transplantation at 24 wk			Significantly decreased ALP levels; Reduced AST levels (by at least 30%)	[107]
Probiotics	*Lactobacilli* and *Bifidobacteria*	Three months of: *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus salivarius*, and *Lactococcus lactis*, *Bifidobacterium bifidum*, *Bifidobacterium*	Reduced ALP levels (by 15%)	[113]
	Combined	Prednisolone (30 mg/d), salazosulfapyridine (3000 mg/d), and *Lactobacillus casei* Shirota (3 g/d) for 2 wk	Decreased ALP, ALT, AST, and GGT levels	[114]

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; MRS: Mayo primary sclerosing cholangitis risk score; ESR: Erythrocyte sedimentation rate; GGT: Gamma-glutamyl transpeptidase; CRP: C-reactive protein; TBIL: Total bilirubin.

narrow antibiotic spectrum and specifically targets *Clostridiales*. Consequently, vancomycin affects the abundance of *Clostridiales* in the intestinal flora of the distal small intestine and colon by reducing primary BA dehydroxylation and preventing excessive secondary BA accumulation, thereby reducing PSC activity. In addition, Davies *et al*[97] demonstrated that vancomycin directly attenuates the inflammatory response to periportal inflammation and liver injury during PSC.

Studies in animal models have demonstrated that metronidazole also has a therapeutic effect on liver injury in PSC[60]. For example, Karvonen *et al*[98] found that treating patients with PSC with both UDCA and metronidazole significantly reduces the serum glutamyl transpeptidase and ALP levels, and significantly improves the MRS and pathological staging compared with those treated with only UDCA. Furthermore, Krehmeier *et al*[99] reported that metronidazole reduced intestinal permeability, decreased bacterial endotoxin entry into the blood, inhibited endotoxin-induced TNF-α production, inhibited hepatic Kupffer cells and macrophage activation, reduced chemokine and cytokine secretion by biliary epithelial cells, attenuated liver inflammation, and prevented PSC-like bead-like liver injury. Finally, Silveira *et al*[100] showed that minocycline is a safe and effective PSC treatment, significantly reducing the ALP level and MRS after one year of oral minocycline administration.

FMT

FMT is the transplantation of fecal flora from healthy individuals into a patient's intestine to replenish or restore normal intestinal flora. This procedure aims to reverse intestinal dysbiosis, regulate product metabolism, and improve clinical symptoms to treat the disease (*Clostridium* difficile infection, IBD, diabetes mellitus, cancer, liver cirrhosis, gut-brain disease and others)[101,102]. FMT restores the health of the intestinal flora, further reducing the transport of harmful metabolites, such as endotoxins to the liver, and reducing the damage caused by metabolites to the liver[103]. FMT uses the principle of bacterial therapy to restore the health of the intestinal flora. The transplanted beneficial bacteria (*Bifidobacteria*, *etc*.) are involved in the conversion of polysaccharides to monosaccharides, producing SCFAs such as acetate, propionate, and butyrate[104]. These metabolites regulate normalization of the intestinal flora and reduce intestinal permeability in patients with liver disease, to further reduce the transport of metabolites such as endogenous ethanol and endotoxins to the liver, thus, reducing the

damage to the liver[103,105,106]. Studies have shown intestinal flora normalization, a significant improvement in intestinal flora diversity, reduced cholestasis, and decreased ALP levels in PSC patients after FMT. Allegretti *et al*[107] performed the first human FMT trial in ten patients with PSC who had ALP levels more than three times the normal upper limit. After FMT, 30% of the patients had decreased ALP levels by 50%, and 70% had a 30% reduction in the levels of serum liver transaminases (ALT and aspartate aminotransferase). One week after FMT, the recipients' intestinal flora diversities were higher than the baseline level of all patients and continued increasing for 24 wk. Furthermore, Philips *et al*[108] found that fecal flora diversity improved in patients with PSC after FMT, with a decrease in the relative abundance of *Proteobacteria* and an increase in the abundances of *Bacteroidetes* and *Firmicutes*; this intestinal flora composition was more similar to that of healthy individuals. The blood biochemistry and total BA indicators also significantly improved.

Probiotics

Probiotic is a general term for a group of active microorganisms that have beneficial roles by regulating intestinal flora growth and improving the host's intestinal microecology. They regulate the intestinal microenvironment metabolism, increase SCFAs production, and reduce the permeability of the intestinal barrier[109,110]. Additionally, probiotics upregulate intestinal epithelial tight junction protein expression, improve intestinal motility[110,111], increase adhesion and colonization of intestinal flora, reduce TNF-α production, and maintain tissue homeostasis[112]. One study demonstrated that oral administration of probiotic preparations (consisting of six strains of viable and freeze-dried bacteria: *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus salivarius, Lactococcus lactis, Bifidobacterium bifidum,* and *Bifidobacterium lactis*) decreased the serum alkaline phosphatase level by 15 % in patients with PSC compared to healthy individuals[113]. Furthermore, Shimizu *et al*[114] treated a patient with PSC with a combination of prednisolone, salazosulfapyridine, and probiotics, and reported that the patient's symptoms and tests improved after two weeks. Additionally, repeat pathological biopsies at 30 mo showed significant improvements in liver inflammatory cell infiltration and peribiliary fibrosis. *Lactobacillus plantarum Lp2* has the potential to ameliorate liver injury by inhibiting the activation of LPS-induced inflammatory pathways in the liver, reducing inflammation, and decreasing oxidative damage and apoptosis[115]. Therefore, probiotics have a therapeutic effect on PSC by suppressing intestinal inflammation and maintaining intestinal flora homeostasis.

BAs and other metabolites

Compared to conventional mice, germ-free mice show higher concentrations of BA in the plasma and significantly reduced concentrations in the feces. Additionally, FXR signaling is significantly inhibited, resulting in reduced BA synthesis in germ-free mice[116,117]. Colonization of germ-free mice with human feces activates the expression of FXR target genes and increases the levels of BAs in the liver and ileal tissue[118]. FXR agonists inhibit cholesterol 7α-hydroxylase activity and, thus, intracellular BA synthesis. These agonists can activate transcription of the bile salt export pump on the hepatocyte membrane, enhancing the transport of BAs from hepatocytes to bile ducts and promoting BA excretion. Simultaneously, These agonists can inhibit the expression of extracellular matrix proteins in hepatic astrocytes and, thus, prevent the transformation of liver fibrosis in patients with PSC[119]. Obeticholic acid (OCA) is one of FXR agonists representative drugs that alleviates the cholestatic symptoms of PSC by reducing the BA pool[120]. OCA is also approved for the treatment of PSC[121,122]. In fact, there are phase II clinical trials demonstrating the efficacy and safety of OCA in patients with PSC[123].

Relevant clinical trials

In addition to the above-mentioned studies, there are currently several relevant clinical trials demonstrating the efficacy of treatments targeting intestinal flora and its metabolites in PSC (Table 3). From these clinical studies, we found that oral vancomycin is the most established for the treatment of PSC, and all phase IV clinical trials using vancomycin have been completed. Vancomycin can significantly reduce biochemical indexes such as ALP and ALT and reduce MRS in patients with PSC [92,124]. One case study also described a decrease in serum γ-GGT, which reached normal levels at 195 d, in pediatric patients with PSC-UC who were administered vancomycin[94]. *Fusobacterium, Haemophilus,* and *Neisseria*, which generally have a significantly high abundance in PSC, showed decreased abundance in the saliva and feces of these patients[40,42,43,47]. Results of meta-analyses have also shown vancomycin to be beneficial in patients with PSC[96]. Currently, there are clinical guidelines recommending the use of antimicrobial agents and FXR agonists for the treatment of PSC[125,126]. Clinical trials of UDCA for PSC are also well established[127]. UDCA is a hydrophilic dihydroxy BA, and pharmacological studies have confirmed that UDCA has a strong affinity in bile, promoting bile secretion, protecting bile duct cells from the cytotoxicity of hydrophobic BAs, and protecting hepatocytes from BA-induced apoptosis[128]. It promotes the formation of liquid cholesterol crystal complexes, accelerates cholesterol excretion and clearance to the intestine, acts as a cholagogue, and competitively inhibits endogenous hepatic BA absorption in the small intestine, reducing serum BA levels[129]. 24-norUDCA is a side chain shortened congener of C23UDCA, which makes a bile hepatic shunt possible. Based on its pharmacological properties of relative amidation resistance and reduced

Table 3 Clinical trials related to primary sclerosing cholangitis treatment

Study Phase	Study title	ClinicalTrials.gov identifier	Actual enrollment	Status	Interventions
Phase 1	A Pilot Study of Vancomycin or Metronidazole in Patients with Primary Sclerosing Cholangitis (PSC)	NCT01085760	35 participants	Completed	Drug: vancomycin; Drug: metronidazole
	Minocycline in Primary Sclerosing Cholangitis (PSC)	NCT00630942	16 participants	Completed	Drug: minocycline
	Treating Primary Sclerosing Cholangitis and Biliary Atresia with Vancomycin	NCT02137668	200 participants	Recruiting	Drug: oral vancomycin
	A Pilot Study to Characterize Bile Acid Metabolism and Dysbiosis in Primary Sclerosing Cholangitis	NCT02464020	8 participants	Completed	Drug: vancomycin
	Gastrointestinal Microbiota in Primary Sclerosing Cholangitis and Biliary Atresia with Vancomycin (PSC)	NCT01322386	32 participants	Completed	Drug: vancomycin
	Fecal Microbiota Transplantation for the Treatment of Primary Sclerosing Cholangitis.	NCT02424175	10 participants	Completed	Biological: Fecal microbiota transplantation
Phase 2	Norursodeoxycholic Acid in the Treatment of Primary Sclerosing Cholangitis (NUC-3)	NCT01755507	159 participants	Completed	Drug: norursodeoxycholic acid; Drug: placebo
	Obeticholic Acid (OCA) in Primary Sclerosing Cholangitis (PSC) (AESOP)	NCT02177136	77 participants	Completed	Drug: OCA; Drug: placebo
	Vancomycin for Primary Sclerosing Cholangitis	NCT03710122	102 participants	Recruiting	Drug: vancomycin; Other: placebo
	Trial of High-dose Urso in Primary Sclerosing Cholangitis	NCT00059202	150 participants	Completed	Drug: ursodeoxycholic acid; Drug: placebo
	Fecal Microbiota Transplantation for the Treatment of Primary Sclerosing Cholangitis.	NCT02424175	10 participants	Completed	Biological: fecal microbiota transplantation
Phase 3	Primary Sclerosing Cholangitis with Oral Vancomycin by the Study of Its Antimicrobial and Immunomodulating Effects (PSC)	NCT01802073	34 participants	Completed	Drug: oral vancomycin
	Probiotics in Patients with Primary Sclerosing Cholangitis	NCT00161148	12 participants	Unknown[1]	Drug: probiotics
	Norursodeoxycholic Acid *vs* Placebo in PSC	NCT03872921	300 participants	Recruiting	Drug: norursodeoxycholic acid
	Vancomycin for Primary Sclerosing Cholangitis	NCT03710122	102 participants	Recruiting	Drug: vancomycin; Other: placebo
	Trial of High-dose Urso in Primary Sclerosing Cholangitis	NCT00059202	150 participants	Completed	Drug: ursodeoxycholic acid; Drug: placebo
Phase 4	Effect and Safety of Oral Vancomycin in Primary Sclerosing Cholangitis Patients	NCT02605213	30 participants	Unknown[1]	Drug: vancomycin; Drug: placebo

[1]Study has passed its completion date and status has not been verified in more than two years.

PSC: Primary sclerosing cholangitis; OCA: Obeticholic Acid; AESOP: Aetiology and Ethnicity in Schizophrenia and Other Psychoses.

secondary BA production, norUDCA is a promising drug for a range of cholestatic liver and bile duct diseases[130]. Some clinical trials have shown that norUDCA improved cholestasis and significantly reduced serum alkaline phosphatase levels in patients after 12 wk in a dose-dependent manner. Importantly, norUDCA treatment has shown a good safety profile[131]. OCA is a potent FXR agonist that affects the hepatic transport of conjugated BAs in humans and reduces duration of hepatocyte exposure to potentially cytotoxic BAs[132,133]. Clinical trials have demonstrated the efficacy and safety of OCA in patients with PSC; Treatment with OCA 5-10 mg resulted in a significant reduction in ALP in patients with PSC after 24 wk[123]. In addition, clinical studies of probiotics, FMT, and other approaches targeting intestinal flora for the treatment of PSC are ongoing to highlight their efficacy and safety in PSC and demonstrate their therapeutic potential[108,134].

CONCLUSION

PSC is a chronic progressive autoimmune disease that can develop into cirrhosis or liver failure, thereby severely affecting the patient's quality of life if not actively and effectively treated. Intestinal flora dysbiosis is crucial in the occurrence and development of PSC, as it destroys the intestinal barrier and

prompts intestinal lymphocyte homing and translocation of bacteria and their metabolites, thus aggravating the immune damage to the liver. The intestinal flora also interacts with BAs and participates in PSC development.

Our understanding of the gut flora has expanded with the development of genomics, metabolomics, and high-throughput sequencing technologies. These research approaches help elucidate the complex role of the gut flora in diseases, such as PSC. Technological advances have also provided individualized treatment options for patients with PSC that target the intestinal flora with good clinical results. Treatments, including antibiotics, FMT, and probiotics, have offered new ideas for managing PSC. More precise therapies, such as probiotics, synbiotics, and phages, have shown promising results in PSC patients. However, there remain some challenges in the use of intestinal flora for PSC treatment. The intestinal flora regulation mechanisms for PSC are not fully understood, and the optimal method and timing have not been standardized. Future prospective studies with a large sample size or multi-center studies are warranted to provide direct evidence of the role of the intestinal flora in PSC and establish a therapeutic protocol for the use of the intestinal flora. If these issues are resolved, targeted regulation of the intestinal flora will become a new option for PSC treatment.

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REVIEW

Machine learning insights concerning inflammatory and liver-related risk comorbidities in non-communicable and viral diseases

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Abstract

The liver is a key organ involved in a wide range of functions, whose damage can lead to chronic liver disease (CLD). CLD accounts for more than two million deaths worldwide, becoming a social and economic burden for most countries. Among the different factors that can cause CLD, alcohol abuse, viruses, drug treatments, and unhealthy dietary patterns top the list. These conditions prompt and perpetuate an inflammatory environment and oxidative stress imbalance that favor the development of hepatic fibrogenesis. High stages of fibrosis can eventually lead to cirrhosis or hepatocellular carcinoma (HCC). Despite the advances achieved in this field, new approaches are needed for the prevention, diagnosis, treatment, and prognosis of CLD. In this context, the scientific community is using machine learning (ML) algorithms to integrate and process vast amounts of data with unprecedented performance. ML techniques allow the integration of anthropometric, genetic, clinical, biochemical, dietary, lifestyle and omics data, giving new insights to tackle CLD and bringing personalized medicine a step closer. This review summarizes the investigations where ML

techniques have been applied to study new approaches that could be used in inflammatory-related, hepatitis viruses-induced, and coronavirus disease 2019-induced liver damage and enlighten the factors involved in CLD development.

Key Words: Machine learning; Liver inflammation; Liver disease; Viral diseases; Comorbidity

Core Tip: Chronic liver disease has become a global burden, and new approaches need to be explored to tackle this disease. In this context, machine learning techniques bring a whole new set of opportunities to study novel approaches and biomarkers for prevention, diagnosis, treatment, and prognosis of inflammatory and virus-related liver diseases. The application of machine learning algorithms constitutes a pivotal piece of personalized medicine, allowing the integration of different phenotypical and genotypical data for a precision outcome concerning inflammatory liver comorbidities in non-communicable and viral diseases.

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INTRODUCTION

The liver is a key organ involved in relevant homeostatic metabolic and detoxifying human functions [1]. Thus, the liver is the epicenter of an organ-organ network weaving a series of complex interactions in the organism, which makes liver damage an underlying adverse condition in a whole set of diseases. Chronic liver disease (CLD) can be caused mainly by alcoholic liver-related dysfunctions, hepatitis B virus (HBV), hepatitis C virus (HCV), drug treatments, or non-alcoholic fatty liver disease (NAFLD), as recently updated to the term metabolic-associated FLD (Figure 1)[2,3]. Patients with liver-related diseases need frequent follow-ups and careful monitoring since CLD can eventually lead to cirrhosis or hepatocellular carcinoma (HCC) if not diagnosed on time for treatment or surgery. These CLD-related conditions have become a global burden, whose mortality associated rates have increased over the years reaching more than 2 million deaths worldwide[4].

CLD is usually accompanied by an unhealthy inflammatory environment[5]. The immune response is a fundamental process to maintain homeostasis within the organism defense machinery and is characterized by the secretion of proinflammatory cytokines, like interleukin (IL)-1, tumor necrosis factor-α (TNF-α), and prostaglandin E2, in an acute manner in order to resolve sudden damage[5]. However, if sustained over time, these abnormal levels of inflammatory cytokines cause low-grade inflammation (LGI). LGI is a silent condition that predisposes to the development of metabolic and infectious diseases that has become a worldwide health issue[6]. Patients with CLD, such as non-alcoholic steatohepatitis (NASH), present impaired immune function, dysbiosis, insulin resistance (IR) and LGI, all of which can aggravate infectious disease progression and perpetuate excess of adipose tissue, are characterized by overstimulation of the production of adipose-derived inflammatory molecules[5,7-9].

The liver also secretes important hepatokines that act as signaling proteins modulating functions in other organs and are involved in a wide range of conditions, such as IR and adipogenesis[1]. For instance, fibroblast growth factor-21 (FGF-21) is a mediator participating in glucose metabolism mainly secreted by the liver that modulates adipogenesis, while fetuins, liver-derived plasma proteins, are participating in metabolic impairment and inflammation[1]. A dysregulation in systemic cytokines prompts fat accumulation in hepatocytes, which in turn promotes local secretion of proinflammatory hepatokines, leading to liver steatosis and IR. In addition, immune cells also find difficulty in this inflammatory environment to exert their role appropriately. Persistent inflammatory signals over time also abnormally activate immune cells, impairing the body's ability to fight infection, repair tissue damage, or recover from possible poisoning. Inflammation comes hand in hand with an increase in oxidative stress, a state characterized by an imbalance in favoring the accumulation of higher reactive oxygen (ROS) and nitrogen species. These molecules in unusual concentrations damage the cell and environmental milieu by promoting the expression of proinflammatory genes, resulting in a vicious cycle. Thus, CLD presents an oxidative atmosphere, probably linked to the proinflammatory state[10,11]. This environment is the perfect setting for the fibrogenic process to unfold, an underlying condition of CLD that is characterized by progressive accumulation of fibrillar extracellular matrix in the liver

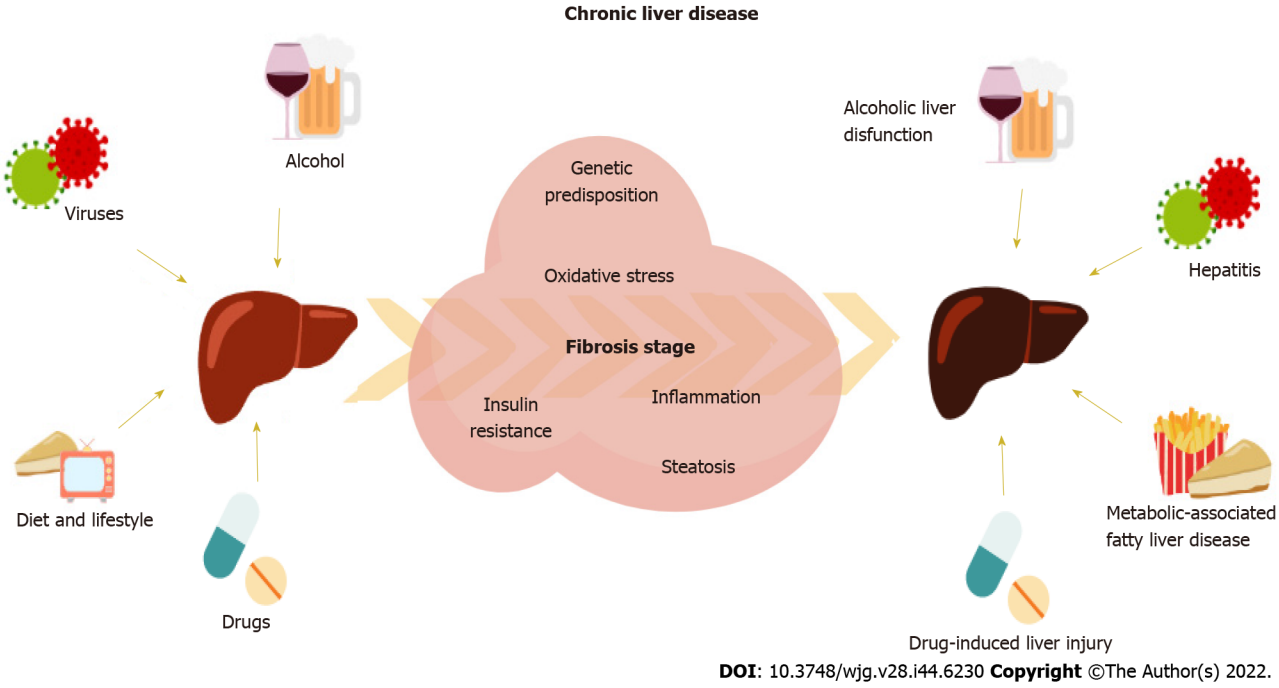

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Figure 1 Factors involved in the development of chronic liver disease triggering associated processes that lead to increased fibrosis stage.

[12]. The stage of hepatic fibrosis has been associated with the risk of mortality and liver-related morbidity in patients with NAFLD[13], virus-induced hepatitis[14,15], and alcoholic-derived liver disease[16], eventually leading to HCC.

In this context, infection by human hepatitis viruses (HHVs) is the most common cause of hepatitis, leading to the activation of the immune system, and the subsequent inflammatory response[17]. HBV and HCV acute infections can be resolved with antiviral and immune therapy. However, in a significant percentage they can progress to chronic hepatitis. This persistent infection can lead to comorbidities outside the liver, like arthritis, vasculitis, myalgia, and peripheral neuropathies[18]. Moreover, another new infectious disease appeared in late 2019 that can cause liver damage: Coronavirus disease 2019 (COVID-19). COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and it has become a global health issue since its outbreak in 2020 was declared a pandemic. Beyond lung function, COVID-19 can affect a wide variety of tissues, like the gastrointestinal tract, kidneys, and liver, with an underlying adverse inflammatory environment[19]. This inflammatory-related condition has been strongly associated to metabolic status and worsening diseases like obesity, diabetes, and hypertension[7,20-22]. For instance, COVID-19 can increase hepatic lipid accumulation by mitochondrial and endoplasmic reticulum (ER) dysfunction or worsen NAFLD if it was already present. A recent systematic review depicted that the parameters normally used for liver impairment screening were significantly increased in COVID-19 patients[23], placing CLD as a risk factor for progressive and severe COVID-19[24,25].

CLD is a global health problem, and new methods are needed to tackle this life-threatening condition. In this line, this review aims to explore machine learning (ML)-based approaches to manage CLD and develop biomarkers for diagnosis and prognosis. Its goal is to shed light on the factors involved in CLD to help health professionals in clinical management with the support of ML and identify new targets that can define therapeutic care lines in viral infections and non-communicable diseases (NCD), with an impact on liver functions with an inflammatory component. This includes the new disease, COVID-19.

MECHANISMS BY WHICH NCD AND INFLAMMATORY/IR PHENOMENA CAN AFFECT LIVER FUNCTION

The incidence of NCD, such as cardiovascular diseases and diabetes, has skyrocketed in the last decades, pressing authorities to establish developmental goals to achieve in the near future in terms of decreasing NCD-caused mortality[26]. Some of the risk factors that contribute to the development of NCD are excess of adipose tissue and high levels of glycemia. In this context, adipose tissue plays a key role in the development of FLD by secreting adipokines and other molecules, like free fatty acids (FFA) [8].

An energy excess prompts fat accumulation in the organism and the subsequent dysregulation of this tissue. This is of relevance since an inflamed adipose tissue results in increased levels of FFA and pro-inflammatory cytokines, IR, and infiltration of macrophages in the liver by the activation of Th1 and Th17 cells[8]. FFA enter the liver through the portal vein and trigger a series of reactions. For instance, they serve as ligands to toll-like receptor-4 complex, stimulating the production of TNF-α through the activation of nuclear factor-kappa B, favoring an inflammatory environment. Moreover, the excess of fat drives the polarization state of this increased number of macrophages from anti-inflammatory M2 to proinflammatory M1 macrophages and prompts fat accumulation in the liver and IR[8]. Adipose-derived macrophages also secrete inflammatory molecules, like TNF-α and IL-6, and adipokines, such as visfatin [also named nicotinamide phosphoribosyl transferase (NAMPT)]. NAMPT has gained relevance as a pivotal molecule linking adipose tissue and FLD. NAMPT is a pleiotropic molecule that can be found in an extracellular (eNAMPT) or an intracellular (iNAMP) form. Studies indicate that eNAMPT has enzyme and cytokine-like activity, stimulating the release of proinflammatory cytokines. Meanwhile, iNAMPT catalyzes the rate-limiting step in nicotinamide adenine dinucleotide (NAD+) formation. Because of this NAD+ boosting property, levels of iNAMPT have been proposed as beneficial for the homeostasis of the cell due to influencing the activity of NAD-dependent enzymes, such as sirtuins (SIRT). Remarkably, SIRT1 plays a key role in the liver by modulating the acetylation status of target molecules in lipid metabolism[27].

Furthermore, IR is characterized by hyperglycemia and the subsequent hyperinsulinemia to counteract high glucose levels, being a risk factor for NCDs, particularly type 2 diabetes, where it has been closely linked to oxidative stress[28]. A normal insulin signaling pathway starts with the activation of the insulin receptor so that it can bind to phosphoinositide 3-kinase to ultimately activate protein kinase B (Akt). Activated Akt drives glucose entry into the cell by promoting GLUT4 expression and glycogen synthesis[29]. Oxidative stress impairs this signal transduction through many different mechanisms, like inhibiting the transcription factors insulin promoter factor 1 and peroxisome proliferator-activated receptor gamma, which mediate insulin and GLUT-4 expression, respectively. Moreover, under hyperglycemic conditions, fetuin A hepatokine inhibits the insulin receptor and promotes inflammation, while FGF-21 inhibits lipid accumulation and increases insulin sensitivity. Dysregulation of this hormones, together with oxidative stress imbalance, lead to impaired insulin signaling[30].

The metabolic conditions underlying the development of NCD are complex, and they often reinforce each other, perpetuating an inflammatory environment and oxidative stress imbalance. As the orchestrating organ, these processes converge in the liver, affecting metabolic functions and setting the basis for the onset of the fibrogenic process characteristic of CLD.

MECHANISMS BY WHICH VIRAL INFECTIONS AND INFLAMMATORY/IR PHENOMENA CAN AFFECT LIVER FUNCTION

Persistent virus-associated liver damage can progress to CLD, which pressures health systems with a big social and economic burden. Although lots of resources have been invested to study the molecular mechanisms that mediate this process, results are diverse and still under investigation by the scientific community. HHVs directly infect hepatocytes, and the internalization into the cell is believed to happen by endocytosis, requiring the interaction with several host cell factors[17]. However, viral entry of HBV and HCV within hepatocytes is unclear, and further research is needed to elucidate this question. Sodium taurocholate co-transporting polypeptide was recently identified as an HBV receptor that would mediate HBV cell entry[31]. In the case of HCV, specific intercellular adhesion molecules appear key to cell adhesion and subsequent internalization[32].

Regarding HBV and HCV replication, it has been found that liver X receptor-α (LXR-α) plays a key role. LXR-α is a transcription factor whose activation triggers the expression of different genes that directly or indirectly modulate these viruses' replication as well as the lipid and inflammatory alterations associated to CLD[33]. This inflammation is also mediated by the nucleotide-binding oligomerization domain-like receptor protein 3, which is activated by the abnormal production of ROS after a viral infection occurs in the liver. This ROS increase is associated with a decreased expression of nuclear factor-e2-related factor-2, a transcription factor that regulates ROS/recepteur d'origine nantais balance by maintaining redox homeostasis. These alterations compromise the normal state of the cell, laying the foundations on which the fibrotic process of CLD begins[11].

In the case of COVID-19, the mechanisms by which liver damage can occur are more unclear, but it is widely accepted that inflammation plays a huge role. This infection can trigger an exaggerated immune response leading to an uncontrolled cytokine release, also known as a "cytokine storm". It is characterized by abnormal levels of IL-6, IL-1, C-C motif chemokine ligand (CCL)-5, chemokine (C-X-C motif) ligand (CXCL)-8, CXCL-1, and TNF-α among others[19]. This inflammatory cascade affects bile duct function since cytokines like TNF-α, IL-1, and IL-6, can induce hepatocellular cholestasis by downregulating hepatobiliary uptake and excretory systems[34].

Furthermore, the presence of this inflammatory environment can upregulate the expression of angiotensin converting enzyme 2 (ACE2) receptor in different tissues, like the adipose tissue and the liver[35-39]. This is of relevance since ACE2 receptors are the main cell entrance of the SARS-CoV-2 virus, and they are present in different tissues. Particularly in the liver, the cholangiocytes (characteristic cells of the bile duct)[40], as well as liver vascular endothelial cells[41], express ACE2 receptors. Hepatocytes and cholangiocytes are permissive to the SARS-CoV-2 virus, mediating subsequent entrance into the liver[42]. Several studies have found that *ACE2* expression in hepatocytes is increased under hypoxia[43], a frequent condition in COVID-19 patients, and fibrotic conditions[44]. Besides ACE2 receptors, transmembrane serine protease 2 (TMPRSS2) and paired basic amino acid cleaving enzyme (FURIN) have been noted as significant for infection in the liver[45,46]. In this context, *ACE2* expression is increased in patients in HCV-related cirrhosis[44], whereas *TMPRSS2* and *FURIN* expression are upregulated in patients with obesity and NAFLD[47]. Moreover, infection by SARS-CoV-2 increases glucose-regulated protein 78 and 94, two biomarkers of ER stress[48,49], and impairs mitochondrial function[50]. This process is of interest since this state has been associated with *de novo* lipogenesis in hepatocytes[51], which could eventually lead to steatosis in these patients.

The use of therapeutic drugs can be another underlying cause of liver damage[3]. Because of detoxifying functions, the liver is subject to drug-induced damage coming from a wide range of approved drugs. Oncology drugs account for most hepatotoxicity cases, followed by those used for infectious diseases[3]. Since the beginning of the COVID-19 pandemic, a wide range of different treatments (antivirals, antibiotics, antimalaria, or corticosteroids) have been used in the absence of an efficient drug to treat severe infections. This pharmacological administration could explain that drug-induced liver injury appears in nearly 25% of COVID-19 patients[23], a consequence to consider when addressing liver damage in this disease.

ML APPROACHES IN INFLAMMATORY AND LIVER-RELATED COMORBIDITIES IN NON-COMMUNICABLE AND VIRAL DISEASES

Despite all the advances in the mechanisms driving the onset of these diseases, new techniques to detect innovative biomarkers for diagnosis and prognosis as well as to discover novel drugs are needed, for example artificial intelligence (AI). AI seeks to mimic human behavior, and within this science, ML is the most common approach[52]. The advances in computational science in the last decades have permitted the development of powerful algorithms based on this science. ML algorithms are particularly relevant for biological research because they allow the processing and integration of the huge amount of data that the latest advances in this field have brought by applying statistical methods to enable machines to improve experiences. This methodological approach can be categorized into two big groups: Supervised and unsupervised learning. In supervised algorithms, data is tagged in order to train the algorithm and fit it appropriately, whereas if it is unsupervised, the algorithm learns patterns from unlabeled data[53]. ML algorithms are generally assessed by simple methodologies like sensitivity, specificity, and accuracy. While sensitivity evaluates the proportion of true positives correctly identified, specificity evaluates the proportion of true negatives. Meanwhile, the accuracy value indicates the number of times the model is correct[54].

Supervised algorithms can be divided into two categories depending on the purpose: Prediction, in which the algorithm is fed and trained predictive models to data; or classification, which consists in clustering data within explanatory groups[55,56]. Predictive algorithms are based on regression models, and the most used are linear and logistic regression (LR), support vector machine (SVM), support vector regression (SVR), extra tree regression (ETR), artificial neural networks (ANN), and decision trees (DT). Regression models analyze the influence of one or multiple variables on a nominal or ordinal categorical outcome. ANN are more complex mathematical models (deep learning algorithms) that mimic the brain neural network, like the convolutional neural network (CNN), in which an input is fed through a hidden layer of many different well connected and structured nodes to produce a final output. In deep neuronal network (DNN) models, a great number of hidden successive layers use the output from the previous layer as input in a more complex algorithm. DT can also classify data, like random forest (RF) or gradient boosting (GB) models. Instead of minimizing error, these models determine thresholds derived from input data, assigning weight values to variables. Other models of classification are the Ada-Boost, Bayesian network (BN), Naïve Bayes (NB), K-Nearest Neighbors (KNN), and linear discriminant analysis (LDA) that group data into clusters[55,56]. All these models can shed light into biological questions and are normally used indistinctively to obtain the best performance with the same dataset. For instance, Mijwil and Aggarwal[57] analyzed and compared 7 ML algorithms to predict appendix illness in the same dataset, revealing that certain models performed better than others, allowing for higher accuracy and results.

In FLD, the common techniques used in diagnostics are based on techniques like ultrasonography and magnetic resonance imagining (MRI). These methods are subjective, and the informed outcome mainly relies on the interpretation of the professional carrying out the procedure. Several investigations have studied the implementation of ML in order to classify FLD and other liver diseases by using

images from ultrasounds, computed tomography (CT), and MRI[58,59]. However, the downside of this approach is that the quality of the images differs from one another because of several factors, such as equipment precision and interpersonal differences, for instance. Therefore, there is a need for ML approaches to help in image segmentation, and some authors have already implemented this technique to improve clinical practice[60,61].

Moreover, ML can help with the integration of more complex information beyond imaging to study and diagnose liver diseases since patients with CLD in the developmental phase require frequent follow-ups to check the progress of the disease and early detection changes in the diagnosis[58]. For example, patients with HHV-induced CLD are normally on antivirals. However, there is no consensus or guidelines about when to stop antiviral therapy or even if quitting these drugs will increase HCC risk. Therefore, new approaches need to be established to classify and prevent the development of more severe illnesses, like cirrhosis or cancer. In this line, ML approaches can be used to measure liver fibrosis, optimize diagnosis, and predict disease progression of CLD[62]. Table 1 summarizes selected studies that have used ML for these purposes, which have been collected for this review, and Table 2 summarizes the most repeated inputs from all compiled ML models along with the most repeated predictive results for the main four inflammation-related liver conditions.

ML in inflammation-related liver disease

In recent years, promising results have been found when applying ML approaches in CLD. Regarding prevention, Fialoke *et al*[63] screened 108139 patients to identify those diagnosed with benign steatosis and NASH, a type of NAFLD, train ML classifiers for NASH and healthy (non-NASH) populations, and predict NASH disease status on patients diagnosed with NAFLD according to aspartate transaminase (AST), alanine transaminase (ALT), and platelet (PLT) levels. In this line, another study detected body mass index (BMI), triglycerides (TG), gamma-glutamyl transpeptidase (GGT), ALT, and uric acid as the top 5 features contributing to NAFLD, with the BN model performing the best[64]. Accordingly, Yip *et al*[65] selected TG, ALT, white blood cell count, high-density lipoprotein cholesterol (HDL-c), glycated hemoglobin A1c (HbA1c), and the presence of hypertension as the six variables to build ML models, of which Ada-Boost outperformed the others individually and described the NAFLD status in 922 subjects.

More recently, Pei *et al*[66] designed a ML model that integrated medical records as a clinical variable to classify FLD. Concretely, they selected the variables of age, height, BMI, hemoglobin, AST, glucose, uric acid, low-density lipoprotein cholesterol (LDL-c), alpha-fetoprotein, TG, HLD-c, and carcinoembryonic antigen. They tested six different ML models in 3419 participants, of which 845 were diagnosed with FLD: LR, RF, ANN, KNN, extreme gradient boosting (XGBoost) (a type of GB model), and LDA. Results from these authors showed that the XGBoost model had the highest performance, followed by LR and ANN, to predict the risk of FLD. BMI, uric acid, and TG levels were the top three variables associated to FLD risk across the six analyzed models.

When it comes to diagnosis and treatment, several ML models have been tested for different purposes obtaining good specificity, sensitivity, and accuracy values[62]. For example, to determine the stage of liver fibrosis, some authors have used CT images processed by segmentation algorithms. Choi *et al*[67] used CNN upon CT images, whereas Chen *et al*[68] employed RF, KNN, SVM, and the NB classifiers with real-time tissue elastography imaging, age, and sex as feeding variables. In both cases, the ML approach outperformed the classical methods. Regarding treatment, different ML models have been used to define the best therapy for liver diseases such as carcinomas and virus-induced hepatitis. Jeong *et al*[69] used DNN to classify intrahepatic cholangiocarcinoma susceptible to adjuvant therapy following resection according to laboratory and clinicopathological markers and found it more accurate than the commonly used staging system.

Wübbolding *et al*[70] studied the prediction of early virological relapse analyzing soluble immune markers using supervised ML approaches like KNN, RF, and LR. This study showed that IL-2, monokine induced by interferon γ/CCL9, RANTES/CCL5, stem cell factor, and TNF-related apoptosis-inducing ligand in combination were more reliable in predicting virological relapse than viral antigens. In the same way, researchers have used ML classifiers to explore new methods able to better predict prognosis of liver diseases[71-74]. The weighted variables are usually CT images and/or biochemical parameters that involved invasive and costly methods. However, researchers have recently proposed volatile organic compounds as new biomarkers for progression and prognosis of liver disease. These researchers monitored isoprene, limonene, and dimethyl sulfide concentrations from a breath sample in liver patients compared to healthy subjects. They used regression ML models (LR, ETR, SVR, and RF) to demonstrate that these approaches together with breath profile data can predict clinical scores of liver disease[75]. These findings are promising and open the way for new, safe, and non-invasive approaches to study liver function and for diagnosis purposes.

ML methods have been employed when studying the comorbidities of liver-related diseases, like obesity, diabetes, and cardiovascular diseases[53,55,76]. For example, ML algorithms have been built to study the risk factors associated to overweight and obesity development, showing that BMI, age, dietary pattern, blood test results, socioeconomic status, and sedentarism were key factors when studying excess of adipose tissue[77]. In this line, further research has revealed by ML techniques that the minutes devoted to physical activity in one week[78], as well as specific species of gut microbiota[79], are also crucial for obesity prediction. ML algorithms have also elucidated the risk factors of childhood

Table 1 Summary of machine learning articles studying virus and inflammatory-related liver damage

Ref.	Objective	Subjects	Variables	ML model	Performance	Observations/remarks
Fialoke *et al* [63]	To predict NASH in NAFLD patients	*n* = 108139, NASH and healthy (non-NASH) populations	Demographic data, type 2 diabetes status, and blood biomarkers	RF, XGBoosting, DT, LR	AUROC of 88% by XGBoosting	The average and maximum value of ALT appeared was the most important variable
Ma *et al*[64]	To predict NAFLD in the general population	*n* = 10508, Subjects who attended a health examination	Age, blood biomarkers, and anthropometric data	LR, RF, SVM, baggin, DT, LR, KNN, BN, hidden NB, AdaBoosting, AODE	83% accuracy, 0.878 specificity, 0.675 sensitivity, and 0.655 F-measure score by BN	BMI, TG, GGT, ALT and uric acid were the top five predictors
Yip *et al*[65]	To detect NAFLD for the general population	*n* = 500, involving NAFLD patients and healthy subjects	Demographic, clinical data and blood biomarkers	LR, RIDGE regression, AdaBoosting, DT	AUROC of 90% by AdaBoosting	ALT, HDL-c, TG, HbA1c and white blood cells to predictors
Pei *et al*[66]	To identify FLD in general patients	*n* = 3419, patients of which 845 had FLD	Age, anthropo-metric, and blood biomarkers	RF, ANN, KNN, XGBoosting, LDA	0.9415 accuracy, 0.9306 AUC, and 0.9091 sensitivity by XGBoosting	Uric acid, BMI, and TG were the top three risk factors
Choi *et al*[67]	To stage liver fibrosis	*n* = 7461, patients with pathologically confirmed liver fibrosis	Age, sex, clinical data, CT images, and liver fibrosis stage	CNN	Overall staging accuracy of 79.4% and an AUROC of 0.96, 0.97, and 0.95 for diagnosing significant and advanced fibrosis, and cirrhosis, respectively	The model outperformed the radiologist's interpretation, APRI, and FIB-4 index
Chen *et al*[68]	To stage liver fibrosis in patients with CHB	*n* = 513, patients with confirmed liver fibrosis	Age, sex, CT liver images	RF, KNN, SVM, NB	0.8118-0.9125 accuracy by RF for all stages	The adopted classifiers significantly outperformed the liver fibrosis index method
Jeong *et al*[69]	To classify susceptible individuals for adjuvant treatment in patients with ICC after resection	*n* = 1421, ICC patients	Age, sex, clinical data, and blood biomarkers	DNN	AUC of 0.78	The model was found to be more accurate than the traditional AJCC stage classifier
Wübbolding *et al*[70]	To identify immune profiles for the prediction of early virological relapse	*n* = 284, patients with CHB and treated with NA antivirals	Age, sex, and analytical and blood biomarkers	KNN, RF, LR	AUC of 0.89	The combination of IL-2, MIG/CCL9, RANTES/CCL5, SCF, and TRAIL was reliable in predicting viral relapse
Hong *et al*[71]	To predict esophageal varices in patients with HBV related cirrhosis	*n* = 197, patients with HBV-related cirrhosis	PLT count, spleen width, and portal vein diameter	ANN	Sensitivity of 96.5%, specificity of 60.4%, accuracy of 86.8%	The model obtained a positive predictive value of 90.00%; and a negative predictive value of 80.85%
Zhong *et al*[72]	To compare the prognostic performance of ALBI and CTP grades for HCC treated with TACE combined with sorafenib as an initial treatment	*n* = 504, HCC patients	ALBI and CTP grades BCLC stage, clinical data and plasma α-fetoprotein	ANN	-	The ALBI grade had higher importance in survival prediction compared to the CTP one
Shi *et al*[73]	To predict in-hospital mortality after primary liver cancer surgery	*n* = 22926, HCC surgery patients	Age, sex, clinical, and hospital data	ANN, LR	97.28% of accuracy and 84.67 % of AUROC by ANN	ANN model had higher overall performance indices and accurately predicted in-hospital mortality
Shi *et al*[74]	To predict 5-yr mortality after surgery for HCC	*n* = 22926, HCC surgery patients	Age, sex, clinical, and hospital data	ANN, LR	96.57 % of accuracy and 88.51 % of AUROC by ANN	Surgeon volume was the top predictor parameter
Patnaik *et al*	To predict liver	*n* = 28, healthy patients	Age, anthropo-	LR, RF, SVR,	R^2 values of 0.78,	Isoprene, limonene and

[75]	function-related scores (MELD, APRI, CTP) using breath biomarkers	compared to *n* = 17, liver patients	metric data, blood biomarkers, breath analysis	ETR	0.82, and 0.85 for CTP score, APRI score, and MELD, respectively, by ETR	dimethyl sulfide can be potential biomarkers for liver disease
Butt *et al*[85]	To diagnose the stage of hepatitis C	*n* = 968, patients with HCV	Age, anthropo-metric data, blood biomarkers, and histological staging	ANN, RF, SVM, XGBoosting	98.89% precision by ANN	The model performed better than previously presented models by other authors
Wei *et al*[87]	To predict HBV and HCV-related hepatic fibrosis	*n* = 490, HBV patients; *n* = 254, and 230 HCV patients	Age, BMI, analytical data (FIB-4 score), and liver biopsy	GB, DT, RF	AUROC of 0.918 by GB	GB outperformed the FIB-4 predictive score
Barakat *et al* [89]	To predict and stage hepatic fibrosis in children with HCV	*n* = 166, children with CHC	Analytical data (APRI and FIB-4 scores)	RF	AUCs of 0.903 for any type of fibrosis	RF outperformed FIB-4 and APRI predictive score
Konerman *et al* [88]	To predict progression of HCV	*n* = 72683, veterans with CHC	Age, BMI, demographic, and blood biomarkers (APRI score)	CS and LGT Cox and boosting	AUROC of 0.830 and 0.77 sensitivity by LGT boosting model for 1 yr follow-up	APRI and PLT count were top predictors in the LGT boosting model
Wong *et al*[86]	To predict HCC in patients with CVH	*n* = 86804, CHV patients, of which 6821 with HCC	Age, sex, clinical data, and blood biomarkers	LR, RIDGE regression, AdaBoosting, RF, DT	AUROC of 0.992 and 0.837 by RF in training and validation cohort, respectively	ML models obtained better AUROCs than HCC traditional risk scores
Feldman *et al* [91]	To predict DAA therapy duration in hepatitis C	*n* = 3943, HCV patients with sofosbuvir/ledipasvir as the first course of DAA, of which *n* = 240, received the prolonged DAA treatment	Age, sex, and clinical data (including hepatitis C record data)	XGBoosting, RF, SVM	AUC of 0.745 by XGBoosting	Results showed age, comorbidity burden, and type 2 diabetes status as new predictors for DAA therapy duration
Kamboj *et al* [92]	To predict repurposed drugs for HCV	*n* = 17968 HCV molecular fingerprints	Experimentally validated small molecules from the ChEMBL database with bioactivity against HCV NS3, NS3/A4, NS5A and NS5B proteins	SVM, ANN, KNN, RF	R^2 value of 0.92 by SVM	Results identified more than 8 repurposed treatments anti-HCV
Tian *et al*[93]	To predict HBsAg seroclearance	*n* = 2235, patients with CHB, of which 106 achieved HBsAg seroclearance	Age, BMI, demographic and clinical data, and blood biomarkers	LR, RF, DT, XGBoosting	AUC of 0.891 by XGBoosting	Level of HBsAg followed by age and HBV DNA were the top predictors
Chen *et al*[94]	To predict HBV-induced HCC using quasispecies patterns of HBV	*n* = 307, CHB patients; *n* = 237, HBV-related HCC patients	*rt* nucleic acid and *rt*/s amino acid sequences	SVM, RF, KNN, LR	AUC of 0.96, and accuracy of 0.90 by RF	HBV *rt* gene features can efficiently discriminate HCC from CHB
Mueller-Breckenridge *et al*[95]	To classify HBeAg status in HBV patients using virus full-length genome quasispecies	*n* = 352, CHB untreated patients	Matrix of allele frequencies (0.1-0.99) and the associated HBeAg status	RF	Range balanced accuracy of 0.8-1	n1896GA, n1934AT, n1753TC mutants were the highest-ranking variables
Kayvanjoo *et al*[96]	To predict HCV interferon/ribavirin therapy outcome based on viral nucleotide attributes	*n* = 76, gene attributes	HCV nucleotide attributes	DT, SVM, NB, DNN	Accuracy of 84.17% by SVM in responder *vs* relapser of subtype 1b sequences	Dinucleotides UA and UU were top predictors in the combination treatment outcome
Li *et al*[98]	To distinguish influenza from COVID-19 patients	*n* = 398, COVID-19 and influenza cases	Age, sex, blood biomarkers, clinical data, and CT and X-ray scans	XGBoosting, RF, and LASSO and RIDGE regression models	AUC of 0.990, sensitivity of 92.5% and a specificity of 97.9% by XGBoosting	Age, CT scan result, and temperature were top three predictors
Bhargava *et al*	To detect novel	*n* = 31454, images	CT or X-ray scans	KNN, SRC,	99.14 of accuracy	SVM model classified with

[99]	COVID-19 and discriminate between pneumonia	acquired from nine distinct datasets of COVID-19 patients		ANN, SVM	by SVM	the highest recognition rate the images as normal, pneumonia, and COVID-19 positive
Bennett *et al* [97]	To predict early severity and clinically characterize COVID-19 patients	*n* = 174568, patients with a positive lab test for COVID-19	Age, sex, demographic, anthropometric and clinical data, and blood biomarkers	RF, LR, XGBoosting	AUROC of 0.87 by XGBoosting	Age, oxygen respiratory rate, and blood urea nitrogen were ranked as top predictor for severity outcome
Günster *et al* [100]	To identify independent risk factors for 180-d all-cause mortality in COVID-19 patients	*n* = 8679, hospitalized COVID-19 patients	Age, sex, BMI, and clinical data	LR	AUC of 0.81	A high BMI and age were strong risk factors for 180-d all-cause mortality, while female sex was protective
Deng *et al*[101]	To identify clinical indicators for COVID-19	*n* = 379, patients, 62 with COVID-19 and 317 with pneumonia	Age, sex, demographic and clinical data, and blood biomarkers	EBM	AUC of 0.948	Variables grouped under liver function was top the predictor category for COVID-19 prediction
Lipták *et al* [102]	To identify gastrointestinal predictors for the risk of COVID-19-related hospitalization	*n* = 680, patients	Age, sex, clinical data, and blood biomarkers	RF	AUC of 0.799	AST was top predictor for hospitalization
Elemam *et al* [103]	To identify immuno-logical and clinical predictors of COVID-19 severity and sequelae	*n* = 37, COVID-19 patients; *n* = 40, controls	Age, sex, BMI, clinical data, and blood biomarkers	Stepwise linear regression	AUC of 0.93 for cytokines as predictors. AUC of 0.98 for biochemical markers as predictors	IL-6 and granzyme B were top potential predictors of liver injury in COVID-19 patients
Mashraqi *et al* [104]	To predict adverse effects on liver functions of COVID-19 ICU patients	*n* = 140, COVID-19 patients admitted to ICU	Blood biomarkers and existence of liver damage	SVM, KNN, ANN, NB, DT	AUC of 0.857 and precision of 0.95 by SVM	AST and ALT were top predictors of liver damage in these patients
Soltan *et al* [106]	To evaluate a laboratory-free COVID-19 triage for emergency care	*n* = 114957, emergency presentations prior to the global COVID-19 pandemic and *n* = 437, COVID-19 positive	Blood biomarkers, blood gas, and vital signs	LR, XGBoosting, RF	AUROC range of 0.9-0.94 by XGBoosting for datasets	The model could effectively triage patients presenting to hospital for COVID-19 without lab results
Gao *et al*[111]	To predict mortality in patients with alcoholic hepatitis	*n* = 210, alcoholic hepatitis patients	Age, clinical data, blood biomarkers, and omics data sets (metage-nomics, lipidomics, and metabolomics)	GB, LR, SVM, RF	AUC of 0.87 by GB for 30-d mortality prediction using the dataset combining clinical data, bacteria and MetaCyc pathways and for and 90-d mortality prediction using the fungi dataset	The model performed better than the currently used MELD score

NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease; CHB: Chronic hepatitis B virus infection; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; CHC: Chronic hepatitis C virus infection; CVH: Chronic viral hepatitis; RF: Random forest; DT: Decision trees; LR: Logistic regression; SVM: Support vector machine; KNN: K-nearest neighbors; BN: Bayesian network; NB: Naïve Bayes; AODE: Aggregating one-dependence estimators; FLD: Fatty liver disease; ANN: Artificial neural networks; LDA: Linear discriminant analysis; CNN: Convolutional neural network; DNN: Deep neuronal network; SRC: Sparse representative classifier; EBM: Explainable boosting machine; CS: Cross-sectional; LGT: Longitudinal; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B virus e antigen; BMI: Body mass index; ALT: Alanine transaminase; AST: Aspartate transaminase; APRI: Aspartate transaminase/platelet ratio index; COVID-19: Coronavirus disease 2019; CT: Computed tomography; GB: Gradient Boosting; AUC: Area under the curve; AUROC: Area under the receiver operating characteristic curve; ICU: Intensive care unit; IL-6: Interleukin 6; DAA: Direct-acting antiviral; MELD: Model for end-stage liver disease; TG: Triglycerides; HbA1c: Glycated hemoglobin A1c; ICC: Intrahepatic cholangiocarcinoma; ML: Machine learning; ETR: Extra tree regression; AJCC: American Joint Committee on Cancer; CXCL: chemokine (C-X-C motif) ligand; CCL: C-C motif chemokine ligand; SVR: Support vector regression; MIG: Monokine induced by interferon γ; SCF: Stem cell factor; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; PLT: Platelet; GGT: Gamma-glutamyl transpeptidase; HDL-c: High density lipoprotein cholesterol; FIB-4: Fibrosis-4; HBV: Hepatitis B virus; TACE: Transarterial chemoembolization; BCLC: Barcelona Clinic Liver Cancer.

Table 2 Summary of the most repeated inputs of the machine learning models with the most repeated predictor outcomes for the four main inflammatory-related liver conditions

Inflammatory-related liver condition	Inputs	Most repeated predictors
FLD	Age, sex, blood biomarkers, and demographic, anthropometric, and clinical data	BMI, uric acid, TG, and ALT levels
Liver fibrosis	Age, sex, and CT images	Better diagnosis compared to classical methods like APRI and FIB-4 indexes
Virus-induced hepatitis	Age, sex, blood biomarkers, and demographic, anthropometric, and clinical data	AST, PLT levels, APRI index, and age
COVID-19	Age, sex, blood biomarkers, CT images, and demographic, anthropometric, and clinical data	Age, BMI, CT images, oxygen rate, AST, and ALT levels

FLD: Fatty liver disease; CT: Computed tomography; BMI: Body mass index; TG: Triglycerides; ALT: Alanine transaminase; AST: Aspartate transaminase; PLT: Platelet; APRI: Aspartate transaminase/platelet ratio index; COVID-19: Coronavirus disease 2019; FIB-4: Fibrosis-4.

obesity, of which parental BMI and the upbringing environment play a huge role[80-82]. Furthermore, researchers have observed by training a multivariate LR model with a dataset of 3634 children and adolescents' vitamin intake that vitamins A, D, B1, B2, and B12 were associated in a negative manner with obesity in this cohort[83]. These results are of interest since new insights are needed to discover novel targets to tackle comorbidities that affect liver function.

ML in hepatitis virus-induced liver damage

HBV and HCV infections can dangerously become chronic if not treated early and with the right treatment[84]. While scientists are still relentlessly working on an effective vaccine against HCV, a good and efficient diagnosis is key to prevent chronic HCV infection (CHC), and ML algorithms have been elucidated for this purpose. Thus, Butt *et al*[85] designed an ANN model and trained it with a dataset of 19 variables, among which age, sex, BMI, transaminase, and PLT count levels were included. The algorithm was able to better identify the stage of hepatitis C compared to other XGBoost, RF, and SVM models tested by other researchers with a higher precision rate and a decreased miss rate.

ML algorithms have been applied and compared to traditional methods used to follow HHV-induced advanced liver disease[86-88]. For instance, Wei *et al*[87] used a GB model trained with the same variables that the formula fibrosis-4 (FIB-4) uses, which are age, AST, ALT and PLT levels in a cohort of 490 HBV patients, and two cohorts of HCV patients (n = 240 each). The GB model outperformed FIB-4 score in classifying hepatic fibrosis and the existence of cirrhosis. Barakat *et al*[89] designed an RF model that also outperformed the FIB-4 score, as well as the AST/PLT ratio index (APRI), for prediction and staging of fibrosis in children with hepatitis C. In this line, data of 72683 veterans with CHC were used to predict the progression of the disease. GB models were used and compared with cross-sectional or linear models fed with variables like transaminases levels, alkaline phosphatase (ALP), PLT, AST, APRI, albumin, bilirubin, glucose, white blood cells, and BMI were included in the dataset. Results showed that APRI, PLT, AST, albumin, and AST/ALT ratio were the best predictors for featuring CHC progression[88].

Regarding therapy, CHC can be effectively treated with direct-acting antiviral (DAA) therapy, a novel treatment that targets viral non-structural proteins. Although it has null side effects compared to standard treatment, it has some downsides. Treatment failure in a low percentage of the cases, a very high cost, and no treatment duration established[90]. New methods to define this therapy duration are needed to optimize adherence and success. Feldman *et al*[91] studied the prediction of DAA treatment duration in hepatitis C patients using XGBoost, RF, and SVM models. They used the dataset of 240 patients with prolonged first course of DAA against another dataset of 3478 patients on standard duration. Age, sex, comorbidities, and previous hepatitis C treatment record were considered. The predictive model constructed with XGBoost obtained the best performance in predicting prolonged DAA treatment, in which the presence of cirrhosis, type 2 diabetes, age, HCC, and previous standard treatment were the most determining variables. Meanwhile, Kamboj *et al*[92] used ML approaches in the search of repurposed drugs that could target non-structural proteins, developing regression-based algorithms able to identify inhibitors of these proteins, and proposing new drugs to test in CHC.

A huge milestone when treating chronic HBV infection (CHB) is seroclearance of HBV surface antigen (HBsAg)[84]. It has been demonstrated that seroclearance of HBsAg is associated to a better prognosis in CHB. Some authors used ML models to predict HBsAg seroclearance in a cohort of 2235 patients, of which 106 achieved it. They used XGBoost, RF, and LR, among other models, and tested a total of 30 categorical and continuous variables, including sex, drinking history, initial diagnosis and treatment, age, BMI, and serum and radiological indicators. Results revealed that the XGBoost model showed the best predictive performance, indicating that HBsAg levels were the best predictor for

HbsAg seroclearance, followed by age, and the DNA level of HBV[93].

Interestingly, ML has also contributed to personalized medicine in this field. HHVs evolve and adapt to different cellular environments in order to escape immune responses and drugs to survive. These adaptations rely on high mutagenetic activity, especially within the target genes of antivirals. Regarding HBV, Chen *et al*[94] used ML to identify patients with HCC or CHB based solely on genetic differences and found that the RF model impressively discriminated both cases based on the *rt* gene sequence of HBV. Moreover, Mueller-Breckenridge *et al*[95] ultra-deep sequenced 400 HBV samples and used an RF model to classify the status of a particular HBsAg according to the novel viral variants encountered. Results showed five genotypes that could benefit from personalized healthcare. In the case of HCV, Kayvanjoo *et al*[96] built several ML algorithms and trained them with two datasets of responders *vs* non-responders of antiviral therapy in HCV infection caused by two different strains. These investigations reported novel genetic markers that could predict therapy response with high accuracy. These results are very promising since they contribute to bringing personalized medicine to the public system.

ML in COVID-19-induced liver damage

A recent systematic review depicted that the parameters normally used for liver impairment screening were significantly increased in COVID-19 patients[23]. Particularly, several studies showed that levels of AST and/or ALT can increase in these patients up to 20%, bilirubin up to 14%, ALP up to 6%, and GGT levels up to 21%. Prothrombin is a protein synthesized in the liver that results in thrombin, a protein with a critical role in coagulation function. Prolonged prothrombin is a symptom of decreased production of coagulation factors, characteristic of liver disease. For this reason, the prolonged prothrombin time (PT) is another parameter usually checked when screening for liver injury, and it has been described that COVID-19 patients present nearly a 10% increase in PT[23]. Besides biochemical alterations, COVID-19 illness can lead to hypoxemia, impaired cardiac function, and secondary damage due to multiple organ dysfunction, which can result in liver injury in patients with or without prior liver disease. Therefore, new insights of the relationship between this recent infectious illness and liver disease are expected.

The use of ML approaches has been encouraged by the National COVID Cohort Collaborative Consortium for early detection, prediction, and follow-up of severe COVID-19 cases since the pandemic started[97]. For instance, some researchers used the XGBoost approach and found that age, CT scan result, body temperature, lymphocyte levels, fever, and coughing can classify influenza patients from COVID-19 patients[98]. Bhargava *et al*[99] tried different ML approaches to detect novel COVID-19 and discriminate between pneumonia using CT and *X*-ray scans as inputs. These authors pre-processed the images by normalization and then segmented them by fuzzy c-means clustering. Results showed that the SVM model was the one that better classified patients in COVID-19 positive, pneumonia, and healthy groups, obtaining a very high accuracy.

In this same line, obesity and liver disease were identified as risk factors for higher clinical severity in a cohort of 174568 adults with severe acute respiratory syndrome associated with SARS-CoV-2 infection by a multivariable LR model[97]. Interestingly, a German study of 8679 patients used an LR model and identified liver disease and BMI as determinant risk factors for 180-d all-cause mortality in hospitalized COVID-19 patients[100]. A case-control study with COVID-19 patients compared to patients with community-acquired pneumonia showed how, by applying a GB model, the category of liver function appeared as one of the top systematic predictors for COVID-19 risk factors, with albumin, total bilirubin, and ALT among the most important input variables[101]. Furthermore, a study with 710 enrolled patients diagnosed with COVID-19 identified AST levels as the top predictor for COVID-19-related hospitalization based on an RF algorithm, followed by age and diabetes mellitus[102].

A stepwise linear regression model identified IL-6 and granzyme B as potential predictors of liver dysfunction, characterized by an elevation in the levels of ALT and/or AST[103]. Other authors designed a model for detecting liver damage testing different ML approaches with laboratory parameters as the input variables. SVM was the model with the best accuracy, and AST and ALT levels were the variables with the best predictive scores[104]. In this context, the newest version of the CURIAL model was developed to identify COVID-19 patients using vital signs, blood gas, and laboratory blood tests. It showed greater sensitivity, making this model a potential emergency workflow [105,106]. All these ML-based methods would dramatically improve the time to diagnosis, free hospital laboratories and rooms of potential positive subjects, and reduce costs if implemented in the public health system.

AI has also been employed to discover potential efficient new drugs to tackle SARS-CoV-2 infection [107]. Baricitinib is a drug initially approved for rheumatoid arthritis that was selected by ML as a potential drug to treat COVID-19. Researchers proved the anti-inflammatory and antiviral properties of this drug in human liver spheroids infected with live SARS-CoV-2 to check any potential drug-induced liver injury[107]. Due to the good results, researchers moved on to a clinical trial where they tested baricitinib in a few COVID-19 patients. Levels of liver enzymes were not altered, except for a transient increase in liver aminotransferases in all patients that remitted in the following 72 h without interrupting treatment. The authors stated that this might be reflective of disease severity rather than a drug-induced injury, showing overall good tolerance and results in this pilot study[108]. In summary, ML approaches support liver biochemistry as a prognostic tool in COVID-19 disease.

PERSONALIZED MEDICINE IN LIVER-RELATED DISEASES SUPPORTED BY ML

In the early 21st century, the Human Genome Project started the genomic era in which new disciplines like precision medicine appeared. Precision medicine aims to deliver targeted treatments based on a group of individual factors that greatly influence the onset and progression of a disease, like omics sciences. This approach covers a great number of patients, overcoming potential adverse effects and ensuring effectiveness of the treatment. In this context, computational advances have greatly contributed to the escalation of this science by lowering the costs of omics analysis and allowing the processing and integration of an enormous amount of data based on ML algorithms (Figure 2).

ML has permitted the development of diagnostics and therapeutics based on the integration of omics data (genomics, epigenomic, transcriptomics, proteomics, metabolomics, and metagenomics) with clinical data. The ultimate goal is to bridge these omics data with the phenotype to bring molecular accuracy to the diagnosis, treatment, prognosis, and recurrence process of a pathological condition. This methodology has been used in a wide range of diseases in the search for more efficient and effective approaches, like heart and liver diseases[109,110]. For example, ML algorithms fed with omics data have been able to predict mortality in patients with alcoholic hepatitis. In this study, routine clinical variables of 210 patients with this disease were used to build six different datasets to assess mortality at 30 d and 90 d. Five different ML models were tested, obtaining the best performance in predicting 30-d mortality with a GB model using bacteria, MetaCyc pathways, and clinical data, as well as LR using viral and clinical data[111].

In hepatitis B, it has been found that ML algorithms can be very useful in assessing HBV-associated HCC progression. Ye *et al*[112] analyzed 67 HBV-positive HCC samples with or without intrahepatic metastases and discovered key genes for metastatic progression and survival training ML models. The majority of them were inflammatory or related to the inflammation process, like IL-2 receptor and osteopontin, which encodes an extracellular cytokine ligand whose overexpression favors metastasis. These authors were able for the first time to draw a molecular signature useful to classify metastatic HBV-HCC patients, opening the way for early detection and new treatments to increase patient survival. In hepatitis C, the CC and CT genotypes of the rs12979860 polymorphism in the *IL28B* gene have been associated with liver fibrosis progression, being able to predict antiviral treatment effectiveness[113].

Moreover, ML algorithms have allowed the diagnosis of advanced liver fibrosis according to the rs12979860 genotype with higher performance compared to APRI and FIB-4 scores[114]. In this study, patients were divided into two groups according to HCV-related liver fibrosis stage: None to moderate fibrosis (n = 204); or with advanced fibrosis (n = 223). ML algorithms revealed the *IL28B* genotype as the first predictor, while the second predictor depended on the mentioned genotype. For instance, in CT patients, PLT, albumin, and age were the determining variables, while for patients with the TT genotype, white blood cell count was the decisive feature to assess advanced fibrosis probability.

ML approaches have also helped to categorize obesity in different subtypes based on metabolic status [115-117]. For example, Masi *et al*[115] studied a cohort of 2567 subjects suffering from obesity and made clusters of metabolically healthy or metabolically unhealthy patients based on clinical and biochemical variables using two ML models. The first model showed that IR, body fat, HbA1c, red blood cells, age, ALT, uric acid, white blood cells, insulin growth factor-1, and GGT were the top predictors of a metabolically healthy obesity, revealing the importance of liver function.

Other authors have also used ML models to classify 882 obese patients in subtypes of obesity according to glucose, insulin, and uric acid levels[116]. Results showed four stable metabolic clusters in this cohort, which were characterized by a healthy metabolic status, or by hyperuricemia, hyperinsulinemia, and hyperglycemia, respectively. Furthermore, Lee *et al*[117] explored three-way interactions between genome, epigenome, and dietary/lifestyle factors using GB and RF models in a subset (n = 394) of the exam 8 of the Framingham Offspring Study cohort. Interestingly, GB obtained the best performance, revealing 21 single nucleotide polymorphisms, 230 methylation sites in relevant genes (like *CPT1A*, *ABCG1*, and *SREBF1*), and 26 dietary factors as top predictors for obesity. Intake of processed meat, artificially sweetened beverages, French fries, and alcohol intake, among other dietary factors, were highly associated with overweight/obesity.

Personalized and precision medicine aims to harmonize the greatest number of factors so that diagnosis, prognosis, and treatment are based on the greatest number of decision elements. Much remains to be investigated to establish guidelines in the context of personalized medicine. However, it is safe to say that precision medicine will drive modern medicine, combining the most classic variables with the newest digital variables. Health professionals must be prepared to understand and implement these new technologies in the near future.

CONCLUSION

In summary, ML science can process and integrate a vast amount of different data with unprecedented outstanding performance. The objective of this article was to collect the information derived from ML

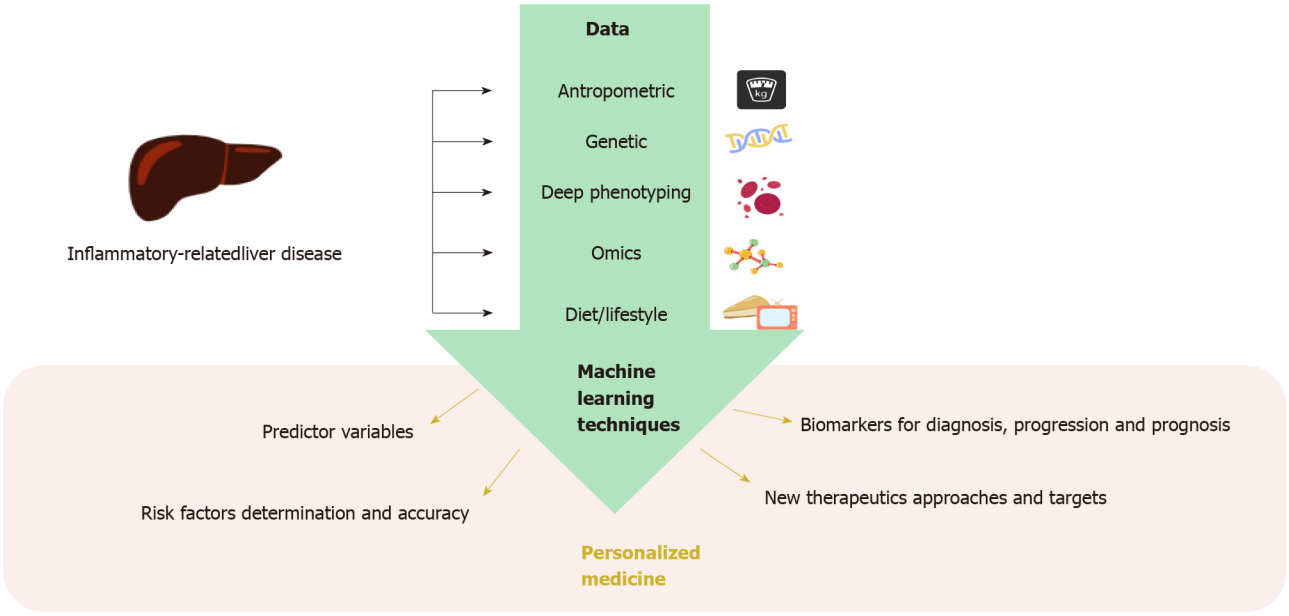

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Figure 2 Data implicated in the onset of inflammatory-related liver diseases can be used to train machine learning algorithms for prediction, diagnosis, treatment, and prognosis of chronic liver disease, leading the way to personalized medicine.

techniques in liver damage induced by inflammatory conditions, including the new disease COVID-19. The main role of ML in liver pathologies is to help identify high risk patients for referral to specialized centers. Results show that the use of ML models have brought new insights into biology and medicine questions that can be very useful in determining the next directions for research in diagnosis, prognosis, and treatment of inflammatory and virus-related liver diseases, leading the way to personalized medicine. Also inflammation/IR biomarkers related to liver disease can be boosted by ML strategies. This review clarified and compiled the importance of the different factors involved in CLD and analyzed by ML algorithms, which can be useful information for clinicians, like endocrinologists and gastroenterologists, and other healthcare professionals with a focus on hepatology and bioinformatics.

FOOTNOTES

Author contributions: Martínez JA and Alonso-Bernáldez M contributed equally to this work as first co-authors. Martínez JA and Ramos-Lopez O conceived and designed the study; Martínez JA, Alonso-Bernáldez M, and Ramos-Lopez O performed the search of articles and wrote the draft of the manuscript; Martínez-Urbistondo D, Vargas-Nuñez JA, Dávalos A, and Ramos-Lopez O contributed to the analysis and critical interpretation of the data; and all authors read and approved the final manuscript.

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MINIREVIEWS

Development of Epstein-Barr virus-associated gastric cancer: Infection, inflammation, and oncogenesis

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Abstract

Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC) cells originate from a single-cell clone infected with EBV. However, more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection, and *H. pylori* is a major causative agent of gastric cancer. Therefore, it has long been argued that *H. pylori* infection may affect the development of EBVaGC, a subtype of gastric cancer. Atrophic gastrointestinal inflammation, a symptom of *H. pylori* infection, is observed in the gastric mucosa of EBVaGC. Therefore, it remains unclear whether *H. pylori* infection is a cofactor for gastric carcinogenesis caused by EBV infection or whether *H. pylori* and EBV infections act independently on gastric cancer formation. It has been reported that EBV infection assists in the oncogenesis of gastric cancer caused by *H. pylori* infection. In contrast, several studies have reported that *H. pylori* infection accelerates tumorigenesis initiated by EBV infection. By reviewing both clinical epidemiological and experimental data, we reorganized the role of *H. pylori* and EBV infections in gastric cancer formation.

Key Words: *Helicobacter pylori*; Epstein-Barr virus; Epstein-Barr virus-associated gastric cancer; Coreceptor; Inflammation; Oncogenesis

Core Tip: Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC) tumor cells originate from a single cell clone infected with EBV. In contrast, it is reported that more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection. Accordingly, it has long been argued that *H. pylori* infection may have some effect on the development of EBVaGC, a subtype of gastric cancer. It is also a mystery that the number of gastric cancer patients is higher in Asia, South America, and the Middle East. We will reorganize the role of *H. pylori* and EBV infections in gastric cancer formation.

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INTRODUCTION

Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC) accounts for 10% of all gastric cancers. At the same time, more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection. Thus, the question arises as to whether *H. pylori* and EBV infections promote gastric cancer formation in a dependent or independent manner. The high prevalence of gastric cancer in Asia, South America, and the Middle East is also intriguing.

EBV IS AN ONCOGENIC HUMAN HERPESVIRUS

EBV infects B lymphocytes and epithelial cells and is an oncogenic virus that assists in the proliferation of latently infected cells, resulting in the development of Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, and EBVaGC[1].

More than 90% of adults are latently infected with EBV; however, cytotoxic T lymphocytes that recognize EBV antigens suppress the proliferation of viral antigen-positive cells. When the local or systemic immune function is compromised, EBV-positive cells begin to proliferate. B lymphocytes that migrate to local areas where immune surveillance is weak often transition to lytic infection, resulting in viral production. Under such conditions, EBV appears to be transmitted to and infects gastric epithelial cells. The expression of EBV genes causes epithelial cells to acquire proliferative properties and resist apoptosis, and cells that escape immunological elimination may begin proliferating[2].

EBV-ASSOCIATED GASTRIC CANCER

Molecular features

Classification of gastric cancer by molecular mechanism was performed through an exhaustive analysis of next-generation sequencing data from numerous cases. The results divided gastric cancer into the following four molecular subtypes: Microsatellite instability (MSI), chromosomal instability (CIN), genomically stable (GS), and EBV[3]. These classifications have facilitated the identification of specific therapeutic candidates for each subtype of gastric cancer, and have revealed that each of these four subtypes is driven by a specific developmental mechanism that needs to be clarified individually. In particular, the molecular biology of EBVaGC is characterized by frequent and extensive methylation of the promoter regions of tumor cell genes[4]. *De novo* EBV infection induces DNA methylation in more than 3000 gene promoter regions within 4 wk[4]. However, methylation of the promoter of the mismatch repair gene *MLH1*, which is frequently observed in MSI, is not observed in EBVaGC[5]. In addition to inactivation by DNA methylation, the EBV genome binds to heterochromatin, a region of inactivation that causes aberrant activation of the region (enhancer infestation) and increases the expression of surrounding proto-oncogenes[6].

Clinical features

In EBVaGC, which accounts for 5%–10% of all gastric cancers, all tumor cells are infected with EBV. Endoscopy is the most informative method for diagnosing gastric cancer. EBVaGC is observed as a superficial depressed lesion in the upper part of the stomach. Using endoscopic biopsy specimens, EBV-

encoded RNA *in situ* hybridization (EBER-ISH), stains all gastric cancer cells positive for EBER, even in the intramucosal cancer stage[7]. The histological hallmark of EBVaGC is lymphoepithelioma-like carcinoma, in which a diffuse lymphocytic infiltrate is observed around EBER-positive epithelial tumor cells[8]. Furthermore, EBVaGC tumor cells are derived from the proliferation of a single EBV-infected epithelial cell[8,9].

Many studies have shown a male predominance (2-fold) of EBVaGC, suggesting that the risk may exist in male lifestyle and occupational factors[10]. The percentage of patients with EBVaGC to those with total gastric cancer is higher in younger patients. In men, the proportion of EBVaGC decreases with increasing age, especially in patients with pyloric gastric cancer. In women, the decrease in the proportion of EBVaGC with increasing age is unclear. Consumption of salty foods that cause mechanical damage to the gastric epithelium as well as exposure to wood and iron filings are associated with a higher EBVaGC risk[11].

EBVaGC is a gastric cancer with a relatively good prognosis. A Dutch study reported that EBVaGC is characterized by fewer lymph node metastases, less residual disease, and younger patient age, which results in longer disease-free survival[12]. Cohort study data from TCGA also reported that EBVaGC has the best recurrence-free period and overall survival compared to MSI, GS, and CIN subtypes[13].

EBVaGC tumors are frequently found in non-antral parts of the stomach[10,14]. In contrast, *H. pylori*-associated gastric cancer mostly occurs in the antral region[10]. Because moderate to severe atrophic gastric mucosa due to *H. pylori* infection was characteristically observed surrounding early gastric cancers, gastritis may play an important role in the tumorigenesis of EBVaGC[14]. Development of gastric cancer is supposed to follow the "infection, inflammation, and carcinogenesis" route, which consists of *H. pylori* infection followed by chronic gastritis, intestinal metaplasia, and cancer. In contrast, in the case of EBVaGC, it is controversial whether tumor formation is initiated by EBV-infected normal mucosal cells or promoted by EBV-infected cells in precancerous lesions[15]. Abe *et al*[16] performed EBER-ISH on 1110 sections of non-neoplastic gastric mucosal tissue from 300 cases and found 2 (0.18%) ductal-level EBER-positive lesions.

The mutual contribution of EBV and *H. pylori* in the carcinogenesis will be discussed later in the chapter “Inflammation and carcinogenesis”.

EBV INFECTION OF EPITHELIAL CELLS

EBV infects B lymphocytes through the binding of the viral glycoprotein gp350 to the high-affinity receptor CD21, followed by binding of gp42 to HLA class II molecules, resulting in membrane fusion [17]. In contrast, when low-affinity co-receptors are used to infect CD21-negative epithelial cells, the infection efficiency is extremely low (Figure 1).

The CD21-independent routes of epithelial cell infection include the following: (1) The viral envelope glycoprotein gp350/220 binds to CD35; (2) Integrins αVβ5, αVβ6, and αVβ8 interact with the viral envelope glycoprotein gH/gL complex to fuse the viral envelope with the epithelial cell membrane; (3) The BMRF2 membrane protein expressed during EBV lytic infection binds to α3, α5, αV, and β1 integrins; and (4) EphA2 and NMHC-IIA bind to gH/gL produced by many herpesviruses and enhance infection efficiency.

A previous study reported that a boy with X-linked agammaglobulinemia who did not have mature B lymphocytes due to a genetic enzymatic deficiency did not develop an EBV infection[18]. EBV infection of epithelial cells was considered to occur after EBV infection of B lymphocytes because the epithelial cells of the affected boy were intact. EBV-infected B lymphocytes are believed to carry and deliver EBV to the epithelial cells *via* cell-to-cell transfer. In the case of CD21-independent infection, the efficiency of epithelial cell infection by cell-to-cell transfer is more than 1000 times higher than that of direct epithelial cell infection by EBV particles[19]. It is speculated that infection of epithelial cells *via* B lymphocytes is promoted when viral activation and lymphocyte infiltration are accompanied by inflammation (Figure 1).

EBV-associated gastric cancer-derived cell lines

In EBVaGC, all tumor cells are infected with EBV. However, cell lines established from gastric cancer tissues, similar to those in nasopharyngeal carcinoma, are almost entirely EBV-negative[20]. The EBV genome in EBVaGC tumor cells exists as a plasmid-like episome that does not integrate into the host chromosomes. However, the presence of the virus does not appear to favor cell growth *in vitro*. Rather, it may be more convenient for *in vitro* cell growth to avoid the use of extra energy to maintain the episomes. Alternatively, the expression of viral genes such as microRNAs may be crucial for tumor cells to evade elimination by the *in vivo* immune system. In fact, EBV-positive KT cells established from EBVaGC can only be passaged by transplantation into SCID mice and cannot be expanded in an *in vitro* culture system[21]. SNU-719, YCCEL1, and NCC-24 are rare cells established from EBVaGC and can be propagated *in vitro*. These cell lines appear to be unique because the presence of EBV episomes is essential for their growth. Experiments with hydroxyurea and EBNA1 siRNAs were not successful in shedding the EBV episome from SNU-719 cells[22].

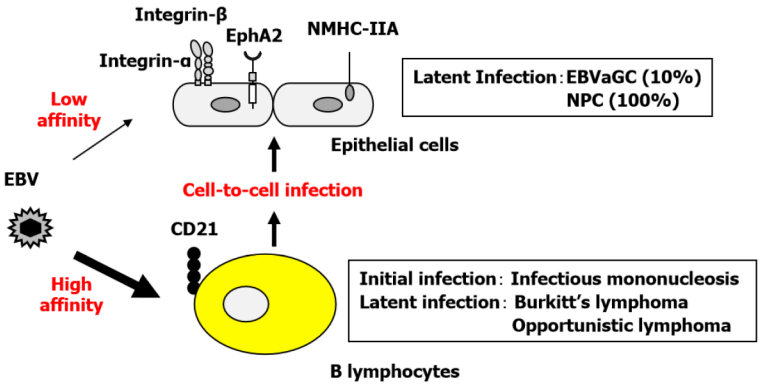

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Figure 1 Epstein-Barr virus infects B lymphocytes and epithelial cells to form tumors. Epstein-Barr virus (EBV) infects B lymphocytes and epithelial cells *via* the CD21 receptor and co-receptors, respectively. Although the efficiency of epithelial cell infection is extremely low, approximately 1000000 times lower than that of B lymphocytes, cell-to-cell EBV infection by B lymphocytes increased the efficiency of EBV infection by more than 1000-fold. The squares show the EBV infection status and disease names that are established differently depending on the cell type. EBV: Epstein-Barr virus; EBVaGC: Epstein-Barr virus-associated gastric cancer; NPC: Nasopharyngeal carcinoma.

Gastric epithelial cell lines infected with recombinant EBV

We established gastric epithelial cells infected with recombinant EBV, where a drug-resistant gene was inserted into the nonessential *BXLF1* (thymidine kinase) gene (Figure 2). It is possible to elucidate the oncogenic molecular mechanism of EBV-infected epithelial cells by comparing EBV-positive cells with EBV-negative cells. EBV infection markedly promotes the proliferation of gastric epithelial cells[23].

EBV-infected gastric epithelial cells also exhibit type I latent infection that expresses EBNA1 and LMP2A, similar to that in EBVaGC *in vivo*. EBNA1 promotes tumorigenesis *via* p53 ubiquitination, suppresses transforming growth factor-β signaling, and enhances the transcription of the anti-apoptotic protein survivin[24]. In contrast, LMP2A activates PI3K/Akt signaling similar to that activated by B-cell receptor stimulation, increases survivin expression, and resists apoptosis[25]. LMP2A also induces DNA methyltransferases, resulting in epigenetic changes in infected cells[26]. *BARF1* is strongly expressed as a latent gene in EBV-associated epithelial tumors[27]. Nasopharyngeal carcinoma-derived cells infected with recombinant EBV constitutively expressing *BARF1* exhibit resistance to apoptosis[28].

In addition to the oncogenic activity of EBV proteins expressed in type I latent infections, non-coding RNAs (miRNAs and EBERs) that are not translated into proteins have been investigated. Multiple BART miRNAs cooperatively repress lytic replication[29]. BART miRNAs also downregulate pro- and anti-apoptotic mediators such as caspase 3[30]. EBERs bind to protein kinase R and disrupt innate immune function[31]. Elimination of EBER2 from the EBV genome reduces the efficiency of B lymphocyte transformation[32].

INFLAMMATION AND CARCINOGENESIS

Clinical observation

It is very difficult to collect EBVaGC cases without *H. pylori* infection, because most patients with gastric cancer are infected with *H. pylori*[33,34]. However, a clinical study was conducted to investigate the relationship between EBV infection, *H. pylori* infection, and atrophic gastritis in 468 patients with chronic gastritis[35]. This study confirmed that patients who were EBV-positive had a lower pepsinogen I/pepsinogen II ratio than patients who were EBV-negative. EBV infection significantly increases the risk of atrophic gastritis, especially in *H. pylori*-negative patients. However, a report from Mexico mentioned that EBER1 *in situ* hybridization showed that EBV infection of epithelial cells could be detected in gastric cancers as well as in one-third of non-atrophic gastritis samples[36]. This study showed that EBV infection affected early cancer precursor lesions. However, it is difficult to determine whether EBV causes cancer directly or indirectly by triggering inflammation.

Inflammation and initiation of innate immune mechanisms promote EBV activation, although it is difficult to assess the extent to which these mechanisms are involved in tumorigenesis of EBV-infected cells (Figure 3). EBV proliferation occurs at the early stage of EBVaGC formation because early antigens-immunoglobulin G (IgG) and viral capsid antigen-IgG antibodies against early viral antigens and capsids are elevated in the sera of patients with EBVaGC. In addition, while the incidence of EBVaGC is approximately 10% worldwide, the incidence of gastric cancer after surgical invasion by gastric anastomosis increases by three times (30%)[8].

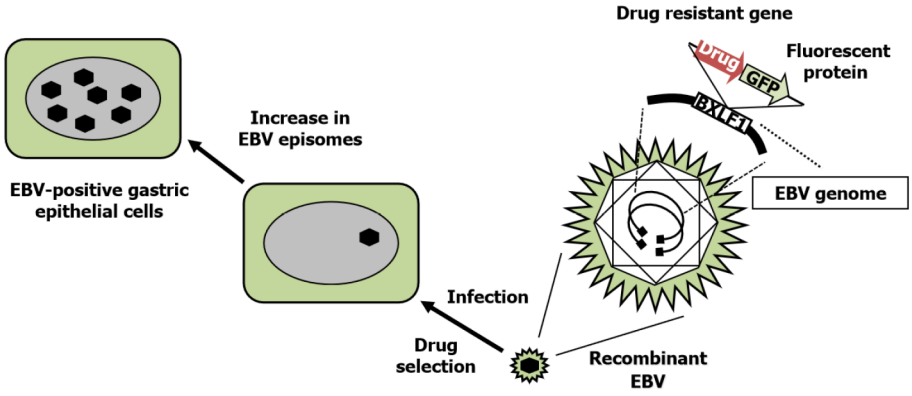

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Figure 2 Isolation of recombinant Epstein-Barr virus-infected gastric epithelial cells. A drug resistant gene and fluorescent protein gene were inserted into the viral genome (*BXLF1*) using gene recombination technique. *BXLF1* is an *EBV* gene that does not affect viral production or infectivity by disruption through the insertion of marker genes. It is possible to isolate only recombinant virus-infected cells by infecting cells with a recombinant virus and selecting them for a drug. When recombinant virus-infected cells are cultured in the presence of the drug, the viral plasmid copy number in the nucleus of infected cells increases[52]. EBV: Epstein-Barr virus; GFP: Green fluorescent protein.

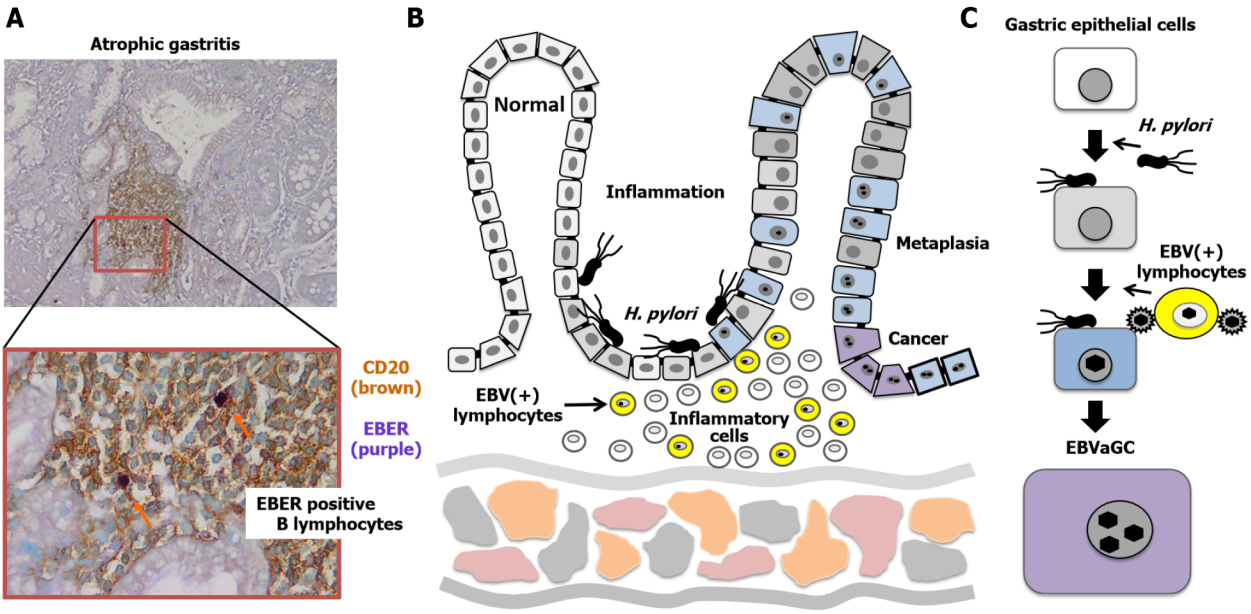

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Figure 3 Interaction of *Helicobacter pylori* and Epstein-Barr virus in the formation of Epstein-Barr virus-associated gastric cancer. A: Infiltration of Epstein-Barr virus (EBV)-infected B lymphocytes in non-tumor areas of EBV-associated gastric cancer. Numerous CD20-positive B lymphocytes infiltrate the submucosal lesions of atrophic gastritis. CD20 is stained brown and EBV-encoded RNA (EBER) is stained purple. EBER-positive B lymphocytes are indicated by arrows. Chronic gastritis in the background is counterstained by Hematoxylin-Eosin staining; B: Induction of inflammatory cytokine production by bacterial adhesion to epithelial cells and tumorigenesis of EBV-infected epithelial cells. *Helicobacter pylori* adhesion induces production of inflammatory cytokines from gastric epithelial cells. Inflammation of gastric mucosa leads to an accumulation of various immune cells. EBV-positive B lymphocytes localized in the submucosa are activated by inflammation and transition from latent to lytic EBV infection. The viral particles produced are transferred to gastric epithelial cells, and the infected epithelial cells eventually form tumors; C: EBV transfer infection and tumorigenesis of epithelial cells *via* activated EBV-positive B lymphocytes. Infectious viral particles produced during inflammation adhere to CD21-positive B lymphocytes and transferred to epithelial cells expressing EBV coreceptors. Thus, gastric epithelial cells infected with EBV form Epstein-Barr virus-associated gastric cancers over time. EBV: Epstein-Barr virus; EBVaGC: Epstein-Barr virus-associated gastric cancer; *Helicobacter pylori*: *H. pylori*; EBER: EBV-encoded RNA.

Here, we investigated the relationship between *H. pylori*-associated gastritis and EBV propagation in the stomach. Gastric biopsy specimens were collected from patients with chronic atrophic gastritis and categorized into three histopathological stages: Mild, moderate, and severe. The specimens were subjected to DNA extraction and quantitative polymerase chain reaction to quantify EBV genome copy numbers[37]. More than 900 copies of the EBV genome have been frequently detected in patients with moderate atrophic gastritis. In other words, EBV frequently activates proliferation in patients with *H. pylori* infection with moderate chronic atrophic gastritis and strong histological inflammation.

In contrast, EBVaGC is significantly associated with marked mucosal atrophy and moderate to marked lymphocytic infiltration, but there is no direct association with intestinal metaplasia[7]. Although this appears to indicate that EBVaGC is not directly associated with *H. pylori* infection, this result is consistent with our findings. This is because the intestinal metaplastic epithelium resulting from prolonged gastritis is an unsuitable mucosal environment for the growth of both *H. pylori* and EBV [38].

Experimental observation

Several studies have been investigated the interaction between EBV and *H. pylori* in gastric epithelial cell lines. Because it is difficult to infect the epithelial cells with the two microorganisms simultaneously, experiments have been conducted on sequential infection with EBV first and *H. pylori* second, or vice versa.

Persistent infection of the gastric mucosa by CagA-positive *H. pylori* strains causes gastric cancer. This is because the tyrosine-phosphorylated CagA protein binds to the tyrosine phosphatase SHP2 in gastric epithelial cells, activating *Ras* oncogene. In contrast, SHP1, which competes with SHP2 weakens the oncogenic activity of SHP2. Saju *et al*[39] showed that EBV infection of gastric epithelial cells activates host cell promoter methylation and decreases SHP1 expression[39]. In other words, SHP2 activity is relatively higher and EBV infection promotes carcinogenesis of *H. pylori* associated gastric carcinoma. The induction of DNA methylase by EBV infection in gastric epithelial cells also decreases the expression of tumor suppressor genes such as *APC*, *breast cancer susceptibility gene 1*, and *phosphatase and tensin homolog deleted from chromosome 10* (*PTEN*)[40].

Furthermore, activation of innate immune signals by *H. pylori* attachment enhances the expression of the EBV co-receptor EPHA2 in gastric epithelial cells, thereby increasing the frequency of EBV infection in epithelial cells[41]. Another study demonstrated that organoids derived from gastric cancer cells were infected with EBV but did not infect those derived from the normal gastric epithelium[42]. The probable reason for this is that gastric organoids maintain cell polarity and express EPHA2 only between cells. Therefore, the localization of EPHA2 might change due to gastric epithelial cell injury caused by *H. pylori* infection or by a prior gene mutation, which subsequently facilitates EBV infection.

TUMORIGENIC MECHANISM OF EBV-INFECTED EPITHELIAL CELLS

At present, it is difficult to infect primary gastric epithelial cells with EBV and immortalize them. Instead, gastric epithelial cell lines persistently infected with EBV have been used to elucidate the tumorigenic mechanisms of EBV genes during latent infections.

EBV genes that encode untranslational RNA

The EBV genome contains two miRNA clusters, consisting of four BHRF1 miRNAs and 40 BART miRNAs. Although BHRF1 miRNA is poorly expressed in epithelial cells, BART miRNAs are highly expressed in latently infected epithelial cells and play a substantial role in tumorigenesis[43].

Epigenetic changes of gene expression in EBV-infected epithelial cells

Modification of gene expression *via* methylation is frequently observed in patients with EBVaGC. Tumor suppressor genes, such as *p14*, *p16*, *p73*, *PTEN*, *APC*, *RASSF1A*, and *CXXC4*, are repressed by promoter methylation. And the expression of molecules important for cell invasion, including THBS1, E-cadherin (CDH1), and TIMP2, is also repressed by promoter methylation. The decreased expression of these molecules may be involved in carcinogenic processes[44].

Multiple EBV episomal DNAs have been shown to approach enhancer sites in the genome, alter the surrounding chromatin structure (enhancer infestation), and activate genes such as transcription factors [6]. Although epigenetic analyses have been conducted to understand tumorigenesis, the overall mechanism remains unclear.

Model of tumorigenesis for epithelial EBV infection

Viral gene products transcribed in cells latently infected with EBV confer resistance to apoptosis. *EBV* gene products also accumulate mutations in the genes of the infected cells. Genetic changes in infected cells further affect *EBV* gene expression and alter intercellular communication, including the cross-talk between EBV-infected epithelial cells and immune cells[45] or epithelial-mesenchymal transition[46]. In other words, changes induced by persistent EBV infection in host cell signaling and host immune responses advance the tumorigenic stage[47].

FUTURE PROSPECTS

With the progress in research on EBER, miRNA, and long non-coding RNA, the functions of these

molecules in latent EBV-infected cells are being elucidated. A highly tumorigenic B81 EBV strain was isolated from a patient with nasopharyngeal carcinoma[48]; however, an EBV strain unique to gastric cancer has not yet been isolated.

Host gene mutations frequently observed in EBVaGC, including changes in *PIK3CA*, *ARID1A*, *PD-L1*, and *PD-L2*[3] are considered to affect histological characteristics, clinical course, and response to treatment. EBV-induced tumorigenesis is believed to be affected by environmental factors such as previous infections; however, the molecular basis that characterizes EBVaGC remains to be elucidated.

Considering that EBVaGC most strongly expresses *PD-L1* and *PD-L2* among the four molecular subtypes of gastric cancer, immune checkpoint inhibitors are expected to be effective therapeutic agents for EBVaGC[49,50]. *PIK3CA* mutations and *JAK2* amplification are frequently observed in EBVaGC. Therefore, PI3K and JAK2 inhibitors may be effective. Other EBNA-1 inhibitors are also expected to be EBV-specific therapeutic agents[51].

CONCLUSION

Several clinical and experimental data support the etiological role of *H. pylori* in EBV-associated gastric cancer.

FOOTNOTES

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MINIREVIEWS

Glucagon-like peptide-2 analogues for Crohn's disease patients with short bowel syndrome and intestinal failure

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Abstract

Short bowel syndrome (SBS) with intestinal failure (IF) is a rare but severe complication of Crohn's disease (CD), which is the most frequent benign condition that leads to SBS after repeated surgical resections, even in the era of biologics and small molecules. Glucagon-like peptide-2 analogues have been deeply studied recently for the treatment of SBS-IF. These drugs have a significant intestinotrophic effect and the potential to reduce the chronic dependence of SBS-IF patients on parenteral support or nutrition. Teduglutide has been approved for the treatment of SBS-IF, and apraglutide is currently in clinical development. The use of these drugs was examined with a focus on their use in CD patients.

Key Words: Short bowel syndrome; Intestinal failure; Crohn's disease; Glucagon-like peptide-2 analogues; Teduglutide; Apraglutide; Glepaglutide

Core Tip: Short bowel syndrome with intestinal failure and chronic dependency on parenteral support are rare but severe complications of Crohn's disease (CD) after repeated intestinal resections. New therapeutic options are available, including glucagon-like peptide-2 analogues. Their use in CD appears safe and efficacious, but more data from specifically designed studies are needed.

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INTRODUCTION

Short bowel syndrome (SBS) in adults is a condition in which the normal length of the bowel (which ranges from 3 to 8 metres) is reduced to less than 2 metres[1]. It is classified anatomically into: (1) Type 1: End-jejunostomy; (2) Type 2: Jejuno-colonic anastomosis; and (3) Type 3: Jejuno-ileal anastomosis[2]. The onset of SBS may lead to intestinal failure (IF), which is defined according to the European Society for Clinical Nutrition and Metabolism as the reduction of gut function below the minimum necessary for the absorption of macronutrients and/or water and electrolytes, and intravenous supplementation is required to maintain health and/or growth. A reduction in gut absorptive function that does not require intravenous supplementation to maintain health and/or growth is considered intestinal deficiency. IF is categorised according to temporal and functional evolution into: (1) Type 1 - acute, short term and self-limiting; (2) Type 2 - a prolonged acute condition, often in metabolically unstable patients, that requires complex multidisciplinary care and intravenous supplementation over periods of weeks or months; and (3) Type 3 - a chronic condition in stable patients[3].

However, not all patients with SBS develop IF, and not all patients with IF have underlying SBS[1]. The anatomical classification of SBS is mirrored by different clinical presentations: (1) Type 1 (end-jejunostomy with no colon in continuity) shows a higher risk of dehydration and electrolyte imbalance, especially immediately after surgical resection; (2) Type 2 (jejuno-colonic anastomosis) patients may develop malnutrition in a long-term setting (months to years); and (3) Type 3 (jejuno-ileal anastomosis with ileo-caecal valve conservation) has a lower risk of malnutrition[4].

The causes of SBS-IF in 2919 patients with benign chronic IF were CD (22.4%), mesenteric ischaemia (17.7%), surgical complications (15.8%), primary chronic pseudo-obstruction (9.75%) and radiation enteritis (7.3%). The pathogenetic mechanisms of IF were heterogeneous in the same population, with most patients presenting (38.6%) with end jejunostomy, and approximately 20% of patients presenting with jejuno-colonic anastomosis[5]. The remainder of the cohort was divided into intestinal dysmotility (17.5%), fistulas (7%), mucosal disease (6.8%), jejuno-ileal anastomosis (5.9%), and mechanical obstruction (4.4%)[5]. However, other reports, such as the United States intestinal transplant (IT) registry, describe mesenteric ischaemia as the first cause of IT (24%) and CD as the second most frequent cause (11%)[6].

Following a large bowel resection, humans undergo a wide range of functional and anatomical modifications due to the reduction of the intestinal area dedicated to the absorption of nutrients[7]. Structural and anatomical changes include crypt hyperplasia, angiogenesis, bowel dilation and bowel elongation. Functional changes include accelerated crypt differentiation, a slower transit time, an increase in the number of transporters and a consequent increase in nutrient absorption[8]. After an early phase immediately following surgery, the adaptation phase starts 48 h after resection and lasts at least 1-2 years. Most of the intestinal adaptation described above occurs in this phase. Nutritional homeostasis may be achieved in the maintenance phase *via* oral autonomy or with parenteral support (PS)[3].

The clinical consequences of SBS are very heterogeneous and may considerably impact the patient's quality of life. One frequent symptom is diarrhoea due to accelerated intestinal transit, intestinal and gastric increased secretion[9], intestinal bacterial overgrowth[10], and the malabsorption of fats and bile salts[11,12]. One common consequence of SBS is the formation of stones. Asymptomatic gallbladder stones were reported in a population of SBS patients[13]. Most of these stones consist of calcium bilirubinate. The formation of gallbladder stones is favored by altered enterohepatic circulation[14], gallbladder hypomobility[15], and the reduced secretion of cholecystokinin after meals[16]. The formation of calcium oxalate kidney stones is also common, especially in patients with SBS and colon in continuity[17]. Within the colon, unabsorbed long-chain fatty acids compete with oxalate for available luminal calcium, and a larger amount of free oxalic acid is absorbed *via* passive diffusion and ultimately excreted by the kidney[13]. Other mechanisms may be involved, such as regional differences in oxalate absorption[17].

A relatively rare but underestimated complication of patients with SBS, especially patients with colon preservation, is so-called D-lactic acidosis. When a carbohydrate meal is consumed, colonic bacteria metabolise non-absorbed carbohydrates to short-chain-fatty acid and lactate. Lactate lowers the intraluminal pH, which leads to the overgrowth of D-lactate-producing bacteria, *Bifidobacterium* and *Lactobacillum*. D-lactate is absorbed into the systemic circulation and is responsible for the onset of neurological symptoms[18]. From a clinical point of view, this condition is characterized by neurological symptoms, such as ataraxia, movement disorders and altered mental status[19].

The prevalence of IF due to benign disease ranges from 5 to 80 cases of patients on chronic home parenteral nutrition (HPN) per million population in Europe[3]. Benign chronic IF and secondary SBS are in the directory of rare diseases ORPHANET (ORPHA:294422 and ORPHA:95427).

SBS-IF IN CROHN'S DISEASE

A survey in 64 centres from 22 countries enrolling 2919 patients on HPN described CD as the most frequent benign cause of SBS-IF[5]. However, a long-term study from a single IF centre in the United Kingdom described a change in the causes of IF over time, and the prevalence of CD decreased from 44% in 1978-1988 to 22% in 2006-2012[20].

Surgery remains a common treatment for CD during the course of the disease[21]. The indications for small intestinal resection in CD range from complications, such as stenosis, fistulas and abscesses, to the treatment of medically refractory disease[22]. Surgery rates in CD declined in recent decades due to multifactorial reasons, including earlier diagnosis and treatment, the use of biological agents, a decline in smoking rates, and improved patient education[23,24].

Although a United States population study demonstrated that biological agents reduced the proportion of CD patients undergoing resection, this reduction was not observed in patients with SBS-IF, which likely represents a subgroup of CD patients characterized by a more severe disease course and resistance to treatments[25]. Predictors of SBS-IF in CD were reviewed and roughly correspond to predictors of severe disease course. Notably, the CD phenotype characterized by an ileocolonic location is associated with a greater risk, and the absence of the ileocecal valve increases the risk of HPN dependence. Patients with perianal disease at diagnosis have a higher risk of disabling disease course, including bowel resections, and patients with penetrating disease have longer bowel resections and more frequently depend on HPN. Patients younger than 40 years at the time of CD diagnosis were more likely to have a "very short bowel" (< 100 cm), and an older age at first surgery was associated with decreased odds of IF. Patients who had ever smoked were more likely to develop IF. A family history of inflammatory bowel disease (IBD), frequent corticosteroid use for flares, and the number of bowel resections and complications of surgery were also identified as risk factors for SBS-IF[26].

A recent case-control study of 410 CD patients (41 with SBS) at a single centre demonstrated that subjects with SBS underwent significantly more bowel resections than controls. Patients treated with IV steroids were at higher risk of SBS, and Montreal B1 (inflammatory) behavior and treatment with budesonide characterized patients at a lower risk of SBS[27].

Another study of 2456 IBD patients identified 25 patients who required long-term HPN (1%, 24 CD). They described that HPN use was significantly associated with smoking, narcotic use, IBD-related surgeries, and lower quality-of-life scores. They found that these refractory patients had a 15-fold increase in annual median health care charges compared to control IBD patients[28].

MEDICAL TREATMENT OF SBS-IF AND COMPLICATIONS

The management of SBS-IF is complex, expensive and requires a multidisciplinary approach. A multidisciplinary team consisting of at least gastroenterologists, nutritionists, surgeons, radiologists, stoma therapists, care managers, pharmacists, and home care nurses is useful to provide patients with the best management possible[29]. The process of intestinal rehabilitation may be improved with medical management and includes spontaneous adaptation (dietary intervention and oral rehydrating solutions) and pharmacological therapies to improve symptoms. Gastric hypersecretion may be reduced with Proton-pump inhibitors. The accelerated transit time may be slowed with loperamide. The reduced reabsorption of bile acids may be targeted with cholestyramine, and bacterial overgrowth may be modulated with antibiotics, particularly rifaximin. Other drugs commonly used in the symptomatic management of diarrhoea are diphenoxylate, atropine, codeine, and antihistamines (*e.g.*, ranitidine and cimetidine).

Although symptom management provides an initial benefit in quality of life and the patient's perception of disease, it does not provide any improvement in prognosis[30]. The cornerstone in the treatment of SBS-IF is HPN. HPN ensures the correct intake of micro- and macronutrients and provides a significant improvement in prognosis. HPN is a life-saving treatment for these patients, but it significantly lowers patients' quality of life and is not free of complications[31].

HPN is generally administered in a single dose *via* a nutrition bag and infused over 10 to 12 h, typically overnight. Patients are encouraged to continue oral feeding with HPN[32]. Catheter infections [also called catheter-related blood stream infections (CRBSIs)] and central venous catheter (CVC) thrombosis are the most feared complications of parenteral nutrition. The rates of CRBSI range from 0 to 11.89 episodes per 1000 catheter days in systematic reviews and meta-analyses[33]. No significant difference was shown in catheter infection rates between peripherally inserted central catheters (PICCs) and tunneled catheters. However, PICCs showed lower CRBSI rates than ports[34]. The more frequently implicated pathogens are Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus species*, and *Strepto-*

coccus species), Gram-negative bacteria (*Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae*), and fungi (*Candida parapsilosis*, *Candida albicans*, and *Candida glabrata*)[35]. Antibiotic therapy is the mainstay of CRBSI management. An empiric regimen with broad-spectrum drugs is generally used until the responsible agents are identified[36]. Bacterial infections are generally treated with the catheter in place, but fungal infections are treated by removing the catheter[37]. Locking the catheter with ethanol[38,39], taurolidine[40] or antibiotics[41] has been proposed as a strategy for the prevention of CRBSI. However, the results from randomized controlled trials (RCTs) are nonunivocal, and no clear guidelines have been generated on this topic[42].

The incidence of CVC thrombosis is approximately 0.12 events/1000 catheter days[43]. The management of catheter-related thrombotic events is based on the administration of low-molecular-weight heparins (LMWH)[44] or thrombolytic agents, such as streptokinase[45]. Flushing the CVC with a saline solution or LMWH has been proposed as a measure to prevent the occurrence of thrombotic events[46,47].

One possible complication of HPN is progressive steatohepatitis and liver damage, also known as "IF-associated Liver Disease" (IFALD). Up to half of the patients receiving total parenteral nutrition develop severe liver disease after 5 years[48,49], and higher rates of incidence and prevalence are observed in children[50]. IFALD manifests as steatosis, steatohepatitis or a cholestatic pattern. It may lead to progressive liver damage with fibrosis and cirrhosis in some cases[51]. Therapeutic strategies include fish oil-based lipid emulsions[52], ursodeoxycholic acid[53], and lecithin administration[54].

Malabsorption-related anaemia may develop into microcytic anaemia due to iron deficiency or macrocytic anaemia due to malabsorption of vitamin B12 and folate, which lead to the need for iron and vitamin supplementation[3,55]. The occurrence of metabolic bone disease must be monitored[56]. Bone densitometry, the biochemical dosing of vitamin D and calcium profiles play a role in preventing the insurgency of metabolic bone disease[57]. Manganese toxicity must be noted. Manganese is a central element of HPN, but its requirement is low. This low requirement leads to an increase in blood concentration and accumulation in the central nervous system, which is detectable using magnetic resonance imaging. Manganese toxicity presents with neurological symptoms that are similar, but not identical, to Parkinson's disease[58].

NEW THERAPIES: GLUCAGON-LIKE PEPTIDE-2 ANALOGUES

Hormonal manipulation may also increase intestinal absorption[59] (Figure 1). Only two molecules have been approved for SBS-IF therapy, growth hormone in the United States and the glucagon-like peptide-2 (GLP-2) analogue teduglutide (TED) in the United States and Europe[60,61]. TED is a recombinant analogue of GLP-2 that is resistant to degradation by DPD4. The elimination half-life of TED is 2 to 6 h, which allows administration as a daily subcutaneous injection. Notably, the elimination half-life of endogenous GLP-2 is approximately 7 min[62].

The mechanism of action of TED is complex and involves direct and indirect effects of interaction with a GLP-2 receptor and includes the following most relevant factors: Crypt cell proliferation, increase in bowel weight and villous growth, enhancement of intestinal barrier function, inhibition of motility of the gastrointestinal tract and gastric acid secretion, and increase of intestinal blood flow[60]. The effect of TED on intestinal epithelial stem cells is of particular relevance in the possible risk of the drug being tumorigenic[63].

The first open-label trial on TED was performed and published in 2005. Sixteen patients with end-jejunostomy or colon in continuity received s.c. TED for 21 d. Patients treated with TED showed an increased absorption of nutrients, urine output and sodium excretion. TED significantly increased villus height, crypt depth and mitotic index in the small intestines of patients with end-jejunostomy. Leg oedema and enlargement of the stoma nipple were the most common, but not severe, side effects[64].

The most significant RCT of TED effectiveness was performed from 2008 to 2011 and published in 2012 (STEPS study). Eighty-six patients receiving PS were enrolled: 43 patients underwent management with TED, and 43 were treated with placebo (PBO) for 24 wk. In the TED group, 63% of patients were responders who reduced their PS by at least 20% compared to 30% of patients in the PBO group (P = 0.02). The secondary outcomes of this study were the reduction of parenteral nutrition volume at week 24 (2.1 litres per week difference between the TED group and PBO, $P < 0.05$) and the number of patients achieving at least 1 d off parenteral nutrition per week (21/39 in the TED group *vs* 9/39 in the PBO group)[65].

The STEPS study was followed by STEPS-2, which was a 2-year, open-label extension of the prior study. The enrolled patients completed 24 wk of TED (TED/TED) or PBO (PBO/TED) in the initial PBO-controlled study or qualified for the original study but were not treated (NT/TED) because of full enrolment. Patients received 0.05 mg/kg/d subcutaneous TED for up to 24 mo (NT/TED and PBO/TED) or up to 30 mo (TED/TED). In patients who completed the study, clinical response (as defined in the STEPS study) was achieved in 28/30 (93%) TED/TED, 16/29 (55%) PBO/TED, and 4/6 (67%) NT/TED patients. The mean PS volume reductions from baseline were 7.6 (66%), 3.1 (28%), and 4.0 (39%) litres/week in the TED/TED, PBO/TED, and NT/TED groups, respectively. Thirteen patients

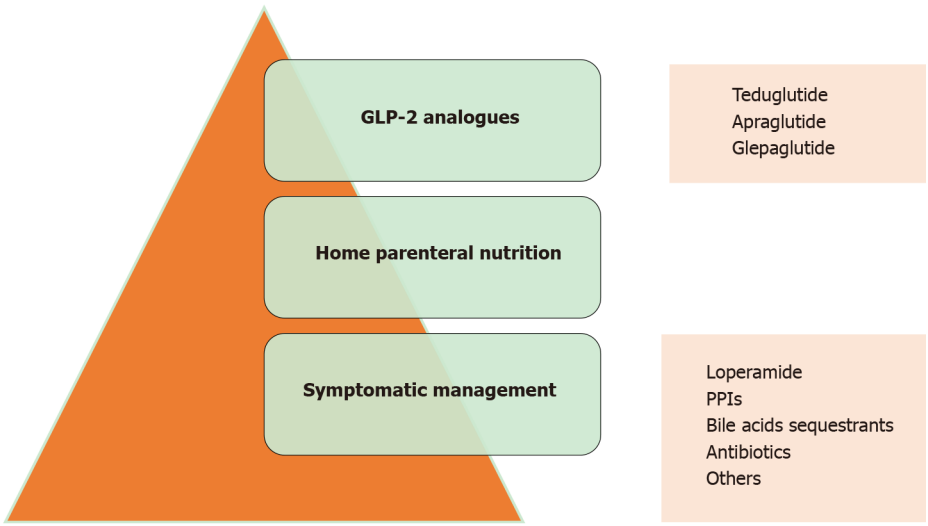

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Figure 1 Step-up approach in the management of short bowel syndrome-intestinal failure. GLP-2: Glucagon-like peptide-2; PPIs: Proton-pump inhibitors.

achieved full enteral autonomy. This trial demonstrated the long-term effectiveness and safety of TED [66].

A further extension of STEPS and STEPS-2 studies is the STEPS-3 study. Long-term treatment with TED in this 1-year, open-label extension trial showed a safety profile consistent with previous studies, with prolonged effectiveness and a further reduction in HPN necessity[67]. A post hoc analysis determined factors associated with the response to TED. Notably, the remaining bowel anatomy, rather than the remaining bowel length, primarily affected the outcome of TED therapy. Patients with jejunostomy or ileostomy (SBS-IF type 1 according to the anatomical classification) experienced the most pronounced benefit from TED compared to patients with colon continuity. The same study outlined how patients with higher baseline PS volume had higher reductions in PS, which means that SBS–IF patients with more severe malabsorption benefit the most from therapy with TED[68].

Safety data from clinical trials of TED reported abdominal distension and gastrointestinal stoma complications as the most frequent adverse events (AEs), and the AE frequency was higher early in the course of treatment and declined thereafter[69]. The capacity of GLP-2 to accelerate the growth of experimental intestinal tumors was known before the introduction of TED[70,71]. A post hoc analysis of STEPS studies found that 50 of 65 patients with remnant colon (77%) underwent a protocol-mandated postexposure colonoscopy. Colon polyps were reported in 12% (9/73) of patients at baseline and 18% (9/50) of patients postexposure. Two patients had polyps at baseline and postexposure. Histology was available for 7 patients: 5 had adenomas (1 serrated, 4 tubular), and none had malignancies or high-grade dysplasia[72].

A large population analysis characterizing 170 patients treated with TED from the United States from 2015 to 2020 found that an overall 5.9% of patients developed posttreatment polyps of the large intestine. Twenty events (12%) of catheter infection were described in the same study within 1 year from the start of administration. Other documented adverse effects were abdominal pain (70 cases, 41.2%), nausea (40 cases, 23.5%), intestinal obstruction (30 cases, 17.6%) and stoma complications (20 cases, 11.8%). Ten patients (6%) developed colon polyps[73].

A French real-life study reported a population of 54 patients with SBS-IF treated with TED. The effectiveness and safety were fully confirmed. Twenty-four weeks after the start of treatment, 85% of patients experienced a reduction in PS of at least 20%, and 24% of patients were weaned off PS[74]. Another real-life cohort of 18 patients treated with TED for a median of 3.2 years was reported. Twelve of these patients achieved a clinical response at 12 mo, which was maintained in most of these patients at 36 mo. Five patients were able to wean off PS, but no predictor was identified due to the small sample[75].

New GLP-2 analogue molecules were studied recently. Apraglutide (TA799-007), which is characterized by high protein binding, resistance to DPP4 cleavage, low clearance and slow absorption after subcutaneous administration, has been highlighted[76]. These features make apraglutide clinically appealing, which suggests the possibility of more delayed dose administration[77].

Apraglutide showed efficacy in studies of animal models of SBS-IF in terms of increases in intestinal length and weight, crypt weight and villous height and lower faecal fat and energy losses[78,79]. The effects on intestinal length and mucosal hypertrophy after discontinuation of TED and apraglutide were also studied in piglets. These two molecules apparently showed similar results in this case: Intestinal growth appeared to be a lasting outcome of treatment with long-acting GLP-2 and persisted for at least 7 d after discontinuation. In contrast, mucosal hypertrophy appeared to regress 7 d after the end of

treatment with both agents[80]. However, these results must be interpreted with caution and need further confirmation. The once weekly administration of apraglutide to neonatal piglets showed superior intestinotrophic effects than a single daily dose of TED and led to increased bowel length and villus height[81].

A recent PBO-controlled, randomized, phase II clinical trial treated eight adults with SBS-IF with once-weekly 5-mg apraglutide or PBO for 4 wk, followed by once-weekly 10-mg apraglutide doses for 4 wk, with a washout period of 6-10 wk between treatments. No severe AEs related to the drug were detected, and comparable safety profiles were observed between the low-dose and high-dose regimens. The main AE detected was an increase in urine output, and the following less frequent AEs were reported in decreasing order: Stoma complications, decreased thirst, oedema, increased weight, and injection site reactions[82]. A phase 3 study in SBS-IF patients treated with apraglutide *vs* PBO is currently ongoing[83].

Glepaglutide is another long-acting analogue of GLP-2 that chemically differs from native GLP-2 by 9 amino acid substitutions and a C-terminal tail consisting of six lysine residues. It has a half-life of approximately 50 h and has been evaluated in a phase II study, where it demonstrated good tolerance and the ability to improve intestinal absorption in SBS patients treated with 1 mg and 10 mg daily glepaglutide[84]. A phase 3 study comparing weekly or twice weekly glepaglutide and PBO administration to SBS patients is currently ongoing[85].

Other molecules are in the preclinical phase of their development. Elsiglutide is a GLP-2 analogue with a long half-life that reduced diarrhoea induced by lapatinib, a tyrosine kinase inhibitor, in a rat model[86]. Dapiglutide is a dual GLP-1 and GLP-2 agonist that showed beneficial effects in a rat model of SBS by improving body weight, promoting intestinal growth, increasing villous height and intestinal length, and reducing watery stool losses[87].

For the duration of therapy with GLP-2 analogues in patients with SBS-IF, the available data indicate that these drugs must be administered over a long life because reversal of the previous need for PS occurs if TED is discontinued[64]. A recent report described 13 patients (one with CD and one with ulcerative colitis) who discontinued TED after a successful clinical outcome. The volume of PS remained stable in the first 4 years but later increased in 12/13 patients up to 9 years after withdrawal[88]. These data support further studies exploring the possibility of the periodic use of GLP-2 analogues for selected SBS-IF patients.

GLP-2 ANALOGUES IN CROHN'S DISEASE

Knowledge of GLP-2 analogue use in patients with CD is limited. No RCTs specifically address the efficacy and safety of GLP-2 in CD patients with SBS-IF. However, 18 patients with CD were included in the 86 subjects of the STEPS study, 10 in the TED arm and 8 in the PBO arm[65]. Sixteen CD patients were included in the STEPS-2 long-term study[66]. The CD patients in these studies were in the inactive phase of their inflammatory disease, and the concomitant use of immunosuppressants and biologics were exclusion criteria. The subsequent post hoc analysis of the STEPS studies analysing factors associated with the response to TED in patients with SBS-IF found that the effects of TED on PS volume reduction were more pronounced in patients with jejunostomy/ileostomy than in patients with partial colon continuity. Only 10.5% of CD patients had colon in continuity in this study. Therefore, CD patients were likely among the best responders according to this analysis[68].

Subsequent data from real-life studies reported the experiences of TED treatment for CD patients treated with immunosuppressants and biologics and patients with active inflammation. A retrospective cohort study published in 2017 analysed 13 patients with CD and SBS-IF treated with TED for an average of 1 year. Nine of these patients were under parenteral nutrition before beginning TED therapy, and only 1 of them needed parenteral nutrition after TED. All of these patients required intravenous fluids before TED, but only 7 of them required this supplementation after TED. Eight of these patients were treated with biological therapies and/or immunosuppressants. TED was well tolerated in most of these CD patients, with the exception of one patient who intermittently presented with obstructive symptoms that required the cessation of therapy[89].

Two other active CD cases treated with TED were described. The first case was a 38-year-old patient with relapsing perianastomotic disease treated with ustekinumab. This patient received PN daily for SBS and was weaned off after 7 mo of TED. The second patient was 39 years old and had received PN daily since 2003. This patient was treated with vedolizumab and 6-mercaptopurine. After multiple resections, he was left with a jejunocolic anastomosis and 60 cm of residual small bowel length. PN was reduced to 1 night per week after TED[90].

One case of long-term TED use (54 mo) was reported in a patient with multidrug-resistant CD, and beneficial effects on nutritional status, a significant anti-inflammatory effect and reduction in CD clinical activity were observed[91]. A recent real-life cohort of 52 patients successfully treated with TED found that 16 had CD[74]. Another real-life series of 18 patients on TED found that 10 patients had SBS-IF caused by CD, and the 5 who were able to withdraw PS were all CD patients[75]. The findings of these studies are presented in Table 1.

Table 1 Main studies and case reports on glucagon-like peptide-2 analogues in Crohn's disease with short bowel syndrome

Ref.	Molecule tested	Number of patients	Number of CD patients	Study type	Main results	Adverse events
Jeppesen *et al*[64], 2005	Teduglutide	16	12 (in clinical remission)	Pilot open label, phase II	Increased wet weight absorption; decreased urine weight and urine sodium excretion; decreased fecal wet weight and fecal energy content; increased villus height, crypt depth and mitotic index in end-jejunostomy patients	Enlargement of the stoma nipple; mild lower leg oedema; severe AE (dehydration, sepsis, CS) in 4/16, not judged to be related to the drug
Jeppesen *et al*[65], 2012	Teduglutide	43 (+ 43 PBO)	10 (in clinical remission)	Multicenter, randomized, double blind, PBO-controlled phase III	63% of TED patients had a ≥ 20% reduction of PS volume at week 24 (significant versus 30% of the PBO group); increased serum citrulline (index of intestinal mucosa mass)	Mostly mild GI symptoms (abdominal pain, nausea, stoma complication, or abdominal distension); 7/43 CS; not different from PBO
Schwartz *et al*[66], 2016	Teduglutide	88	16	2 yr open label extension study	Clinical response (≥ 20% reduction of PS volume) in 28/30 (93%) and 66% of PS volume reduction in TED/TED group; 13 reached enteral autonomy	34%abdominal pain; 25% episodes of weight decrease; 39% infections (SAE); 2 CD exacerbations (12% of CD, SAE)
Kochar *et al* [89], 2017	Teduglutide	13	13 (8 on biologics and/or IS)	Retrospective cohort study (median duration 1 yr)	9 patients on PN at the beginning of therapy; 1 patient still on PN at the end of therapy; PS reduced from median 9000 mL/wk to 3100 mL/wk, 6 patients no PS at the end	Among non-immunosuppressed (5) only 2 minor AE and 1 CS; among immunosuppressed (8) minor AE, 3 CS and 2 pancreatitis
Barberio *et al*[94], 2019	Teduglutide	1	1 (on EN and adalimumab)	Case report	EN reduction of 50% after 24 wk of TED; EN suspension after 72 wk of treatment	Transient nausea and mild abdominal pain and nausea
Al Draiweesh *et al*[90], 2019	Teduglutide	2	2 (on biologics)	Case report	Weaning off from PS after 7 mo of TED; improvement in oral intake, reduced stool output, and weight gain; reduction of PN to 1 night/wk	Any reported
Naimi *et al* [84], 2019	Glepaglutide	16	8	Double-blind randomised phase II trial	1 mg daily glepaglutide reduces the fecal output by 592 mg/d; 10 mg daily glepaglutide reduces the fecal output by 833 mg/d	Stoma complications (73%); injection site reactions (61%); peripheral edema (56%); nausea and abdominal pain (44%); SAEs (stoma obstruction, sepsis) in 4 patients
Joly *et al* [74], 2020	Teduglutide	54	16	Retrospective multicenter real life cohort	85% of patients were responders (PS reduction ≥ 20%) at week 24; 24% weaned off PS at week 24	Not specifically collected
Borghini *et al*[91], 2020	Teduglutide	1	1 (clinically active)	Case report	At least 20% reduction in PS; daily Kcal intake reduction of 15%; reduction in CD activity and severity	Two mild CVC-related infections
Mouillot *et al*[93], 2020	Teduglutide	1	1	Case report	Reduction of PS and increase of nutrients absorption; hypertrophy of villi at capsule endoscopy; increase in the size of intestinal villi and crypts assessed by biopsy	Not specifically assessed
Puello *et al* [75], 2021	Teduglutide	18	10	Retrospective single center real life cohort	Response (reduction of > 20% PS) in: 16 (75%) patients at 12 mo; 10 of 13 (76.9%) patients at 24 mo; 7 of 10 (70%) patients at 36 mo; 3 of 3 (100%) patients at 60 mo; 5 patients (all CD) weaned off PS	Abdominal pain or cramping (3); volume overload (1); taste loss (1); legs edema (2); increase in ostomy size (2)
Eliasson *et al*[82], 2022	Apraglutide	8	3 (in clinical remission)	Open label phase I-II trial	Weekly 5 mg apraglutide increases urinary output by 714 mL/d; weekly 10 mg apraglutide increases urinary output by 795 mL/d	Polyuria (7/8); any stoma complication (6/8); thirst decrease (4/8) and appetite decrease (3/8); edema (4/8); no SAE related to the drug

CD: Crohn's disease; PN: Parenteral nutrition; PS: Parenteral support; EN: Enteral nutrition; TED: Teduglutide; PBO: Placebo; GI: Gastrointestinal; SAEs: Severe adverse events; CS: Catheter-related sepsis; CVC: Central venous catheter; IS: Immunosuppressives; AE: Adverse events.

Other retrospective and prospective cohorts of SBS-IF patients treated with TED (including a variable proportion of CD patients) were described and recently summarized in a meta-analysis that confirmed the efficacy of TED in adult SBS-IF patients[92]. This analysis suggested a non-significant trend of CD aetiology of SBS-IF as a positive predictor of response and weaning from PS.

A case of intestinal adaptation induced by TED in CD was recently described. The case was a 40-year-old female patient suffering from SBS-IF due to CD since 2001. The patient underwent terminal jejunostomy, with 100 cm of jejunum remaining. SBS and IF resulted, and the woman needed continuous PS. She was recommended to start treatment with TED, and her intestinal status and disease activity were studied at baseline. No active CD was detected, and the intestinal villi appeared normal. After 1 year of continued TED treatment, a new biopsy of intestinal villi was performed and showed an increase of 33% in the length of villi[93].

The efficacy of off-label TED use in a 65-year-old CD patient treated with adalimumab with SBS and enteral nutrition was also described, and the patient was weaned off enteral nutrition after 72 wk[94]. Concerning TED discontinuation, a report described 2 CD patients on chronic TED treatment who were not able to tolerate even a few days withdrawal of the drug[95].

One line of research attempted to determine whether TED had anti-inflammatory activity in CD based on the results of the use of glucagon-like peptides in animal models of colitis[96]. An RCT was performed on 100 CD patients with moderately to severely active disease who were treated with TED (0.05, 0.10, or 0.20 mg/kg) daily for 8 wk or PBO. Seventy-one patients completed the study, and the results showed numerically higher response and remission rates in all treatment groups compared to PBO, but these differences were not statistically significant. The results were more substantial in the group treated with the higher dose (44% response and 32% remission *vs* 32% response and 20% remission in the PBO group). It is questionable whether the clinical disease activity indices modifications described in this study were mostly due to the effect of TED on diarrhoea because no significant modification of C-reactive protein was detected. AEs were not different between the PBO and treatment groups[97]. This last result is particularly relevant, because it came from a controlled study on a relevant number of patients with active CD, although not affected by SBS-IF. The good safety profile in this setting justifies the use of GLP-2 analogues in patients with SBS-IF and active CD, but more data on this specific population are needed. Data on the use of the new GLP-2 analogues (apraglutide and glepaglutide) in CD are lacking.

CONCLUSION

SBS-IF is a rare condition that affects patients who undergo several intestinal resections due to CD and other gastrointestinal conditions. Although surgery rates in CD have declined in recent decades due to improved diagnostic and pharmacological modalities, the rate of CD patients developing SBS-IF (albeit low) has not been reduced. Several mechanisms are adopted by organisms to adapt to intestinal functional reduction. However, these adaptations are often not sufficient to avoid the clinical impact and long-term complications of SBS-IF, and several patients require HPN. The main therapeutic approaches consist of PS and symptomatic therapy. Yet, PS is associated with several AEs that complicate patient management, such as catheter-related complications, liver and metabolic disease, iron deficiency anaemia, and manganese toxicity.

New molecules and therapeutic approaches have been studied in recent years. The GLP-2 analogue TED demonstrated a relevant clinical utility for SBS-IF patients, and it significantly reduced HPN volume and/or days of infusion, allowing enteral autonomy in some patients. However, because this therapy is likely life-long or of long duration, more data are needed on the long-term safety and cost-effectiveness. Recent further consideration has been given to new GLP-2 analogues that have undergone phase I and II studies, including apraglutide and glepaglutide. These agents showed low clearance and slow absorption after subcutaneous administration, which allowed for a single weekly administration. Relatively scant data are available on the use of GLP-2 analogues in CD patients with SBS-IF. However, signals from RCTs and real-life observations indicate that TED is efficacious and well tolerated by CD patients, even if they are being treated with immunosuppressants and/or biological agents. More data are needed on the use of GLP-2 analogues in patients with active CD to clarify their safety and efficacy in this setting. This new pharmacological approach may improve the quality of life of patients with CD and SBS-IF and reduce their dependence on artificial nutrition. Clinical studies specifically addressing this peculiar population are warranted.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Postoperative outcomes and recurrence patterns of intermediate-stage hepatocellular carcinoma dictated by the sum of tumor size and number

Xin-Sheng Hu, Hui-Yuan Yang, Chao Leng, Zhi-Wei Zhang

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Abstract

BACKGROUND

The selection criteria for Barcelona Clinic Liver Cancer (BCLC) intermediate-stage hepatocellular carcinoma (HCC) patients who would truly benefit from liver resection (LR) remain undefined.

AIM

To identify BCLC-B HCC patients more suitable for LR.

METHODS

We included patients undergoing curative LR for BCLC stage A or B multinodular HCC (MNHCC) and stratified BCLC-B patients by the sum of tumor size and number (N + S). Overall survival (OS), recurrence-free survival (RFS), recurrence-to-death survival (RTDS), recurrence patterns, and treatments after recurrence in BCLC-B patients in each subgroup were compared with those in BCLC-A patients.

RESULTS

In total, 143 patients who underwent curative LR for MNHCC with BCLC-A (n = 25) or BCLC-B (n = 118) were retrospectively analyzed. According to the N + S, patients with BCLC-B HCC were divided into two subgroups: BCLC-B1 (N + S ≤ 10, n = 83) and BCLC-B2 (N + S > 10, n = 35). Compared with BCLC-B2 patients, those with BCLC-B1 had a better OS (5-year OS rate: 67.4% *vs* 33.6%; P < 0.001), which was comparable to that in BCLC-A patients (5-year OS rate: 67.4% *vs* 74.1%; P = 0.250), and a better RFS (median RFS: 19 mo *vs* 7 mo; P < 0.001), which was worse than that in BCLC-A patients (median RFS: 19 mo *vs* 48 mo; P = 0.022). Further analysis of patients who developed recurrence showed that both BCLC-B1 and BCLC-A patients had better RTDS (median RTDS: Not reached *vs* 49 mo; P =

0.599), while the RTDS in BCLC-B2 patients was worse (median RTDS: 16 mo *vs* not reached, $P < 0.001$; 16 mo *vs* 49 mo, $P = 0.042$). The recurrence patterns were similar between BCLC-B1 and BCLC-A patients, but BCLC-B2 patients had a shorter recurrence time and a higher proportion of patients had recurrence with macrovascular invasion and/or extrahepatic metastasis, both of which were independent risk factors for RTDS.

CONCLUSION

BCLC-B HCC patients undergoing hepatectomy with N + S ≤ 10 had mild recurrence patterns and excellent OS similar to those in BCLC-A MNHCC patients, and LR should be considered in these patients.

Key Words: Hepatocellular carcinoma; Multinodular; Intermediate-stage; Liver resection; Recurrence pattern; Prognosis

Core Tip: Subgroups of Barcelona Clinic Liver Cancer (BCLC) intermediate-stage hepatocellular carcinoma (HCC) patients who would truly benefit from liver resection (LR) remain undefined. We demonstrated that the sum of tumor size and number (N + S) can predict not only prognosis in BCLC-B patients undergoing LR, but also the recurrence patterns and recurrence-to-death survival (RTDS) in these patients. In addition, we indicated that BCLC-B patients undergoing hepatectomy with N + S ≤ 10 had mild recurrence patterns, good RTDS and excellent overall survival similar to those in BCLC-A multinodular HCC patients. The results of this study are helpful in selecting BCLC-B patients more suitable for LR.

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INTRODUCTION

As the sixth most common cancer globally, primary liver cancer accounted for 906,000 newly confirmed cancer cases and 830,000 cancer-related deaths worldwide in 2020, of which 75%-85% were hepatocellular carcinoma (HCC)[1].

Barcelona Clinic Liver Cancer (BCLC) staging system, which was proposed in 1999, has been widely used to guide treatment decisions in patients with HCC[2,3]. The 2022 version of the BCLC strategy recommends liver transplantation (LT), transarterial chemoembolization (TACE), and systemic therapy, respectively, for BCLC intermediate-stage HCC patients based on their expected survival time[4].

In addition, emerging studies have suggested that liver resection (LR) may also be a good treatment option for BCLC-B HCC patients[5,6]. Nevertheless, the subgroups of BCLC-B HCC patients who would truly benefit from LR have yet to be defined. Several previous studies found that some BCLC-B HCC patients undergoing LR had favorable long-term overall survival (OS) rates (5-year OS rates: 50%-75%); however, these selected patients still had high postoperative recurrence rates (2-year recurrence rate: ≥ 50%), which means that many of these patients had good recurrence-to-death survival (RTDS)[7,8]. Both recurrence patterns and treatments after recurrence can affect the RTDS of HCC patients who develop recurrence after LR[9-11]. However, previous studies did not analyze the main reasons why these selected patients had good RTDS, which may affect the judgment of the role of LR in these patients[7,8].

In this study, we retrospectively included patients undergoing curative LR for BCLC stage A or B multinodular HCC (MNHCC) and stratified the BCLC-B patients by the sum of tumor size and number (N + S), which combines the two main prognostic factors of BCLC-B patients into a continuous variable [7,8]. BCLC-B patients more suitable for LR were identified by comparing the outcomes, recurrence patterns, and treatments after recurrence in BCLC-B patients in each subgroup with those in BCLC-A patients.

MATERIALS AND METHODS

Patients

We enrolled BCLC stage A or B MNHCC patients who underwent curative LR in Tongji Hospital from January 2010 to May 2018. The inclusion criteria were: (1) MNHCC pathologically diagnosed with two or more nodules, in which lesions less than 1 cm in diameter and less than 2 cm away from the main nodule were defined as satellite nodules[12]; (2) Curative resection, defined as complete macroscopic removal of all tumors with negative histologic resection margins for the tumors (R0 resection)[13,14]; and (3) No preoperative anticancer treatment other than TACE. The exclusion criteria were: (1) Recurrent HCC or combined HCC and cholangiocarcinoma; and (2) Complicated with other malignancies.

Data collection

Patient data at the time of initial hepatectomy including sex, age, body mass index, hepatitis B antigen status, liver function, tumor characteristics, surgical procedure, and preoperative treatment were recorded. Liver function in this study was classified by the albumin-bilirubin score[15]. Maximum tumor size was defined as the maximum diameter of the largest tumor. Microvascular invasion was defined as tumor within a vascular space lined by endothelium that was visible only on microscopy[16].

In addition, the recurrence patterns, which consisted of recurrence time and tumor characteristics at the time of recurrence, and treatments after recurrence in those patients who developed recurrence during follow-up were also recorded. Recurrence time was defined as the time between initial LR and the first recurrence.

Initial hepatectomy

In our center, we routinely estimated the residual liver volume in MNHCC patients before hepatectomy, and only patients with residual liver volume of more than 40% of the standard liver volume (for patients with liver cirrhosis) or more than 30% (for patients without liver cirrhosis) would receive LR [17,18]. The decision to perform anatomical or non-anatomical hepatectomy depended largely on the tumor distribution, and major resection was defined as the resection of three or more Couinaud liver segments[19]. Intraoperative ultrasound was routinely used to locate the tumor and screen the nodules. All nodules were completely resected intraoperatively and negative margin was determined according to postoperative pathology.

Follow-up

Patients were followed every month with measurement of serum alpha-fetoprotein (AFP), chest radiography and ultrasound or computed tomography (CT) or magnetic resonance imaging (MRI) in the first 6 mo after discharge from hospital, and every 3-6 mo thereafter. When HCC recurrence was confirmed by CT or MRI, patients were treated with repeated hepatectomy, ablation, TACE or systemic therapy. Follow-up was terminated on May 15, 2022.

Recurrence-free survival (RFS) was calculated from the date of hepatectomy until recurrence or last follow-up. OS was defined as the time from LR to death or last follow-up, and RTDS was defined as the time from recurrence to death or last follow-up.

Statistical analysis

Continuous variables were presented as mean ± SD or median (interquartile range; IQR). Categorical variables were described by frequency and percentage. In the comparison of different subgroups, continuous variables were compared using the Student's t or Mann-Whitney U test, and categorical variables using the χ^2 or Fisher's exact test, as appropriate. Survival was analyzed by the Kaplan-Meier method, and survival curves were compared by the log-rank test. Univariate and multivariate analyses were based on the Cox proportional analysis. Variables with P values less than 0.1 identified by the univariate analysis were included in multivariate analysis. The cutoff value of N + S was determined by X-tile, a bioinformatics tool produced by Camp and colleagues[20]. The area under receiver operating characteristic (ROC) curve (AUC) was compared using DeLong test[21]. $P < 0.05$ was considered to indicate statistical significance. Both SPSS (version 23.0, SPSS, Inc., Chicago, IL, United States) and MedCalc software (version 20.115, MedCalc Software, Ostend, Belgium) were used for the analysis.

RESULTS

Variables and outcomes of the entire cohort

A total of 143 patients who underwent curative LR for BCLC stage A or B MNHCC were enrolled. Their mean age was 52.1 years and most patients were male (n = 134, 93.7%) and were hepatitis B surface antigen positive (n = 131, 91.6%). Median maximum tumor size in the entire cohort was 5.6 cm (IQR: 3.4–7.6) and tumor number in the vast majority of patients was ≤ 3 (n = 136, 95.1%). Overall, 17.5% (n = 25) of patients had BCLC-A MNHCC, and 82.5% (n = 118) had BCLC-B MNHCC (Table 1).

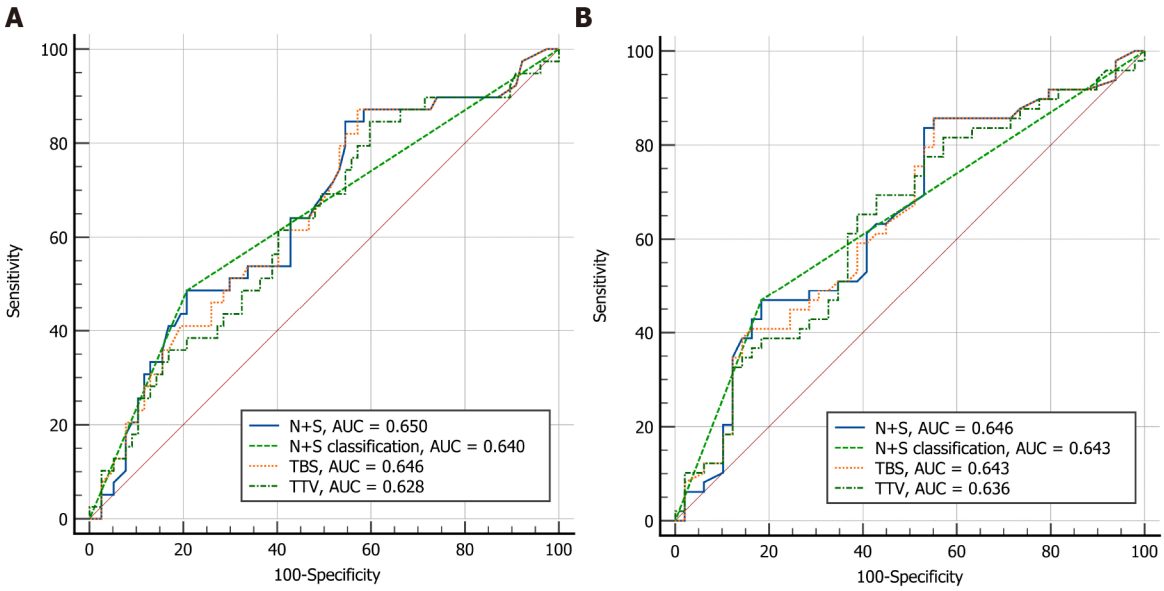

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Figure 1 The receiver operating characteristic analysis of the sum of tumor size and number, the classification of the sum of tumor size and number, tumor burden score and total tumor volume in intermediate-stage hepatocellular carcinoma patients. A: 3-year overall survival (OS); B: 5-year OS. N + S: The sum of tumor size and number; TBS: Tumor burden score; TTV: Total tumor volume; AUC: Area under receiver operating characteristic curve.

After a median follow-up of 54 mo (IQR 27–79), 5-year OS and RFS after R0 resection in all patients were 60.2% and 23.2%, respectively. Of note, BCLC-B patients had worse 5-year OS (57.2% *vs* 74.1%, P = 0.028, Supplementary Figure 1A) and RFS (19.4% *vs* 41.6%, P = 0.002, Supplementary Figure 1B).

Stratification of BCLC-B patients based on N + S

Among patients undergoing LR for BCLC-B HCC, the median maximum tumor size was 6.2 cm (IQR: 4.1–8.4) and tumor number in 111 (94.1%) patients was ≤ 3. Of note, 43.2% (n = 51) of patients had bilateral disease and 14.4% (n = 17) of patients underwent TACE before initial LR (Table 1).

Using the X-tile program[20], patients with BCLC-B HCC were divided into two groups by N + S: BCLC-B1 (≤ 10, n = 83, 70.3%), BCLC-B2 (> 10, n = 35, 29.7%) (Supplementary Figure 2).

The prognostic ability of N + S and the rationality of the cut-off value of 10 were then verified by time-dependent ROC curves and Cox-regression analysis. The AUCs for 3-year and 5-year OS in BCLC-B patients were 0.650 and 0.646, respectively, for N + S, and 0.640 and 0.643, respectively, for stratification according to N + S (Figure 1). Multivariate analysis showed that N + S > 10 was an independent risk factor for OS [hazard ratio (HR) 2.996, 1.779 to 5.045; P < 0.001] (Table 2) and RFS (HR 1.657, 1.057 to 2.596; P = 0.028) (Table 3) in BCLC-B patients.

In addition, we compared the predictive accuracy of N + S with those of tumor burden score (TBS) and total tumor volume (TTV), both of which were previous prognostic models based on tumor size and number of HCC patients[22,23]. The results showed that the AUCs of N + S at 3 and 5 years were both similar to those of TBS (3-year AUC, 0.650 *vs* 0.646, P = 0.552; 5-year AUC, 0.646 *vs* 0.643, P = 0.762) and TTV (3-year AUC, 0.650 *vs* 0.628, P = 0.171; 5-year AUC, 0.646 *vs* 0.636, P = 0.535) (Figure 1).

Comparison of the clinical characteristics, OS, and RFS among BCLC-A, BCLC-B1 and BCLC-B2 patients

Clinical characteristics, OS and RFS of patients with BCLC-B1, BCLC-B2, and BCLC-A were compared (Figure 2, Supplementary Table 1). The results showed that BCLC-B2 patients had a higher serum AFP level and a larger proportion of bilateral tumor distribution, compared to patients with BCLC-A and BCLC-B1. With an increase in N + S, the maximum tumor size gradually increased, and a larger proportion of patients underwent major resection (Supplementary Table 1).

Both BCLC-A patients and BCLC-B1 patients had good 5-year OS (74.1% *vs* 67.4%, P = 0.250), which was better than that in BCLC-B2 patients (74.1% *vs* 33.6%, P < 0.001; 67.4% *vs* 33.6%, P < 0.001) (Figure 2A). Compared with BCLC-A patients, BCLC-B1 patients had a worse RFS (median RFS: 19 mo *vs* 48 mo; P = 0.022), which was better than that in BCLC-B2 patients (median RFS: 19 mo *vs* 7 mo; P < 0.001) (Figure 2B).

Table 1 Characteristics of patients with Barcelona Clinic Liver Cancer stage A or B multinodular hepatocellular carcinoma, *n* (%)

Variables	Total (*n* = 143)	BCLC-A (*n* = 25)	BCLC-B (*n* = 118)	*P* value
Male gender	134 (93.7)	25 (100)	109 (92.4)	0.330
Age (yr)	52.1 ± 12.7	50.5 ± 14.5	52.4 ± 12.4	0.490
BMI	22.97 ± 3.15	23.05 ± 3.35	22.96 ± 3.12	0.895
HBs-Ag positive	131 (91.6)	24 (96)	107 (90.7)	0.635
Albumin (g/L)	38.89 ± 4.51	39.95 ± 5.15	38.65 ± 4.34	0.194
Bilirubin (μmol/L)	13.8 (9.9, 18)	12.9 (10.2, 20.9)	13.9 (9.7, 17.8)	0.568
ALBI grade				0.680
1	69 (48.3)	13 (52)	56 (47.5)	
2/3	74 (51.7)	12/0 (48/0)	62/0 (52.5/0)	
AFP (μg/L)	239 (13, 2338)	74 (6, 390)	483 (16, 2944)	0.011
Maximum tumor size (cm)	5.6 (3.4, 7.6)	2.5 (2.1, 2.9)	6.2 (4.1, 8.4)	< 0.001
Tumor number				0.460
≤ 3	136 (95.1)	25 (100)	111 (94.1)	
> 3	7 (4.9)	0	7 (5.9)	
Tumor distribution				0.506
Unilateral	83 (58)	16 (64)	67 (56.8)	
Bilateral	60 (42)	9 (36)	51 (43.2)	
Presence of microvascular invasion	15 (10.5)	1 (4)	14 (11.9)	0.420
Edmondson-Steiner grade				0.337
I-II	85 (59.4)	17 (68)	68 (57.6)	
III-IV	58 (40.6)	8 (32)	50 (42.4)	
Major resection	64 (44.8)	4 (16)	60 (50.8)	0.001
Anatomical hepatectomy	22 (15.4)	4 (16)	18 (15.3)	1.000
Preoperative TACE				0.690
No	121 (84.6)	20 (80)	101 (85.6)	
Yes	22 (15.4)	5 (20)	17 (14.4)	

BMI: Body mass index; HBs-Ag: Hepatitis B surface antigen; ALBI: Albumin-bilirubin; AFP: Alpha-fetoprotein; IQR: Interquartile range; TACE: Transarterial chemoembolization.

Comparison of recurrence patterns, treatments after recurrence, and RTDS in BCLC-A, BCLC-B1 and BCLC-B2 patients

During follow-up, 14 (56%) BCLC-A, 66 (79.5%) BCLC-B1 and 34 (97.1%) BCLC-B2 patients developed recurrences ($P < 0.001$). Nine BCLC-B1 and 4 BCLC-B2 patients who lacked information on tumor characteristics at the time of recurrence and treatments after recurrence were excluded from the analysis. Ultimately, 14 BCLC-A, 57 BCLC-B1 and 30 BCLC-B2 patients with recurrence were included in the analysis. The recurrence patterns and treatments after recurrence in these patients are summarized in Supplementary Table 2.

Compared with BCLC-A and BCLC-B1 patients, BCLC-B2 patients had a shorter recurrence time and a higher proportion of recurrence with macrovascular invasion and/or extrahepatic metastasis. However, there were no significant statistical differences in recurrence patterns and treatment after recurrence between BCLC-B1 and BCLC-A patients. Fewer BCLC-B2 patients underwent curative treatments after recurrence than BCLC-A patients, but the treatment after recurrence was similar between BCLC-B2 patients and BCLC-B1 patients (Supplementary Table 2).

Both BCLC-B1 and BCLC-A patients had good RTDS (median RTDS: Not reached, *vs* 49 mo for BCLC-B1 and BCLC-A patients, respectively; $P = 0.599$), while BCLC-B2 patients had a worse RTDS (16 mo *vs* not reached, $P < 0.001$; 16 mo *vs* 49 mo, $P = 0.042$) (Figure 3).

Table 2 Univariate and multivariate analysis of overall survival in patients with Barcelona Clinic Liver Cancer stage B hepatocellular carcinoma

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Gender (male)	1.128 (0.409-3.117)	0.816		
Age (> 65 yr)	0.668 (0.285-1.562)	0.352		
BMI > 25	0.954 (0.513-1.772)	0.880		
HBs-Ag positive	1.084 (0.466-2.525)	0.851		
ALBI grade				
1	1.00 (Reference)		1.00 (Reference)	
2	1.891 (1.112-3.217)	0.019	2.279 (1.236-4.201)	0.008
AFP > 400 ng/mL	1.969 (1.165-3.327)	0.011		
Maximum tumor size > 5 cm	2.510 (1.354-4.651)	0.003		
Tumor number > 3	3.806 (1.716-8.444)	0.001	5.519 (2.207-13.803)	< 0.001
N + S > 10	3.403 (2.031-5.702)	< 0.001	2.996 (1.779-5.045)	< 0.001
Bilateral tumor distribution	2.201 (1.312-3.694)	0.003		
Presence of MVI	1.816 (0.855-3.858)	0.120		
Edmondson-Steiner III-IV	2.084 (1.248-3.480)	0.005	2.051 (1.219-3.449)	0.007
Major resection	1.886 (1.115-3.191)	0.018		
NAH	0.905 (0.458-1.788)	0.775		
Preoperative TACE				
No	1.00 (Reference)			
Yes	0.494 (0.198-1.238)	0.132		

BMI: Body mass index; HBs-Ag: Hepatitis B surface antigen; ALBI: Albumin-bilirubin; AFP: Alpha-fetoprotein; N + S: The sum of tumor size and number; MVI: Microvascular invasion; NAH: Non-anatomical hepatectomy; TACE: Transarterial chemoembolization.

Independent risk factors for RTDS

We further conducted a multivariate analysis of the factors affecting RTDS of BCLC stage A or B MNHCC patients undergoing LR. Multivariate analysis showed that initial tumor with BCLC-B2 (N + S > 10) (HR 2.696, 1.468 to 4.953; $P = 0.001$), recurrence within 2-year (HR 4.353, 1.024 to 18.503; $P = 0.046$), recurrent tumor number > 3 (HR 3.247, 1.629 to 6.474; $P = 0.001$), recurrence with macrovascular invasion and/or extrahepatic spread (HR 2.894, 1.458 to 5.746; $P = 0.002$) and noncurative treatments after recurrence (HR 2.423, 1.209 to 4.854; $P = 0.013$) were independent risk factors for RTDS (Supplementary Table 3).

DISCUSSION

The role of LR in BCLC-B HCC patients is unclear. Although the latest BCLC staging system still does not recommend LR for BCLC-B patients, the results of emerging studies have indicated that LR resulted in a good 5-year OS for BCLC-B HCC patients[4-6]. In this study, patients who underwent LR for BCLC-B HCC had an overall 5-year OS rate of 57.2%, which demonstrated that LR was a good treatment option in these patients.

To select BCLC-B patients more suitable for LR, we stratified these patients according to N + S, which has been used to select HCC patients who are more suitable for LT and for TACE[24,25]. In fact, Matsukuma *et al*[26] suggested that N + S was a good prognostic factor for BCLC-B HCC patients undergoing hepatectomy. The present study increased the cutoff point of N + S from 8 to 10, which may be related to different study cohorts and different calculation methods used for the cutoff value[26]. Nevertheless, the results of this study and in the study by Matsukuma *et al*[26] demonstrated that N + S could predict the recurrence and OS of BCLC-B HCC patients undergoing hepatectomy. In addition, the present study showed that N + S had a predictive accuracy similar to TBS and TTV in predicting OS in

Table 3 Univariate and multivariate analysis of recurrence-free survival in patients with Barcelona Clinic Liver Cancer stage B hepatocellular carcinoma

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
Gender (male)	1.135 (0.526-2.450)	0.747		
Age (> 65 yr)	0.881 (0.514-1.511)	0.646		
BMI > 25	1.157 (0.729-1.836)	0.536		
HBs-Ag positive	1.109 (0.558-2.202)	0.769		
ALBI grade				
1	1.00 (Reference)			
2	1.474 (0.988-2.198)	0.057		
AFP > 400 ng/mL	1.458 (0.984-2.162)	0.060		
Maximum tumor size > 5 cm	1.253 (0.830-1.891)	0.283		
Tumor number > 3	2.449 (1.123-5.343)	0.024		
N + S > 10	2.113 (1.385-3.224)	0.001	1.657 (1.057-2.596)	0.028
Bilateral tumor distribution	2.104 (1.409-3.140)	< 0.001	1.820 (1.187-2.791)	0.006
Presence of MVI	1.757 (0.973-3.171)	0.062		
Edmondson-Steiner III-IV	1.709 (1.151-2.539)	0.008	1.676 (1.127-2.493)	0.011
Major resection	1.285 (0.867-1.904)	0.211		
NAH	1.126 (0.650-1.950)	0.673		
Preoperative TACE				
No	1.00 (Reference)			
Yes	0.784 (0.437-1.405)	0.414		

BMI: Body mass index; HBs-Ag: Hepatitis B surface antigen; ALBI: Albumin-bilirubin; AFP: Alpha-fetoprotein; N + S: The sum of tumor size and number; MVI: Microvascular invasion; NAH: Non-anatomical hepatectomy; TACE: Transarterial chemoembolization.

BCLC-B patients. However, compared with the complicated calculation of TBS and TTV, the calculation of N + S is simpler and more suitable for clinical application.

Previous studies have focused on the OS when selecting BCLC-B patients for LR, and ignored that those selected patients still had a high postoperative recurrence rate[7,8]. In order to demonstrate that the selected BCLC-B HCC patients truly benefit from LR rather than remedial treatments after recurrence, and to better understand the tumor characteristics of the selected patients, we compared not only the OS and RFS, but also the RTDS, recurrence patterns, and treatments after recurrence.

In the present study, BCLC-B1 (BCLC-B with N + S ≤ 10) HCC patients were considered as BCLC-B HCC patients who likely benefitted most from LR. Although BCLC-B1 HCC patients were still more likely to develop recurrence after LR than BCLC-A MNHCC patients, these BCLC-B1 patients had mild recurrence pattern, good RTDS and excellent OS similar to BCLC-A MNHCC patients.

However, BCLC-B2 (BCLC-B with N + S > 10) HCC patients not only had a high postoperative recurrence rate, but also an aggressive recurrence pattern. Although the treatment after recurrence was similar between BCLC-B2 patients and BCLC-B1 patients, the BCLC-B2 patients still had a worse RTDS. The long-term OS of BCLC-B2 patients undergoing LR is not satisfactory.

To the best of our knowledge, this study is the first to demonstrate that N + S could predict not only prognosis in BCLC-B HCC patients, but also the recurrence patterns and RTDS in these patients.

In addition, it is interesting to note that patients with BCLC-B1 HCC had worse RFS but comparable OS than patients with BCLC-A MNHCC in this study. In fact, previous studies comparing LT *vs* LR in HCC patients found a similar phenomenon. These studies showed that although patients receiving LR had a higher rate of postoperative recurrence, the 5-year OS between LR and LT was comparable[27,28]. Previous studies have suggested that the reasons for this phenomenon may be related to noncancerous death in the LT group and treatment after recurrence in the LR group, and our results suggest that it may also be related to the recurrence patterns after LR.

As a single-center retrospective study, the present study has some limitations. Firstly, the sample size was small, which may have affected the accuracy of the results. Secondly, there was a lack of

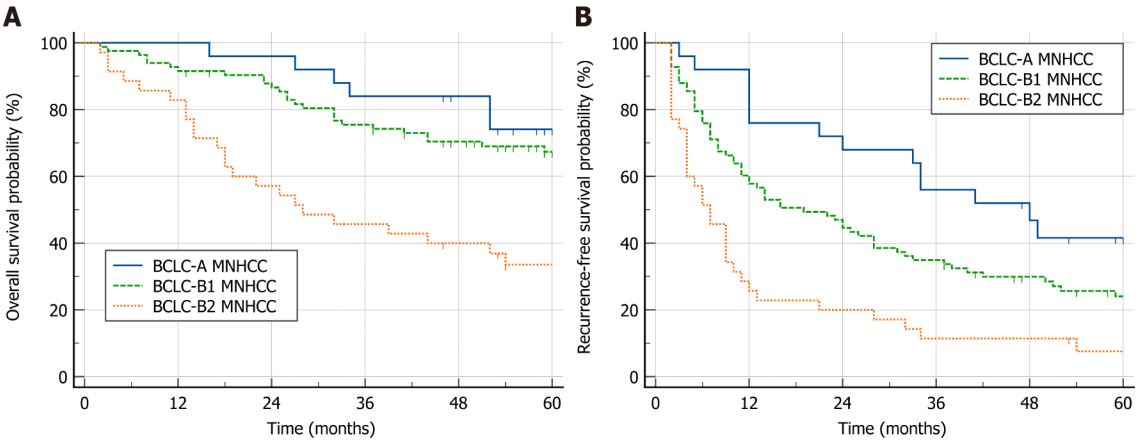

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Figure 2 Comparison of overall survival and recurrence-free survival in multinodular hepatocellular carcinoma patients with Barcelona Clinic Liver Cancer stage A, B1 and B2. A: overall survival; B: recurrence-free survival. BCLC: Barcelona Clinic Liver Cancer; MNHCC: Multinodular hepatocellular carcinoma.

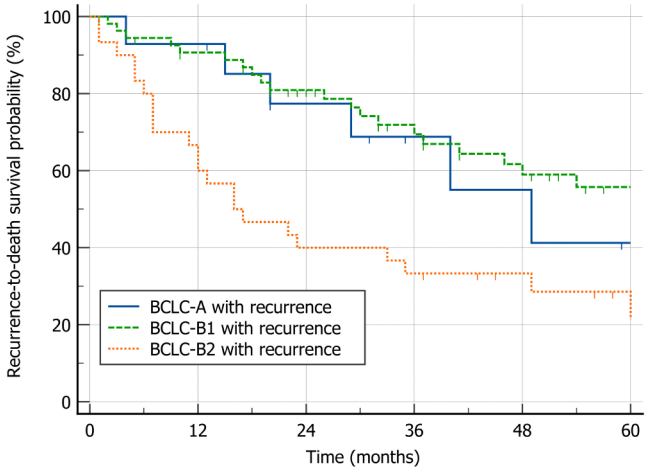

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Figure 3 Comparison of recurrence-to-death survival in Barcelona Clinic Liver Cancer stage A, B1 and B2 multinodular hepatocellular carcinoma patients with recurrence. BCLC: Barcelona Clinic Liver Cancer.

comparison of treatment options other than LR. Some patients with BCLC-B HCC and N + S ≤ 10 would meet the 'Extended Liver Transplant criteria', and the best treatment option for these patients remains to be explored[29]. Finally, the results of this study need to be verified by an external cohort.

CONCLUSION

N + S is a good measure that could predict the OS, RFS, RTDS and recurrence patterns in BCLC-B HCC patients undergoing LR. In particular, BCLC-B patients with N + S ≤ 10 had survivals similar to those of BCLC-A MNHCC patients. Given the computational simplicity of N + S, it is worth exploring the application of N + S to guide decision-making in the treatment of BCLC-B patients.

ARTICLE HIGHLIGHTS

Research background

Emerging studies have shown that Barcelona Clinic Liver Cancer (BCLC) intermediate-stage hepatocellular carcinoma (HCC) patients had a good prognosis after liver resection (LR), but the subgroups of BCLC-B patients more suitable for LR have yet to be defined.

Research motivation

There is a lack of studies on whether the sum of tumor size and number (N + S) can be used to select BCLC-B patients who are more suitable for LR. The effect of recurrence patterns on long-term survival in BCLC-B patients undergoing LR is also poorly explored.

Research objectives

The present study aimed to identify BCLC-B patients more suitable for LR and to further analyze the reasons why these patients could benefit from LR.

Research methods

BCLC stage A or B multinodular HCC (MNHCC) patients undergoing curative hepatectomy were enrolled. Overall survival (OS), recurrence-free survival (RFS), recurrence-to-death survival (RTDS), recurrence patterns, and treatments after recurrence in BCLC-B patients in each subgroup according to N + S were compared with those in BCLC-A patients.

Research results

N + S could predict not only the OS and RFS in BCLC-B HCC patients undergoing hepatectomy, but also the recurrence patterns and RTDS in these patients. BCLC-B patients with N + S ≤ 10 had mild recurrence patterns, good RTDS and excellent OS similar to those in BCLC-A MNHCC patients.

Research conclusions

N + S can be used to select BCLC-B HCC patients who are more suitable for LR, and LR should be considered in BCLC-B patients with N + S ≤ 10.

Research perspectives

As a measure that can be easily obtained and calculated in clinical practice, N + S can help with the clinical decision-making in the treatment of BCLC-B HCC patients.

FOOTNOTES

Author contributions: Hu XS performed the research and wrote the paper; Yang HY performed the follow-up; Leng C designed the research and supervised the report; Zhang ZW provided clinical advice and supervised the report; and all authors read and approved the final version.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Tongji Hospital of Tongji Medical College of Huazhong University of Science and Technology (Approval No. TJ-IRB20210918).

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

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ORIGINAL ARTICLE

Observational Study

Virological and histological evaluation of intestinal samples in COVID-19 patients

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Abstract

BACKGROUND

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the pathogen responsible for pandemic coronavirus disease 2019 (COVID-19). It is a highly contagious virus which primarily affects the respiratory tract, nevertheless, the lungs are not the only target organs of the virus. The intestinal tract could represent an additional tropism site for SARS-CoV-2. Several observations have collectively suggested that enteric infections can occur in COVID-19 patients. However, the detection of viral RNA in gastrointestinal (GI) tissue samples has not been adequately investigated and results are conflicting.

AIM
To detect the presence of SARS-CoV-2 RNA in intestinal mucosa samples and to evaluate histological features.

METHODS
The COVID-19 patients hospitalized at an Italian tertiary hospital from April 2020 to March 2021 were evaluated for enrollment in an observational, monocentric trial. The study population was composed of two groups of adult patients. In the first group (biopsy group, 30 patients), patients were eligible for inclusion if they had mild to moderate disease and if they agreed to have a rectal biopsy; in the second group (surgical specimen group, 6 patients), patients were eligible for inclusion if they underwent intestinal resection during index hospitalization. Fifty-nine intestinal mucosal samples were analyzed.

RESULTS
Viral RNA was not detectable in any of the rectal biopsies performed (0/53). Histological examination showed no enterocyte damage, but slight edema of the lamina propria with mild inflammatory lymphoplasmacytic infiltration. There was no difference in inflammatory infiltrates in patients with and without GI symptoms. SARS-CoV-2 RNA was detected in fecal samples in 6 cases out of 14 cases examined (42.9%). In the surgical specimen group, all patients underwent emergency intestinal resection. Viral RNA was detected in 2 surgical specimens of the 6 examined, both of which were from patients with active neoplastic disease. Histological examination also pointed out abundant macrophages, granulocytes and plasma cells infiltrating the muscular layer and adipose tissue, and focal vasculitis.

CONCLUSION
Mild-moderate COVID-19 may not be associated with rectal infection by the virus. More comprehensive autopsies or surgical specimens are needed to provide histological evidence of intestinal infection.

Key Words: COVID-19; SARS-CoV-2; Intestinal infection; Intestinal samples; Intestinal tropism; Rectal samples

Core Tip: The detection of viral RNA in gastrointestinal tissue samples has not been adequately investigated. In this trial, 53 rectal biopsies and 6 surgical specimens from coronavirus disease 2019 patients were analyzed, with the primary objective of detecting severe acute respiratory syndrome coronavirus-2 RNA, using real time reverse transcriptase-polymerase chain reaction, and evaluating the histological features. Viral RNA was not detectable in any of the rectal biopsies performed (0/53). Histological examination of rectal biopsies showed no enterocyte damage, but mild inflammatory infiltration. Viral RNA was detected in 2 surgical specimens of the 6 examined, both of which were from patients with active neoplastic disease. Histological examination also pointed out mild inflammatory infiltration and focal vasculitis.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the pathogen responsible for pandemic coronavirus disease 2019 (COVID-19). It is a highly contagious virus which primarily affects the respiratory tract, typically causing symptoms, such as fever, dry cough and dyspnea, up to respiratory failure and death[1]. Nevertheless, the lungs are not the only target organs of the virus. Several studies have suggested that the intestinal tract could represent an additional tropism site for SARS-CoV-2[2]. Gastrointestinal (GI) symptoms, including diarrhea, nausea, vomiting, anorexia and abdominal pain are present in a substantial number of COVID-19 patients; the incidence can vary from

10% to 55%[3-6]. Many studies have reported that viral nucleic acids are detected in stool samples of COVID-19 patients with rates varying from 15.3% to 66.7%[7-11]. Viral receptor angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine-type 2 are highly expressed in the epithelial cells of the intestinal mucosa[7,12]. Moreover, some studies have demonstrated that SARS-CoV-2 virus can actively infect and replicate in human enteroids and *in vitro* models of human intestinal epithelium[13, 14]. These observations have collectively suggested that enteric infection can occur in patients with COVID-19. However, more robust evidence is limited. The detection of viral RNA in GI tissue samples or the isolation of the virus in stool samples have been reported in studies which enrolled only a limited number of cases[15-18]. In the present study, a greater number of intestinal mucosal samples from COVID-19 patients (as compared to previous studies) were analyzed, with the primary objective of detecting SARS-CoV-2 RNA and evaluating histological features.

MATERIALS AND METHODS

The COVID-19 patients hospitalized at IRCCS Sant'Orsola Hospital, University of Bologna, Bologna, Italy from June 2020 to March 2021 were evaluated for enrollment in a monocentric trial. SARS-CoV-2 infection was diagnosed at admission using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) on pharyngeal swab specimens. The study was approved by the local hospital ethics committee and informed consent was obtained from all of the patients.

Inclusion/exclusion criteria

The study population was composed of two groups of adult patients (≥ 18 years of age) hospitalized for COVID-19. In the first group (biopsy group), patients were eligible for inclusion if they had mild to moderate disease (mild: Only mild symptoms with no radiological signs; moderate: Characterized by fever, respiratory symptoms and radiological signs of pneumonia)[19] and if they agreed to have a rectal biopsy; in the second group (surgical specimen group), patients were eligible for inclusion if they underwent intestinal resection during index hospitalization.

In the biopsy group, patients who had severe (dyspnea, respiratory frequency ≥ 30 breaths per minute, blood oxygen saturation ≤ 93%, partial pressure of arterial oxygen to fraction of inspired oxygen ratio < 300, and/or lung infiltrates > 50% within 24-48 h) and critical (respiratory failure, septic shock, and/or multiple organ dysfunction or failure) disease[19], contraindications to rectal biopsies (anticoagulant therapy with the exception of therapy with low molecular weight heparin and antiplatelet therapy), rectal disease (*e.g.*, chronic inflammatory disease and proctitis), previous abdominoperineal resection, recent anal surgery, anal stenosis or anal pain were excluded. In the surgical specimen group, those patients undergoing surgical treatment, cases without intestinal resection were excluded.

Interventions and design overview

Patients enrolled in the biopsy group were asked to collect a stool sample and undergo anoscopy with biopsy during their hospital stay. The anoscopy was performed at the bedside with the patient in the left lateral decubitus position using a disposable anoscope 18 mm diameter, lubricated with an anesthetic gel. During each anoscopy, 2 biopsies were performed at different sites of the rectal mucosa for viral RNA detection and stored, one in RNA Preservation Medium (RNAlater™Stabilization Solution, Thermo Fisher Scientific, United States) and then fresh frozen, and one in 10% buffered formalin. The biopsy specimen fixed in formalin was subsequently paraffin embedded, cut into 4-μm-thick sections and then stained with hematoxylin and eosin. Multiple sections were obtained to assess the extent of the inflammation. Serial paraffin sections, 3-μm-thick, mounted on precoated slides were processed using standardized automated procedures with prediluted anti-CD68 antibody (clone PG-M1; DBS, Pleasanton, CA). The stool samples and all the biopsies were tested for the detection of SARS-CoV-2 RNA using real-time RT-PCR. The extraction of nucleic acids, reverse transcription reaction and real-time PCR amplification were performed using a SARS-CoV-2 ELITe MGB® Kit (ELITechGroup, Italy) on an ELITeInGenius® instrument. The assay detects the RNA of two SARS-CoV-2 specific genomic regions: RdRp gene and ORF8 gene. The tissue viral load was reported as number of copies/microgram RNA. The limit of detection of the test is 2 copies/reaction (10 copies/microgram RNA). Positive results below the lower limit of quantification (5 copies/reaction) were reported as < 25 copies/microgram RNA. Qualitative data were provided for the fecal samples.

Patients enrolled in the surgical specimen group were asked for consent in order to take a tissue sample from the surgical specimen to identify the presence of both inflammatory cells infiltrated and virus SARS-CoV-2 RNA using the same methodology described above. For each patient, data were collected regarding sex, age, comorbidities, disease severity, symptoms on admission, radiological features and clinical outcomes.

Outcomes

The primary aim was to evaluate the presence of SARS-CoV-2 RNA in intestinal mucosa samples using real-time RT-PCR. The secondary aims were to detect both SARS-CoV-2 RNA in stool samples using

RT-PCR and the inflammatory state in all tissue samples.

Statistical analysis

Sample size analysis was not carried out due to the exploratory nature of the study. Statistical analysis was carried out using SPSS 20 software (SPSS Inc, Chicago, IL). Continuous data were expressed as means ± SDs (for data with normal distribution), or median and range (for data with nonnormal distribution); discrete data were expressed as percentages. The descriptive analyses were carried out using parametric methods, depending on the distribution of the variables under examination. Variables between the 2 groups were compared using the following tests as appropriate: *t*-test and Fisher's test. The differences were considered statistically significant for values of < 0.05.

RESULTS

From June 2020 to March 2021, 957 patients hospitalized for confirmed COVID-19 were screened. The diagram in Figure 1 shows the flow of participants in the trial. The biopsy group consisted of 30 patients and the surgical specimen group had 6 patients. The clinical characteristics of the patients in both groups are shown in Tables 1 and 2. In the biopsy group, GI symptoms were present in 19 patients (63.3%). Diarrhea was the most common GI symptom; it was reported in 14 cases (73.7%), anorexia in 8 cases (42.1%), nausea and vomiting in 7 cases (36.8%) and abdominal pain in 4 cases (21.0%). There was no statistically significant difference in the general demographics or clinical outcomes between patients with and without GI symptoms (Table 1); SARS-CoV-2 RNA was detected in the stool in 36.4% of the patients with GI symptoms and in 66.7% of those without GI symptoms. Nevertheless, the number of positive fecal cases did not show significant difference between the two groups. Considering only patients who had diarrhea on admission, viral RNA was found in half of the fecal samples examined. Overall, SARS-CoV-2 RNA was detected in fecal samples in 6 cases out of 14 cases examined (42.9%). The greatest number of positive cases was found in the fecal samples collected in the 2nd wk after the first positive nasopharyngeal swab (NPS) (3/3, 100%), 2 cases in the 1st wk (2/5, 40%) and the remaining case in the 3rd wk (1/4, 25%). There was no significant difference in time interval between sampling and the first positive NPS in positive and negative viral RNA fecal samples (9.0 ± 4.6 *vs* 16.4 ± 14.7 respectively, $P = 0.26$). Viral RNA was not detectable in any of the 53 rectal biopsies performed. The histological examination of the rectal samples showed that the mucosal epithelium of the rectum did not have any major damage in patients with and without GI symptoms. The glandular architecture was always normal. In no case was there any enterocyte damage. Moreover, microscopy revealed slight expansion of the lamina propria by moderate edema in 26 cases out of 30 (86.7%) and an inflammatory lymphoplasmacytic infiltration in the lamina propria, varying from mild to moderate, in 28 and 2 cases (93.3% and 6.7%), respectively. There was no difference in inflammatory infiltrates in patients with and without GI symptoms (Table 1). Rare eosinophilic and neutrophil granulocytes were identified in the lamina propria in 20 and 2 cases (66.7% and 6.7%), respectively.

In the surgical specimen group, all patients underwent emergency intestinal resection (Table 2). Three patients were hospitalized for COVID-19 symptoms and, 8-20 d after the first positive NPS, underwent bowel resection for iatrogenic perforation of the rectum, diverticular perforation and ischemic colitis, respectively. One patient was hospitalized for intestinal obstruction secondary to bridles and underwent segmental resection of the small intestine within 1 d after a positive pharyngeal swab for SARS-CoV-2. One patient with plurimetastic colon cancer (liver, lung, peritoneum and brain), during hospitalization for a spontaneous pneumothorax developed symptoms of COVID-19 and, soon after, had an intestinal perforation for which the patient underwent a right hemicolectomy. The remaining patient affected by acute myeloid leukemia developed COVID-19 symptoms while hospitalized for chemotherapy; a week later, the patient underwent an appendectomy and colonic resection for peritonitis secondary to an inflammatory mass englobing the appendix and the sigmoid colon. All the patients died during hospitalization. SARS-CoV-2 RNA was detected in 2 cases out of 6 (33.3%). In both cases, the virus RNA was positive in the colonic tissue of the 2 patients with active neoplastic disease. In both cases, the viral load was very low. In one intestinal specimen, the viral load was less than the limit of quantification of the test (< 25 copies/microg RNA) and, in the second, it was 29 copies/microgr RNA. Histological examination of the apparently healthy tissue of all the cases showed normal glandular architecture, no enterocyte damage, a slight expansion of the lamina propria by edema and inflammatory lymphoplasmacytic infiltration in the lamina propria varying from mild to moderate. However, in the two cases positive for viral RNA, histological examination also pointed out abundant macrophages, granulocytes and plasma cells infiltrating the muscular layer, and adipose tissue, focal vasculitis and some macrophages in the vascular lumen (Figures 2A and B).

Table 1 Baseline characteristics of the biopsy group

Characteristic	All patients, *n* = 30	Patients with GI symptoms at onset, *n* = 19	Patients without GI symptoms at onset, *n* = 11	*P* value
Median age in yr, mean (range)	65 (41-90)	60 (44-90)	78 (41-89)	NS
Sex as women:men (%)	7:23 (23.3:76.7)	4:15 (21.0:79)	3:8 (27.3:72.7)	NS
COVID-19 classification				
Mild, *n* (%)	0	0	0	NS
Moderate, *n* (%)	30 (100)	19 (100)	11 (100)	NS
Coexisting illness				
Hypertension	9 (30.0)	6 (31.6)	3 (27.3)	
Diabetes mellitus	4 (13.3)	3 (15.8)	1 (9.1)	
Cardio-cerebrovascular disease	5 (16.7)	3 (15.8)	2 (18.2)	
Previous malignant tumor	2 (6.7)	1 (5.3)	1 (9.1)	
Chronic obstructive pulmonary disease	3 (10.0)	2 (10.5)	1 (9.1)	
Chronic kidney disease	3 (10.0)	2 (10.5)	1 (9.1)	
Obesity	2 (6.7)	2 (10.5)	0	
Chest CT				
Negative, *n* (%)	4 (13.3)	3 (15.8)	1 (9.1)	NS
Bilateral distribution of GGO with or without consolidation, *n* (%)	16 (53.3)	9 (47.4)	7 (63.6)	NS
Unilateral distribution of GGO, *n* (%)	2 (6.7)	1 (5.2)	1 (9.1)	NS
Bilateral interlobular septal thickening, *n* (%)	8 (26.7)	6 (31.6)	2 (18.2)	NS
Median time from first positive NPS (range), d	10 (1-25)	8.0 (1-25)	12.0 (3-24)	NS
SARS-CoV-2 RNA in feces[1], *n* (%)	6/14 (42.9)	4/11 (36.4)	2/3 (66.7)	NS
SARS-CoV-2 RNA in RNA-preservation medium rectal mucosa biopsy[2], *n* (%)	0	0	0	-
SARS-CoV-2 RNA in FFPE rectal mucosa biopsy[3], *n* (%)	0	0	0	-
Histological examination				
Glandular architecture: Normal/altered, *n* (%)	30/0 (100/0)	19/0 (100/0)	11/0 (100/0)	NS
Edema of the lamina propria: Absent/slight, *n* (%)	4/26 (13.3/86.7)	3/16 (15.8/84.2)	1/10 (9.1/90.9)	NS
Inflammatory lymphoplasmacytic infiltration in the lamina propria: Mild/moderate, *n* (%)	28/2 (93.3/6.7)	18/1 (94.7/5.3)	10/1 (90.9/9.1)	NS
Eosinophilic granulocytes in the lamina propria: Absent/occasional/scattered, *n* (%)	10/18/2 (33.3/60.0/6.7)	7/10/2 (36.8/52.6/10.7)	3/8/0 (27.3/72.2/0)	NS
Neutrophil granulocytes in the lamina propria: Absent/rare, *n* (%)	28/2 (93.3/6.7)	18/1	10/1 (90.9/9.1)	NS
Enterocyte damage: Absent/present, *n* (%)	30/0 (100/0)	19/0	11/0 (100/0)	NS
Clinical outcome, *n* (%)				
Discharged	30 (100)	19 (100)	11 (100)	NS
Died	0	0	0	NS

[1]Available in 14 cases.
[2]Available in 23 cases.
[3]Available in 30 cases.
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; GI: Gastrointestinal; NPS: Nasopharyngeal swab; SD: Standard deviation; FFPE: Formalin-fixed paraffin-embedded; GGO: Ground glass opacities; COVID-19: Coronavirus disease 2019; CT: Computed tomography.

DISCUSSION

In the present study, SARS-CoV-2 RNA was identified in only two cases out of the 59 intestinal samples (3.4%). In both cases the viral load was very low; the intestinal samples consisted of colonic tissue of patients with active neoplastic disease (a patient with acute myeloid leukemia who was receiving immunosuppressive therapy and a patient with metastatic colon cancer), and there was a nosocomial transmission of SARS-CoV-2. Interestingly, in the biopsy group, two patients had previously undergone surgery for cancer (prostate and cervix) and in both cases, no viral RNA was identified in the rectal samples. Several studies have suggested that people with neoplastic disease are more likely to contract COVID-19 and to develop more severe disease or die from it than the general population. A recent systematic review on COVID-19 patients with active malignancy, defined as current malignant disease or treatment for malignancy within the last 12 mo, showed that cancer constitutes a co-morbidity in 2.6% [95% confidence interval (CI): 1.8%-3.5%, I^2: 92.0%] of hospitalized COVID-19 patients and that the pooled in-hospital mortality risk was 14.1% (95%CI: 9.1%-19.8%, I^2: 52.3%)[20]. Nahshon *et al*[21] suggested a severe clinical course of 50.6% and a mortality rate of 34.5% in COVID-19 patients with cancer. The worst COVID-19 outcomes include acute respiratory distress syndrome, septic shock, acute myocardial ischemia and death[22]. These severe events occurred more frequently in patients with stage IV cancer as compared to those with non-stage IV cancer (70.0% *vs* 44.4%, respectively) and if the last antitumor treatment was within 14 d (hazard ratio = 4.079, 95%CI: 1.086-15.322, P = 0.037)[22]. The pathogenesis of severe COVID-19 in cancer patients may be due to the aggravation of inflammatory cytokine storms, the imbalance of immune responses, and multiple organ damage[23]. In a multicenter retrospective cohort study on 232 patients with cancer and 519 statistically matched patients without cancer, Tian *et al*[23] identified elevated levels of interleukin (IL)-6, tumor necrosis factor α (TNF-α) and N-terminal pro-B-Type natriuretic peptide (NT-proBNP) and a reduced level of CD4+T cells and albumin-globulin ratio as risk factors of COVID-19 severity in patients with cancer. Similarly, in a retrospective cohort study which included 2052 patients hospitalized with COVID-19 (cancer, n = 93; non-cancer, n = 1959), Cai *et al*[24] reported that immune dysregulation was an important feature in cancer patients with COVID-19, which might account for their poorer prognosis; they found that COVID-19 patients with cancer had ongoing and significantly elevated inflammatory factors and cytokines (C-reactive protein, procalcitonin, IL-2 receptor, IL-6, IL-8) as well as a decreased number of immune cells (CD8 + T cells, CD4 + T cells, B cells, nature killer cells, T-helper and T- suppressor cells) than those without cancer. In patients with weakened immune systems, SARS-CoV-2 could infect vascular epithelial cells and organs, such as the lungs, heart, kidneys, liver, and intestine, expressing high levels of ACE2[25]. Autopsy data have reported viral infection in several organs, indicating hematogenic spread of the virus[26,27]. Moreover, serum SARS-CoV-2 nucleic acid (RNAemia) was associated with COVID-19 severity [odds ratio (OR) = 5.43, 95%CI: 3.46-8.53], increased risk of multiorgan failure (OR = 7.33, 95%CI: 2.46-21.88) and mortality (OR = 11.07, 95%CI: 5.60-22.88)[28]. Notably, viral RNA was undetectable in any of 53 rectal biopsies performed on the 30 patients hospitalized for moderate COVID-19 (biopsy group). The inability of the authors to detect viral RNA in the rectal samples contrasted with some data which have identified SARS-CoV-2 in intestinal samples[8,9]. Xiao *et al*[8] evaluated the viral nucleocapsid protein in the GI tissues of a COVID-19 patient who developed severe respiratory distress and an upper GI bleed. At endoscopy, they observed mucosal damage in the esophagus and found viral nucleocapsid protein in the cytoplasm of gastric, duodenal, and rectal glandular epithelial cells with immunofluorescent staining[10]. Lin *et al*[9] detected SARS-CoV-2 RNA at endoscopy in esophageal, gastric, duodenal, and rectal specimens in 2 out of 6 COVID-19 patients having GI symptoms. In this study, the presence of viral RNA in the GI tissue was also associated with severe disease; in fact, SARS-CoV-2 RNA was found in the samples of 2 patients with severe disease but not in those of 4 patients with non-severe disease[9]. Therefore, although the authors' failure to detect SARS-CoV-2 in the rectal samples could have been due to the mild disease course of the cases selected, the possibility that the viral load was below the detection limit of their RT-PCR assay cannot be excluded. This could justify the presence of a mild to moderate inflammatory infiltrate in the lamina propria in all the rectal samples at histological examination. In fact, plasma cells, lymphocytes, and granulocytes can migrate to the extravascular space to reach the possibly infected tissues. These findings are in line with the low number of endoscopic and histological examinations of intestinal samples of COVID-19 patients which showed inflammatory infiltration in the lamina propria. Endoscopy and biopsy samples of the esophagus, stomach, duodenum and rectum were taken from a 78-year-old patient with COVID-19 who showed symptoms of upper GI bleeding. Numerous infiltrating plasma cells and lymphocytes with interstitial edema were found in the lamina propria of the stomach, duodenum and rectum of this patient[8]. In a surgical rectal specimen obtained during the incubation period in a COVID-19 patient with rectal adenocarcinoma, histological examination showed prominent lymphocytes and macrophages infiltrating the lamina propria without significant mucosal damage. T lymphocytes and macrophages were found to be more numerous than B lymphocytes in the lamina propria[29].

Six patients out of the 14 cases examined (42.9%) tested positive for SARS-CoV-2 RNA in the stool. Multiple studies have reported the positive detection of viral nucleic acids in the fecal samples of COVID-19 patients, finding rates varying from 15.3% to 66.7%[6,14]. In a meta-analysis, the authors

Table 2 Baseline characteristics of the surgical specimen group

Characteristic	Patient no. 1	Patient no. 2	Patient no. 3	Patient no. 4	Patient no. 5	Patient no. 6
Age in yr	93	76	78	96	56	62
Coexisting illness	COPD, CVD	COPD, metastatic colon cancer	COPD, CVD, chronic kidney disease	COPD, hypertension	Psychiatric disease, obesity	Acute myeloid leukemia
COVID-19 classification	Moderate	Moderate	Mild	Mild	Moderate	Moderate
Thorax CT imaging	Bilateral distribution of GGO with consolidation	Unilateral distribution of GGO with consolidation	Bilateral distribution of GGO with consolidation	Unilateral distribution of GGO with consolidation	Bilateral distribution of GGO with consolidation	Bilateral distribution of GGO with consolidation
Time from first positive NPS	10	1	8	1	20	7
Reason for admission	COVID-19	Spontaneous pneumothorax	COVID-19	Intestinal occlusion	COVID-19	Chemotherapy for acute myeloid leukemia
Reason for surgical treatment	Rectal perforation after enema	Fecal peritonitis from perforated colon cancer	Fecal peritonitis from perforated diverticulitis	Small bowel obstruction secondary to bridle	Ischemic colitis	Acute abdomen
Surgical treatment	Colorectal resection and end-colostomy	Right colectomy and end-ileostomy	Sigmoid resection and end-colostomy	Segmental intestinal resection	Total colectomy and ileostomy	Appendectomy and sigmoid resection
GI symptoms at onset	No	No	No	No	No	Diarrhea
SARS-CoV-2 RNA in FFPE tissue/viral load (*n*° copies/microg)	No	29	No	No	No	< 25
Histological examination						
Glandular architecture	Normal	Normal	Normal	Normal	Normal	Normal
Edema of the lamina propria	Slight	Absent	Slight	Slight	Slight	Absent
Inflammatory lymphoplasmacytic infiltration in the lamina propria	Mild	Mild	Mild	Mild	Mild	Moderate
Eosinophilic granulocytes in the lamina propria	Occasional	Occasional	Occasional	Occasional	Occasional	Occasional
Enterocyte damage	Absent	Absent	Absent	Absent	Absent	Absent
Vasculitis	Absent	Focal	Absent	Absent	Absent	Focal
Granulocyte, macrophage and plasma cell infiltrate in the muscle wall and adipose tissue	Absent	Moderate	Absent	Absent	Absent	Severe
Clinical outcome	Died	Died	Died	Died	Died	Died
Time from surgical treatment in d	6	4	4	35	17	20

GI: Gastrointestinal symptoms; COPD: Chronic obstructive pulmonary disease; CVD: Cardiovascular disease; NPS: Nasopharyngeal swab; FFPE: Formalin-fixed paraffin-embedded; GGO: Ground glass opacities; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; CT: Computed tomography.

showed that viral RNA may be present in the feces in 48.1% of patients[30]. The mechanism of diarrhea in patients with COVID-19 is still largely unknown. Various etiopathogenetic hypotheses have been advanced to explain the occurrence of diarrhea in COVID-19 patients including alterations in gut microbiota, osmotic diarrhea due to malabsorption or inflammation, release of virulent proteins or toxins, and viral-induced intestinal fluid and electrolyte secretion by activation of the enteric nervous system[31,32].

Currently, the exact mechanism of intestinal involvement in COVID-19 is not yet well understood. Intestinal epithelial cells could be primarily infected by SARS-CoV-2 *via* the oral-fecal route or SARS-CoV-2 may invade the enteric cells after respiratory infection *via* lympho hematogenic spread. As COVID-19 is also associated with the involvement of different organs and systems, such as the liver,

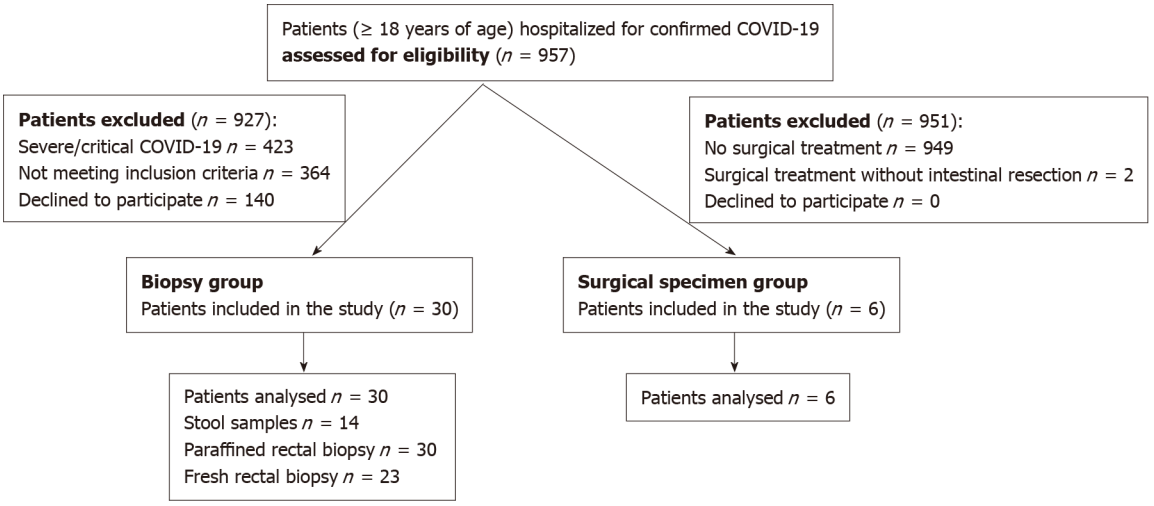

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Figure 1 Flow diagram of the participants in the trial. COVID-19: Coronavirus disease 2019.

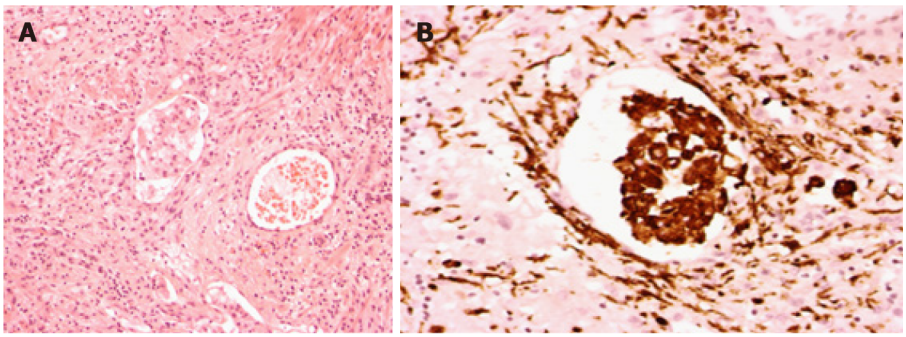

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Figure 2 Histological examination of intestinal samples. A: Histological examination showed many macrophages in the vascular lumen (hematoxylin and eosin, magnification 10 ×); B: CD68 antibody highlighted the macrophages (magnification 20 ×).

kidneys, heart, blood, and nervous system; it has been hypothesized that in severe COVID-19 patients and in those with compromised immunity, SARS-CoV-2 has not been successfully eradicated and can spread from the lungs to target organs, such as the intestine[33]. Although the present data are unable to support the observations suggesting that enteric infection can occur in COVID-19 patients, in the two cases positive for viral RNA, histological examination showed an inflammatory infiltrate characterized by the presence of macrophages, granulocytes, plasma cells, and focal vasculitis. Thus, it could be hypothesized that in these cases, there were both a direct viral infection and immune hyperactivation. Hyperactivation of the immune system in response to infection can cause severe complications and organ damage. The host immune response is thought to play a vital role in the pathogenesis of COVID-19[34-36]. The present study has several limitations. The biopsies were performed only in the rectum of patients with moderate COVID-19. On the other hand, due to the high risk of viral spreading during endoscopic procedures, it is difficult to obtain samples from the gastric, intestinal and colic mucosa in patients who do not complain of GI symptoms, especially in cases of severe illness. Moreover, it is not possible to exclude that the two positive colon samples could be contaminated by SARS-CoV-2 positive stool or blood. Another limitation of the present study was its observational nature which made it difficult to identify the causes of the observed phenomena. Nonetheless, the detection of the viral RNA observed and the inflammatory cell infiltration to the colonic tissue of patients with active cancer could serve as hypothesis generators, leading to the analyzing of more comprehensive autopsy or surgical specimens in order to assess the potential link between SARS-CoV-2 and enteric infections in this population. The strengths of this study include the use of RT-PCR which is the gold standard for detecting SARS-CoV-2 infection. Moreover, the rectal biopsies were performed in two different sites and stored in both RNA preservation medium and in 10% buffered formalin to reduce the risk of false negatives.

CONCLUSION

SARS-CoV-2 RNA was found in only a small percentage of the intestinal samples analyzed (3.4%). Nevertheless, more comprehensive autopsy or surgical specimens are needed to provide histological evidence of intestinal infection.

ARTICLE HIGHLIGHTS

Research background

Although some observations provide evidence for intestinal infection of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the mechanisms leading to this infection are not known.

Research motivation

The detection of viral RNA in gastrointestinal (GI) tissue samples has not been adequately investigated and results are conflicting. More GI tissue samples, comprehensive autopsy and surgical specimens are needed to provide histological evidence of intestinal infection.

Research objectives

Intestinal mucosal samples from mild-moderate coronavirus disease 2019 (COVID-19) patients were analyzed with the primary objective of detecting SARS-CoV-2 RNA and evaluating histological features.

Research methods

This is a monocentric trial in which real time reverse transcriptase-polymerase chain reaction and histological features were used to detect SARS-CoV-2 RNA in intestinal mucosal samples. The study population was composed of two groups of patients hospitalized for COVID-19. In the first group (biopsy group), the patients were eligible for inclusion if they had mild to moderate disease and if they agreed to have a rectal biopsy regardless of the presence or absence of GI symptoms; in the second group (surgical specimen group), patients were eligible for inclusion if they underwent intestinal resection during index hospitalization. The data obtained in this study are valuable because rectal biopsies were carried out on 30 patients who did not need the procedure to frame their disease status. The study therefore provides data that are not only more numerous but also qualitatively different from those available up to now.

Research results

Overall, we analyzed 53 rectal biopsies and 6 surgical specimens. Viral RNA was not detectable in any of the rectal biopsies performed (0/53). Histological examination showed no enterocyte damage, but slight edema of the lamina propria with mild inflammatory lymphoplasmacytic infiltration. Viral RNA was detected in 2 surgical specimens of the 6 examined, both of which were from patients with active neoplastic disease. Histological examination also pointed out abundant macrophages, granulocytes and plasma cells infiltrating the muscular layer and adipose tissue, and focal vasculitis.

Research conclusions

Mild-moderate COVID-19 may not be associated with rectal infection by the virus. Although the present data are unable to support the observations suggesting that enteric infection can occur in COVID-19 patients, the detection of the viral RNA observed and the inflammatory cell infiltration to the colonic tissue of patients with active cancer could serve as hypothesis generators, leading to the analyzing more comprehensive autopsy or surgical specimens in order to assess the potential link between SARS-CoV-2 and enteric infections in this population.

Research perspectives

Does intestinal infection lead to increased expression of inflammatory cytokines in the intestine and/or serum? Since the two positive samples were both from patients with active cancer, could a weakened immune system, induced by the neoplastic disease, increase the risk of the intestinal infection of SARS-CoV-2?

FOOTNOTES

Author contributions: Lazzarotto T and Poggioli G contributed equally to this work. Cuicchi D, Lazzarotto T, D'Errico A and Poggioli G participated in the conceptualization and design of the study; Cuicchi D collected data and carried out the initial analyses; Cuicchi D, Gabrielli L and Tardio ML drafted the initial manuscript; Gabrielli L and Rossini G carried out the virological evaluation; Tardio ML, D'Errico A and Poggioli G carried out the histological evaluation;

D'Errico A, Poggioli G and Lazzarotto T made substantial contributions to all aspects of the writing of the manuscript, which included contribution to conception, design, analysis and interpretation of the article; Lazzarotto T contributed to the review and revision of the manuscript; Poggioli G interpreted data, reviewed and revised the manuscript, and supervised and provided mentorship throughout all stages of the project and writing of the manuscript; and all authors approved the final version to be submitted.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: All the authors report having no relevant conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at dajana.cuicchi@aosp.bo.it. Participants gave informed consent for data sharing in anonymized form.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Randomized controlled trial to evaluate the efficacy and safety of fexuprazan compared with esomeprazole in erosive esophagitis

Kang Nyeong Lee, Oh Young Lee, Hoon Jai Chun, Jin Il Kim, Sung Kook Kim, Sang Woo Lee, Kyung Sik Park, Kook Lae Lee, Suck Chei Choi, Jae-Young Jang, Gwang Ha Kim, In-kyung Sung, Moo In Park, Joong Goo Kwon, Nayoung Kim, Jae Jun Kim, Soo Teik Lee, Hyun Soo Kim, Ki Bae Kim, Yong Chan Lee, Myung-Gyu Choi, Joon Seong Lee, Hwoon-Yong Jung, Kwang Jae Lee, Jie-Hyun Kim, Hyunsoo Chung

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Abstract

BACKGROUND

Fexuprazan, a novel potassium-competitive acid blocker, reversibly suppresses the K^+/H^+-ATPase enzyme in proton pumps within gastric parietal cells. Fexuprazan's suppression of gastric acid was maintained in healthy individuals for 24 h in a dose-dependent manner.

AIM

To compare fexuprazan to esomeprazole and establish its efficacy and safety in patients with erosive esophagitis (EE).

METHODS

Korean adult patients with endoscopically confirmed EE were randomized 1:1 to receive fexuprazan 40 mg or esomeprazole 40 mg once daily for eight weeks. The primary endpoint was the proportion of patients with healed EE confirmed by endoscopy at week 8. The secondary endpoints included the healing rate of EE at week 4, symptom response, and quality of life assessment. Safety profiles and serum gastrin levels were compared between the groups.

RESULTS

Of the 263 randomized, 218 completed the study per protocol (fexuprazan 40 mg, n = 107; esomeprazole 40 mg, n = 111). Fexuprazan was non-inferior to esomeprazole regarding the healing rate at week 8 [99.1% (106/107) *vs* 99.1% (110/111)]. There were no between-group differences in the EE healing rate at week 4 [90.3% (93/103) *vs* 88.5% (92/104)], symptom responses, and quality of life assessments. Additionally, serum gastrin levels at weeks 4 and 8 and drug-related side effects did not significantly differ between the groups.

CONCLUSION

Fexuprazan 40 mg is non-inferior to esomeprazole 40 mg in EE healing at week 8. We suggest that fexuprazan is an alternative promising treatment option to PPIs for patients with EE.

Key Words: Gastroesophageal reflux; Esophagitis; Proton pump inhibitors; Heartburn; Quality of life

Core Tip: A mainstay therapy of erosive esophagitis (EE) is acid suppression using proton pump inhibitors (PPIs), which have shortcomings such as their slow absorption and variability in metabolism. Acid suppression can be competitively and reversibly achieved by a novel potassium-competitive acid blocker, fexuprazan. We compared the efficacy and safety of fexuprazan and esomeprazole (each 40 mg once daily) in patients with EE for 8 wk. We conclude that fexuprazan is a new alternative to PPIs, by showing that fexuprazan is not inferior to esomeprazole in endoscopic healing rate of EE at week 8 and in safety profiles.

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INTRODUCTION

Gastro-esophageal reflux disease (GERD) is characterized by heartburn and acid regurgitation symptoms resulting from abnormal gastric reflux into the esophagus[1]. GERD prevalence is increasing in Asian and Western countries[2]. A recent report documented the worldwide prevalence of GERD as 13.3% (10.0% in Asia, 15.4% in North America, and 17.1% in Europe)[3]. The percentage change in age-standardized GERD prevalence in South Korea was 7.6% between 1990 and 2017[2]. GERD is classified as erosive esophagitis (EE) or non-erosive reflux disease (NERD) based on the presence of esophageal mucosal breaks *via* endoscopic examination[4]. Approximately one-third to half of the patients with EE complain of the typical symptoms of GERD[5]. In addition to typical symptoms, atypical and extraesophageal symptoms in patients with EE may impair health-related quality of life (HRQL)[6,7]. However, a poor HRQL is more likely associated with symptom frequency and severity rather than the presence or absence of EE[8].

A main treatment of GERD has been the use of proton pump inhibitors (PPIs). Current practical guidelines recommend PPIs as the first-line therapy for patients with EE[9]. PPIs irreversibly inhibit (H^+/K^+)-ATPase within the parietal cells of the gastric mucosa[10]. Studies have demonstrated that PPIs are 40%-50% more effective than placebo in healing of EE and resolving GERD symptoms[11,12]. Furthermore, complete healing of EE has been reported in 80% to 90% of patients after four and eight weeks of PPI treatment, respectively[11]. However, there are shortcomings of PPIs in GERD treatment, including unsatisfactory efficacy in atypical and extraesophageal symptoms and typical symptoms[13]. This might be due to the pitfalls of PPIs: The variability in PPI metabolism based on cytochrome P450 (CYP) 2C19 polymorphisms and the delayed onset of PPIs owing to their slow absorption associated with enteric coating to prevent degradation by acid.

As an alternative to PPI in GERD treatment, a novel potassium-competitive acid blocker (P-CAB), fexuprazan (Daewoong Pharmaceutical Co., Ltd., Seoul, South Korea), was developed[14]. In contrast to PPIs, metabolism of fexuprazan is independent of CYP 2C19; enteric coating is not needed because of acid stability. While PPIs bind irreversibly to only the active forms of the proton pump, fexuprazan can bind to both the active and inactive forms of the proton pump competitively and reversibly. A previous study on healthy individuals demonstrated the effect of fexuprazan's acid suppression and tolerability, observing that gastric pH > 4 was reached within 2 h and maintained for 24 h in a dose-dependent manner[14].

Nevertheless, the effectiveness and safety of fexuprazan compared to esomeprazole, one of the most widely used PPIs in GERD, have not been confirmed among patients with EE. Therefore, this phase III, double-blind, randomized, active-controlled, multi-center study was conducted to compare the efficacy and safety between fexuprazan and esomeprazole in patients with EE.

MATERIALS AND METHODS

Study design and treatments

This randomized, double-blind, parallel-group, multicenter, and phase III trial was performed in 25 institutions in South Korea between December 2018 and August 2019. Adult patients provided written informed consent prior to enrolment, and then screening test including the endoscopy was performed. Eligible participants were randomized 1:1 to receive either fexuprazan 40 mg or esomeprazole 40 mg following the screening test. At this point, participants were stratified according to Los-Angeles (LA) Classification Grade classified by the result of upper gastrointestinal endoscopy.

To ensure the double-blinded nature of the study, patients were administered once daily with two tablets of the study medication (fexuprazan 40 mg or esomeprazole 40 mg with its matching placebo in the study and control groups, respectively) for eight weeks.

Compliance of the study medication was ascertained at each visit by participants returning the unused portion and empty packaging, and was calculated using the total numbers of tablets to be taken, of tablets actually taken, and of returned and unreturned tablets in each participant.

This study was approved by the institutional review boards of each institution, conducted in compliance with the relevant ethics guidelines, and registered at ClinicalTrials.gov (NCT03736369). All the study medications and procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Participants

Eligible participants were male or female patients (20-75 years old) with EE (LA Classification Grades A to D) confirmed by endoscopy at the same institution within 14 d of study treatment initiation. The major exclusion criteria were Barrett's esophagus (> 3 cm); esophageal stricture; active peptic ulcers; ulcer-related stenosis; gastrointestinal bleeding; eosinophilic esophagitis; Zollinger-Ellison syndrome; inflammatory bowel diseases; irritable bowel syndrome; pancreatitis; psychiatric disorders; acquired immune deficiency syndrome (AIDS); viral hepatitis; history of gastric acid suppression surgery; significant morbidities in the cardiovascular, respiratory, hepatic, renal, neurologic, endocrine, hematologic, and urologic systems; history of malignancies within five years; drug or alcohol abuse; and hypersensitivity to drugs containing active constituents of esomeprazole or other similar drugs (benzimidazoles and antibiotics). Also excluded were those who had abnormal laboratory values, including alanine aminotransferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), total bilirubin, creatinine, and blood urine nitrogen > 2´ upper limits of the normal range, and women with child-bearing potential who did not consent to appropriate contraceptive methods use during the study.

Protocol

Endoscopy was performed at the start of the screening period and at weeks 4 and 8. EE healing was defined as the complete absence of mucosal breaks. If mucosal breaks did not heal at week 4, the patients continued to receive the study drug until week 8, when endoscopy was performed again. Two weeks after the confirmation of EE healing from the centralized endoscopic evaluation, the patients were evaluated for safety *via* telephone interviews, and where applicable, they underwent additional tests and procedures (Figure 1).

The primary efficacy endpoint was the proportion of patients with endoscopically confirmed EE healing at week 8. The secondary efficacy endpoints were EE healing rate at week 4; the patients' reported symptom outcomes, symptom assessment by reflux disease questionnaire (RDQ), and GERD-health related quality life (GERD-HRQL). Symptoms were evaluated based on patients' symptom diaries. Symptom severity in the daytime and at night were measured according to the five-point scale (0: none, 1: mild, 2: moderate, 3: severe, 4: very severe).

The parameters for assessing symptom responses were the first day of the complete resolution of symptoms (heartburn, acid regurgitation, and heartburn/acid regurgitation) after treatment, the proportion of patients without symptoms in the first 7 d and through the 8 wk of treatment, and the proportion of symptom-free days in the first 7 d and through the 8 wk of treatment. Changes in symptoms and GERD-HRQL from baseline at weeks 4 and 8 were evaluated using the RDQ and GERD-HRQL scales, respectively. The RDQ is a self-administered questionnaires comprising of 12 items to assess the frequency and severity of heartburn, acid regurgitation, and dyspepsia. Each item for frequency and severity was scored from 0 to 5; the higher score, the more severe or frequent symptoms [15]. The RDQ demonstrated the validity and reliability for diagnosis of GERD in primary care and community settings[16]. The GERD-HRQL scale comprises 11 items for the symptoms of heartburn and dysphagia, medication effects, and the patients' health conditions[17]. Each item was scored from 0 to 5; the higher the score, the lower the quality of life. The GERD-HRQL was validated and considered as an appropriate instrument to evaluate typical GERD symptoms[18]. Therefore, previous clinical studies performed in South Korea have used the RDQ and GERD-HRQL to evaluate the therapeutic effect in patients with GERD[19,20].

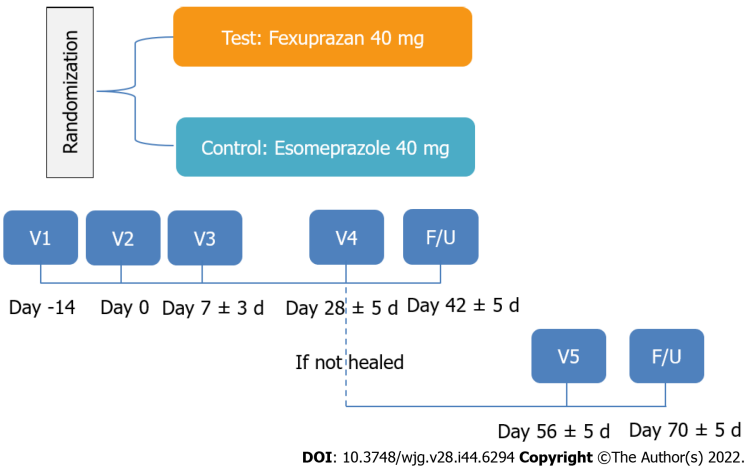

Figure 1 Study schema. V: Visit; F/U: Follow-up.

Additional analyses included heartburn and extraesophageal symptoms of GERD (chronic cough and throat irritation) in terms of the proportion of patients without symptoms and the proportion of symptom-free days in the first 3 d, 7 d and through the 8 wk of treatment. Patients with moderate/severe heartburn (RDQ ≥ 3) were also compared between the groups in terms of the proportion of patients without symptoms and the proportion of symptom-free days in the first 3 d, 7 d and through the 8 wk of treatment.

The patients' baseline characteristics included age, sex, body mass index (BMI), smoking history, drinking history, LA grade, *Helicobacter pylori* (*H. pylori*) infections, and CYP2C19 extensive metabolizer (EM)/poor metabolizer (PM) status. Serum gastrin levels were measured at weeks 4 and 8. Safety outcomes were measured by the analysis of adverse events (AEs), vital signs, physical examination, electrocardiogram (ECG) findings and laboratory tests. Adverse events (frequency, severity and seriousness) and concomitant medications were monitored throughout the study. Treatment-emergent adverse event (TEAE) was defined as an AEs newly occurred after the randomization and the first administration of study medication, and adverse drug reaction (ADR) was defined as any untoward and unintended response to the study medication of which causal relationship cannot be excluded.

Sample size

Based on previous studies, we estimated the sample size, assuming that the complete healing rate of mucosal breaks was 94.8% at week 8 after treatment with fexuprazan 40 mg and esomeprazole 40 mg [21,22]. Based on this threshold parameter, the sample size was 104 patients per treatment group, using the following conditions of the PASS program: non-inferiority margin of 10%[23], a one-sided significance level of 2.5%, 90% statistical power, and 1:1 randomization.

Randomization

This study was used stratified block randomization method base on LA grades (A/B/C/D) by the result of upper gastrointestinal endoscopy. An independent statistician generated a randomization list based on stratification factor (LA grades) using the PLAN (Proc Plan) procedure of SAS (ver. 9.4, SAS Institute, Cary, NC, United States). Eligible participants were randomly assigned by the investigators in a ratio of 1:1 *via* an interactive web-response system (IWRS). Neither participants nor relevant investigators were aware of these assignments.

Statistical analysis

Efficacy was evaluated by both the full analysis set (FAS) and per-protocol set (PPS), and PPS findings were interpreted as the main results. For the safety assessment, statistical analysis was performed on the safety set (SS). The FAS, based on the intention-to treat principle, included patients who received at least one dose of the study drug after randomization and had at least one primary efficacy assessment. The PPS included patients in the FAS who completed the study without any major protocol deviation. The SS group included all patients who received the study drug at least once after randomization.

For symptoms responses daily (day-time and night-time) assessment in the efficacy analysis, missing symptom in day-time or night-time was imputed using the last observation carried forward. Except for this, missing value was set to missing without imputation, and the results of patients who were completed the study early as mucosal breaks were completely healed up to week 4 were used as the results of the week 8.

Summaries of baseline characteristics of patients were presented in FAS. To assess the difference between the treatment group, the two sample t-test or Wilcoxon rank-sum test were used after normality evaluation in continuous baseline characteristics variables, and the chi-square test or Fisher's exact test were used in categorical baseline characteristics variables.

The primary objective of the study was to demonstrate the non-inferiority of fexuprazan 40 mg compared with esomeprazole 40 mg. The cumulative healing rate of EE and corresponding two-sided 95% confidence interval (CI) were presented for visit (up to week 4 or week 8) by treatment group. The common risk difference of the healing rate of EE up to week 8 between the treatment groups (fexuprazan 40 mg group - esomeprazole 40 mg group) and corresponding two-sided 95% CI using the Cochran-Mantel-Haenszel method adjusted by a stratification factor (baseline LA grade) were presented in the PPS. The non-inferiority of fexuprazan 40 mg to esomeprazole 40 mg was determined the lower limit of its two-sided 95%CI is larger than the non-inferiority margin of -10%. The same analyses were performed for the non-inferiority of healing rate of EE up to week 4. Furthermore, continuous data were analyzed using an analysis of covariance (ANCOVA) model, including treatment group as treatment effect, and baseline score (included if evaluation data were changed from baseline) and baseline LA grade as covariates. The changes from baseline within-treatment group were used the paired t-test or Wilcoxon signed rank test after normality evaluation as a post hoc analysis. Categorical data were analyzed using the Cochran-Mantel-Haenszel method adjusted by baseline LA grade. For the safety analysis, the chi-square test or Fisher's exact test was used to compare the difference in the incidence of AEs between the treatment groups. All statistical analyses were performed using SAS (ver. 9.4, SAS Institute, Cary, NC, United States) with a two-sided significance level of 5% for all tests.

RESULTS

Baseline characteristics of the participants

Of the total of 470 patients screened, 263 patients with EE were randomized to receive either fexuprazan 40 mg or esomeprazole 40 mg (Table 1). In total, 231 patients [152 men (65.8%) and 79 women (34.2%); 54.4 ± 12.7 mean age] were included in the FAS and completed the study (n = 116 and 115 in the fexuprazan and esomeprazole groups, respectively). Thirteen patients with study medication-related deviation, visit window deviation and consent withdrawal were excluded from the FAS (9 and 4 in the fexuprazan and esomeprazole groups, respectively), and 218 patients completed the study on the PPS (n = 107 and 111 in the fexuprazan and esomeprazole groups, respectively). The SS included 131 patients each in the fexuprazan and esomeprazole groups (Figure 2).

There were no significant differences in baseline characteristics between both groups, except CYP2C19 genotypes (EM or PM). A statistically significant difference was seen in the classification of CYP2C19 genotype (P = 0.007), but the result was obtained from only some of the participants who agreed to genotyping (n = 51 and 56 in the fexuprazan and esomeprazole groups, respectively).

The mean compliance rates were 98.6% ± 8.1% and 99.0% ± 2.6% at weeks 4 and 8, and the overall compliance rate with study medication exceeded 95% in all treatment groups without between-group differences.

Efficacy

Healing rate of EE: In the PPS, the proportions of patients with complete absence of mucosal breaks at week 8 were 99.1% (106/107) and 99.1% (110/111) in the fexuprazan and esomeprazole groups, respectively. The difference in proportions of patients with complete absence of mucosal breaks adjusted by baseline LA grade [fexuprazan 40 mg group – esomeprazole 40 mg group] was 0.9% (95%CI, -0.9 to 2.6) (Figure 3). The lower limit of two-sided 95%CI at week 8, -0.9%, was greater than the non-inferiority margin of -10%, indicating the non-inferiority of 8-week treatment of fexuprazan 40 mg to esomeprazole 40 mg in EE healing in GERD. At week 4, the healing rates of EE were not different between the two groups [90.3% (93/103) in the fexuprazan group and 88.5% (92/104) in the esomeprazole group, respectively] with a difference of 2.6% (95%CI: -5.7 to 10.9). The lower limit of 95%CI, -5.7%, was also greater than the non-inferiority margin of -10%. These results demonstrate that fexuprazan 40 mg was non-inferior to esomeprazole 40 mg in EE healing in GERD at weeks 4 and 8.

As the results in the exploratory analysis, healing rates of EE were not statistically significantly different according to CYP2C19 genotype (EM or PM) and *H. pylori* infection (positive or negative). Healing rates of EE at weeks 4 and 8 in EM participants (n = 88) were not different between fexuprazn and esomeprazole groups [91.7% (33/36) *vs* 89.4% (42/47) at week 4; 100.0% (36/36) *vs* 98.1% (51/52) at week 8]. EE healing rates at weeks 4 and 8 in PM participants (n = 14) were not different between the treatment groups [70.0% (7/10) *vs* 100.0% (3/3) at week 4; 90.9% (10/11) *vs* 100.0% (3/3) at week 8]. Healing rates of EE in *H. pylori*-positive participants (n = 47) were 100.0% (17/17) and 88.46% (23/26) in the fexuprazan and esomeprazole groups at week 4, and all of *H. pylori*-positive participants were completely healed at week 8. Healing rates of EE in *H. pylori*-negative participants (n = 169) were 88.2% (75/85) *vs* 88.3% (68/77) at week 4, and 98.9% (87/88) *vs* 98.8 (80/81) at week 8 in the fexuprazan and esomeprazole groups.

Table 1 Baseline characteristics of the patients (full analysis set)

Variables		Fexuprazan 40 mg (*n* = 116)	Esomeprazole 40 mg (*n* = 115)	*P* value
Age, yr (mean ± SD)[5]		53.70 ± 12.44	55.05 ± 12.89	0.343[w]
Sex, *n* (%)[6]				
	Men	78 (67.2)	74 (64.3)	0.643[c]
	Women	38 (32.8)	41 (35.7)	
BMI, kg/m^2 (SD)[5]		24.42 ± 3.08	24.81 ± 3.25	0.529[w]
Smoking history, *n* (%)[6]				
	Non-smokers	67 (57.8)	66 (57.4)	0.978[c]
	Current smokers	25 (21.6)	26 (22.6)	
	Past smokers	24 (20.7)	23 (20.0)	
Drinking history, *n* (%)[6]				
	Non-drinkers	15 (12.9)	15 (13.0)	0.992[c]
	Current drinkers	77 (66.4)	77 (67.0)	
	Past drinkers	24 (20.7)	23 (20.0)	
LA classification[1], *n* (%)[6]				
	Grade A	75 (64.7)	76 (66.1)	0.630[f]
	Grade B	33 (28.4)	31 (27.0)	
	Grade C	6 (5.2)	8 (7.0)	
	Grade D	2 (1.7)	0 (0.0)	
Helicobacter pylori[2], *n* (%)[6]				
	Positive	20 (17.4)	31 (27.2)	0.075[c]
	Negative	95 (82.6)	83 (72.8)	
CYP2C19[3], *n* (%)[6]				
	EM	39 (76.5)	53 (94.6)	0.007[c]
	PM	12 (23.5)	3 (5.4)	
Severity for heartburn[4], *n* (%)[6]				
	Mild	53 (45.7)	50 (43.5)	0.735[c]
	Moderate/severe	63 (54.3)	65 (56.5)	

Values are mean SD or the number of the patients with percentage, where appropriate.
[1]LA Classification (Grade A: One (or more) mucosal break(s) no longer than 5 mm, that does not extend between the tops of two mucosal folds, Grade B: One (or more) mucosal break(s) more than 5 mm long, that does not extend between the tops of two mucosal folds, Grade C: One (or more) mucosal break(s) that is continuous between the tops of two or more mucosal folds, but which involve(s) less than 75% of the oesophageal circumference, Grade D: One (or more) mucosal break(s) which involve(s) at least 75% of the oesophageal circumference).
[2]Two subjects (Fexuprazan 40 mg: 1 subject, Esomeprazole 40 mg: 1 subject) did not have *H. pylori* results at baseline.
[3]CYP2C19 genotype results were collected only from the subjects who agreed to the informed consent for genetic testing.
[4]Severity for heartburn was defined based on the baseline Reflux disease questionnaire (RDQ). Mild: RDQ ≤ 2 (Less than or equal to 1 d out of 7 d), Moderate/Severe: RDQ ≥ 3 (Greater than or equal to 2 d out of 7 d).
[5]Testing for difference between treatment groups after normality evaluation [Wilcoxon rank-sum test (w)].
[6]Testing for difference among treatment groups [Chi-square test (c) or Fisher's exact test (f)].
BMI: Body mass index; LA: Los-Angeles; CYP2C19: Cytochrome P 2C19; EM: Extensive metabolizer; PM: Poor metabolizer.

Symptom response: Fexuprazan exhibited an overall symptom relief comparable to esomeprazole. The differences between the groups were not significant with respect to the first day of the complete resolution of symptoms (resolution of typical symptoms for 7 d) after treatment: the median values of days to complete resolution for heartburn, acid regurgitation, and heartburn/acid regurgitation were 13, 8, and 18 d *vs* 10, 6, and 16 d in the fexuprazan and esomeprazole groups, respectively. There were no statistically significant differences in the proportions of patients without symptoms in the first 7 d (26.2%, 25.2%, and 15.0%, *vs* 21.6%, 27.9%, and 11.7%, for heartburn, acid regurgitation, and

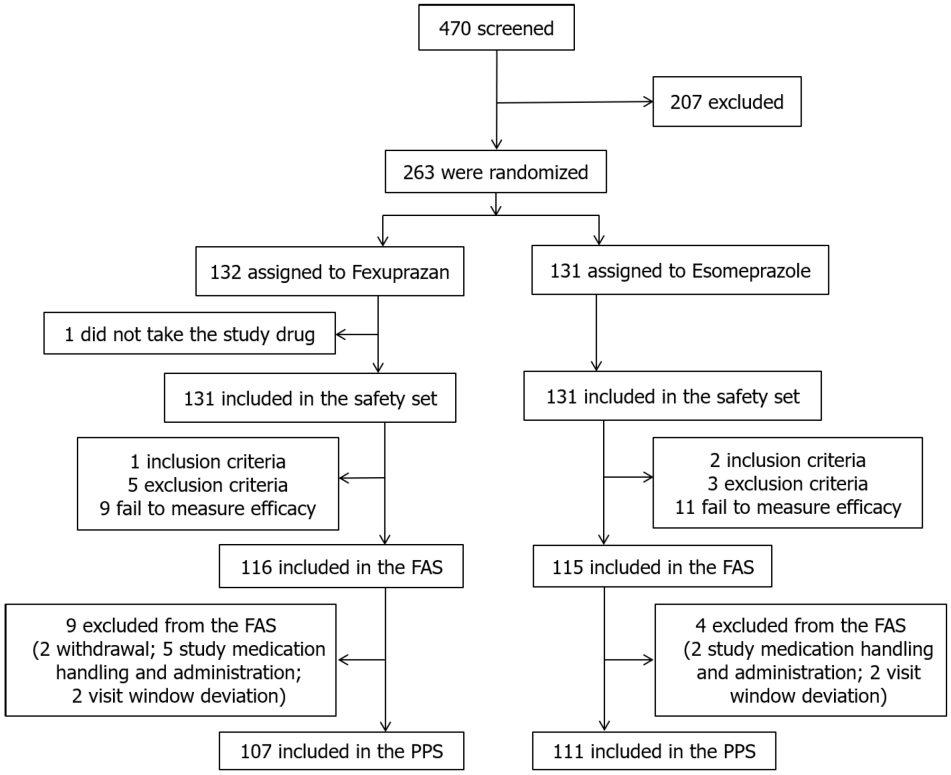

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Figure 2 Flowchart of the study patients. SS: Safety set; FAS: Full analysis set; PPS: Per-protocol set.

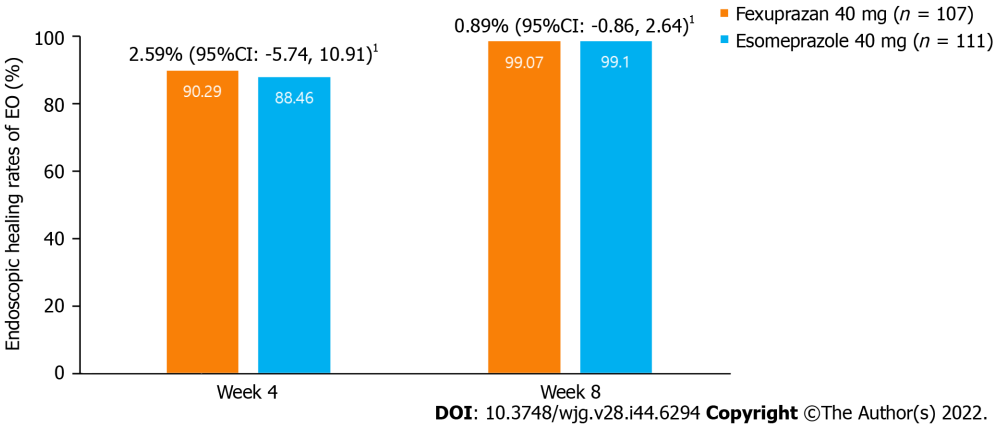

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Figure 3 Erosive esophagitis healing rate at weeks 4 and 8 (per protocol set). Erosive esophagitis (EE) healing was defined as the complete absence of mucosal breaks confirmed by the endoscopy. [1]Common risk difference of the healing rate of EE between the treatment groups (two-sided 95%CI) using the Cochran-Mantel-Haenszel method adjusted by baseline Los Angeles grade. EE: Erosive esophagitis; CI: Confidence interval.

heartburn/acid regurgitation, in the fexuprazan and esomeprazole groups, respectively) and through the 8 wk (20.6%, 21.5%, and 10.3%, *vs* 17.1%, 27.0%, and 9.9%). Similarly, there were no statistically significant differences in the proportion of symptom-free day/night-time in the first 7 d and through the 8 wk between both groups. (Supplementary Tables 1-4).

In the RDQ and GERD-HRQL, the frequency and severity of heartburn and acid regurgitation improved in both groups, with no significant difference in changes from baseline at weeks 4 and 8 (Tables 2 and 3).

In the results of subgroup analyses, fexuprazan demonstrated better heartburn relief in patients with moderate-to-severe symptoms who had experienced heartburn for 2 or more days in the week before treatment: the proportions of those without heartburn on the first day 3 were significantly greater in the fexuprazan group than in the esomeprazole group (22.4% *vs* 7.9%, P = 0.026 at the day/night-time; 29.3% *vs* 12.7%, P = 0.037 at the day-time; 34.5% *vs* 17.5%, P = 0.035 at the night-time) (Supplementary Table 5). The extraesophageal symptom of chronic cough improved better with fexuprazan:

Table 2 Change in reflux disease questionnaires symptom scores from baseline at weeks 4 and 8 (per protocol set)

	Fexuprazan 40 mg			Esomeprazole 40 mg		
	Baseline (*n* = 107)	Week 4 (*n* = 103)	Week 8 (*n* = 107)	Baseline (*n* = 111)	Week 4 (*n* = 104)	Week 8 (*n* = 111)
Frequency						
Heartburn						
mean ± SD	1.92 ± 1.23	0.91 ± 1.37	0.86 ± 1.33	2.12 ± 1.42	0.80 ± 1.29	0.70 ± 1.28
Change from baseline (mean ± SD)	-	-0.96 ± 1.50	-1.06 ± 1.49	-	-1.33 ± 1.69	-1.42 ± 1.64
P value[1]	-	< 0.001[w]	< 0.001[w]	-	< 0.001[w]	< 0.001[w]
LS mean difference from esomeprazole	-	0.19	0.22	-	-	-
P value[2]	-	0.280	0.184	-	-	-
Reflux						
mean ± SD	2.14 ± 1.29	0.93 ± 1.48	0.92 ± 1.49	1.95 ± 1.29	0.61 ± 1.07	0.59 ± 1.12
Change from baseline (mean ± SD)	-	-1.19 ± 1.58	-1.22 ± 1.53	-	-1.32 ± 1.44	-1.36 ± 1.40
P value[1]	-	< 0.001[w]	< 0.001[w]	-	< 0.001[w]	< 0.001[w]
LS mean difference from esomeprazole	-	0.28	0.28	-	-	-
P value[2]	-	0.112	0.101	-	-	-
Severity						
Heartburn						
mean ± SD	1.81 ± 1.18	0.57 ± 0.78	0.53 ± 0.77	2.10 ± 1.24	0.45 ± 0.75	0.42 ± 0.79
Change from baseline (mean ± SD)	-	-1.23 ± 1.26	-1.28 ± 1.26	-	-1.63 ± 1.29	-1.68 ± 1.27
P value[1]	-	< 0.001[w]	< 0.001[w]	-	< 0.001[w]	< 0.001[w]
LS mean difference from esomeprazole	-	0.16	0.16	-	-	-
P value[2]	-	0.116	0.121	-	-	-
Reflux						
mean ± SD	2.06 ± 1.22	0.61 ± 0.92	0.58 ± 0.90	1.97 ± 1.21	0.40 ± 0.69	0.39 ± 0.73
Change from baseline (mean ± SD)	-	-1.43 ± 1.23	-1.48 ± 1.20	-	-1.55 ± 1.23	-1.58 ± 1.19
P value[1]	-	< 0.001[w]	< 0.001[w]	-	< 0.001[w]	< 0.001[w]
LS mean difference from esomeprazole	-	0.20	0.18	-	-	-
P value[2]	-	0.066	0.089	-	-	-

[1]Testing for change within-treatment groups [paired *t*-test (t) or Wilcoxon signed rank test (w)].
[2]Testing for difference between treatment groups (ANCOVA model with treatment group as a factor, baseline score and stratification factor (baseline LA classification) as covariates).
Note: If subjects did not have any RDQ assessment data by week 4, were treated as missing at week 4. RDQ: Reflux disease questionnaires; LS mean: Least square mean.

the least squares (LS) means of days without chronic cough were significantly greater in the fexuprazan group than in the esomeprazole group on the days 3, 7, and week 8 (P = 0.006, P = 0.003, and P = 0.002, respectively). The extraesophageal symptom of throat irritation improved in both groups on days 3, 7, and week 8 without significant differences between the treatment groups (Supplementary Table 6).

Table 3 Change in gastroesophageal reflux disease-health related quality of life score from baseline at weeks 4 and 8 (per protocol set)

GERD-HRQL	Fexuprazan 40 mg			Esomeprazole 40 mg		
	Baseline (*n* = 107)	Week 4 (*n* = 102)	Week 8 (*n* = 106)	Baseline (*n* = 111)	Week 4 (*n* = 104)	Week 8 (*n* = 111)
mean ± SD	11.88 ± 8.11	4.21 ± 6.17	4.01 ± 6.20	12.98 ± 9.62	3.42 ± 5.04	3.32 ± 5.54
Change from baseline (mean ± SD)	-	-7.71 ± 8.37	-7.90 ± 8.56	-	-9.84 ± 8.70	-9.67 ± 8.56
P value[1]	-	< 0.001[w]	< 0.001[t]	-	< 0.001[w]	< 0.001[w]
LS mean difference from esomeprazole	-	1.06	1.05	-	-	-
P value[2]	-	0.137	0.151	-	-	-

[1]Testing for change within-treatment groups [paired *t*-test (t) or Wilcoxon signed rank test (w)].
[2]Testing for difference between treatment groups (ANCOVA model with treatment group as a factor, baseline score and stratification factor (baseline LA classification) as covariates).
Note: If subjects did not have any Gastroesophageal reflux disease-health related quality of life (GERD-HRQL) assessment data by week 4, were treated as missing at week 4. One subject in fexuprazan 40 mg did not have GERD-HRQL assessment data post baseline. GERD-HRQL: Gastroesophageal reflux disease-health related quality of life; LS mean: Least square mean.

Safety

Safety analyses were performed for 262 patients who received at least one dose of the study meditation. The overall incidences of TEAEs and ADRs were not significantly different between the treatment groups; TEAEs were reported by 22 patients (16.8%) and 25 (19.1%), and ADRs were reported by 9 patients (6.9%) and 7 patients (5.3%) in the fexuprazan and esomeprazole groups, respectively (Table 4). The severity of TEARs was mostly mild (61 events), with six moderate events (diarrhea, nausea, dysgeusia, pruritus, pain, and cystitis) and only one severe event (influenza). All ADRs were either mild (21 events) or moderate (3 events). There were 2 patients (1.5%) with ADRs (diarrhea and pruritus) leading to discontinuation of the study medication in the fexuprazan group, not esomeprazole group. However, there was no statistically significant difference in the incidence of ADRs leading to discontinuation between both groups. No serious TEARs or ADRs were reported in either group of patients (Supplementary Table 7). The most frequently occurring (≥ 2%) TEAEs were shown in Table 4.

The serum gastrin levels intended to increase, and their differences between the treatment groups were not significant at weeks 4 and 8 (Figure 4). There were no clinically significant changes in the laboratory test, vital signs, physical examination and ECG findings, and no liver enzyme elevations were reported.

DISCUSSION

This study demonstrated the non-inferior efficacy and safety of fexuprazan 40 mg once daily to esomeprazole 40 mg once daily in the healing of EE at week 8 in patients with EE. The rates of healing EE were not different between the two groups at week 4. No differences between the groups were found in the secondary endpoints regarding symptom responses, including the first day of the complete resolution of symptoms (heartburn and acid regurgitation) and the proportions of patients without symptoms along with the proportions of symptom-free days in the first 7 d and throughout 8 wk of the treatment period. Furthermore, the two groups did not differ in the changes in RDQ and in GERD-HRQL from baseline at weeks 4 and 8. Serum gastrin levels and safety-related TEAEs and ADRs did not differ.

Our results were consistent with those of other P-CABs (tegoprazan and vonoprazan) in comparison with PPIs. Studies in patients with GERD and healthy volunteers have revealed the efficacy and safety of tegoprazan and vonoprazan, to be similar to those of PPIs. In a phase I study of tegoprazan, which has been used since 2018 after approval in South Korea, it safely inhibited acid secretion compared to esomeprazole[24]. In a multicenter, randomized, double-blind, and parallel-group non-inferiority study on 302 patients with endoscopically confirmed EE[19], tegoprazan 50 mg and 100 mg indicated cumulative healing rates of 98.9% and 98.9% at week 8, respectively, compared to the 98.9% healing rate of esomeprazole 40 mg. Regarding vonoprazan, its efficacy has been identified in clinical and pharmacological factors, including healing EE, symptom responses, maintenance treatment effect after healing EE, efficacy in refractory GERD, the effect of intermittent therapy, and the pH 4 holding time ratio[25-28]. A study of short-term symptom response at week 4 was similar: 88.0% and 81.8% in the esomeprazole 20 mg and vonoprazan 20 mg groups, respectively[29]. In a dose-ranging study,

Table 4 Overall Summary of treatment-emergent adverse events (safety set)

	Fexuprazan 40 mg (*n* = 131)	Esomeprazole 40 mg (*n* = 131)	Total (*n* = 262)
	n (%) [number of event]		
Subjects with TEAEs	22 (16.8) [34]	25 (19.1) [34]	47 (17.9) [68]
95%CI	[10.4, 23.2]	[12.4, 25.8]	[13.3, 22.6]
P value[1]			0.629[c]
Subjects with ADRs	9 (6.9) [13]	7 (5.3) [11]	16 (6.1) [24]
95%CI	[2.5, 11.2]	[1.5, 9.2]	[3.2, 9.0]
P value[1]			0.606[c]
Subjects with serious TEAEs	0	0	0
Subjects with serious ADRs	0	0	0
Most frequently occurring (≥ 2%) TEAEs by system organ class and preferred term			
System organ class preferred term			
Gastrointestinal disorders			
Diarrhoea	4 (3.1) [4]	2 (1.5) [2]	6 (2.3) [6]
Nervous system disorders			
Dizziness	1 (0.8) [1]	3 (2.3) [3]	4 (1.5) [4]

[1]Testing for difference among treatment groups [Chi-square test (c)].
Note: Denominator of percentage is the number of subjects in each treatment group. TEAEs: Treatment-emergent adverse events; ADRs: Adverse drug reactions.

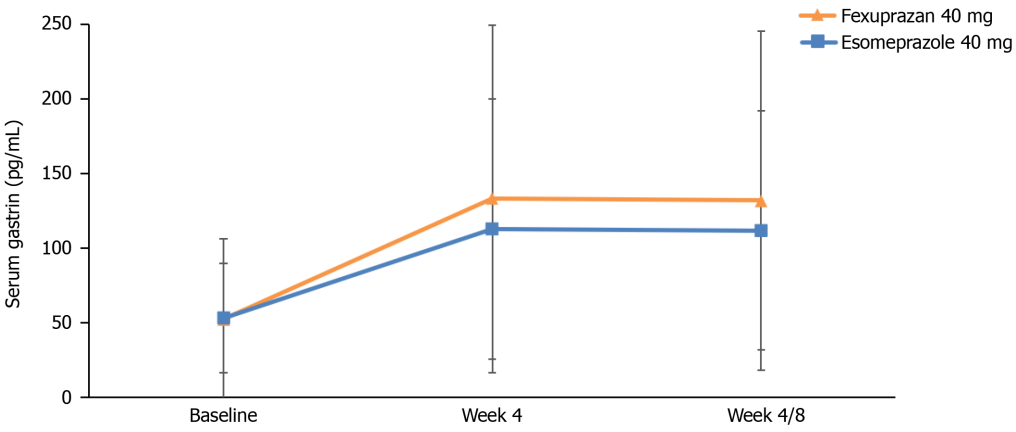

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Figure 4 Changes from baseline in serum gastrin levels at weeks 4 and 8 (per-protocol set).

vonoprazan 5, 10, 20, and 40 mg exhibited non-inferior efficacy to lansoprazole 30 mg in the healing rates of EE at week 8[30]. In those with severe grades of EE and extensive metabolizers, treatments with vonoprazan 20 mg and lansoprazole 30 mg for 8 wk did not differ in the rates of EE healing[23]. Additionally, the recurrence rates of EE were significantly lower after a 24-wk treatment using 10 mg and 20 mg vonoprazan than with lansoprazole 15 mg[31]. Regarding the effect of vonoprazan on gastric acidity, the pH 4 holding time ratio significantly increased from 73.21% to 96.46% and from 69.97% to 100.00% in the 20 mg and 40 mg groups, respectively[26].

In this study, fexuprazan led to rapid treatment response in patients with moderate-to-severe heartburn. The proportion of patients without heartburn on day 3 who had moderate-to-severe symptoms was significantly higher with fexuprazan than with esomeprazole, in both day-time and night-time, and also at night-time only. Nocturnal heartburn was reportedly presented in approximately 80% of patients with frequent heartburn and impaired sleep quality and daytime HRQL[6,32]. Moreover, the continuous use of PPIs was not effective for nocturnal heartburn in 30% of patients with

reflux esophagitis[33], and in over 50% of patients with symptomatic EE[34]. Thus, this study suggests that fexuprazan may provide rapid symptom resolution in patients with nocturnal heartburn and refractory response to PPI treatment. The rapid response of symptoms to P-CABs was identified in another study revealing that vonoprazan 20 mg relieved heartburn symptoms on day 1 in more patients than lansoprazole 30 mg[35]. Although the present study did not demonstrate faster healing of EE, there have been studies showing rapid healing of EE after 2-week treatment of vonoprazan than PPIs[23,36]. Accordingly, in conjunction of this faster healing in EE with our finding of rapid symptom response by fexuprazan, it is cautiously suggested that patients with EE may need a relatively short-term treatment period by fexuprazan than PPIs. Further studies on shorter treatment in EE by fexuprazan are needed.

The pharmacodynamic and pharmacokinetic profiles of fexuprazan explain the rapid onset and sustained inhibition of acid secretion in GERD. Studies of fexuprazan in healthy individuals revealed that the mean percentage of time of gastric pH > 4 was achieved in 80% of 24 h and even at night. However, esomeprazole achieved a lower mean percentage time of gastric pH > 4, which was also lower at night[14]. With regard to the pharmacokinetic parameters, C_{max} was reached within 1-4 h after dosing, and the mean elimination half-life was approximately 9 h. Fexuprazan also exhibited dose-response relationships. Plasma concentrations increased proportionately with the doses ranging from 10-320 mg, whereas multiple doses did not cause significant accumulation. The elimination pathway of fexuprazan was not a renal route but probably *via* the liver or gut. Furthermore, in contrast to PPIs, food intake was not necessary for optimal action, as the parameters of gastric pH and plasma concentrations of fexuprazan did not change with a high-fat diet. Adverse drug effects on the liver were not higher with fexuprazan than with placebo, in contrast to the 0.2% potential liver toxicity in the pre-clinical experiment of vonoprazan[37]. Moreover, the gastrin-increasing effect of fexuprazan was similar to that of other PPIs, and was less frequent than that of vonoprazan[38]. Furthermore, the effects on gastric acid suppression, serum gastrin elevation, and dose response relationship were also consistent in different populations including Korean, Caucasian, and Japanese ethnicities[39].

In our study, fexuprazan improved one of the extraesophageal symptoms of GERD better than esomeprazole. Despite its unknown pathophysiology, patients with GERD-related chronic cough have been treated with PPIs with unsatisfactory symptom control. The superior efficacy of PPIs over placebo has not been confirmed in patients with GERD-related chronic cough in recent randomized controlled trials (RCTs)[40,41]. In addition, a meta-analysis of 5 RCTs did not suggest any evidence in favor of PPI therapy[42]. Taken the effect of fexuprazan in this study with the overall inadequate efficacy of PPIs in chronic cough, we suggest that fexuprazan could provide a better solution than PPIs for GERD-related chronic cough.

This study revealed elevated serum gastrin levels, but these were not significantly different between both groups. Previous reports have revealed higher serum gastrin levels in the P-CAB group than in the PPI group[37,43]. In the study of 212 outpatients, the serum gastrin in the P-CAB group had 2-3 fold and 1-2 fold increases than the normal and PPI groups, respectively[43]. However, increased serum gastrin levels were limited, particularly in patients with normal mucosa or mild atrophic gastritis. Additional limitations were the treatment periods of less than one year and the sampling time at pre-meal rather than at the peak level of 30 min after meals.

This study had some limitations. First, the number of patients classified as LA grade C/D was small. Actually, those with LA grades C/D accounted for only 6.2% of the fexuprazan and 7.0% of the esomeprazole groups. Therefore, it was difficult to confirm the advantage of fexuprazan, better clinical performances due to unique pharmacokinetics and pharmacodynamics of fexuprazan in severe EE than PPIs, as in other P-CAB studies[30]. Future fexuprazan studies need to be focused on significantly larger number of patients with severe EE (LA grades C/D). Second, the treatment period was only eight weeks, and studies on the long-term safety or recurrence rates after EE healing are required in the future, considering the insufficient data regarding the long-term safety of P-CABs. Third, when evaluating symptom severity, the possible effects of comorbidities such as chronic obstructive pulmonary disease were not considered[44].

CONCLUSION

We concluded that fexuprazan 40 mg once daily has non-inferior efficacy and safety to esomeprazole 40 mg once daily in healing EE at weeks 4 and 8. From the symptom evaluation through the symptom diary, RDQ and GERD-HRQL, it was confirmed that fexuprazan improved symptoms of heartburn and acid regurgitation and quality of life similarly to esomeprazole. The increase in serum gastrin levels by fexuprazan was not different from that of esomeprazole. Future research on fexuprazan is needed to evaluate the long-term efficacy and safety of fexuprazan in GERD including EE, PPI-refractory GERD, and other acid-related diseases along with the long-term maintenance therapy including on demand or intermittent treatment.

ARTICLE HIGHLIGHTS

Research background

Currently, a mainstay therapy of erosive esophagitis (EE) is proton pump inhibitors (PPIs), which have disadvantages like their delayed absorption and variable efficacy due to differences in drug metabolism A novel potassium-competitive acid blocker, fexuprazan, suppresses the K^+/H^+-ATPase enzyme reversibly and competetively in proton pumps within gastric parietal cells.

Research motivation

A previous study of fexuprazan on healthy individuals demonstrated the effect of its acid suppression and tolerability, by showing that gastric pH > 4 was reached within 2 h and maintained for 24 h in a dose-dependent manner. However, the efficacy and safety of fexuprazan in EE have not been compared to esomeprazole, one of the most widely used PPIs in gastro-esophageal reflux disease (GERD) including EE.

Research objectives

The aim of this phase III, double-blind, randomized, active-controlled, multi-center study was to compare the efficacy and safety between fexuprazan and esomeprazole in patients with EE.

Research methods

Adult patients who have EE confirmed by endoscopy were randomized 1:1 to receive fexuprazan 40 mg or esomeprazole 40 mg once daily for eight weeks in South Korea between December 2018 and August 2019. The primary endpoint was healing rates confirmed by endoscopy at week 8. The secondary endpoints included the proportion of patients with healed EE at week 4, symptom response, and GERD-related quality of life assessed from the evaluation through the symptom diary, reflux disease questionnaire (RDQ) and GERD-health related quality life (GERD-HRQL) questionnares. We also compared safety profiles and serum gastrin levels between the groups.

Research results

This study shows that fexuprazan 40 mg once daily is non-inferior to esomeprazole 40 mg once daily in healing rates of at weeks 4 and 8 and in symptom improvement of heartburn and acid regurgitation and RDQ and GERD-HRQL. In 218 participants who completed the study per protocol (fexuprazan 40 mg, n = 107; esomeprazole 40 mg, n = 111), fexuprazan was non-inferior to esomeprazole regarding the healing rate at week 8 [99.1% (106/107) *vs* 99.1% (110/111)], and at week 4 [90.3% (93/103) *vs* 88.5% (92/104)], symptom responses, and quality of life assessments. Also, serum gastrin levels at weeks 4 and 8 and drug-related side effects were not significantly different between the groups.

Research conclusions

This study results indicate that fexuprazan 40 mg once daily can be an alternative of esomeprazole 40 mg once daily for patients with erosive esophagitis in terms of efficacy and safety.

Research perspectives

Further research on fexuprazan should be directed to evaluate the long-term efficacy and safety of fexuprazan in various acid-related gastrointestinal diseases including NERD, PPI-refractory GERD, H. pylori infection, peptic ulcer diseases, and so on.

FOOTNOTES

Author contributions: Lee OY contributed to study design, acquisition and interpretation of data, and critically reviewed and edited the manuscript; Lee KN contributed to the data interpretation, and drafting and editing the manuscript; All authors contributed to enrolment of patients, agreed to be responsible for every aspect of this work, reviewed and finally approved the manuscript.

Institutional review board statement: This study was approved by the institutional review boards (IRBs) of each institution, conducted in compliance with the relevant ethics guidelines. All the study medications and procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

Clinical trial registration statement: This study is registered at ClinicalTrials.gov. The registration identification number is NCT03736369.

Informed consent statement: All study participants provided informed written consent prior to study enrollment.

Conflict-of-interest statement: The corresponding author, Lee OY, had been a member of outside directors at the Daewoong Pharmaceutical Co., Ltd, form the period March 24 2018 to Nov 2 2021. All other authors declare no conflict of interests regarding this study.

Data sharing statement: Data are available upon reasonable request.

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

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LETTER TO THE EDITOR

Comment on "Prognostic value of preoperative enhanced computed tomography as a quantitative imaging biomarker in pancreatic cancer"

Jian Yang, Ying Liu, Shi Liu

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies because of its high invasiveness and metastatic potential. Computed tomography (CT) is often used as a preliminary diagnostic tool for pancreatic cancer, and it is increasingly used to predict treatment response and disease stage. Recently, a study published in *World Journal of Gastroenterology* reported that quantitative analysis of preoperative enhanced CT data can be used to predict postoperative overall survival in patients with PDAC. A tumor relative enhancement ratio of ≤ 0.7 indicates a higher tumor stage and poor prognosis.

Key Words: Pancreatic ductal adenocarcinoma; Computed tomography; Tumor relative enhancement ratio; Diagnostic imaging; Quantitative analysis; Prognosis

Core Tip: Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal malignancies because of its high invasiveness and metastatic potential. The purpose of this letter is to highlight that a quantitative parameter based on enhanced computed tomography, namely the tumor relative enhancement ratio, can reveal the correlation between high malignant potential because of hypervascularity and poor prognosis in PDAC.

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TO THE EDITOR

The stroma of pancreatic ductal adenocarcinoma (PDAC) is a fibroproliferative microenvironment mainly composed of fibroblasts, and its low vascular supply severely limits the tumor utilization of oxygen and nutrients[1,2]. In such a situation, invasion into fertile tissue becomes an acquired behavior of the tumor in response to severe metabolic stress[3,4]. We were extremely interested in a retrospective study by Gao *et al*[5] published in the June 2022 issue of *World Journal of Gastroenterology*. This was a moderate-quality observational study with a Newcastle-Ottawa Quality Assessment Scale score of 6 (3, 1, 2) that was assessed independently by two of our authors[6]. The importance of this study was that it revealed the ability to predict the overall survival of patients with resectable pancreatic cancer (PC) from an imaging perspective, providing assistance in developing early treatment plans and improving patient prognosis. Gao *et al*[5] initially found that enhanced computed tomography (CT) characterizing vascular perfusion could be used as a quantitative imaging biomarker (QIB) of the malignant potential of PC. Based on this innovative idea and combined with data analysis, the authors demonstrated the value of QIB for predicting the prognosis of patients with PC. In addition, the authors proposed some new concepts to calculate the difference between the region of the overall tumor of the portal venous (PV) phase and that of the non-enhancement phase as the tumor enhancement amplitude (TEA), and the difference between the pancreatic tissue outside the tumor of the PV phase and that of the non-enhancement phase was used as the pancreatic enhancement amplitude (PEA) outside the tumor[5]. The tumor relative enhancement ratio (TRER) was then derived as TEA/PEA. Based on a retrospective analysis of 67 patients with resectable PC, the conclusions drawn by the authors properly summarize the data in the study. Furthermore, this study provided the unique insight that preoperative enhanced CT is a simple and effective predictive tool for overall survival in patients with PDAC and highlighted the need for close monitoring of patients with a TRER ≤ 0.7 because their prognosis is likely to be poor. We would like to thank Gao *et al*[5] for this study, which helped to advance clinical diagnosis and treatment.

In recent years, QIB has become more widely used in clinical practice because the objective features obtained from *in vivo* images measured on a scale of proportions or intervals can serve as indicators of normal biological processes, pathogenic processes, or responses to therapeutic interventions[7]. We therefore use an open multidisciplinary citation analysis database based on artificial intelligence techniques termed *Reference Citation Analysis*. We used "quantitative imaging biomarker" and "pancreatic cancer" as search terms to find the most recent (last 5 years) and relevant cutting-edge research. Overall, the application of QIB is mainly combined with a clinical perspective, and it plays an important role in characterizing tissue, detecting disease, identifying phenotypes, defining longitudinal changes, or predicting outcomes[7]. As previously mentioned, the highly invasive and metastatic nature of PC makes the search for prognostic biomarkers with high accuracy challenging. Numerous studies developed different QIB models that, in addition to characterizing microvascular density[8], significantly compensate for the survival prediction rate of clinical models[9] and contribute to clinical decision making. Next, we provide a brief analysis of PC survival prediction based on the study by Gao *et al*[5] and in the context of the current state of research.

At present, radiomics research concerning the prediction of the prognosis of resectable PC mainly focuses on the analysis of tumor texture features based on CT images[10,11]. Low-attenuation radiomic features of tumors are associated with poorer survival[12,13]. In addition, current radiomics data suggest that first-order entropy is associated with overall survival in PDAC patients and can significantly improve prediction accuracy[14]. Gao *et al*[5] revealed that PDAC hypervascularity was positively associated with poorer survival based on a quantitative analysis of vascular perfusion imaging, which is consistent with the aforementioned low blood supply of highly invasive PDAC[1,2]. In addition, TRER is calculated using CT, which is simple and more easily accepted by clinicians and supports its strong practicability.

We are extremely concerned about the study of PDAC invasion and metastasis because high invasion and metastasis are the characteristics of PDAC itself[15]. Several current radiomics studies identified several predictors of survival following treatment in patients with unresectable or advanced PDAC, including the mean value of positive pixels and kurtosis[16], age and homogeneity on unenhanced CT [17], skewness[18], and cluster tendency with a square root filter[19]. Gao *et al*[5] cited several limitations, including the absence of patients with metastasis. We anticipate future research by Gao *et al* [5] on the use of TRER based on enhanced CT to predict the treatment response and survival of patients with metastatic PDAC after treatment, which will bring great benefits concerning the diagnosis and treatment of patients. In conclusion, quantitative analysis based on enhanced CT imaging (TRER) has

good acceptability and utility for predicting the prognosis and survival of patients with PDAC.

FOOTNOTES

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