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REVIEW

Coronavirus disease–2019 and the intestinal tract: An overview

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Abstract

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection can progress to a severe respiratory and systemic disease named coronavirus disease–2019 (COVID-19). The most common symptoms are fever and respiratory discomfort. Nevertheless, gastrointestinal infections have been reported, with symptoms such as diarrhea, nausea, vomiting, abdominal pain, and lack of appetite. Importantly, SARS-CoV-2 can remain positive in fecal samples after nasopharyngeal clearance. After gastrointestinal SARS-CoV-2 infection and other viral gastrointestinal infections, some patients may develop alterations in the gastrointestinal microbiota. In addition, some COVID-19 patients may receive antibiotics, which may also disturb gastrointestinal homeostasis. In summary, the gastrointestinal system, gut microbiome, and gut-lung axis may represent an important role in the development, severity, and treatment of COVID-19. Therefore, in this review, we explore the current pieces of evidence of COVID-19 gastrointestinal manifestations, possible implications, and interventions.

Key Words: COVID-19; SARS-CoV-2; Gastrointestinal; Microbiota; Antibiotics

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Core Tip: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection can progress to a severe respiratory and systemic disease named coronavirus disease–2019 (COVID-19). Nevertheless, SARS-CoV-2 can also generate a gastrointestinal infection. In this review, we explore the impact of COVID-19 on the gastrointestinal system, gut microbiome, and the gut-lung axis and the severity and possible implications and interventions in COVID-19 patients.

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INTRODUCTION

The coronavirus disease–2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Coronaviruses (CoVs) are a family of single-stranded ribonucleic acid (RNA) viruses; currently six subtypes of CoVs can infect humans. Two CoVs, the Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1), are the etiologic agents of the previous epidemics and have caused over 2000 deaths worldwide[1,2].

COVID-19 can cause a systemic and respiratory infection that can lead to death[3]. Since November 2019, SARS-CoV-2 has infected over 80 million people and killed over 1.5 million people worldwide, being declared a pandemic by the World Health Organization[4]. Several comorbidities have been postulated as risk factors for severe COVID-19, such as high age[5], smoking, chronic obstructive pulmonary disease[6], obesity[7], pregnancy[8], and co-infections[9].

SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) to invade the host's cells. ACE2 and TMPRSS2 are expressed in many different organs in the human body, such as the lungs, heart, liver, kidney, and brain, intestine luminal cells, colonic epithelial cells, and small intestinal enterocytes[10-13]. In addition, SARS-CoV-2 infection has been described in multiple organs, including the lungs, pharynx, heart, liver, brain, kidneys, and gastrointestinal tract[14,15].

COVID-19 AND THE GASTROINTESTINAL TRACT

Gastrointestinal infections have been reported[16] (Table 1), with a lower frequency in comparison with the previous SARS-CoV-1 infection[17]. Nevertheless, SARS-CoV-2 can remain positive in fecal samples after nasopharyngeal clearance and may remain infective[18].

The most frequent gastrointestinal symptoms in COVID-19 are diarrhea, nausea, abdominal pain, and lack of appetite[19]. After a viral gastrointestinal infection, some patients may develop alterations on the gastrointestinal microbiota such as an increase of Proteobacteria and a simultaneous reduction of Bacteriodetes[20]. A recent report by Zuo *et al*[21] identified alterations in the intestinal microbiota of hospitalized patients with COVID-19, with a reduction in beneficial commensals bacteria and an increase in opportunistic pathogens[21]. In addition, Gupta *et al*[18] verified a reduction in the community richness and microbial diversity in COVID-19 patients with and without diarrhea. The intestinal tissue and feces may also be acting as a reservoir; recent reports have identified that even after negative nasopharyngeal and oropharyngeal swabs test for SARS-CoV-2 RNA, patients could still possess SARS-CoV-2 RNA in the stool samples[16,22]. Another report identified that the duration of COVID-19 symptoms was prolonged in patients with diarrhea and that the stool samples from patients with diarrhea were more frequently positive for virus RNA[23].

Although is not clear the mechanisms responsible for the development of diarrhea in COVID-19, the current hypothesis is that the direct viral infection on the intestinal tissue and local immune response to the virus may be involved. In fact, the detection

Table 1 Manuscripts describing patients with severe acute respiratory syndrome coronavirus-2 ribonucleic acid detection in rectal swabs or fecal samples

COVID-19 respiratory manifestations	COVID-19 gastrointestinal clinical manifestations	Percentage of patients with gastrointestinal clinical manifestations	Rectal swabs or fecal samples positive for SARS-CoV-2	Ref.
Yes	Yes	65.38%	53.42%	[16]
Yes	Yes	60%	50%	[122]
Yes	Yes	80%	90%	[123]
Yes	Yes	11%	22%	[124]
Yes	Yes	33%	80%	[125]
Yes	Yes	No data	39%	[126]
Yes (89 only respiratory/69 respiratory and gastrointestinal)	Yes (48 only gastrointestinal/69 respiratory and gastrointestinal)	56%	54% of a cohort of 22 individuals	[127]
Yes	Yes	No data	29%	[128]
Yes	Yes	31%	55%	[24]
Yes	Yes	No data	83%	[129]
Yes	Yes	No data	25%	[130]

COVID-19: Coronavirus disease–2019; SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2.

of SARS-CoV-2 RNA in stool COVID-19 patients may indicate the possibility of fecal-oral transmission[24].

In addition to the SARS-CoV-2 impact on the gut immune response, bacterial co-infections and secondary infection could also occur in COVID-19 patients, implicating the necessary usage of antibiotics[25]. Nevertheless, even in the absence of bacterial co-infections in COVID-19 patients some reports highlighted that the usage of antibiotics is a common clinical practice in COVID-19 patients[25,26], which could also disrupt the gastrointestinal microbiome. Bacterial communities are present in numerous body sites such as the gut, the respiratory system, and skin; therefore, the unnecessary usage of antibiotics may predispose COVID-19 patients to opportunistic infections inside and outside of the gastrointestinal environment.

GUT MICROBIOTA

The microbiota is influenced by environmental factors, food, drugs, and infections[27]. Each microbiota possesses unique characteristics[28,29], differing its composition according to the site in the human body. Many factors influence the microbiome composition such as local pH, temperature, and nutrients[30]. Microorganisms can be found in nearly every niche of the human body[31], but the gastrointestinal tract is the largest interface between the host and microorganisms in the human body. There are approximately 10^{13}-10^{14} microorganisms in the gastrointestinal tract, with greater genomic content than in the human genome[32].

Microbes and humans have a symbiotic relationship. Commensal microbes are crucial for human health, regulating many physiological functions, degradation of substances, production of metabolites, and immune response[33]. Microorganisms can activate and stimulate the differentiation of T helper cells (Th) 1, Th2, Th17, and T regulatory cells (Treg), which in consequence can regulate the immune response[34,35]. A low-diversity in the intestinal microbiota can increase the susceptibility to local[36] and pulmonary disorders[37].

The microbiome's environment is in constant regulation, modulated by external microorganisms and other non-bacterial compounds, for example, food in the intestinal microbiota and viruses. An abrupt change in the microbiota can generate an imbalance in the commensal bacteria and/or increase opportunistic microbes, increasing the susceptibility to diseases[38,39].

The microbiota is essential for the development of the human immune system and can influence both local and non-local immune responses, such as the gut-lung axis[40].

It is well established that alterations in the gut microbiota can modulate the development of respiratory disease[41].

In patients with gut dysbiosis such as patients with an established intestinal inflammatory disease or obese patients, the intestinal microbiota may be a secondary risk factor for the development of severe COVID-19[7]. Besides, patients with COVID-19 may develop a dysbiosis in the gastrointestinal microbiota[21] and a reduction in short-chain fatty acid-producing bacteria[42].

GUT MICROBIOTA DYSBIOSIS

Disruption of the gut microbiota can trigger inflammatory events that are associated with metabolic dysfunction, obesity, cancer, and neurological disorders[43,44]. The proliferation or reduction in certain microorganisms can increase the stimulation of innate immune receptors, like nucleotide-binding oligomerization domain-like receptors and Toll-like receptors[45]. The stimulation of this receptor triggers several pro-inflammatory signals and the production of cytokine and chemokine, which modulate the adaptive immune system, influencing both local and systemic immune response[43,44].

The activation of Toll-like receptors on immune cells by the microbiota can generate a low-grade systemic inflammation in the host that is associated with a change in metabolic and immunological responses[46]. Alterations in the microbiome are related to the development of diseases such as obesity, inflammatory bowel disease, and cancer[47]. Therefore, SARS-CoV-2 gastrointestinal infections and alteration of gut homeostasis may be implicated in the development of disease and impact immune response to oral vaccines and medicines and the pathogen immune response[48,49].

NUTRITIONAL INTERVENTION

Several reports have highlighted the potential role of nutrients in the modulation of the immune response to SARS-CoV-2 or a direct anti-viral and/or anti-SARS-CoV-2 properties[50-53]. During this pandemic, nutritional aspects such as obesity[7,54], malnutrition[55], and micronutrient deficiency[56,57] have been postulated as risk factors for severe COVID-19. Nevertheless, the composition of the human microbiota is influenced by many factors, including dietary components[41]. Some bacteria can ferment nondigestible carbohydrates (prebiotics), like soluble fibers, to produce short-chain fatty acids (SCFAs). SCFAs can stimulate the growth and/or activity of commensal bacteria and are associated with health benefits. SCFAs can induce the regulation of the intestinal barrier, reduce oxidative stress, control diarrhea, and modulate intestinal motility and also induce a local and systemic anti-inflammatory effect[58,59].

High-fiber diets can induce the proliferation of beneficial commensal microbes, such as *Lactobacillus* spp. and *Bifidobacterium* spp. in the gastrointestinal tract[60]. In fact, high-fiber diets may increase immunoglobulin A production and modulate the secretion of interferon-gamma and interleukin (IL)-10[61-63], which could aid in the control of gastrointestinal infections.

Prebiotics may alter the microbiota composition by a mechanism called cross-feeding, when the product of a prebiotic's fermentation by a microorganism in the microbiota can be used as a substrate by another microorganism[64-66]. Another mechanism that prebiotics can alter the microbiota is through pH alterations. The fermentation products are predominantly acids, which may cause a decrease in the intestinal pH, restraining the growth of acid-sensitive bacteria, such as *Bacteroides* spp., and promoting butyrate-producing bacteria[67].

SCFAs are divided into acetate, propionate, and butyrate. All SCFAs have potential anti-inflammatory effects with the reduction of prostaglandin E2 and inflammatory cytokines[68]. Acetate can curb the activation of the NLR family pyrin domain containing 3 inflammasome[69]; propionate can inhibit histone deacetylase and reduce lipopolysaccharide-induced inflammation[70]. Butyrate has been associated with anti-cancer properties and reduces pulmonary inflammation[71,72].

Overall, research has demonstrated a potential anti-inflammatory role for SCFAs in both local (intestinal) and non-local inflammation *via* direct anti-inflammatory effects or modulation of the microbiota[71,73,74].

SCFAs can induce the release of anti-inflammatory cytokines such as IL-10[75,76], promoting the regulation of Th cells and inflammatory diseases[77], including

inflammatory bowel disease[78].

Another intervention for the modulation of the gastrointestinal microbiome is *via* the consumption of probiotics. Probiotics are bacteria that can be ingested and provide a beneficial interaction to the host[79]. Several studies have investigated the effects of probiotics on the gut microbiota[80], with conflicting results involving their ability to graft on the commensal microbiota[81-84]. However, probiotics can produce metabolites that can modify and influence the commensal microbiota, intestinal barrier, and immune system[85,86].

Probiotics can also aid in the prevention or treatment of bacterial[87] and viral infections[88]. The administration of probiotics increases the survival of mice infected with the influenza virus[87]. Besides the influenza virus, studies have demonstrated beneficial protection against respiratory syncytial virus infection[89].

The heath benefit of probiotics in respiratory viral infections is due to the modulation of cytokine production and oxidative stress[90]; therefore, they may possibly be an adjuvant treatment for the aberrant release of pro-inflammatory cytokines, chemokines, and oxidative stress during severe COVID-19[91].

The most used probiotics are *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*[92]. Although there is extensive research demonstrating their health benefits, currently there is a gap in knowledge involving the ideal dosage and comparison among strains of probiotics[93].

DISCUSSION

COVID-19 is a potentially deadly disease, which can infect intestinal cells[13]. SARS-CoV-2 gastrointestinal infections[16] can generate diarrhea, pain, and vomiting[19]. To date, few reports have investigated the possible consequences of gastrointestinal infection by SARS-CoV-2; nevertheless, viral infections can alter the gastrointestinal microbiota[20]. A report by Xu *et al*[94] identified a reduction in *Lactobacillus* and *Bifidobacterium* in fecal samples from COVID-19 patients[94]. Also, the microbiome of COVID-19 patients can be disturbed by the necessary or unnecessary use of antibiotics[34].

Yeoh *et al*[95] identified that the alteration on the gastrointestinal microbiome in COVID-19 patients was independent of medications. The alterations on the gut microbiome included a reduction in *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *bifidobacteria* for up to 30 d after SARS-CoV-2 clearance[95].

The microbiota dysbiosis in COVID-19 may be involved in the inflammatory response and may be a persistent problem after COVID-19 resolution, indicating a possible role for nutritional interventions to curb the inflammatory response and reestablish the gastrointestinal homeostasis of COVID-19 patients.

Dietary and nutritional intervention can modulate the immune response, increasing or dampening the anti-viral response[59-62]. Western-style diets (low fiber content) can increase *Bacteroidetes* and reduce *Firmicutes*[96] and are linked to the development of obesity[97], a risk factor for severe COVID-19[7]. Although reports have identified an increase in SCFAs in fecal samples from obese individuals[96], SCFAs have been associated with control of appetite[98] and increase energy expenditure[99].

In addition, very low fiber-diets can lower mucus production on the intestine and increase the susceptibility to gastrointestinal infections[39]. Importantly, a change in diet can modify the microbiota composition[100]. The microbial communities are in constant change and are also affected seasonally by food consumption[101]. In fact, a reduction in the consumption of fiber can change the microbiota in as little as 1 d, reducing SCFAs production[102].

In opposition, high fiber-diets increase *Firmicutes* and *Actinobacteria* on the gut microbiota[103] and increase the production of SCFAs, which can aid in the reduction of pulmonary inflammation, *via* the gut-lung axis[41,104,105] and promote a local and systemic anti-inflammatory response *via* IL-10 production and Treg cells[75,76,106]. The ingestion of probiotics may stabilize or alter the gastrointestinal microbiome, especially after a perturbation of the microbiota such as post usage of antibiotics or gastrointestinal infections[107].

Probiotic treatments with *Bacillus subtilis* and *Enterococcus faecalis* have been demonstrated to reduce ventilator-associated pneumonia[108]. Treatment with *Lactobacillus rhamnosus* can reduce ventilator-associated pneumonia and *Clostridium difficile*-associated diarrhea in mechanically ventilated patients[109], making it a possible addition to the treatment of patients with severe COVID-19 in intensive care units with assisted mechanical ventilation. Treatment with *Lactobacillus* may be of particular

importance, because respiratory infections may cause a reduction in *Lactobacillus*, and an increase in *Enterobacteriaceae* and intestinal IL-17 inflammation[110].

Targeting IL-17 has been postulated as a treatment for COVID-19[111] because of the increase in IL-17 in severe COVID-19 patients compared to moderate COVID-19 patients[111]. IL-17 and IL-17-producing T helper cells (Th17), type three innate lymphoid cells, invariant natural killer cells, and γδ T cells are involved in the immune response of COVID-19[112]. IL-17 receptor is expressed on the surface of many different cells such as neutrophils, eosinophils, epithelial cells, keratinocytes, and fibroblasts[112]. In addition, IL-17 can directly influence the expression of ACE2, SARS-CoV-2 entry's receptor[113].

The usage of IL-17 blockade, such as monoclonal antibodies against IL-17A and/or IL-17 receptor A, may represent a possible therapeutic option for COVID-19[112]. Nevertheless, IL-17 is an important cytokine in the immune response against *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*, common pathogens in respiratory and intestinal tract infections[112,114]. Secondary bacterial infections can occur in the respiratory system following SARS-CoV-2 infection, especially in patients with invasive mechanical ventilation[34]. IL-17 is especially important for intestinal homeostasis[115]. Therefore, treatment with anti-IL-17 should consider the possible risk for an increase in susceptibility for bacterial infections both respiratory and intestinal.

COVID-19 patients may also develop a cytokine storm syndrome, which may induce multi-organ failure and lead to death or long-term consequences[91]. In this context, probiotics or prebiotics treatment have been previously demonstrated anti-inflammatory effects in respiratory infections *via* the increase in SCFAs[71,73,74,88,116].

SCFAs production and health benefit can be increased by the ingestion of highly fermentable fiber diets[117], probiotics[73], oral administration of drugs like tributyrin (a prodrug of butyrate)[118], or SCFAs directly[119-121].

In this context, the intake of prebiotics and/or probiotics can represent a significant prophylactic intervention and/or recovery of COVID-19 patients.

CONCLUSION

The SARS-CoV-2 infection on the gastrointestinal tract and the long-term consequences of COVID-19 in gastrointestinal homeostasis still needs further investigations. It is clear that SARS-CoV-2 can infect the gastrointestinal tract and impact the intestinal immune response and the gut microbiome. Currently, there is no specific treatment for COVID-19, but investigations on the impact of nutritional intervention *via* modulation of the immune response or *via* microbiota are been investigated and may represent a significant prophylactic intervention and/or recovery of COVID.

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REVIEW

Metabolic complications of hepatitis C virus infection

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Abstract

Hepatitis C virus (HCV) infection is a systemic disease that is implicated in multiple extrahepatic organ dysfunction contributing to its protean manifestations. HCV is associated with diverse extrahepatic disorders including atherosclerosis, glucose and lipid metabolic disturbances, alterations in the iron metabolic pathways, and lymphoproliferative diseases over and above the traditional liver manifestations of cirrhosis and hepatocellular carcinoma. The orchestration between HCV major proteins and the liver-muscle-adipose axis, poses a major burden on the global health of human body organs, if not adequately addressed. The close and inseparable associations between chronic HCV infection, metabolic disease, and cardiovascular disorders are specifically important considering the increasing prevalence of obesity and metabolic syndrome, and their economic burden to patients, the healthcare systems, and society. Cellular and molecular mechanisms governing the interplay of these organs and tissues in health and disease are therefore of significant interest. The coexistence of metabolic disorders and chronic hepatitis C infection also enhances the progression to liver fibrosis and hepatocellular carcinoma. The presence of metabolic disorders is believed to influence the chronicity and virulence of HCV

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leading to liver disease progression. This comprehensive review highlights current knowledge on the metabolic manifestations of hepatitis C and the potential pathways in which these metabolic changes can influence the natural history of the disease.

Key Words: Chronic hepatitis C infection; Insulin resistance; Metabolic syndrome; Cardiovascular diseases; Fatty liver; Diabetes mellitus

Core Tip: Available evidence proves a strong association between hepatitis C virus (HCV) and metabolic complications such as hyperlipidemia, hepatic steatosis, insulin resistance, metabolic syndrome, and diabetes mellitus. *De novo* development of insulin resistance and hepatic steatosis in chronic HCV infection influences the disease progression in the liver and enhances overall morbidity and mortality. The influence of metabolic diseases on HCV infection can increase disease severity. The interplay between HCV major proteins and the liver-muscle-adipose axis is complex and still not fully elucidated. Coexistence of metabolic diseases such as diabetes mellitus and HCV infection are also known to result in adverse outcomes of both disorders. There is evidence that successful treatment halts the progression of liver disease, but more studies are required on how treatment influences the metabolic manifestations.

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INTRODUCTION

Hepatitis C virus-beyond a hepatotropic infection

Hepatitis C virus (HCV) is a major cause of liver disease worldwide, with 130-170 million people infected according to the World Health Organization[1]. Approximately 10%-20% of chronically infected patients experience persistent inflammation and develop liver cirrhosis and eventually hepatocellular carcinoma[1]. Well recognized extrahepatic manifestations include but not limited to; glucose and lipid metabolic disorders, atherogenic disease, mixed cryoglobulinemia, lymphoproliferative disorders, renal disease, insulin resistance (IR), type 2 diabetes (T2DM), sicca syndrome, rheumatoid arthritis-like polyarthritis, and autoimmune diseases[2,3]. Moreover, patients with chronic HCV are at an increased risk of developing metabolic bone disease and osteopenia as it was observed in more than 50% of infected subjects[4]. The deleterious effects of metabolic complications as a result of HCV infection is mainly due to impairments in glucose and lipid metabolism[5]. Independent of the stage of hepatic fibrosis, IR and DM are more prevalent in the course of HCV infection and post liver transplantation in chronic HCV (CHC) infected patients[6-8]. Interestingly, prevalence of HCV infection among diabetic patients is higher than in the age-matched general population[9]. CHC induces systemic and hepatic inflammation that contribute to the development of atherosclerosis through elevated levels of pro-atherogenic cytokines and chemokines[10]. Atherosclerosis is also found to be related to the high tumor necrosis factor alpha (TNF-α)/adiponectin ratio that is found in HCV-infected patients and is related to IR[11].

The systemic burden of hepatitis C infection surpasses its liver disease burden due to its metabolic spectrum and it is plausible that, the virus, through disruptions of glucose and lipid homeostasis, stimulates other mechanisms of liver damage and contributes to the pathogenesis of extrahepatic disorders[12]. Therefore, it is imperative to have thorough understanding on the metabolic impact of this enigmatic disease on human body for optimal management of the disease and its consequences. This evidence-based review highlights the current understanding on CHC infection and its metabolic complications.

IMPACT OF CHRONIC HEPATITIS C ON MAJOR METABOLIC ORGANS

Most HCV infected people are unaware of their condition due to its asymptomatic nature. About one-third of infections resolve spontaneously in the first year, the remaining infections persists and become chronic. CHC infection can progress to end-stage liver disease, including cirrhosis and hepatocellular carcinoma (HCC). HCV can also cause serious problems in organ systems other than the liver, including cryoglobulinemic vasculitis, metabolic bone disease, kidney disease, cardiovascular disease, and hematologic malignancies. In the United States, HCV is one of the leading causes of end-stage liver disease requiring liver transplantation, and the mortality attributed to HCV is expected to continue rising during the next 10 years. Figure 1 illustrates the HCV-related metabolic disturbances mainly targeting the liver, skeletal muscle and adipose tissue as current evidence suggests[13].

METABOLIC EFFECTS OF CHRONIC HEPATITIS C INFECTION ON LIVER

Hepatic steatosis in the setting of HCV viral infection is considered as a distinct entity with specific clinical and prognostic implications. The prevalence of hepatic steatosis in patients with HCV infection is 55.54%, which remains higher than that in non-infected individuals[14]. Contrary to non-alcoholic fatty liver disease being associated with hyperlipidemia, CHC is linked with hypolipidemia including hypo-cholesterolemia, hypo-triglyceridemia and lower low-density lipoprotein (LDL) cholesterol levels. Co-existence of non-alcoholic fatty liver disease in HCV infected patients is associated with features of metabolic syndrome (MetS), and is an independent risk factor for advanced liver fibrosis[15-17].

In patients with CHC, genotype 3 has the highest prevalence of hepatic steatosis (40%-86%) while patients with other genotypes is around 50%[18,19]. A direct association exists between genotype 3 HCV (G3-HCV) and the development of steatosis, while in non-genotype 3 CHC, IR plays a key role in the pathophysiology of hepatic steatosis[20,21].

The term "viral steatosis" is used particularly with G3-HCV infection when hepatic steatosis is related with viral load and not MetS and "metabolic steatosis" occurs secondary to IR/MetS in the genotypes G1, G2, and G4[22-24]. Studies in patients infected with G3-HCV have demonstrated that steatosis can improve and even disappear following successful antiviral treatment with interferon and ribavirin; but data from directly acting antivirals (DAAs) are limited[25,26]. The risk of progression of fibrosis is increased with pre-existing steatosis, and a reduced rate of response to antiviral treatment[27-29]. CHC is implicated in the development and progression of non-alcoholic steatohepatitis (NASH). NASH is a chronic state of liver injury that can be caused by CHC infection or coexist with HCV and biopsies have proven that the association leads to advanced fibrosis and is a predictor of liver disease progression in patients with CHC regardless of the genotype[30,31]. Both "viral" and "metabolic" steatosis, stimulates the progression of fibrosis and liver disease influenced by actions of increased insulin levels and inflammatory cytokines on hepatic stellate cells (Figure 2).

HEPATITIS C VIRUS AND IRON METABOLISM

Iron is a central component for HCV virus replication and translation, though it is debatable whether iron promotes or suppresses HCV viral replication. High serum ferritin levels, associated with hepatic iron overload, were evident in CHC patients and considered an independent risk factor for advanced liver fibrosis[32,33]. Alterations of iron metabolism in CHC is believed to be caused by a reduction in the level of hepcidin. Hepcidin, a peptide hormone, is critical in the strict regulation of iron levels under homeostatic states. Though the underlying mechanisms remains unclear, the current opinion propose that all HCV proteins regulates hepcidin expression through signal transducer and activator of transcription 3, mitogen-activated protein kinase, or bone morphogenetic protein/Sma and Mad proteins signaling pathways, and the altered expression of other related genes[34]. Due to the ability of different factors influencing the levels of serum ferritin levels, it serves as an important indicator of hepatic iron overload but only a liver biopsy can confirm the diagnosis. It is vital to further our understanding on how HCV affects iron metabolism and whether it can be used as a therapeutic tool to prevent disease progression.

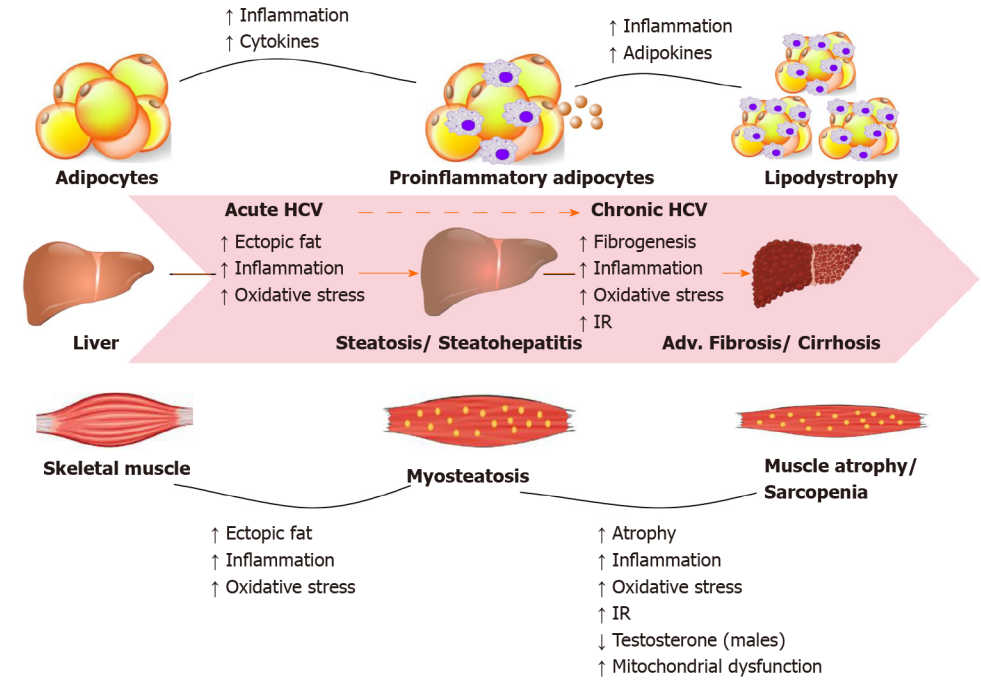

Figure 1 Changes in liver, adipose tissue, and muscle with hepatitis C virus infection. Adv: Advanced; HCV: Hepatitis C virus; IR: Insulin resistance.

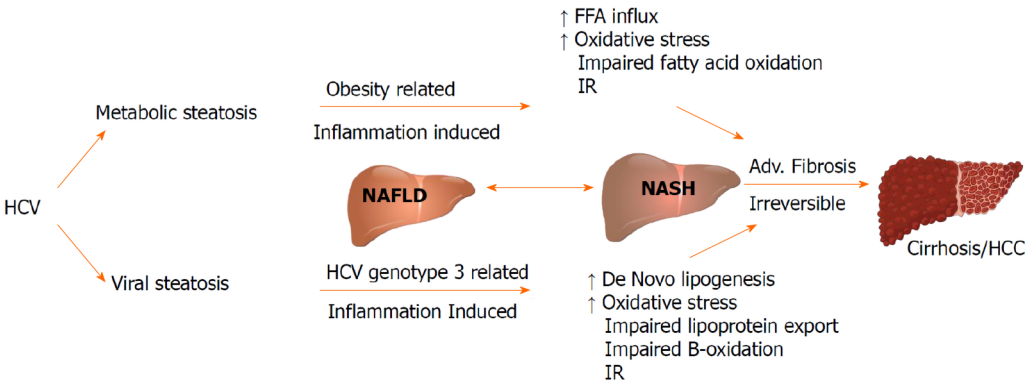

Figure 2 Hepatic steatosis development in hepatitis C virus infection. Adv: Advanced; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; FFA: Free fatty acid; IR: Insulin resistance; HCC: Hepatocellular carcinoma.

EFFECTS OF CHRONIC HEPATITIS C INFECTION ON SKELETAL MUSCLE

CHC infection is associated with skeletal muscle mass loss, sarcopenia, increased intramyocellular lipid deposition, myosteatosis, and low muscle strength[35-37]. A recent Japanese study by Fukui *et al*[38] demonstrated that skeletal muscle mass decreases in accordance with liver disease progression in male patients with CHC. Sarcopenia, loss of muscle mass and function, has a high incidence rate in patients with cirrhosis (30%-70%) favoring atrophy of type II fast-twitch glycolytic fibers[39,40]. Myosteatosis, is characterized by increased lipid accumulation within muscle fibers and is a complication of hepatitis C Cirrhosis predisposing individuals to muscle atrophy over time[41]. High BMI, IR, diabetes, steatosis, inflammation, increased oxidative stress and lipotoxicity are all independent risk factors that predispose CHC patients to skeletal muscle disorders[42-47]. Therefore, a comprehensive sarcopenia evaluation assessing skeletal muscle mass, muscle strength and physical performance can provide clinicians the optimal risk assessment tools and therapeutic strategies for improving patient's quality of life and to halt the disease progression.

It is widely recognized that testosterone is highly associated with increased muscle

strength and mass[48]. Chaudhury *et al*[49] demonstrated that male CHC patients with hepatic dysfunction/cirrhosis suffered from low levels of free and total testosterone regardless of the treatment regimens (interferon and ribavirin (6%); interferon, ribavirin, and DAAs (18%); ribavirin and DAAs (14%); and DAAs only (62%)) received by patients[50]. The decrease in testosterone was sustained after viral clearance suggesting that testosterone metabolism in the liver is most likely impaired by liver dysfunction in these patients. Neff *et al*[51] was able to show that administration of testosterone hormone to HCV-patients suffering from liver failure increases muscle strength, enhance albumin synthesis, and improve survival post-liver transplant[49,52-54].

The association between CHC infection of the liver as well as hepatic dysfunction and muscle loss is well documented, though not fully understood. The triad complex association can be linked through increased inflammation, oxidative stress, alterations in the endocrine system (IR, testosterone levels), hepatic steatosis, and multiple factors involved in muscle depletion. A better understanding of the etiology of sarcopenia and skeletal muscle mass loss in HCV infected patients and the appropriate management strategies are expected to improve patient outcomes.

HEPATITIS C VIRUS INFECTION AND ADIPOSE TISSUE

Following liver and skeletal muscles, adipose tissue is another major site of IR in CHC infections[51]. Adipose tissue is considered as an important endocrine organ which releases biologically active polypeptides including adipose tissue specific adipokines like leptin and adiponectin and non-adipose tissue specific adipokines such as plasminogen activator inhibitor I (PAI-1)[55]. Adiponectin (an insulin-sensitizing hormone in muscle and liver) and leptin are most abundant in subcutaneous fat while PAI-1 is found in high levels in extracellular matrix[56,57]. Together, leptin, adiponectin and PAI-1 are the abundant adipokines which regulate the body lipid and glucose metabolism *via* the adipo-insular axis[58]. Alterations in adipokine levels/function are a major culprit for multiple complications including the risk of developing T2DM, cardiovascular disease and neurodegenerative disorders.

An interplay between HCV virion and adipocytes is suggested by a strong relationship between HCV viral load and subcutaneous (not visceral) fat[59]. Patients with CHC have significant subcutaneous adipose tissue insulin resistance in comparison with BMI-matched controls. Lim *et al*[58] suggested that that viral eradication improves global, hepatic, and adipose tissue insulin sensitivity. Serum levels of adiponectin and leptins even though associated with IR, HCV associated IR is predominantly a cytokine-independent direct virus-specific effect[60].

Though data is limited, new adipokines have an important role in the regulation of insulin sensitivity in CHC. Vaspin (visceral adipose tissue-derived serpin; seroinA12) has been found to improve insulin sensitivity and glucose tolerance and down regulate TNF-α synthesis[61-63]. The levels of serum vaspin are significantly decreased in chronic HCV infected patients without advanced fibrosis and increased in cases of advanced fibrosis suggesting vaspin to be a compensatory mechanism switch in HCV associated IR. However, no such association was found between serum vaspin level and viral load or homeostatic model assessment of IR values[64]. The level of another adipokine, visfatin, also increases in CHC and is inversely associated with inflammatory activity[65]. Visfatin exerts insulin like effect stimulating insulin receptor substrate-1 (IRS-1) phosphorylation and the peroxisome proliferator-activated receptors (PPARγ) expression but it also potentiates expression of interleukin-6 and TNF-α like adhesion molecules[65,66]. Chimerin, another adipokine thought to be related to IR in CHC infection, inhibits the synthesis of TNF-α and interleukin-6 and ameliorates IRS-1 phosphorylation, to improve insulin sensitivity of adipocytes and to enhance adiponectin synthesis[67,68].

HEPATITIS C VIRUS AND LIPID METABOLISM

HCV regulates the host factor diacylglycerol acyltransferase-1 to promote the biogenesis of lipid droplets, and the HCV core protein recruit nonstructural protein 5A (NS5A) that carries HCV ribonucleic acid from the replication complex on the endoplasmic reticulum-derived membranous web to lipid droplets[69]. The enveloped viral particles may be simultaneously packaged into endoplasmic reticulum luminal lipid droplets in the very low-density lipoprotein cholesterol (VLDL) precursor and eventually the VLDL-dependent pathway secretes it into the circulation as lipo-viral

particles[70,71]. Therefore, HCV infection is implicated in disrupted lipoprotein homeostasis due to impairment of the VLDL-releasing pathway which is one of the driving mechanisms of hepatic steatosis[72]. Derangement of lipid metabolism in HCV infection plays a role in hepatic steatosis, dyslipidemia, and hypobetalipoproteinemia. The HCV core and NS5A proteins interact with apolipoprotein (apo) A, apo A II and apo E. An increased levels of ApoC-III has been found in HCV lipo-viral particles that may inhibit the activity of lipoprotein lipase, disturbing the intravascular catabolism of triglyceride-rich lipoproteins[73-75]. HCV may modulate lipid metabolism and promote the synthesis of saturated fatty acids by regulating the enzymes involved in lipid synthesis[76,77]. Poly-unsaturated fatty acids supplementation counteracts the effects of HCV-induced lipid alterations and inhibits HCV formation[78]. Previous treatment with interferon-based antiviral therapy could reverse the hypocholesterolaemia induced by HCV. It has been shown that cholesterol and low-density lipoprotein cholesterol (LDL-C) levels rapidly recover following viral clearance or sustained virologic response indicating that large amounts of lipids released from the liver into blood circulation may increase levels of cholesterol and LDL-C, resulting in the hydrolysis of triglycerides[79]. More studies are needed to delineate the pathways and potential therapeutic targets.

CHRONIC HEPATITIS C VIRUS AND GLUCOSE METABOLISM

CHC infection is closely associated with alterations of glucose metabolism from the early stages of infection, prior to the development of significant hepatic fibrosis[80]. In patients infected with HCV, total serum fasting blood glucose levels are found to be higher than that in healthy controls[81]. Clinical studies, both prospective and retrospective, showed a two-fold increased risk of developing insulin resistance and T2DM in HCV-infected patients even after correction of confounding factors[82].

The mechanism by which HCV causes glucose metabolism derangement resulting in T2DM is not fully understood. The proposed mechanisms from experimental studies in mice suggest downregulation of glucose transporter 2 receptors causing impaired hepatic glucose uptake, down-regulation of insulin receptor substrate 2 expression [suppression of cytokine signaling (SOCS)-3-dependent mechanism] causing altered hepatic insulin receptor cascade and impaired insulin-driven hepatic neoglucogenesis switch to glycolysis, as well as a defective insulin-driven shutdown of gluconeogenesis resulting in higher endogenous glucose production. Also alterations of the forkhead box O1 phosphorylation and nuclear exclusion by HCV proteins is implicated in T2DM development[83].

There is some evidence to suggest that HCV infected individuals are at risk of developing Type 1 diabetes mellitus through autoimmunity with some studies pointing towards pancreatic autoimmunity developing after Interferon treatment[84]. Many epidemiologic studies have found an association between T2DM and HCV infection possibly due to an interplay of multiple factors including direct viral effects, cytokine milieu, IR *etc*.[85] T2DM can accelerate the natural course of HCV-induced liver disease causing higher risk of fibrosis, cirrhosis, and hepatocellular carcinoma[86]. In patients with CHC infection and T2DM, significant improvement was noted in their diabetes status after HCV eradication with treatment[87-90]. The patients who did not achieve sustained virological response had a higher risk of T2DM[91-96]. Figure 3 illustrates the deleterious effects of HCV on glucose and lipid metabolism.

HEPATITIS C VIRUS AND INSULIN RESISTANCE: CENTRAL AND PERIPHERAL PATHWAYS

HCV proteins that are mainly involved in IR include core proteins, Serine protease and nonstructural viral proteins NS5A and NS5B that contribute to the formation of capsid, down-regulation of interferon-stimulated genes and polymerase activity. HCV induces IR both through multiple pathways and appears to depend on viral load and specifically genotypes G 1, 2 and 4[97].

In the direct mechanism, HCV proteins interact with various components of the insulin signaling pathway disrupting the signaling process of insulin in the hepatocytes. This leads to overexpression of protein phosphatase 2A and SOCS-3, and down regulates the expression of PPAR and IRS causing IR directly. HCV infection enhances hepatic gluconeogenesis through forkhead box O1-dependent pathway and

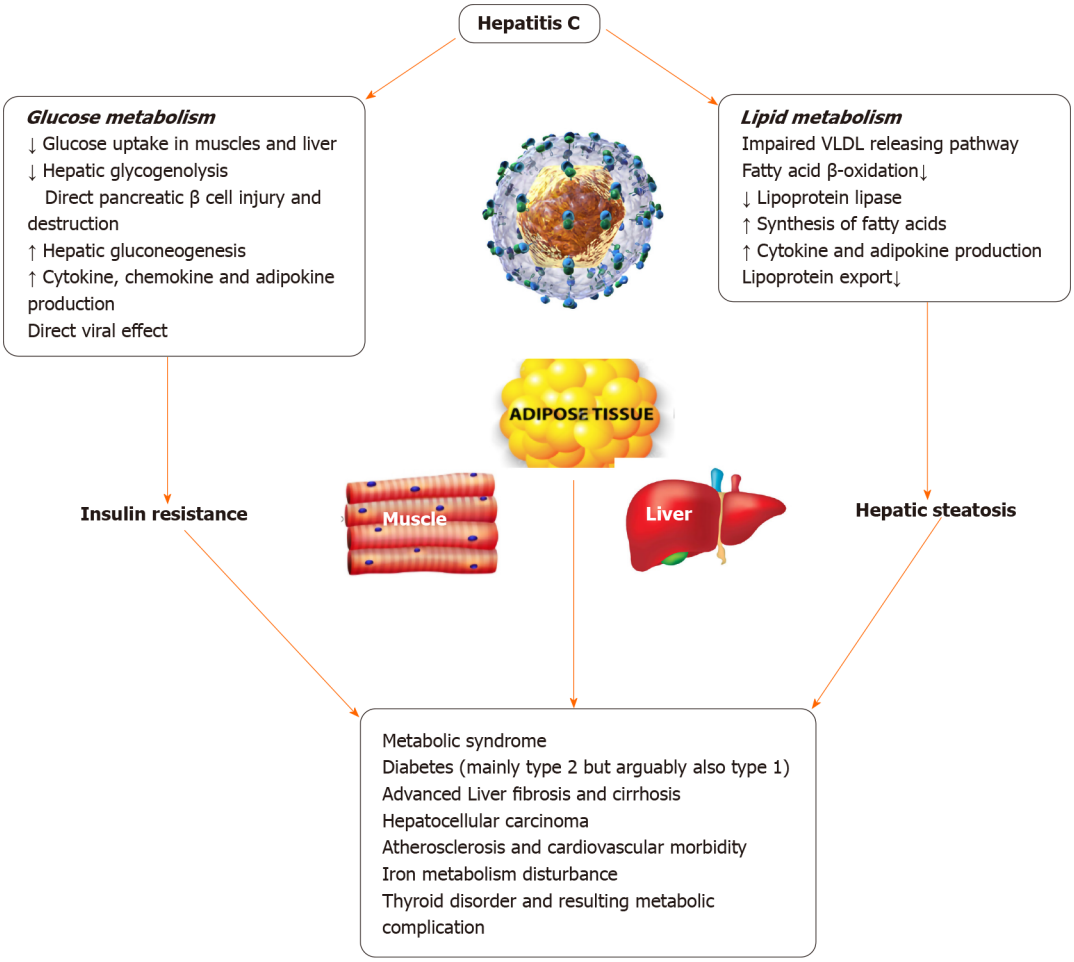

Figure 3 Impact of hepatitis C virus on glucose and lipid metabolism.

suppresses the cell surface expression of glucose transporter 2, resulting in reduced glucose uptake with the help of HCV NS5A protein that plays important roles in these two independent pathways. Any disruption in insulin signaling results in various pathophysiological changes, including glucose intolerance, obesity, dyslipidemia, hypertension, and development of T2DM[98-100]. The mechanism of insulin impairment appears HCV genotype specific, as core protein expression of G3 led to upregulation of SOCS-7 and downregulation of PPARγ, while the core protein from G1 activated mammalian target of rapamycin and induced phosphorylation of IRS-1 at inhibitory serine residues[101]. However, activation of SOCS family appeared to be common mechanism for all major genotypes to induce IR, including genotype 1 as the mammalian target of rapamycin activating variant appeared to be infrequent among known isolates[102-107].

Peripheral IR is the deficits in insulin induced glucose uptake into target tissues (adipose and muscle tissues). IR in CHC infection is thought to primarily affect muscle tissue making it a notable paradigm of IR. Mangia *et al*[106] showed that peripheral insulin resistance and resulting T2DM does not develop for at least first 5 years of infection with HCV suggesting the role of chronic infection in the development of IR rather than acute infection[107]. The pathways of lipid metabolism derangements and IR induced by HCV proteins and host cells opens up potential future treatment options scope for more studies[107,108].

The main PPAR nuclear receptors expressed in the liver that regulates glucose and lipid metabolism, influence cellular differentiation and proliferation as well as regulate the inflammatory process, include PPARα and PPARγ alongside retinoid X receptor. PPARα gene expression in the liver is decreased by 86% in the course of CHC infection[109]. Studies demonstrated a sharp decrease in the hepatic PPARγ expression in G3 CHC patients compared to those with G1 HCV[109-114].

The major pathways by which CHC infection induce insulin resistance are shown in the Table 1.

Table 1 The role of major hepatitis C virus proteins in insulin resistance development

HCV core protein	Nonstructural protein 3 (NS3)	Nonstructural protein 5 (NS5)
Activates members of SOCS family; Genotype 1: Activates mTOR and induces phosphorylation of IRS-1; Genotype 3: Upregulates SOCS-7 and downregulates PPARγ	NOX2 activation	Increasing the ROS within the mitochondria
↓ PPARα gene expression in the liver	↑ ROS	Induces ER stress → ↑ protein phosphatase 2A (PP2A)
↓ Assembly of VLDL	Through ROS → Activation of transcriptional factors such as NF-κB and STAT-3 → more advanced stages of chronic hepatitis → oncogenesis	Dephosphorylation and inactivation of Akt
Induces lipogenesis and gluconeogenesis		Stimulates the NF-κB-mediated increase in proinflammatory cytokines
↓ IFN-α production; ↓ IFN-α stimulated genes		Up-regulation of PP2Ac → hypomethylation of STAT-1 → ↑ association of STAT-1 with PIAS1PIAS1 → impairing the transcriptional activation of IFN-stimulated genes
Activation of the pattern-recognition receptor TLR2 → ↑ production of profibrotic factors. *i.e.* TGF-β, procollagen 1, and MMPs		Stimulates the NF-κB → increase in proinflammatory cytokines (*i.e.* IL-6, TNF-α)
Activates the PA28γ → ↓ IRS-1 tyrosine phosphorylation and IRS-2 expression and TNF-α promoter activation		Dephosphorylation of PKB/Akt → inhibition of insulin signaling
Induces TNF α → portal or periportal inflammation		
Impedes insulin-mediated FoxO1 translocation affecting glucose metabolism		

Akt: Protein kinase B; PKB: Protein kinase B; ER: Endoplasmic reticulum; FoxO1: Forkhead box protein O1; IFN-α: Interferon alpha; IL-6: Interleukin-6; IRS-1: Insulin receptor substrate 1; IRS-2: Insulin receptor substrate 2; MMPs: Matrix metalloproteinases; mTOR: Mammalian target of rapamycin; NF-κB: Nuclear factor kappa light chain; NOX2: NADPH oxidase 2; NS3: Serine protease; NS5: Nonstructural viral proteins NS5A and NS5B; PA28γ: Proteasomal activator; PIAS1: Protein inhibitor of activated STAT 1; PP2Ac: Catalytic subunit of protein phosphatase 2A; ROS: Reactive oxygen species; SOCS: Suppressors of cytokine signaling; SOCS-7: Suppressor Of cytokine signaling 7; STAT-1: Signal transducer and activator of transcription 1; TGF-β: Transforming growth factor beta; TNF-α: Tumor necrosis factor a; TLR2: Toll-like receptor 2; VLDL: Very low density lipoprotein.

HEPATITIS C VIRUS AND METABOLIC SYNDROME

According to the Joint Scientific Statement in 2009, patients who exhibit three of the five following characteristics are diagnosed with the metabolic syndrome (MetS): (1) Abnormal waist circumference as population and country-specific definitions; (2) Systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or currently taking blood pressure-lowering agents; (3) High-density lipoprotein of cholesterol < 40 mg/dL in males or < 50 mg/dL in females; (4) Fasting blood sugar ≥ 100 mg/dL or currently taking diabetes medications; and (5) Triglyceride ≥ 150 mg/dL[115,116]. Both CHC infection and the MetS have common basic pathogenesis which is glucose metabolic derangement and IR resulting in close association and overlapping manifestations[117]. Amongst HCV infected patients, particularly older subjects, the prevalence of MetS ranges from 13.2% to 31.5%[118]. The prevalence increases in patients with CHC, older than 60 years of age, having an odds ratio of eight times of developing MetS than those under 40 years of age. A study by Lonardo *et al*[118] exploring the association between HCV genotypes and MetS prevalence found no significant difference compared to controls[119]. This could be due to the small sample size and patient selection criteria.

More studies show that African Americans infected with HCV are more prone to develop MetS than other ethnic groups[120,121]. An aggressive and severe liver disease is common in CHC patients with MetS. Liver fibrosis is notably more advanced in CHC patients with MetS than those without[122]. The prevalence of liver cirrhosis in CHC-MetS patients was 30% compared to 18.4% in CHC patients without MetS[123,124]. As MetS may worsen the progression of liver diseases in HCV infected patients, further research is needed to assess the impact of viral clearance and sustained virologic response on MetS.

IMPACT OF METABOLIC DISEASE ON CHRONIC HEPATITIS C DISEASE PROGRESSION

It is crucial to understand the impact of underlying metabolic diseases on the natural history of CHC infection as much as to understand the risk of developing metabolic complications from CHC. Given the rising prevalence of obesity and metabolic syndrome observed in the United States and globally, recognition of the impact of obesity and IR in disease progression among patients with CHC is particularly important[125].

In a recent systemic review by Dyal *et al*[124] 20 cohort studies were identified to evaluate the impact of metabolic diseases on disease progression among patients with CHC[125]. The authors evaluated the effect of obesity, diabetes, and steatosis as risk factors for developing advanced fibrosis in patients with chronic HCV infection and demonstrated that the presence of concurrent diabetes among patients with CHC infection was associated with a significantly higher risk of developing advanced fibrosis, with effect measures ranging from odds ratios of 2.25 to 9.24. Hepatic steatosis was also strongly associated with an increased risk of developing advanced fibrosis, with odds ratios of 1.80 to 14.3[126]. However, further studies are needed to assess the association between obesity and advanced fibrosis in CHC patients.

Available evidence aimed to better clarify the impact of concurrent metabolic diseases on disease progression and the natural history of chronic HCV infection; however, several limitations while interpreting the results must be acknowledged. The standard method for exploring the effects of metabolic diseases on disease progression would be to recognize patients with pre-existing metabolic diseases who eventually acquired HCV infection. However, a number of these studies were observational in nature and either utilized a case-control or retrospective cohort study design, which inherently limit the true ability to understand causal links. Significant advancements in HCV therapy were introduced subsequent to these studies which may influence the metabolic milieu and disease progression differently and we need more evidence before we extrapolate the current data to the presently available regimens.

The presence of metabolic diseases may influence the sequela of HCV infection. Patients with diabetes, hepatic steatosis, NASH, and insulin resistance were at more risk of developing CHC compared to patients without underlying metabolic disorders[127,128]. These patients were also found to have a faster progression to liver fibrosis through the same mediators that are inducing inflammation. A study by Paradis *et al*[129] investigated the risk of developing HCC in patients with underlying metabolic syndrome disorders and found there is a profound role of MetS in HCC development and progression. This can be further aggravated by the presence of HCV. The presence of NASH is a major contributor to liver cirrhosis in the presence/absence of HCV[130,131]. These findings elucidate the strong association between MetS disorders, HCV and their complex involvement in liver disease progression. This evidence contributes to a shift in HCC etiology which signifies further research.

MANAGEMENT OF METABOLIC COMPLICATIONS IN CHRONIC HEPATITIS C

Advances in treatment options with highly effective DAAs therapy for CHC aimed for eradication of HCV will not only improve HCV-related liver disease but will most likely impact the incidence and prevalence of HCV-related metabolic diseases, given the strong association between the two conditions. The efficacy of current regimens for CHC has improved the sustained virologic response reaching almost > 95%-100%. It is important to know whether the patient is treatment naïve or experienced, the genotype, the fibrosis level, and underlying comorbidities for personalized medicine and best tailored treatment options.

Concurrent management of coexistent metabolic disorders is also important for optimal control of CHC related liver injury and complications such as HCC as mentioned earlier. However, a detailed discussion of management of these conditions is beyond the scope of this review.

CONCLUSION

Hepatitis C infection has emerged as a systemic infection with impacts beyond the primary site of infection, causing a wide range of clinical manifestations in patients. It is crucial to understand the systemic effects of CHC along with its hepatic complications as it relates to metabolic manifestations and complications. HCV virion exploits the multiple functions of lipid droplets to sustain its multiplication and replication within host cells. The orchestration between HCV major proteins and the liver-muscle-adipose axis, poses a major burden on all the health systems of human body if not adequately addressed. The close and inseparable associations between chronic HCV infection, metabolic disease, and cardiovascular disorders are specifically important considering the increasing prevalence of obesity and metabolic syndrome and their economic burden to patients, the healthcare systems, and society. Cellular and molecular mechanisms governing the interplay of these three organs in health and disease are therefore of significant interest. As indicated by the extent of the metabolic comorbidities discussed, HCV increases the disruption in the liver–muscle–adipose triangle resulting in myriad clinical outcomes in patients. Further studies are needed to assess the association between metabolic derangement and patient quality of life. Physicians are encouraged to pay close attention to the metabolic parameters in HCV patients especially cirrhotics and patients listed for liver transplant.

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MINIREVIEWS

Artificial intelligence for early detection of pancreatic adenocarcinoma: The future is promising

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a worldwide public health concern. Despite extensive research efforts toward improving diagnosis and treatment, the 5-year survival rate at best is approximately 15%. This dismal figure can be attributed to a variety of factors including lack of adequate screening methods, late symptom onset, and treatment resistance. Pancreatic ductal adenocarcinoma remains a grim diagnosis with a high mortality rate and a significant psychological burden for patients and their families. In recent years artificial intelligence (AI) has permeated the medical field at an accelerated pace, bringing potential new tools that carry the promise of improving diagnosis and treatment of a variety of diseases. In this review we will summarize the landscape of AI in diagnosis and treatment of PDAC.

Key Words: Pancreatic adenocarcinoma; Artificial intelligence; Neural network; Future perspectives; Early diagnosis; Improved performance

Core Tip: Pancreatic adenocarcinoma is one of the deadliest malignancies in the world. Several factors are responsible for this but delayed diagnosis is one of the most important. Despite improvements in diagnostic methods, early lesions are still missed in clinical practice. Artificial intelligence (AI)-assisted diagnostic methods have the potential of improving the clinical outcomes of these patients. However, major improvements in AI technology and its implementation need to occur before potential

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benefits can be attained.

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INTRODUCTION

Current modalities for the diagnosis and treatment of pancreatic ductal adenocarcinoma (PDAC) remain disappointing. With an overall survival rate of 3%-15%, it is one of the deadliest malignancies in the world[1]. Although currently it is the 7th leading cause of cancer death worldwide, recent trends suggest that in North America and Europe PDAC will soon become 2nd and 3rd respectively[2,3]. Several factors contribute to the poor survival statistics of PDAC: Lack of adequate screening tests, delayed diagnosis, and sub-optimal treatment options. Consequently, improvements in all these areas are desperately needed.

Recent technological advances have led to the increased application of artificial intelligence (AI) in different disciplines. Because computers can store and analyze larger amounts of data than the human brain, AI has the potential to achieve unmet needs in medicine. Since improving outcomes for PDAC is an area of urgent need, this review will provide a summary of current and future applications of AI in the diagnosis and management of PDAC.

AI, MACHINE LEARNING, AND ARTIFICAL NEURAL NETWORKS

AI is a branch of computer science dedicated to developing models aimed at performing functions comparable to those accomplished by the human brain. Machine learning (ML) is the area of AI that deals with developing computer models capable of learning specific tasks through the repetition of calculations derived from large amounts of data[4]. These computer models analyze data through repetitive calculations using mathematical self-derived algorithms that are constantly adjusted until the model produces the desired outcome. Once the combination of adjustments necessary to achieve the outcome has been discovered, the computer then "learns" how to perform that specific task[5].

Broadly speaking, ML can be either supervised or unsupervised. The difference lies in whether the desired outcome of interest is previously known by the computer. In supervised learning, a computer is first introduced to a training dataset (the "input") as well as the desired outcome of interest (the "output"). The computer then analyzes the input making the necessary adjustments to the algorithm until it consistently produces the desired output[6]. This type of learning requires large amounts of training data that has been pre-labeled ("curated") by a human operator. Once the training of the machine is completed, a different dataset is used to test its performance (testing data). In unsupervised learning, the computer is introduced to unlabeled data. The machine then sorts it using the algorithm to identify features within it that can be grouped and analyzed further to reach a specific outcome[7]. Because the data in unsupervised ML is not curated, larger amounts of training data are required than for supervised ML.

To date, most of the ML used in medicine has been supervised; and it has been made possible due to the emergence of a relatively new discipline called radiomics. Radiomics studies the conversion of digital medical images into data that can be then subjected to statistical analysis[8]. Using computer technology, predefined quantitative features are extracted from computed tomography (CT), magnetic resonance Imaging (MRI) or other imaging modalities. An essential step prior to this data extraction however, is lesion "segmentation". Segmentation is in simple terms, delineation of the lesion within an image. In supervised ML a human operator delineates the lesion prior to the algorithm operating on the data. In unsupervised ML, the algorithm learns how to segment the lesion of interest by itself.

Once the quantitative information of the lesion in the image is extracted, it is

analyzed utilizing artificial neural networks (ANN). An ANN is a group of interconnected computers with a structure similar to a neural network in the human brain. Each computer represents a neuron or "node", and each connection a synapse or "weight" (Figure 1). ANNs are organized in "layers" (groups of nodes) and the most basic model contains three: (1) Input layer (which receives the data), (2) Hidden layer (which performs the calculations and analyses), and (3) Output layer (which produces the final output)[9]. Deep neural networks contain more than one hidden layer and therefore can learn to analyze data with higher complexity levels; this is termed "deep learning"[10]. The different layers work in a hierarchical structure to produce the desired output.

AI-ASSISTED ANALYSIS OF ENDOSCOPIC ULTRASOUND IMAGES

Endoscopic ultrasound (EUS) is currently one of the most useful imaging modalities in the diagnosis of PDAC. The overall sensitivity of EUS guided biopsies for the diagnosis of PDAC reaches 98%, but its specificity can be as low as 20%[11]. The accuracy of EUS depends on both operator and lesion-related factors. The most important operator-related factor is the amount of experience performing EUS. On occasions, variations in gastroduodenal anatomy, (*i.e.* after partial gastric resection, or presence of a duodenal stricture) can significantly limit the ability of operator to visualize the pancreas. On the other hand, lesion-related factors include patient's body habitus, the presence of acute inflammation (when EUS is done immediately after an episode of acute pancreatitis) or the presence of chronic pancreatitis (CP), particularly in the presence of parenchymal calcifications. Sensitivity of EUS for PDAC in the presence of CP can be as low as 54%[12,13]. In addition, CP and autoimmune pancreatitis can occasionally form pseudotumors, which may complicate image analysis by the endosonographer.

Several studies have reported on the application of AI in the analysis of EUS images of pancreatic diseases[14-22] (Table 1). For the most part, these studies have focused on evaluating the accuracy of ANNs in differentiating CP from PDAC. Norton *et al*[14] analyzed still EUS images previously selected by experts who did not perform the procedure and were blinded to the final diagnosis using an ANN. A total of 21 patients with PDAC and 14 with CP were included. Four features were analyzed in each image by the ANN, achieving an overall accuracy of 89%.

In a similar study, Das *et al*[17] retrospectively analyzed the performance of an ANN in differentiating PDAC from normal pancreas and CP. A total of 56 patients (22 normal, 12 CP and 22 PDAC) were studied. Their AI algorithm identified PDAC with an area under the curve of 0.93. The differences in accuracy in this study may have been secondary to the more stringent criteria in the definition of CP and the higher number of image features analyzed by the ANN. A larger study performed by Zhu *et al*[18], analyzed 262 patients with PDAC and 126 with CP and reported that their algorithm reached an overall accuracy of 94%.

Elastography is an imaging method developed to establish the differences in consistency ("strain") between normal and abnormal tissue during EUS. Such differences are portrayed in a color-coded overlay on the EUS image, with red correlated with softer tissue and blue with harder[19]. A multicenter study reported an overall accuracy, sensitivity and specificity of 84%, 88% and 83% respectively in differentiating PDAC from CP[20]. This study used ANN analysis of histograms from elastography images previously selected by experts blinded to the patients' diagnoses. Another similar small study of 68 patients reported an accuracy rate of 90%[21].

Contrast agents have also been developed to aid in the differentiation between PDAC and CP. Săftoiu *et al*[22] reported that ANN analysis of contrast-enhanced EUS images could establish a difference with an area under the curve of 94%. Few studies have focused on using standard EUS B mode images to diagnose pancreatic tumors in the absence of CP. These have reported accuracies up to 99%[15,16].

AI-ASSISTED ANALYSIS OF COMPUTERIZED TOMOGRAPHY IMAGES

Computerized tomography (CT) is perhaps the most common medical imaging modality being explored with AI. The analysis of CT images of neoplastic lesions involves three main steps: detection, characterization and monitoring of change over time[23]. Most of the available studies have focused on AI-assisted characterization of lesions, which is equivalent to the previously defined concept of segmentation. Three

Table 1 Studies exploring artificial intelligence in the diagnosis of pancreatic ductal adenocarcinoma

Ref.	Study design	Data source	AI instrument	Patient	Aim	Accuracy
Norton *et al*[14], 2001	Retrospective	Standard EUS	ANN	21	PDAC *vs* CP	89%
Ozkan *et al*[15], 2015	Retrospective	Standard EUS	ANN	332	PDAC *vs* Nl	89%-92%
Zhang *et al*[16], 2010	Retrospective	Standard EUS	ANN	216	PDAC *vs* Nl	98%
Das *et al*[17], 2008	Retrospective	Standard EUS	ANN	56	PDAC *vs* Nl *vs* CP	93%
Zhu *et al*[18], 2013	Retrospcective	Standard EUS	ANN	388	PDAC *vs* CP	94%
Săftoiu *et al*[20], 2012	Prospective	EUS w/ elastography	ANN	258	PDAC *vs* CP	91%
Săftoiu *et al*[21], 2008	Prospective	EUS w/elastography	ANN	68	PDAC *vs* CP	90%
Săftoiu *et al*[22], 2015	Prospective	EUS w/contrast	ANN	167	PDAC *vs* CP	95%[1]
Fu *et al*[24], 2018	Retrospective	CT	ANN	59	Pancreatic tumor segmentation	76%[1]
Chu *et al*[25], 2019	Retrospective	CT	Computer derived forest algorithm	380	PDAC *vs* Nl	99%
Liu *et al*[26], 2019	Retrospective	CT	ANN	338	PDAC *vs* Nl	76%
Chu *et al*[29], 2019	Retrospective	CT	ANN	456	Segmentation of PDAC *vs* Nl	94%
Devi *et al*[32], 2019	Retrospective	MRI	ANN	168	Nl *vs* Abnormal pancreas	96%
Gao *et al*[33], 2020	Retrospective	MRI	ANN	504	Identify pancreatic disease	77%
Liang *et al*[34], 2020	Retrospective	MRI	ANN	27	Segmentation of panc tumors	Not explicitly stated
Muhammad *et al*[42], 2019	Retrospective	Clinical variables	ANN	800114	PDAC prediction	85%
Klein *et al*[43], 2013	Retrospective	Clinical variables	Computer derived model	7003	PDAC risk	61%
Hsieh *et al*[45], 2018	Retrospective	Clinical variables	ANN	> 1 million	NOD predicting PDAC	72%
Zhao *et al*[46], 2011	Retrospective	Clinival variables + Pubmed data	Bayesian network inference	N/A	PDAC prediction	85%
Sanoob *et al*[47], 2016	Retrospective	Clinical variables	ANN	120	PDAC detection	Not explicitly stated
Momeni-Boroujeni *et al*[56], 2017	Retrospective	FNA samples	ANN	75	PDAC diagnosis	77%
Bhasin *et al*[58], 2016	Retrospective	PDAC genes	Computer vector model	5[2]	PDAC detection	92%
Almeida *et al*[59], 2020	Retrospective	PDAC genes	ANN	40[2]	PDAC detection	86%

[1]Sensitivity.

[2]Genes. AI: Artificial intelligence; ANN: Artificial Neural Network; CT: Computerized tomography; EUS: Endoscopic ultrasound; MRI: Magnetic resonance imaging; FNA: Fine needle aspiration; PDAC: Pancreatic ductal adenocarcinoma; CP: Chronic pancreatitis; NOD: New onset diabetes.

studies have applied AI to the analysis of CT images in PDAC for diagnostic purposes[24-26]. In a small study of 15 healthy patients and 44 with a variety of pancreatic tumors, Fu *et al*[24] reported that their algorithm achieved an overall sensitivity of 76%. In a retrospective case control study of 380 patients (190 cases and 190 controls) Chu *et al*[25] reported an accuracy of 99% in their computer-derived algorithm. Meanwhile, a prospective study by Liu *et al*[26] reported an accuracy of 76%. AI has also been utilized to establish correlations between the CT images of PDAC and their subsequent biological behaviors[27,28].

Two important ongoing projects are worth mentioning. The Felix Project funded by the Lustgarten Foundation is a multidisciplinary study carried out by a group at Johns Hopkins University. Using deep learning computer models with manually segmented images from 156 PDAC cases and 300 normal controls, the group reported a sensitivity

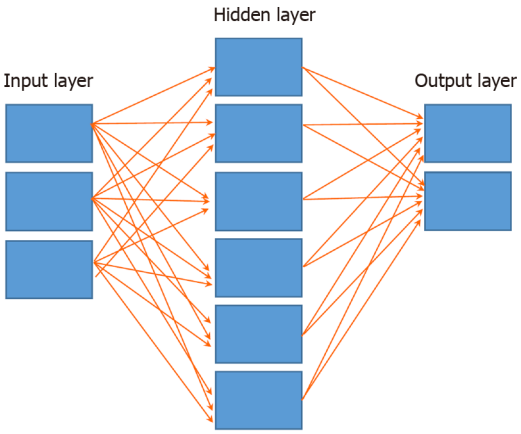

Figure 1 Basic anatomy of an artificial neural network. Input layer, hidden layer (may have more than one) and output layer. All nodes are interconnected through wieghts (arrows).

and specificity of 94% and 99% respectively in their initial report[29]. Based on these encouraging results, the group's next step is to expand their analysis to 575 normal and 750 PDAC patients. The group's ultimate goal is to fine tune the performance of their algorithm prior to expanding it to larger externally derived datasets.

The second ongoing study is being conducted by the Alliance of Pancreatic Cancer Consortium Imaging Working Group (2). Their objective is to collect pre- and post-diagnosis CT, magnetic resonance imaging (MRI) and transabdominal ultrasound images from patients ultimately diagnosed with PDAC. These images will be used to create a repository that will later be shared and analyzed. The ultimate goal of the project is to develop AI models that can predict the appearance of PDAC and diagnose the disease in its early stages.

AI-ASSISTED ANALYSIS OF MAGNETIC RESONANCE IMAGES

Segmentation of MRI images by AI has been reported to be more technically challenging than CT images[30,31]. A few studies have reported that ML models can be trained to accurately identify pathology in the pancreas, although the literature on PDAC is scarce. Devi *et al*[32] reported that an ANN could accurately identify a variety of abnormal pancreatic findings with a 96% accuracy. Similarly, Gao *et al*[33] reported that an AI model differentiated normal from abnormal pancreas at a level comparable to humans (77% *vs* 82% respectively). In the only published study aimed specifically at identifying PDAC in MR images, Liang *et al*[34] reported that a convoluted neural network (a variety of ANN) performed similarly to humans in identifying the lesion.

Artificial Intelligence processing of MRI images has been also applied in the context of PDAC therapy. Spieler *et al*[35] reported that an ANN accurately delineated pancreatic tumors prior to radiotherapy. Zhao *et al*[36] reported similarly positive results. In a study utilizing AI aiming to automatically calculate the dose of stereotactic body radiation therapy, Campbell *et al*[37] demonstrated that an ANN-calculated dose was comparable to a human-calculated one. By applying radiomics to MRI images, investigators have reported that their quantitative data can be correlated with aspects such as tumor subtype, survival and response to chemotherapy[38,39]. This same technology has made possible other studies showing that MR images data can be used to predict relapse after PDAC treatment[40,41].

AI ANALYSIS OF CLINICAL DATABASES

The evolution of AI has resulted in models with the capacity of analyzing data beyond quantitative image features. This type of ML requires a higher complexity in the architecture of the neural networks given the broad range of variables analyzed at any given point in time. These models have been applied in the development of algorithms that can accurately identify patients with or at risk of developing PDAC based on several clinical variables.

Muhammad *et al*[42] utilized an ANN to analyze a large patient population derived from the National Health Interview Survey and the Prostate, Lung, Colorectal and Ovarian trial. The authors developed and trained the ANN with > 800000 patients of which 898 had PDAC. Analyzing variables such as demographics, comorbidities, race and family history, the model predicted the development of PDAC with an AUC of 0.85. In a similar manner, albeit with a lower accuracy, Klein *et al*[43] utilized data from the PanScan Consortium to develop a model that predicted high risk of PDAC among patients of European ancestry with an AUC of 0.61.

New onset diabetes has been adopted as a marker for patients with high risk of developing PDAC within the following 3 years[44]. As such, it has been the subject of analysis by AI techniques. Hsieh *et al*[45] compared the PDAC prediction accuracy of new onset diabetes when analyzed by a regular logistic regression or an ANN. A total of 3092 PDAC cases were identified from a population of > 1000000 patients. Interestingly, the logistic regression slightly outperformed the ANN (AUROC 0.7 and 0.64% respectively). In a complex study combining PubMed data and clinical information, Zhao *et al*[46] utilized an innovative weighted Bayesian network that accurately predicted PDAC with an AUROC of 0.91. In a similar but simpler study, Sanoob *et al*[47] reported that an ANN can accurately diagnose PDAC based on a combination of signs and symptoms[47]. ANNs that analyze clinical data have also been used in determining patient survival and performance after PDAC treatment[48,49].

AI-ASSISTED ANALYSIS OF PATHOLOGICAL AND MOLECULAR FEATURES OF PDAC

Currently, pathologists are responsible for interpretation of histology specimens, and this process is dependent on their previous training, level of experience and individual skills. In an attempt to standardize interpretation and reduce human bias, AI techniques have been applied to pathology specimen analysis[50].

Application of AI in pathology is dependent on creation of a high-resolution digital image from the glass slide. This step is called "whole slide imaging" (WSI). WSI is accomplished by use of glass slide scanners and the technology to support them[51]. WSI and the necessary IT infrastructure to support its clinical use is referred to as "digital pathology". It is expected that eventually, pathology workflow will move away from pathologists looking at glass slides through a microscope to pathologists reviewing digital images of slides on high resolution computer screens. Food and Drug Administration clearance for these devices is relatively new (2017), and widespread adoption of digital pathology technology is still in its early phases. There is an increasing amount of data on validation of WSI compared to microscope viewing of glass slides, for example a recent study showed good concordance of slide interpretation of frozen sections done with glass slides/microscope compared to WSI/digital pathology workstation[52] Similar excellent intraobserver concordance between glass slides and digital pathology has been shown for routine clinical workload in surgical pathology[53].

There is already significant work and available commercial devices that can bring AI computing power to aid in the interpretation and screening of biopsies, although commercial expansion of this is in its infancy[54]. Much of the work regarding AI in pathology concerns prostate and breast malignancy, since the incidence of these malignancies is fairly high, and the clinical need and potential commercial applications present a more attractive corporate opportunity for device sales. However, with continued digitization of glass slides, particularly of pancreatic malignancy (both FNA specimens and surgical pathology) it is hoped that an enlarging curated group of cases can serve as a training set for AI analysis regarding pancreatic cancer.

There has been only a limited number of investigations of AI in pathology for the diagnosis of pancreatic cancer. Although the sensitivity and specificity of EUS guided FNA samples is in average > 90%[55], some specimens still fall under the "atypical cells" category, posing a considerable diagnostic dilemma. In a recent study, Momeni-Boroujeni *et al*[56] studied the performance of an ANN in reclassifying EUS-FNA specimens originally labeled as "atypical" by pathologists. Among a group of 31 patients in whom the final diagnosis had been previously established by other diagnostic methods, the ANN's overall accuracy for adequately reclassifying the specimen as malignant or benign was 77%.

Two of the main factors driving the high mortality of PDAC are suboptimal understanding of its malignant behavior and its unpredictable treatment response rate.

Advances in AI-assisted genetic and molecular profiling of PDAC have recently broadened insight on these factors[57]. Recent data showed that early diagnosis could be possible through AI analysis of the transcription products of certain PDAC genes. These studies have reported sensitivity and specificity ranging from 88%-95% and 83%-95% respectively[58,59]. AI has also been utilized to match PDAC biological information with chemical properties of specific drugs in order to develop models capable of predicting response to these specific agents[60,61].

FUTURE CONSIDERATIONS

Technological advances in the last 50 years have exponentially increased the amount and quality of data available for medical decision making. Hence, it is becoming increasingly evident that new methods for storage and analysis of it are necessary. Although the concept of AI or its applications in medicine may still seem foreign for most practitioners, it is rapidly positioning itself as an indispensable tool to reduce human error. As sophisticated and elegant our diagnostic and therapeutic capacities may be currently, they remain inevitably limited by our subconscious and conscious bias, as well as our wide range of intellectual and technical skills. William Osler's famous quote: "medicine is a science of uncertainty and an art of probability" will likely never be proven false.However the degree of uncertainty and probability considered tolerable in modern medicine is constantly shrinking. The advent of AI brings, in theory, the promise of reducing and even eliminating these shortcomings

Nevertheless, this promise is one that needs to be taken cautiously. There are several hurdles that must be overcome before AI can see widespread adoption in medical care. One limitation is the current lack of adequate standardization. Uniform protocols for data collection, processing, storage, reproduction and analysis must be established and standardized. Furthermore, different types of data may require different AI technologies. For example, ANNs trained to adequately classify histologic slides of pancreatic biopsies which have been fixed and stained with a specific method, may underperform, or not perform at all, when presented with slides prepared in a different manner. Creating such universal protocols, although possible, will be laborious and expensive.

Another concern is with the ethical handling of information. AI systems require vast amounts of data, and therefore, its implementation demands reliable methods of patient data de-identification. This is indispensable to ensure patient confidentiality, since one of the pillars of AI is data sharing. On the other hand, de-identified data needs to maintain its traceability, in order to allow individual practitioners to retrieve it and make the necessary decisions at the bedside. Three different models have been developed for data sharing in AI, and all have their advantages and disadvantages[2,62-65].

Centralized models require sharing of large amounts of data by different sources (*i.e* institutions). This data is uploaded into a central server that carries out the algorithmic adjustments. Once trained, the central server shares the finalized algorithm with the individual sources for internal use. The main drawback of this model is that centralization of the information in the server may increase the risk of a security breach, as the individual source no longer controls the information (Figure 2). In distributed or federated models, each source develops and adjusts its own algorithm with internal data. Once each source has fine-tuned their algorithm, they share its parameters with a central server. The central server then utilizes all the individual parameters to develop a centralized algorithm that later gets returned to the source for internal use (Figure 3). The main advantage of this model is that data is not shared with the central server. In hybrid models features of both models are present. A data repository is created to be shared by both data providers and the central server. The data repository then develops an algorithm using each individual institution's data before it gets sent to the central server. The central server then updates the master algorithm before it gets sent back to the data repository (Figure 4).

The quality of data currently utilized in AI needs to be improved. Most of the AI systems used in data analysis so far have been trained and tested with rather small datasets originating from within local institutions. This raises the issue of information bias. These datasets lack the degree of diversity necessary to mirror the scenarios human providers face during routine clinical practice. For AI systems to perform adequately, the datasets need to be sufficiently diverse in all the possible variables that come into play when making clinical decisions (demographics, medical and/or family history, physical and laboratory findings and others). Therefore, datasets need to

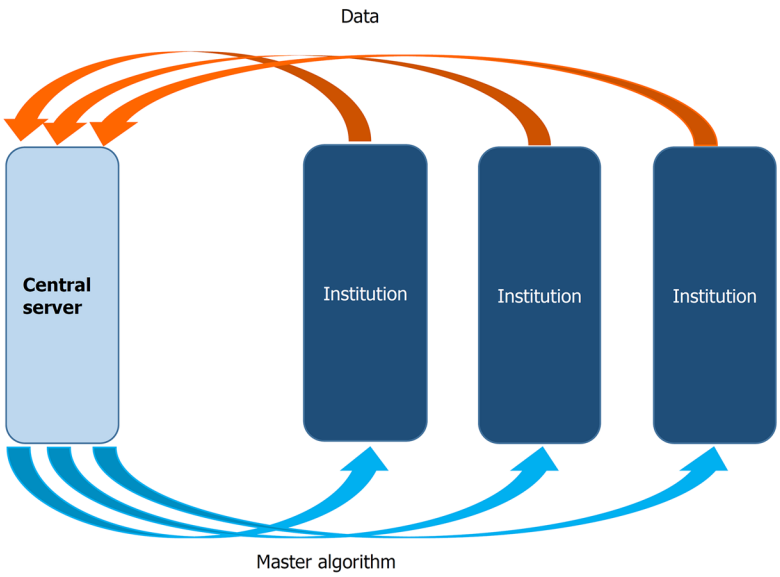

Figure 2 Centralized artificial intelligence information sharing system. Each individual institution provides data to the central server. The server analyzes all the data and develops and algorithm that is sent to each institution. This algorithm is then used by each institution to analyze its own internal data in the future.

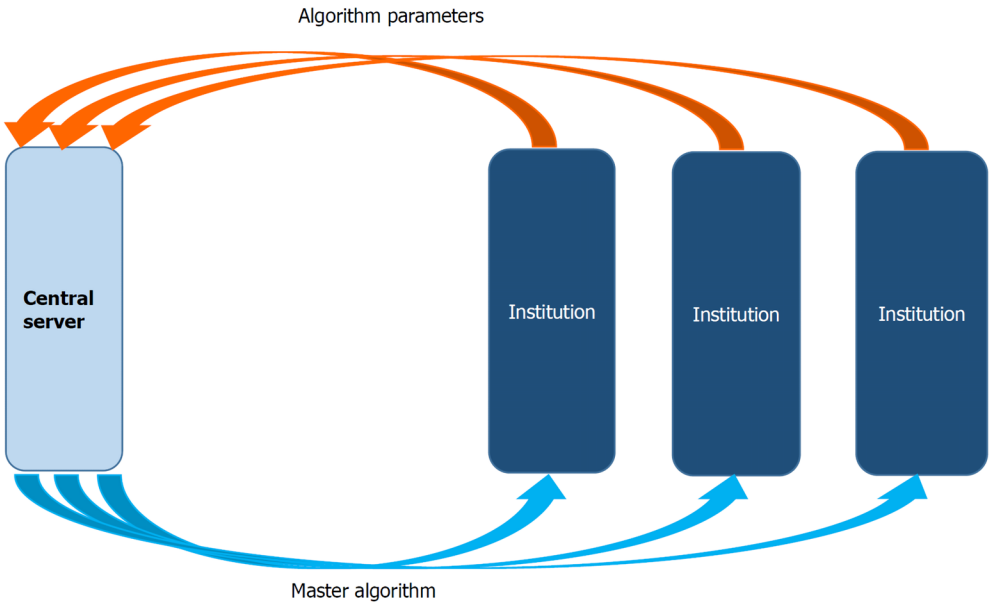

Figure 3 Federated artificial intelligence information sharing system. Each individual institution develops its own algorithm with internal data. Once the algorihms are developed, their parameters are shared with the central server. The server then develops a master algorithm using all the individual parameters. The master algorighm is sent back to the institutions for its internal use.

originate from a variety of sources for them to be representative and inclusive, not from a limited number of large academic medical centers or research institutions.

Another shortcoming is the fact that the average ANN functions as a "black box"[66]. As such, how a specific variable in a dataset is weighted by specific nodes in the network is currently uninterpretable. When evaluating the performance of any ANN, clinicians, mathematicians and computer scientists need to understand the "reasoning" that occurs within the hidden layers. Although questioning of ANNs is possible through mathematical reasoning, it does not reflect clinical decision making. Understanding the way in which ANNs analyze information is paramount for improving their performance and correcting errors that can lead to fatal consequences.

Finally, much of the promise of applying AI in medical image analysis depends on the ability to get actionable results rapidly. The current need for multiple intricate post-processing steps prior to its analysis, indicates that much more work must be

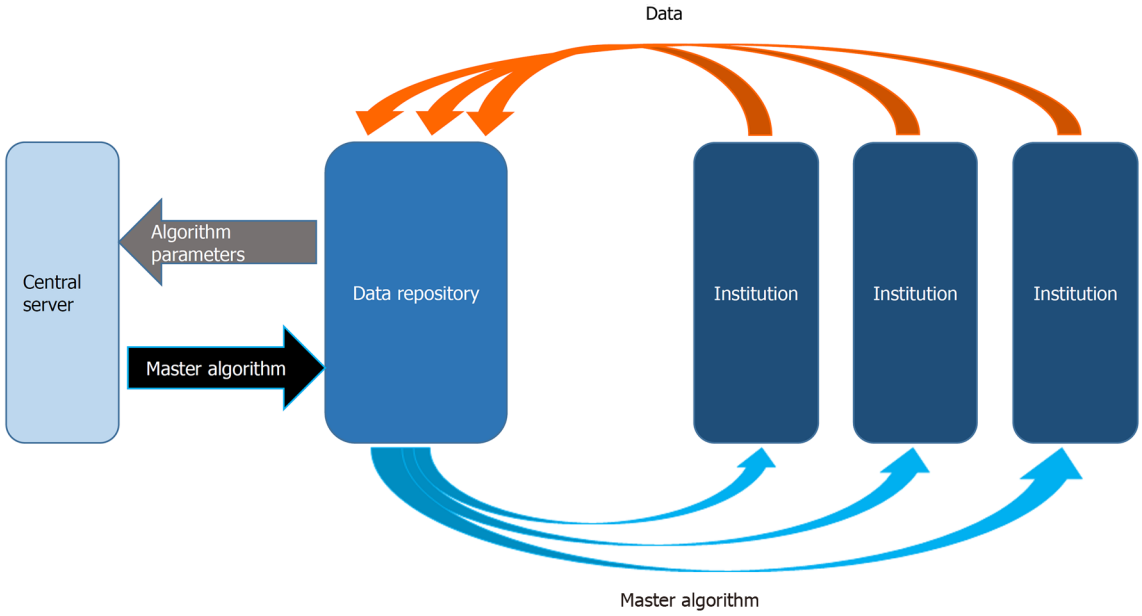

Figure 4 Hybrid artificial intelligence infromation sharing system. A data repository is created as an intermediary between the institutions and the central server. The data repository develops one or more algorithm(s) with the data. The parameters of these algorithms are then shared with the central server to create a master algorithm which is then returned to the repository. New data coming from the institutions is then used by the repository to create new parameters that are then sent to the central server to renew the master algorithm.

done to develop this into a technology that provides "on-the-fly" results[63].

Because of the limitations enumerated above, it is clear that major improvements to the technology need to occur before AI can support everyday activities in clinical practice. It is evident however, that medicine has reached a "point of no return" regarding application of AI. Overcoming these hurdles will require collaboration between academic centers, industry, computer scientists, venture capitalists, regulating organizations and governments (Figure 5). The main goals of this collaboration should be streamlining development of standard data platforms, lowering technology costs and making the technology more "user friendly" so that any provider can use it in real time.

CONCLUSION

With further research, AI could have a large impact on the diagnosis and treatment of PDAC in the future. Novel screening methods are needed, and AI analysis of large comprehensive clinical datasets may yield opportunity for early detection or even predict development of PDAC before a visible lesion can be seen on imaging. An AI protocol which prescreens computed tomography or magnetic resonance imaging prior to a radiologist reading the study could ensure that lesions will not be missed due to human error. "On the fly" AI assistance with endoscopic ultrasound imaging could help the endosonographer optimally target a needle biopsy of a mass. The pathologist can be assisted by an AI algorithm that analyzes the biopsy on the slide that is being read. And AI could prove very useful for following response to treatment and even in suggesting optimal treatment regimens, with personalized treatment strategies based on biological profiling. While AI applications in PDAC are still in the very early stage of development, further investment in research could lead to substantial improvements in screening, early diagnosis, and treatment.

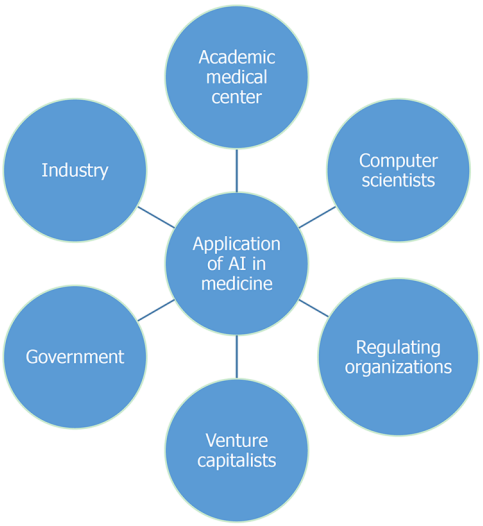

Figure 5 Collaboration to expedite broad artificial intelligence application in medicine. AI: Artificial intelligence.

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MINIREVIEWS

COVID-19 and comorbidities of hepatic diseases in a global perspective

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Abstract

The worldwide outbreak of coronavirus disease 2019 (COVID-19) has challenged the priorities of healthcare system in terms of different clinical management and infection transmission, particularly those related to hepatic-disease comorbidities. Epidemiological data evidenced that COVID-19 patients with altered liver function because of hepatitis infection and cholestasis have an adverse prognosis and experience worse health outcomes. COVID-19-associated liver injury is correlated with various liver diseases following a severe acute respiratory syndrome-coronavirus type 2 (SARS-CoV-2) infection that can progress during the treatment of COVID-19 patients with or without pre-existing liver disease. SARS-CoV-2 can induce liver injury in a number of ways including direct cytopathic effect of the virus on cholangiocytes/hepatocytes, immune-mediated damage, hypoxia, and sepsis. Indeed, immediate cytopathogenic effects of SARS-CoV-2 *via* its potential target, the angiotensin-converting enzyme-2 receptor, which is highly expressed in hepatocytes and cholangiocytes, renders the liver as an extra-respiratory organ with increased susceptibility to pathological outcomes. But, underlying COVID-19-linked liver disease pathogenesis with abnormal liver function tests (LFTs) is incompletely understood. Hence, we collated COVID-19-associated liver injuries with increased LFTs at the nexus of pre-existing liver diseases and COVID-19, and defining a plausible pathophysiological triad of COVID-19, hepatocellular damage, and liver disease. This review summarizes recent findings of the exacerbating role of COVID-19 in pre-existing liver disease and vice versa as well as international guidelines of clinical care, management, and treatment recommendations for COVID-19 patients with liver disease.

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Core Tip: The clinical menace of coronavirus disease 2019 (COVID-19)-related comorbidities of hepatic diseases and severe acute respiratory syndrome-coronavirus type 2 (SARS-CoV-2) tropism for the liver result in liver impairment with increased liver injury markers and cytokine storm. SARS-CoV-2 aggravates liver injury *via* coagulative and fibrinolytic pathways, cytokine-mediated liver injury, ischemia-hypoxia, and immune-mediated cell death pathways owing to adverse outcomes of liver disease such as nonalcoholic steatohepatitis, drug-induced liver injury, nonalcoholic fatty liver disease, metabolic associated fatty liver disease, and hepatocellular carcinoma. This review summarizes diagnostic approaches, therapeutics, clinical guidelines, and vaccines for COVID-19 and liver disease comorbidities.

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INTRODUCTION

Coronavirus belongs to family *Coronaviridae*, subfamily *Orthocoronavirinae*, and order Nidovirales[1]. All over the world, coronaviruses are responsible for causing enteric, neurologic, and hepatic diseases in humans, animals, and other mammals[2]. On the basis of genome and phylogenetic analysis, the subfamily contains four genera named *Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*[3]. Coronaviruses caused epidemics of severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012[2]. The novel coronavirus 2019 (SARS-CoV-2)- belongs to genus *Betacoronavirus*. It is an enveloped virus with a positive-sense, single-stranded RNA genome. SARS-CoV-2 has affected more than 65.8 million people with 1.5 million deaths globally.

In early December 2019, the first documented pneumonia case of unknown origin in Wuhan city of China was confirmed by high-throughput sequencing analysis as a novel *Betacoronavirus* that is currently named SARS-CoV-2. A sudden worldwide outbreak of this virus was declared as health emergency and pandemic of international concern by the World Health Organization (WHO)[4]. In previous epidemics of SARS-CoV and MERS-CoV, bats were considered as the natural host and potential reservoir. Different studies evidenced bats as a reservoir of SARS-CoV-2, however, pangolin species were also considered as natural reservoir of SARS-CoV-2[5].

In the SARS pandemic, the virus was found in patient's stool sample[6], which indicates that fecal samples could be a possible source of transmission of SARS-CoV-2[7]. After clinical recovery, prolonged shedding of SARS-CoV-2 in feces highlighted the possibility of fecal-oral transmission[8]. Positive semen samples containing SARS-CoV-2 have also been observed in two patients who were in recovery and in four who were in the acute stage of infection[9]. Recently, an ocular route of transmission has also been identified in SARS-CoV-2[10].

Symptoms of coronavirus disease 2019 (COVID-19) vary from mild respiratory symptoms to acute respiratory distress syndrome along with multiple organ failure[11] and death, mainly in elderly patients having several comorbidities. The WHO provided timely, effective, and safe supportive management guidelines for COVID-19 patients, which revealed that clinical syndromes associated with SARS-CoV-2 include uncomplicated illness, mild-to-severe pneumonia, acute respiratory distress syndrome, sepsis, and septic shock[12]. However, gastrointestinal symptoms[13] and several extra-pulmonary signs such as liver injury have also been associated with COVID-19[14].

In COVID-19 patients, markers of liver injury may be abnormal[15]. As shown in Table 1, disturbed alanine transaminase (ALT), aspartate transaminase (AST) levels and increased bilirubin have been seen in 14%-53% of COVID-19 patients[16]. A recent study of 1100 patients reported that 18% patients with nonsevere and 56% with severe COVID-19 had elevated serum AST levels. Increased ALT levels were observed in 20% of patients with nonsevere and 28% with severe COVID-19 infection[17]. Moreover, it has been noted that COVID-19 patients with gastrointestinal symptoms showed a high

Table 1 Prevalence of coronavirus disease 2019 patients with altered manifestations of hepatic injury markers

Salient findings	Country	Ref.
Of 417 COVID-19 patients, 76.3% had altered values of liver function tests and 21.5% had liver injury during hospitalization. The use of lopinavir/ritonavir increased the risks of liver injury by 4-fold.	China	Cai *et al*[32], 2020
The prevalence of patients with GI symptoms and elevated level of liver enzymes was 18.6%. The severity of disease increased in patients with digestive symptoms.	China	Pan *et al*[81], 2020
Abnormal liver function tests are common in COVID-19 patients. Of 115 patients, 9.57% had increased ALT levels and 14.78% had increased AST levels.	China	Zhang *et al*[82], 2020
Liver dysfunction at an early stage increases the mortality risk in COVID-19 patients. A total of 151 patients (42.5%) were reported with cholestasis and 101 (28.5%) had hepatocellular injury. Liver dysfunction was more common in critically ill patients.	China	Fu *et al*[83], 2020
About 48.4% of patients with normal liver function had abnormal liver function tests after receiving lopinavir/ritonavir. Liver injury biomarkers (LDH, ALP, GGT, TBiL, prealbumin, and albumin) were dysregulated in a cohort of 288 COVID-19 patients, suggestive of potential as markers of liver injury and a prognosis of severe of COVID-19 disease.	China	Fan *et al*[37], 2020 and Fan *et al*[84], 2020
The presence of acute liver injury was linked with high risk of COVID-19 morbidities and admission to an ICU.	United States	Hajifathalian *et al*[85], 2020
Serum liver enzymes were increased in from 14% to 53% of hospitalized COVID-19 patients.	United States	Fix *et al*[68], 2020
Increased bilirubin level was seen in 16.7% and increased ALT and AST were seen in 15% of COVID-19 patients.	United States	Sultan *et al*[86], 2020

ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; COVID-19: Coronavirus disease 2019; GGT: Gamma-glutamyl transferase; GI: Gastrointestinal; ICU: Intensive care unit. LDH: Lactate dehydrogenase; TBiL: Total bilirubin.

prevalence of liver injury compared with patients with no gastrointestinal symptoms. Correspondingly, the prevalence of chronic liver disease (CLD) was high in patients with gastrointestinal symptoms of COVID-19[18].

Liver cancer is the fourth most common cause of cancer-associated death worldwide[19]. The most common type is hepatocellular carcinoma (HCC), and patients with HCC have underlying CLD, which includes alcoholic liver injury, nonalcoholic fatty liver disease (NAFLD), and chronic hepatitis B or C virus infection[19]. It has been reported that cancer patients are at high risk of COVID-19. A study of three hospitals in Wuhan reported 1276 confirmed cases of COVID-19, among which 28 patients had different types of cancer and two of them had HCC[20]. NAFLD, also called metabolic associated fatty liver disease (MAFLD), is highly prevalent globally[21]. A study reported that patients with NAFLD had increased ALT levels following COVID-19 infection[22]. A study from China showed that patients with NAFLD had an increased risk of COVID-19 compared with patients without it[23]. However, more studies are required to understand the mechanism of liver injury caused by NAFLD and COVID-19. It has been observed that use of immunosuppressant drugs in post liver transplant patients more prone them to SARS-CoV-2 infection. Additionally, the use of these drugs were found to enhance the cytokine storm in COVID-19 patients[18,24].

COVID-19-associated liver injury is manifested by entry of SARS-CoV-2 into the liver that can progress during COVID-19 treatment in patients with or without pre-existing liver disease[25]. SARS-CoV-2-related liver injury can occur by multiple factors, including direct cytopathic effect of the virus on cholangiocytes/hepatocytes, immune-mediated damage, hypoxia[19], sepsis[26], and drugs (*e.g.*, acetaminophen, ritonavir/lopinavir, interferon, Chinese herbs, and antibacterial agents) used in treating COVID-19[27]. However, there is no proof that patients with chronic hepatitis are at risk of COVID-19 unless other comorbidities (*e.g.*, cardiovascular disease, diabetes, and hypertension) that can increase the risk of liver disease are present[26]. The angiotensin-converting enzyme 2 (ACE2) receptor is associated with entry of SARS-CoV-2 and is highly expressed (about 80%) in alveolar cells of the lungs[28], the gut, and the kidneys[29]. The expression of ACE2 receptor has also been observed in myocardial cells, nephron proximal tubule cells, absorptive enterocytes of the ileum and colon, bladder urothelial cells; and the oral, nasal and nasopharyngeal mucosal epithelia [30]. It has been reported that ACE2 cell surface receptors are highly expressed in cholangiocytes and hepatocytes, which indicates tropism of SARS-CoV-2 in the liver[31].

COVID-19-ASSOCIATED LIVER INJURY AND ITS CLINICAL IMPLICATIONS IN A GLOBAL SCENARIO

Abnormal levels of liver injury markers such as AST and ALT or abnormal liver function tests (LFTs) in COVID-19 patients place an additional disease burden on clinicians as well as to scientists in terms of treatment regimens and defining the association of COVID-19-linked hepatic diseases. The data that document liver impairment in COVID-19 patients reveal variable levels of liver injury markers across the globe, as shown in Figure 1[32-36]. In this context, the preliminary data were reported by Cai *et al*[32] from Shenzhen, China from January 11 to February 21, 2020 and followed-up to March 7, 2020. The data in the report was from a local hospital that found 318 of 417 COVID-19 patients (76.3%) with abnormal LFT values and 90 of 417 (21.5%) with liver injury during hospitalization. The incidence of liver injuries in Asia was further evaluated by Vespa *et al*[34] in a cohort of 292 COVID-19 patients until March 30, 2020. Compared with Cai *et al*[32], a cholestatic pattern of liver injury with an increased level of alkaline phosphatase (ALP) > 150 U/L was observed in 9.6% of the selected population. Another study of cholestatic liver injury conducted from January 20 to 31, 2020 at the Shanghai Public Health Clinical Center reported abnormal liver functions as increased levels of ALT and AST, gamma-glutamyl transferase (GGT), ALP, and total bilirubin[37]. Thus, the documented reports describe data related to predictive markers of COVID-19 and associated liver injury, but the underlying causes of liver injury associated with worse outcomes are still elusive.

The clinical significance of COVID-19-associated liver disease needs to be described. Some studies have addressed the clinical challenges. Of relevance is a report by Vespa *et al*[34] of an increase in ALP values because of SARS-CoV-2 liver tropism *via* the ACE2 receptor on hepatocytes and cholangiocytes. Furthermore, they correlated the ALP elevation as a marker of patient frailty or as representing an increased systemic inflammatory response to SARS-CoV-2 infection[34]. The prevalence of altered liver injury markers is highly associated with drug-induced liver injury (DILI) during the COVID-19 pandemic. Several studies[32,38-40] have reported potential harms related to pharmacotherapy with lopinavir/ritonavir in COVID-19 patients and the vulnerability of patients in developing severe pneumonia *via* increased LFTs. Moreover, the pathological characteristics of liver injury *via* autopsy analysis of COVID-19 patients provides evidence in support of DILI. A study revealed moderate microvesicular steatosis with mild hepatic inflammation, indicating the possibility of hepatic injury. However, this pattern of histological injury could be correlated with either DILI or SARS-CoV-2 infection[41].

Several risk factors can be linked to the incidence of COVID-19-associated liver injury. A retrospective study in a French population found a marked prevalence of obesity in confirmed COVID-19 patients. The study included 340 COVID-19 patients, 230 (68%) with noncritical COVID-19 and 110 (32%) with critical COVID-19. It was found that 85 of 340 patients with severe COVID-19 (25%) were obese compared with 15.3% of the general French population. Following standardization by age and sex, the prevalence rates of obesity were 1.35% and 1.89% times higher in patients with severe COVID-19 and in those admitted to intensive care units, respectively, than in the general French population[42]. Likewise, Zheng *et al*[43] reported the key association of obesity with the severity of COVID-19 in MAFLD patients, which provides a rationale for the likelihood of the importance of obesity-related comorbidities of liver diseases[44,45].

PRE-EXISTING LIVER DISEASE AND COVID-19: AN INTERLINKED SETUP AND ITS RELATED CONSEQUENCES

CLDs are an existing threat that accounts for the leading causes of liver-related mortality worldwide[46-48]. The major CLDs, including hepatitis B virus or C virus infection, alcohol-induced liver damage (ALD), and NAFLD, lead to prolonged liver damage and increased incidence of CLD-associated cancers, particularly, HCC[49,50]. Underlying liver diseases were one of the crucial causative factors in the previous SARS outbreak, with high mortality rates in adults and elders[51]. The Centers for Disease Control and Prevention recently included liver diseases as a comorbidity and predisposing factor for contracting SARS-CoV-2 infection. Moreover, the American Association for the Study of Liver Diseases (AASLD) endorses COVID-19 testing on a priority basis for patients who manifest symptoms of liver disease[52]. The COVID-19

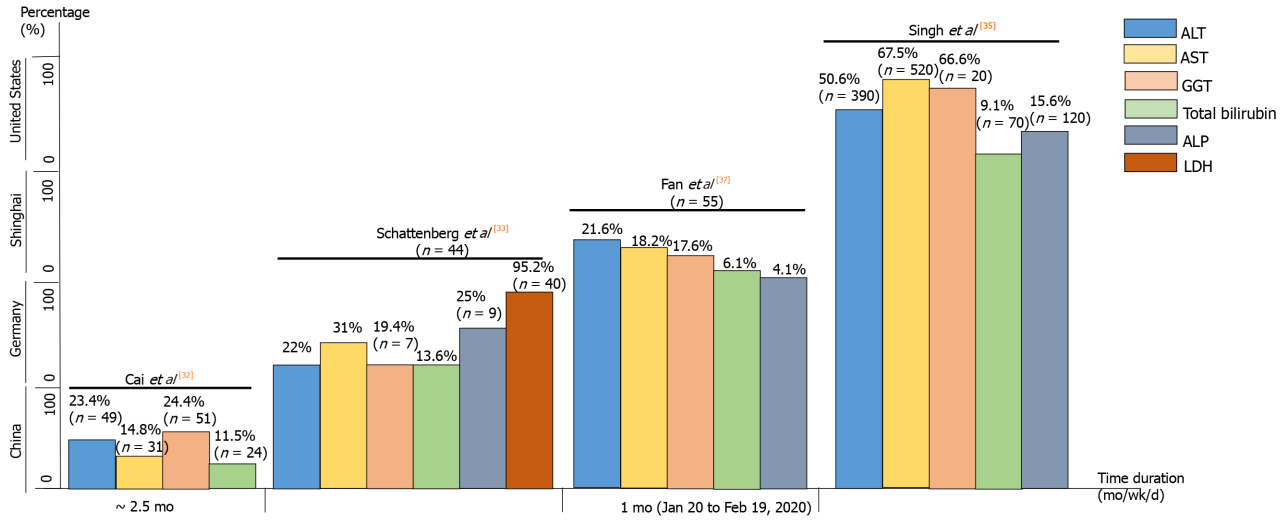

Figure 1 Overview of a global perspective related to incidence of coronavirus disease 2019-associated alterations in liver function tests.
The figure presents only data published in peer-reviewed journals. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; LDH: Lactate dehydrogenase.

pandemic together with the global prevailing menace of CLD further complicates the care of pre-existing liver disease patients as a result of failure of screening, and follow-up. Therefore, the intricate link between pre-existing liver disease and COVID-19 requires additional study and specific disease management. It has to be expected that patients with underlying liver diseases are more vulnerable to exacerbating COVID-19-related effects and vice versa[53] that may account for the high morbidity and mortality rates in the current COVID-19 pandemic.

Relevant to pre-existing liver diseases, alcohol use disorder or ALD is the CLD with the highest hospitalization burden and has seen a more than 2-fold increase in China. The effect is likely to be repeated in the United States, with 12.7% estimated hospitalization rate associated with COVID-19. A gradual increase in the incidence of ALD was accompanied by an increase in patients indicated for liver transplantation prior to COVID-19 pandemic. The direct clinical implications of COVID-19 on ALD are still unknown, but it can be suspected that SARS-CoV-2 infection can serve as a major host-compromising factor with underlying ALD and subsequently results in acute-on-chronic liver failure. The high proportion of ALD among CLD patients is reflected in the number of patients with decompensated ALD during COVID-19 pandemic[52].

Growing evidence of COVID-19-related liver disease comorbidities suggests that MAFLD patients are at higher risk of COVID-19 disease progression[43,54,55]. A retrospective study conducted by Fondevila *et al*[56] described a mechanistic approach in the context of a higher risk of SARS-CoV-2 infection in obese patients. They assessed the hepatic mRNA expression of SARS-CoV-2 cell entry molecules, ACE2 and the cellular transmembrane protease serine 2 (*TMPRSS2*) in obese patients with NAFLD and/or diabetes mellitus type-2. Based on liver mRNA expression of both ACE2 and *TMPRSS2* in obese patients, the results revealed that SARS-CoV-2 entry factors are differently affected in diabetes and NAFLD. Moreover, major alterations in the expression of SARS-CoV-2 entry molecules in in men and women suggest a lower susceptibility of women to liver injury. While obese women with diabetes have unexpectedly lower levels of ACE2 and *TMPRSS2* than obese normoglycemic women, obese patients with nonalcoholic steatohepatitis had a higher expression of those genes, suggesting that advanced stages of NAFLD might predispose to COVID-19.

COVID-19 patients with comorbidities of advanced hepatic complications are generally at an increased risk of infection because of cirrhosis-associated immune dysfunction[57]. A retrospective study by Iavarone *et al*[58] documented a substantial 30-d mortality rate of 34% in a cohort of 50 cirrhotic patients with COVID-19, which was higher than the rate in cirrhotic patients with bacterial infections. Overall COVID-19 mortality from the medical consequences of respiratory failure was correlated with the worsening of liver dysfunction. Other categories of COVID-19 patients with hepatic diseases that are of great concern are liver transplant recipients and patients with autoimmune liver diseases receiving immunosuppressant drugs[57]. However, COVID-19-associated effects in recipients of living donor allografts are still unclear[59]. A case reported by The American Society of Transplantation and the American Society of

Transplant Surgeons described the impact of COVID-19-associated hepatitis during liver transplantation. A patient underwent ABO-incompatible living donor liver transplantation without knowing that the liver donor was infected with COVID-19 during the donation procedure. In that case, donor-derived transmission to the recipient was not identified, and the liver donor was found to be recovering from COVID-19 infection. Donor-derived transmission was not identified[60].

A PLAUSIBLE PATHOPHYSIOLOGICAL TRIAD OF COVID-19, HEPATOCELLULAR DAMAGE AND LIVER DISEASES

Epidemiological studies have described liver dysfunction and the effects of SARS-CoV-2 infection on the liver cells in COVID-19 patients by elevated levels of liver injury markers such as ALT, AST, and bilirubin[32,33,57,61,62]. It has been stated previously that the SARS-CoV and MERS-CoV viruses primarily affect the upper respiratory tract but also affect the liver[62,63]. Despite the immediate and direct action of SARS-CoV-2 *via* potential targets on the epithelial cells of lung alveoli as well as the respiratory tract, emerging evidence suggests that the high expression of ACE2 receptors in the liver renders it susceptible to the pathogenicity of SARS-CoV-2[36,64]. Pathological examination of COVID-19 patients has confirmed cytopathic injury in the lungs[41] and has recently confirmed SARS-CoV-2 infection as an etiology of liver disease. The proposed mechanisms associated with COVID-19-induced liver injury include direct viral insult-linked hepatic derangements, cytokine storm-prompted liver injury, and ischemia related to COVID-19-induced hypoxia as shown in Figure 2[52,65-68].

Liver impairment in COVID-19 might be directly correlated with SARS-CoV-2 infection of liver cells. Approximately 2%-10% COVID-19 patients with diarrhea have confirmed SARS-CoV-2 RNA in stool and blood samples, which implies the possibility of viral exposure in the liver. The affinity of SARS-CoV-2 and SARS-CoV for the ACE2 receptor indicates respective target sites, mainly the upper respiratory tract, lung tissue, and cholangiocytes of the liver, where the virus replicates and manifests COVID-19 associated symptoms[61]. In that context, Zhao *et al*[69] studied a SARS-CoV-2 infection model in human liver ductal organoids. They reported genomic evidence that SARS-CoV-2 virus infection resulted in dysregulation of barrier and bile-acid transporting functions of the cholangiocytes. The study found that altered cholangiocyte functions of could have been the result of a direct SARS-CoV-2 cytopathogenic effect on target cell that expressed ACE2 and *TMPRSS2*.

Cytokine storm is one of the hallmarks of infectious and noninfectious diseases that are capable of causing severe multiple organ injuries. Establishment of the cytokine environment is a multifactorial network that involves an immunological response to an invading antigen along with interplay of activated host immune and inflammatory cells. In line with the concept of cytokine storm in COVID-19 patients, Han *et al*[65] performed a prospective cohort study at a local hospital in Wuhan, China that enrolled 102 COVID-19 confirmed patients and 45 healthy control volunteers. They analyzed the serum profiles of inflammatory cytokines, including tumor necrosis factor-, interferon-, interleukin (IL)-2, IL-4, IL-6, IL-10, and C-reactive protein (CRP) by immunoassays. Significant increases in the levels of the inflammatory markers and CRP were seen in COVID-19 patients compared with the healthy volunteers. Moreover, the levels of IL-6 and IL-10 were significantly higher in critical than severe or moderate COVID-19 patients, suggesting that increased IL-6 and IL-10 may allow rapid diagnosis of patients with increased risk of lethal disease. Furthermore, Wang *et al*[70] reported COVID-19-associated conspicuous cytopathy. The severity of SARS-CoV-2 infection is associated with disturbed levels of liver enzymes, increased alveolar-arterial oxygen gradient and GGT level, and decreased albumin and circulating $CD4^+$ T cells and B lymphocytes. The predominant histological features of COVID-19 liver infection are substantial apoptosis and binuclear hepatocytes[70].

Hepatic dysfunction in severe COVID-19 is accompanied by aberrant activation of the coagulative and fibrinolytic pathways[71], moderately decreased platelet counts, increased neutrophil counts and neutrophil-to-lymphocyte ratios, and high ferritin levels. Such laboratory findings are perceived as nonspecific inflammatory markers, but the altered levels can coincide with a failure of innate immune regulation during progression of severe COVID-19. Indeed, alteration of immune balance activates coagulation and NETosis, and subsequently affects systemic iron metabolism secondary to macrophage activation[38].

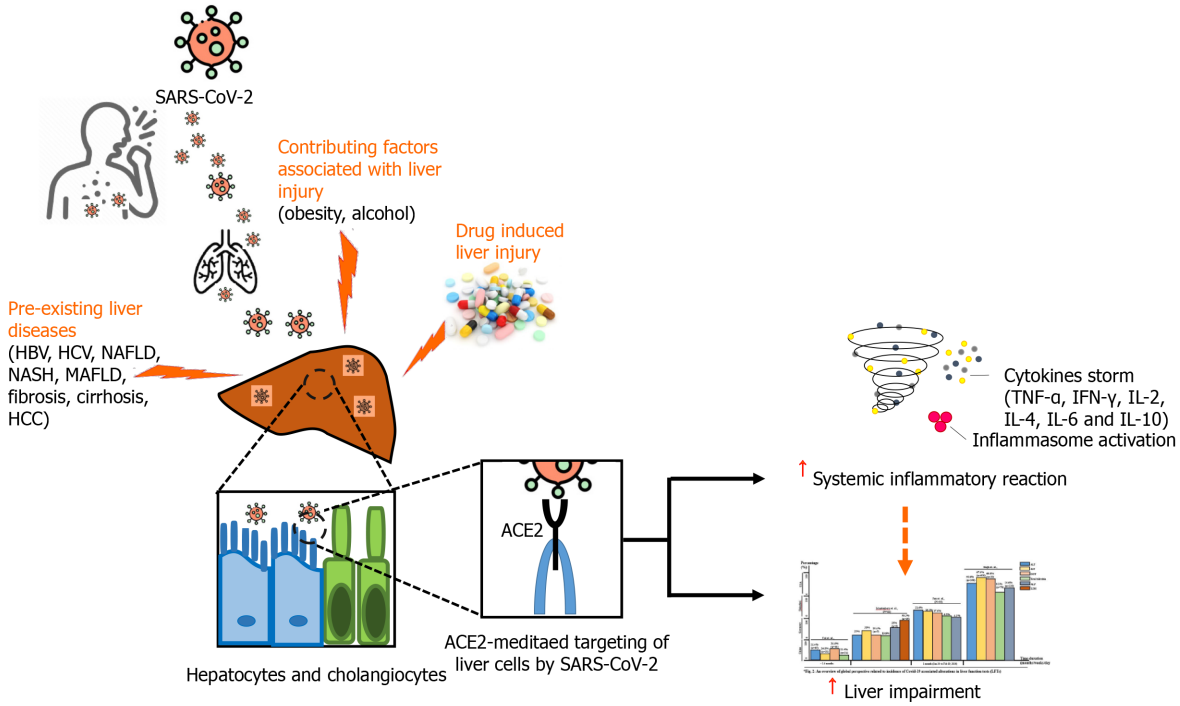

Figure 2 Severe acute respiratory syndrome-coronavirus type 2-associated pathogenesis and immunological response in the liver. ACE2: Angiotensin-converting enzyme 2; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; IFN: Interferon; IL: Interleukin.; MAFLD: Metabolic associated fatty liver disease; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; SARS-CoV-2: Severe acute respiratory syndrome-coronavirus type 2; TNF: Tumor necrosis factor.

DIAGNOSTIC APPROACHES FOR COVID-19

SARS-CoV-2 infection is confirmed by serologic tests that measure the antibody response or antibody titer in the patient. In COVID-19 patients, antibodies are produced in the days to weeks after SARS-CoV-2 infection. Thus, the presence of antibodies indicates that a person was infected with SARS-CoV-2, irrespective of whether the infection caused severe or mild disease or even an asymptomatic infection. Surveillance of antibody seropositivity allows inferences to be made about the extent of infection and the cumulative incidence of infection in the population. Serologic Enzyme-linked immunosorbent assays can be used for COVID-19 serosurveillance and to determine the extent of infection in the population. A confirmed case of SARS-CoV-2 infection is declared by a positive result from nasal and pharyngeal swab specimens by high-throughput sequencing or real-time reverse transcriptase polymerase chain reaction (RT-PCR)[17]. Molecular testing (RT-PCR) confirms infection, often in patients with severe disease, as they are individuals who seek and require health care. A percentage of patients with mild or asymptomatic infections who do not require medical attention may not be tested, and as a result, the full spectrum of the disease will not be known[72]. SARS-CoV-2 infection is associated with epidemiological characteristics, clinical signs, and symptoms of COVID-19 that can be accessed from electronic medical records and laboratory findings. Radiological assessments of COVID-19 patients include chest X-rays or computed tomography. Whereas, laboratory assessments that help to indicate the prognosis of COVID-19 include complete blood counts, blood chemistry, coagulation tests, assays of liver and renal function markers, electrolytes, CRP, procalcitonin, lactate dehydrogenase, and creatine kinase[17].

RECOMMENDATIONS OF THERAPEUTICS FOR COVID-19: VACCINES, LABEL AND OFF-LABEL MEDICATIONS FOR PATIENT CARE

The COVID-19 pandemic has prompted the scientific community and clinicians to develop COVID-19-related therapeutics and vaccines to mitigate as well as control the pathogenicity of SARS-CoV-2. Previous investigations on genomic sequencing of

SARS-CoV and MERS have contributed to vaccination strategies in developing current vaccines against SARS-CoV-2[73]. The characteristics of vaccines in clinical testing are based upon inactivated or live-attenuated viruses, protein subunits, virus-like particles, replicating and nonreplicating viral vectors, and DNA and RNA that may provoke protective immunity to SARS-CoV-2 infection[74,75]. Since July 2, 2020, the global landscape of SARS-CoV-2 vaccine development reported 163 vaccine candidates, 135 of the 163 are in preclinical or exploratory stages of development. Currently, mRNA-1273 (Moderna), Ad5-nCoV (CanSino Biologicals), INO-4800 (Inovio, Inc.), LV-SMENP-DC, a Pathogen-specific aAPC (ShinzenGeno-Immune Medical Institute), ChAdOx1 (Oxford University) BioNtech (Pfizer), Sputnik V (Gamaleya, Russia), and Sinovac (China) have been approved by the WHO under emergency use authorization[76]. India has started mass vaccination with its locally produced Covaxin vaccine. A prolonged time period for approval of vaccines is needed for validation of efficacy and adverse effects in target populations before post-market surveillance. However, adverse reactions to vaccines including fatigue, chills, aches, skin rashes, muscle pain, fever, and joint pain could be a barrier in the global rollout of COVID-19 vaccines[77]. In April, 2020, the Access to COVID-19 Tools accelerator was launched by the WHO and its partners to cooperate in fighting against the COVID-19 pandemic. Moreover, a global collaboration aims to accelerate development, production, and equitable access to COVID-19 tests, treatments, and vaccines among countries, particularly low-to-middle income countries (SAGE; http://www.who.int/).

In terms of therapeutics, COVID-19 medications fall into two categories: Those that target the viral replication cycle and those that aim to control the symptoms of the disease. The aminoquinolines chloroquine and hydroxychloroquine are polymerase inhibitors classically used as antimalarial medications. In malaria, it inhibits heme polymerase, causing the accumulation of toxic heme in the parasite that leads to its death. In COVID-19, it is thought that the drugs keep the virus out of host cells by blocking glycosylation of host receptors and blocking the production of viral proteins by inhibiting endosomal acidification. The WHO recommends the off-label use of hydroxychloroquine or chloroquine and lopinavir/ritonavir for treatment of COVID-19 with any disease severity and any duration of symptoms. Remdesivir and systemic corticosteroids are potential candidates for conditional recommendation of use in hospitalized COVID-19 patients for usual care regardless of disease severity (WHO/2019-nCoV/therapeutics/2020.1). The WHO considers off-label use of medication as country-specific. In many countries, doctors are giving COVID-19 patients medicines that have not been approved for this disease (MEURI; http://www.who.int/). Thus, COVID-19 patients have received off-label and compassionate-use therapies, such as interferon- combined with the repurposed drug Kaletra, an approved combination of the human immunodeficiency virus protease inhibitors ritonavir and lopinavir, chloroquine, azithromycin, favipiravir, remdesivir, steroids, and anti-IL-6 inhibitors, based on either *in vitro* antiviral or anti-inflammatory properties[78]. It is unclear if COVID-19 therapeutics protect against liver injury or disease. It would be interesting to look into protective effects in future.

RECOMMENDATIONS FOR MANAGEMENT OF COVID-19 PATIENTS WITH PRE-EXISTING LIVER DISEASES

The WHO has approved comprehensive guidelines to strengthen the care and management of COVID-19 patients and to provide up-to-date guidance to clinicians and physicians. That report addresses the best practices to manage severe acute respiratory infection (SARI), including infection prevention and control measures and supportive care for COVID-19 patients. Furthermore, the prime considerations focus on recognizing and treating patients with SARI through appropriate diagnosis, early supportive therapy, management of acute respiratory distress and septic shock, prevention of complications, and use of specific COVID-19 treatments[79]. Indeed, patients with CLD do not appear to be over-represented in cohorts of patients with COVID-19, where they make up less than 1% of reported cases. These observations suggest that patients with CLD may have a decreased risk of contracting severe SARS-CoV-2. However, the risk of infection and/or the risk of a severe course of COVID-19 may be different depending on the nature of the CLD and the presence or absence of advanced fibrosis or cirrhosis. In that context, the European Association for the Study of the Liver, European Society of Clinical Microbiology and Infectious Diseases and AASLD provided comprehensive guidance for physicians and clinicians for the care of

patients with CLD during the early stages of the COVID-19 pandemic[68,80]. The salient guidelines are summarized in Table 2 for clinical relevance and management of COVID-19 and liver patients.

CONCLUSION

The chaotic conditions of the global spread of COVID-19 necessitate clinical care and management of patients with pre-existing morbidities, especially highly prevalent liver diseases in developed and developing countries to decrease the economic and health losses globally. Understanding the pathophysiological mechanisms of COVID-19 and its associated adverse effects in hepatic diseases is indispensable for the development of therapeutics and vaccines as well as mitigation of risks factors of disease. The WHO guidelines and associations for the study of liver diseases are timely and helpful in decreasing burden of disease and health education. The WHO needs to play a vital role in equitable and global availability (rollout) of COVID-19 vaccines to low-to-middle income countries to prevent COVID-19 pandemic and associated comorbidities.

Table 2 Summary of specific European Association for the Study of the Liver, European Society of Clinical Microbiology and Infectious Diseases and American Association for the Study of Liver Diseases guidelines and recommendations for the clinical care and management of patients with liver diseases during coronavirus disease 2019 pandemic

	Hospitalization and severe COVID-19	Alterations to standard treatment strategies		Progression of liver disease
	Early administration, laboratory findings and risk of SARS-CoV-2 infection	Treatment of higher risk groups	Resumption of targeted treatment and surveillance	Patient education and intensive lifestyle advice
NAFLD	High prevalence risk of SARS-CoV-2 infection in NAFLD patients with COVID-19 suggest an early admission to the hospital	No side effects related to ACE inhibitors or AR blockers to date, thus, arterial hypertension treatment should continue in accordance to prescribed guidelines	Not well known	Intensive lifestyle interventions including nutritional guidance, weight loss and diabetes management may prevent the risk of severe COVID-19 complications
Chronic Viral Hepatitis	Patients on chronic HBV or HCV medications with poor compliance should observed treatment protocols, directly	(1) In HBV and COVID-19 patients, an alternative agent should be considered rather than interferon-α therapy; (2) COVID-19 patients with high risk of severe acute HCV should consider for an appropriate antiviral therapy on case-by-case basis under the full consultation; and (3) COVID-19 patients with resolved HBV infection, receiving corticosteroids, tocilizumab, or other immunosuppressant agents should be considered for appropriate antiviral therapy to prevent viral reactivation under full consultation	(1) Without COVID-19, the patients should continue the HBV or HCV medications in accordance to general guidelines; and (2) in COVID-19 patients, initiation of HBV or HCV medication should be deferred until full recovery from COVID-19 or on case-by-case basis under the full consultation	Use of telemedicine for patients of on-going chronic HBV or HCV treatment without COVID-19
Autoimmune hepatitis	(1) Immunocompromised patients on corticosteroid treatment during COVID-19 requires respiratory support; And (2) patients on respiratory support may be considered for addition of, or conversion to, dexamethasone treatment	(1) Patients on high doses of corticosteroid may show more susceptibility to SARS-CoV-2 infection or severe COVID-19; (2) Low doses may be considered under special circumstances (*e.g.*, drug-induced lymphopenia, or bacterial/fungal superinfection with severe COVID-19) under consultation with specialist; (3) or may consider budesonide as an alternative first line agent in patient without cirrhosis to induce remission who have a flare of autoimmune hepatitis	Immunocompromised patients with COVID-19 may be considered for dosing of corticosteroid, sufficient for adrenal insufficiency	All patients should receive vaccination of *Streptococcus pneumoniae* and influenza
Alcohol-related liver hepatitis	Alcohol-induced severe hepatitis patients on corticosteroid treatment with COVID-19 require respiratory support	Not well known	Not well known	Increased probability of higher alcohol consumption during social distancing, so, preemptive strategies including patient outreach and telephone alcohol liaison, should be considered
Cirrhosis	(1) Cirrhotic patients with COVID-19 should be considered for early hospitalization; and (2) to avoid admission and to prevent decompensation, guidelines on prophylaxis of spontaneous bacterial peritonitis, gastrointestinal hemorrhage and hepatic encephalopathy should be followed	Vasoconstriction therapy should be considered with great caution for critically ill cirrhotic patients with COVID-19	Cirrhotic patients are vulnerable to both SARS-CoV-2 infection and altered standards of patient care during pandemic. Thus, the best efforts should be made for care of cirrhotic patients according to general guidelines	All patients should receive vaccination of *Streptococcus pneumoniae* and influenza
Hepatocellular carcinoma	Specific risk of HCC patients with COVID-19 remains undefined	In COVID-19 patients, initiation of HBV or HCV medication should be deferred until full recovery from COVID-19 or on case-by-case basis under the full consultation	Full HCC surveillance should resume under specific circumstances	Consider virtual patient visits to discuss diagnosis and management of HCC and other liver tumors
Liver	Patients on the liver transplant	Precautions should be followed to make	Not well known	Patients should

transplant candidates	waiting list with decompensated cirrhosis are at high risk of severe COVID-19 and death following SARS-CoV-2 infection	COVID-19 free liver transplantation process		avoid attending in-person community recovery support meetings, such as Alcoholics Anonymous, and provide alternative telephone or online resources
Liver transplant recipients	Early admission should be considered for all liver transplant recipients who develop COVID-19	Drug levels of calcineurin inhibitors and mechanistic target of rapamycin inhibitors should be closely monitored on administration with COVID-19 medications, particularly hydroxychloroquine, protease inhibitors or new trial drugs for COVID-19	Reduction of immunosuppressant dosing may be considered under special circumstances (*e.g.*, drug-induced lymphopenia, or bacterial/fungal superinfection with severe COVID-19) under consultation with specialist	All patients should receive vaccination of *Streptococcus pneumoniae* and influenza

ACE: Angiotensin-converting enzyme; AR: Adrenoreceptor; COVID-19: Coronavirus disease 2019; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma. HCV: Hepatitis C virus; NAFLD: Nonalcoholic fatty liver disease; SARS-CoV-2: Severe acute respiratory syndrome-coronavirus type 2.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Non-responsive celiac disease in children on a gluten free diet

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Abstract

BACKGROUND

Non-responsive celiac disease (NRCD) is defined as the persistence of symptoms in individuals with celiac disease (CeD) despite being on a gluten-free diet (GFD). There is scant literature about NRCD in the pediatric population.

AIM

To determine the incidence, clinical characteristics and underlying causes of NRCD in children.

METHODS

Retrospective cohort study performed at Boston Children's Hospital (BCH). Children < 18 years diagnosed with CeD by positive serology and duodenal biopsies compatible with Marsh III histology between 2008 and 2012 were identified in the BCH's Celiac Disease Program database. Medical records were longitudinally reviewed from the time of diagnosis through September 2015. NRCD was defined as persistent symptoms at 6 mo after the initiation of a GFD

Gastroenterologues du Quebec; and Phase 2 Award from the Fonds de Recherche Sante Quebec.

Institutional review board statement: This study was reviewed and approved by the Institutional Review Board of Boston Children's Hospital.

Informed consent statement: Patients were not required to give informed consent to the study because this study presented no more than minimal risk to patient privacy and confidentiality.

Conflict-of-interest statement: Leffler DA is employed by Takeda Pharmaceuticals International Co. Kelly CP has acted as a scientific advisor to companies attempting to develop new diagnostic and management approaches for Celiac disease including Cour Pharma, Glutenostics, Innovate, Immunogenx and Takeda. He also acts as Principal Investigator on a research grant on Celiac disease supported by Aptalis. Silvester JA has served on an advisory board for Takeda Pharmaceuticals and has received research funding from Biomedal S.L., Cour Pharma and Glutenostics.

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and causes of NRCD as well as symptom evolution were detailed. The children without symptoms at 6 mo (responders) were compared with the NRCD group. Additionally, presenting signs and symptoms at the time of diagnosis of CeD among the responders and NRCD patients were collected and compared to identify any potential predictors for NRCD at 6 mo of GFD therapy.

RESULTS

Six hundred and sixteen children were included. Ninety-one (15%) met criteria for NRCD. Most were female (77%). Abdominal pain [odds ratio (OR) 1.8 95% confidence interval (CI) 1.1-2.9], constipation (OR 3.1 95%CI 1.9-4.9) and absence of abdominal distension (OR for abdominal distension 0.4 95%CI 0.1-0.98) at diagnosis were associated with NRCD. NRCD was attributed to a wide variety of diagnoses with gluten exposure (30%) and constipation (20%) being the most common causes. Other causes for NRCD included lactose intolerance (9%), gastroesophageal reflux (8%), functional abdominal pain (7%), irritable bowel syndrome (3%), depression/anxiety (3%), eosinophilic esophagitis (2%), food allergy (1%), eating disorder (1%), gastric ulcer with *Helicobacter pylori* (1%), lymphocytic colitis (1%), aerophagia (1%) and undetermined (13%). 64% of children with NRCD improved on follow-up.

CONCLUSION

NRCD after ≥ 6 mo GFD is frequent among children, especially females, and is associated with initial presenting symptoms of constipation and/or abdominal pain. Gluten exposure is the most frequent cause.

Key Words: Celiac disease; Non-responsive celiac disease; Children; Gluten-free diet; Constipation; Abdominal pain

Core Tip: This is a retrospective chart review study of children < 18 years of age diagnosed with celiac disease (CeD) by positive serology and biopsies showing Marsh III histology to characterize non-responsive CeD (NRCD). NRCD was attributed to a wide variety of diagnoses with gluten exposure (30%) and constipation (20%) being the most common causes. Most (64%) patients improved on follow-up. Our study highlights the importance of performing a diligent search for etiologies of NRCD when there are persistent symptoms despite following a gluten-free diet and reinforces the need for close follow up in the first year of the diagnosis of CeD.

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INTRODUCTION

Celiac disease (CeD) is a chronic immune-mediated enteropathy precipitated by exposure to gluten in genetically predisposed individuals[1]. CeD is diagnosed based on clinical symptoms and serological markers in conjunction with specific histological changes found in the duodenum, including villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis[2].

Strict elimination of gluten is currently the only effective treatment for CeD[2,3]. A gluten-free diet (GFD) leads to significant improvement in most people with CeD within weeks[4]. Failure to respond after six to twelve months of a GFD is defined as non-responsive CeD (NRCD), and may occur in both adults and children[5]. NRCD incorporates a range of specific and distinct underlying diagnoses, such as other food intolerance, small intestinal bacterial overgrowth, microscopic colitis, and irritable bowel syndrome[6,7]; nevertheless, the most common etiology of NRCD is continued

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gluten exposure[6,8]. Studies suggest that 7% to 52% of adult CeD patients may have NRCD[2,5,6]. A recent retrospective study showed that 34% of children adhering to a GFD still report at least one symptoms more than 24 mo after their diagnosis[5]; however, little is known about the spectrum and etiologies of NRCD in children.

The objective of this study was to determine the incidence, clinical characteristics and underlying causes of NRCD in children.

MATERIALS AND METHODS

In this retrospective cohort study, we used the Boston Children's Hospital (BCH) Celiac Disease Program database to identify children less than 18 years of age with an initial biopsy-confirmed CeD diagnosis at BCH between January 1, 2008 and December 31, 2012. Celiac antibodies included tissue transglutaminase (tTG) immunoglobulin (Ig) A, deamidated gliadin peptide (DGP) IgG and endomysial antibody (EMA) IgA. Included patients had Marsh III lesions, defined by increased intraepithelial lymphocytes, villous atrophy, and crypt hyperplasia[2]. Children diagnosed elsewhere and seen for a second opinion, and those who were asymptomatic at the time of diagnosis were excluded.

Data extracted from medical records included demographics, clinical presentation, serologic results, comorbidities, family history of CeD and number of dietitian visits at BCH within 120 d of CeD diagnosis. Medical records were reviewed longitudinally from the time of diagnosis through September 2015.

Adherence to the GFD was determined from review of medical records, primarily from dietician documentation/notes of their clinical assessment which included a dietary interview and is reported using a previously published assessment scale graded as follows: (1) Excellent = patient never ate gluten intentionally and/or had rare exposure, (2) Good = inadvertent exposure once per month, (3) Fair = exposure 2-3 times per month, (4) Poor = exposure 1-2 times per week, (5) Noncompliant = not on a GFD, or (6) Unable to assess GFD adherence from the medical record[9].

NRCD was defined as persistent clinical symptoms six months or more after the initiation of a GFD. Children with clinical improvement while on a GFD were considered as responders and compared with the NCRD group. This study was approved by the BCH Institutional Review Board.

Statistical analysis

Categorical data were summarized as frequency (percentage) and comparisons between responders and children with NRCD made with Fisher's exact test. Continuous data were presented as median with interquartile range (IQR) and compared across groups by Wilcoxon rank-sum test. A stepwise logistic regression model was used to investigate symptoms at the time of diagnosis that are independently associated with NRCD signs and symptoms included abdominal pain, constipation (hard or infrequent stools), nausea/vomiting, diarrhea, weight loss or poor weight gain, fatigue, short stature, gassiness, abdominal distension, loss of appetite, arthritis/arthralgia, rash, and mouth ulcers. $P < 0.20$ was required for a symptom to enter the model, and $P < 0.05$ was required for the symptom to remain in the model. All comparisons were two-sided, with statistical significance established a priori as $P < 0.05$. Data analysis was performed with SAS v9.4 (Cary, NC, United States).

RESULTS

A total of 659 children under 18 years of age were diagnosed with biopsy-confirmed CeD at BCH between January 1, 2008 and December 31, 2012 (Figure 1). All had elevated serum tTG IgA except for nine subjects. These nine were diagnosed with CeD according to the presence of other celiac serology, HLA typing compatible with CeD or response to a GFD. Forty-three who did not have symptoms at the time of the diagnosis (evaluated for CeD because of type 1 diabetes, family history of CeD or short stature) were excluded from the primary analysis. Of the remaining 616 children included, 525 (85%) subjects showed complete response as determined by resolution of clinical symptoms in the 6 (± 2) mo following the initiation of the GFD (responders). Ninety-one children (15%) met the criteria for NRCD, with persistent symptoms 6 mo after the diagnosis.

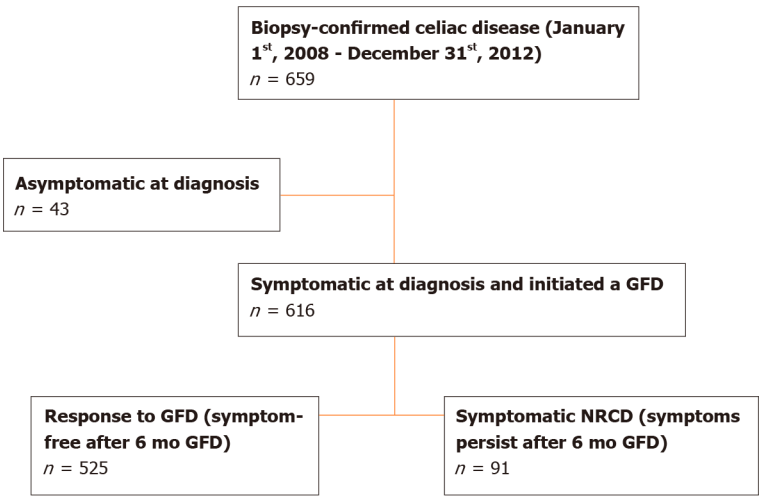

Figure 1 Patient selection flowchart. GFD: Gluten-free diet; NRCD: Non-responsive celiac disease.

Characteristics of responders (n = 525) and NRCD (n = 91) groups are shown in Table 1. Significantly more subjects with NRCD were female (77% *vs* 65%, respectively; P = 0.02). There was no difference in age at time of biopsy, and subjects of all ages were included: < 3 year (9%), 3-6 year (25%), 7-11 year (31%), and 12-17 year (35%). There was a trend towards more Hispanic patients among NRCD subjects compared to responders (5% *vs* 1%, respectively; P = 0.06). Family history of CeD (27%) was less common than family history of other autoimmune disorders (46%), but the incidence for each was similar between NRCD subjects and responders. Finally, at the time of CeD diagnosis, subjects with NRCD had lower body mass index (BMI) z-score compared to responders (median 0.00 (IQR -1.12, 0.58) *vs* 0.18 (IQR -0.48, 0.82); P = 0.02) (Table 1).

Abdominal pain was the most common presenting symptom at diagnosis and was more likely in children with NRCD compared to responders (70% *vs* 54%, P < 0.006). Constipation (hard or infrequent stools) was the second most common presenting symptom and was also more frequent with NRCD (54% *vs* 28%, P < 0.0001). Psychiatric manifestations (anxiety and restrictive eating disorder) were also more prevalent at diagnosis among children with NRCD compared to responders (Table 2).

Symptoms at the time of diagnosis that were determined by stepwise logistic regression to be independently associated with NRCD included constipation, lack of abdominal distension, and abdominal pain. The odds of NRCD were 3.1 (95% confidence interval (CI) 1.9-4.9) times higher for subjects with constipation compared to those with normal bowel movements. Likewise, absence of abdominal distension (odds ratio (OR) for abdominal distension 0.4, 95%CI 0.1-0.98) and presence of abdominal pain (OR 1.8, 95%CI 1.1-2.9) were independently associated with NRCD.

When examining serum tTG IgA antibody levels, 14 (15%) of NRCD patients had a less than 20% decrease in tTG IgA at 6 mo, 11 of whom continued to have tTG IgA levels greater than 1.5 × the upper limit of normal (ULN) at 1 year after diagnosis.

Formal evaluation and counselling by a dietitian within the first 120 d following CeD diagnosis was performed for 75 (82%) of patients with NRCD, compared to 342 (67%) of responders (P = 0.001). Additional nutrition counselling after the first 120 d occurred for 41% (n = 255) children and was more common among NRCD patients than responders (55% *vs* 39%, P < 0.006). GFD adherence was classified as excellent in 54 (66%) of symptomatic NRCD patients and poor in 12 (15%). We were unable to assess adherence to the diet in 9 patients based on medical record review. Seventeen of the 91 patients (19%) had repeat endoscopy as part of their evaluation, a median of 18.9 (IQR 9.5-25.4) months after their initial endoscopy. The majority showed normal villous architecture (82%, n = 14). Two children with persistent villous atrophy had on-going gluten exposure; a third one had follow-up biopsies only 2.8 mo after CeD diagnosis (to follow-up on coexisting eosinophilic esophagitis).

An etiology of NRCD was identified for 88/91 children. Gluten exposure was the most common cause (30%, n = 27) (Figure 2). These children had similar rates of elevated tTG IgA at 6 mo (52% *vs* 46%) and tTG IgA > 1.5 × ULN at one year (15% *vs* 11%) to the rest of the cohort.

Chronic constipation was the underlying cause of NRCD in 18 (20%) patients, and

Table 1 Characteristics of responders to the gluten-free diet and children with non-responsive celiac disease based on persistent symptoms after 6 mo on a gluten-free diet

Characteristic	Responders (*n* = 525)	NRCD (*n* = 91)	*P* value
Female sex, *n* (%)	339 (65)	70 (77)	0.02
Age at biopsy (yr), median (IQR)	9.4 (5.9, 13.4)	9.6 (5.7, 13.4)	0.73
Caucasian race[1] (%)	410/438 (92)	75/85 (91)	1.00
Hispanic[2] (%)	6/438 (1)	4/84 (5)	0.06
Medical history			
Diabetes mellitus, *n* (%)	39 (7)	3 (3)	0.18
Thyroid condition, *n* (%)	20 (4)	1 (1)	0.34
Down syndrome, *n* (%)	12 (2)	0 (0)	0.23
Family history of celiac disease, *n* (%)	144 (27)	22 (24)	0.53
Family history of other autoimmune disease[3], *n* (%)	239 (46)	43 (47)	0.73
BMI at diagnosis[4], median (IQR)	17.0 (15.6, 20.0)	16.4 (15.1, 19.3)	0.03
BMI *z*-score at diagnosis[5], median (IQR)	0.18 (-0.48, 0.82)	0.00 (-1.12, 0.58)	0.02
≥ 1 nutrition visit within first 120 d of GFD, *n* (%)	342 (65)	75 (82)	0.001
Time between diagnosis and first nutrition visit (wk), median (IQR)	4.0 (2.1, 7.0)	3.4 (2.2, 5.6)	0.36

Data unknown:
[1]*n* = 86.
[2]*n* = 84.
[4]*n* = 33.
[5]*n* = 39.
[3]Lupus, rheumatoid arthritis, type 1 diabetes, auto-immune thyroid diseases. *P* value from Fisher's exact test or Wilcoxon rank-sum test. IQR: Interquartile range; GFD: Gluten-free diet; NRCD: Non-responsive celiac disease.

was already present (*n* = 15) and treated with laxatives (*n* = 14) at the time of CeD diagnosis. Other causes for NRCD which were identified are detailed in Figure 2.

Forty-three (64%) of the 67 NRCD children with follow-up had eventual resolution of their clinical symptoms, whereas the remainder (*n* = 24, 26%) were lost to follow-up before symptoms resolved. Of the 11 patients for whom the etiology of NRCD was unclear, 4 were lost to follow-up, and 5 resolved with time. Only 2 children had persistent symptoms of undetermined etiology lasting 3 years or longer. Symptom resolution was frequent in patients with NRCD secondary to gluten exposure (*n* = 14, 67%) and constipation (*n* = 10, 71%). Those with functional abdominal pain (*n* = 6), irritable bowel syndrome (IBS) (*n* = 3), depression or anxiety (*n* = 3) fared worse. Only 1 patient in each group had resolution of symptoms at 3 years of follow-up despite treatment.

DISCUSSION

We report the incidence, etiology, and clinical characteristics of 91 children with NRCD who were diagnosed and treated at BCH between January 2008 and December 2012. The incidence of symptomatic NRCD 6 mo after initiation of a GFD was 15%. Pediatric NRCD was more prevalent in females and associated with a lower BMI *z*-score, greater abdominal pain, constipation and absence of abdominal distension at diagnosis. Etiologies of NRCD mirrored those reported in adults, with gluten exposure as the most common cause; however, there were no cases of refractory CeD.

Although definitions may vary, our results were consistent with previously published data on NRCD in adults of the same geographic region[6]. We found a lower incidence of NRCD than in a recent pediatric cohort study, in which 34% of children had at least one symptom of CeD more than 24 mo after initiating a GFD[5]. It is unclear whether the prevalence of NRCD increases with time, or if the apparently higher rate of NRCD at 24 mo reflects that the responders were lost to follow-up. Our experience

Table 2 Signs and symptoms at diagnosis of celiac disease among responders to the gluten-free diet and non-responsive celiac disease patients

	Responders (*n* = 525)	NRCD (*n* = 91)	*P* value
Abdominal pain, *n* (%)	286 (54)	64 (70)	< 0.006
Hard or infrequent stools, *n* (%)	147 (28)	49 (54)	< 0.0001
Nausea/vomiting, *n* (%)	127 (24)	26 (29)	0.43
Diarrhea, *n* (%)	133 (25)	22 (24)	0.90
Weight loss, poor weight gain, *n* (%)	126 (24)	21 (23)	0.89
Fatigue, *n* (%)	71 (14)	15 (16)	0.51
Short stature, *n* (%)	78 (15)	8 (9)	0.14
Gassiness, *n* (%)	58 (11)	9 (10)	0.86
Abdominal distention, *n* (%)	63 (12)	5 (5)	0.07
Loss of appetite, *n* (%)	53 (10)	14 (15)	0.15
Neurologic[1], *n* (%)	4 (1)	3 (3)	0.07
Psychiatric[2], *n* (%)	0 (0)	2 (2)	0.02
Arthritis, arthralgia, *n* (%)	28 (5)	9 (10)	0.10
Rash, *n* (%)	25 (5)	4 (4)	1.00
Mouth ulcers, *n* (%)	18 (3)	5 (5)	0.37
Other[3], *n* (%)	5 (1)	10 (11)	< 0.0001

[1]Neurologic symptoms include: Headache (*n* = 6), blurry vision (*n* = 1).
[2]Psychiatric symptoms include: anxiety (*n* = 1) and restrictive eating disorder (*n* = 1).
[3]Other signs and symptoms include: Fecal soiling (*n* = 2) dysphagia (*n* = 2), symptomatic anemia (*n* = 2), rectal bleeding (*n* = 3), encopresis (*n* = 2), hair loss (*n* = 2) delayed puberty (*n* = 1), easy bruising (*n* = 1). *P* value from Fisher's exact test. NRCD: Non-responsive celiac disease.

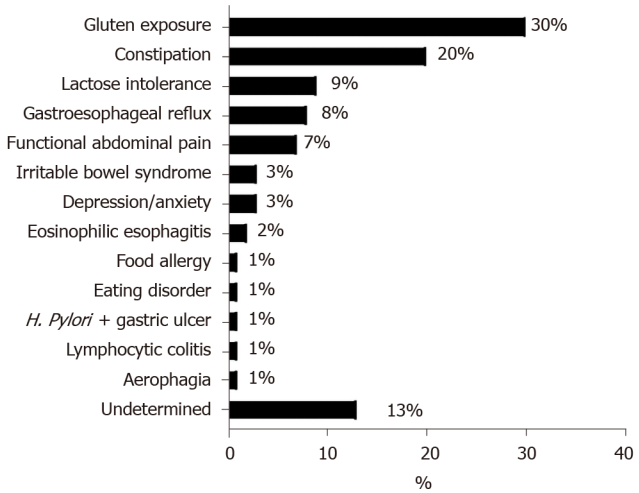

Figure 2 Reasons for non-responsive celiac disease in subjects with persistent symptoms after 6 mo on a gluten-free diet (*n* = 91). *H. Pylori*: *Helicobacter pylori*.

with a pediatric population followed at an academic center that had persisting symptoms six months after the initiation of a GFD may also differ from children who had recurrence of symptoms 12 mo or 18 mo after the diagnosis. Although resolution of symptoms may be slow after starting the GFD, our clinical conclusion is that most children are generally symptom-free after six months on a GFD. Accordingly, 85% of pediatric patients diagnosed at BCH had a good clinical response to the GFD.

Some definitions of NRCD include isolated elevation of tTG[2,6]; however, we did not include such children if they were asymptomatic because tTG is not a reliable proxy

for intestinal histology on a GFD[10]. Coexisting disorders, GFD adherence, the initial degree of tTG elevation and severity of villous atrophy all affect time to normalization of tTG which frequently takes longer than 1 year in children[11,12]. Moreover, the presence of villous atrophy is not a sine qua non for NRCD, since the formal definition is based on signs and symptoms and not histology results[2,6]. On the other hand, by only selecting children with positive serology at the time of the diagnosis of CeD, we may have missed some seronegative NRCD[13]. However, seronegative CD is rare, accounting for less than 1% of cases of pediatric biopsy-confirmed (Marsh III) CD in a recent study, so whether they have a different course than seropositive CD remains unclear[14].

As in previous adult and pediatric studies, females were more likely to be diagnosed with NRCD[5,6]. This trend has also been reported among children with functional abdominal pain, but not constipation[14,15]. Females may be more likely to experience, or report GI symptoms. We did not observe any difference in the age at the time of CeD diagnosis. Intriguingly, an association between longstanding symptoms before the diagnosis of CeD and the risk of NRCD was recently reported[5]. Due to the retrospective design of our study, we unfortunately do not have access to this information; however, we did observe lower BMI *z*-scores at CeD diagnosis among the NRCD group. Lower BMI may be a marker of disease severity, thus explaining a prolonged clinical recovery. This underscores the need to monitor CeD patients after diagnosis because malabsorption due to persistent disease and decreased food intake in relation to GI symptoms may further affect BMI and growth. Moreover, the NRCD group had significantly more symptoms at diagnosis which may reflect visceral hypersensitivity or portend a functional gastrointestinal disorder. Although these manifestations are frequently described among pediatric CeD patients[5], the rate and response to a GFD is variable[16]. Identification of a cause other than gluten exposure for NRCD in most of our patients highlights the importance of considering a coexisting disorder.

Gluten exposure was the most common cause of NRCD in our cohort, as previously reported in adults[6,8]. A strict GFD is the only current treatment for CeD. Consultation with a registered dietitian for specialized GFD education at the time of CeD diagnosis is recommended in clinical practice guidelines and standard practice at our institution[2,3]. Nonetheless, only 65% of the responder group and 82% of the NRCD group had a dietitian visit at our institution within the first 4 mo after diagnosis of CD. Due to the retrospective nature of this study, we were unable to ascertain whether some individuals received GFD education outside of our institution, or if families opted out of dietitian visits because a family member had received GFD education. Nevertheless, additional nutrition counselling after the first 120 days was also more common among NRCD patients than responders, perhaps because ongoing symptoms prompt families to seek out additional education/support. Recent American College of Gastroenterology guidelines recommend reevaluation of diet as a first step in the management of NRCD[2].

Eliminating gluten ingestion, both intentional and unintentional, is a significant challenge for many pediatric CeD patients. Even with specialized GFD education, a sizable number of patients had ongoing gluten exposure. Avoiding gluten cross-contact, which occurs during processing, preparation and serving of foods, is especially difficult. Non-adherence to a strict GFD in children and teenagers has been estimated at 8% when disclosed openly[5], but may be nearer 30% when stools are assessed for gluten immunogenic peptides[17] and may increase with GFD duration[18]. Interestingly, 82% of symptomatic NRCD patients in our study appeared to have excellent or good GFD adherence. This is likely an overestimate due to the inherent challenges associated with assessing GFD adherence through dietary recalls and interviews compared to objective measures such as fecal gluten immunogenic peptides[17].

Fourteen (67%) of the 21 symptomatic NRCD patients with suspected ongoing gluten exposure experienced resolution of their symptoms with improved GFD adherence. It is unclear whether the remaining 7 patients were unsuccessful at fully excluding dietary gluten or if healthcare providers erroneously suspected continued gluten exposure as the cause of NRCD. This cohort was prior to the development of the technology allowing the identification of gluten immunogenic peptides in urine and stools. While these technologies appear promising, currently there is no validated measure of gluten exposure other than dietitian assessment. Although the second step in the management algorithm of NRCD in adults after excluding gluten exposure or food intolerance is to proceed to duodenal biopsies to assess for villous atrophy[2], most patients in this pediatric NRCD cohort did not undergo repeat endoscopy. Moreover, some degree of villous atrophy 6 mo to 12 mo after CeD diagnosis is not uncommon

among patients in clinical remission[19].

The second most common cause of NRCD was constipation, which was also an initial manifestation of CeD for most of these patients. Constipation is a common presenting symptom among children with CeD[5,20]. 196 children included in this cohort were constipated at the time of diagnosis, but only 18 had persisting constipation that was deemed to be the cause of NRCD. The GFD is inherently low in fibers which may precipitate constipation in CeD patients[21]. Thus, some patients with constipation as a cause of NRCD may have had either initially treated constipation that recurred on the GFD after stopping laxative (n = 7), new constipation probably caused by the GFD (n = 3) or persisting constipation despite initial laxative therapy (n = 7) or noncompliance to laxative therapy (n = 1). The latter may have been suffering from functional constipation unrelated to CeD, as it is a quite common disorder in children[22]. Nevertheless, many of these patients subsequently improved, highlighting that early and intensive strategies in addition to the GFD can be effective and may avoid persistent symptoms in children.

Notably, constipation is not typically reported as an etiology of NRCD in adults, possibly because they are diagnosed with IBS. Functional abdominal pain and IBS were the cause of NRCD in 10% of our cohort. In comparison, 18% of adult NRCD patients may have IBS[6]. Other less common etiologies include lactose intolerance, present in 9% of our cohort and 7% of adults with NRCD[6]. Upper GI conditions, including gastroesophageal reflux, peptic ulcer disease, *Helicobacter pylori* infection and eosinophilic esophagitis were also responsible for persistent symptoms in pediatric NRCD. Finally, anxiety and eating disorders were responsible for NRCD and may be associated with gastrointestinal dysmotility. This underscores the need to consider the patient as a whole, and how conditions other than CeD may contribute to symptoms on a GFD.

While most children with NRCD had resolution of their symptoms over the follow-up period, symptoms persisted in 36%. Patients with constipation had higher rates of resolution than those with diagnoses such as functional abdominal pain or IBS. Functional abdominal pain is frequent among children with and without CeD, with a recent multicenter study finding a prevalence of 2.1% to 8.2%[23]. The prevalence of IBS is somewhat lower, from 1.2% to 2.9%[15,24].

Strengths and limitations

This large cohort of children with CeD provides epidemiologic data on the characteristics, causes and evolution of pediatric NRCD thereby addressing an important knowledge gap. Chart reviews conducted by either one of two authors (GV and MD) were reviewed by an expert clinical pediatric gastroenterologist; however, the retrospective nature of this study limited our ability to perform a standardized evaluation of each patient. The large number of clinicians at our institution contributed to variability in documentation and differences in approach to medical evaluation. Also, many etiologies of NCRD are diagnosed based primarily on clinical history. Although inclusion of only children who were diagnosed at BCH reduces referral bias, our cohort may differ from populations in other geographic areas.

CONCLUSION

In summary, NRCD after six months of GFD is frequent among children, especially females. Initial presentation with constipation, abdominal pain and absence of abdominal distension is associated with subsequent diagnosis of NRCD. Although gluten exposure was the most frequent cause, a wide variety of diagnoses are also found among NRCD patients. Our study highlights the importance of performing a diligent search for the above etiologies for NRCD in any celiac child with persistent clinical symptoms despite being on GFD and reinforces the need for close follow up in the first year of a CeD diagnosis.

ARTICLE HIGHLIGHTS

Research background

Non-responsive celiac disease (NRCD) is defined as the persistence of symptoms in individuals with celiac disease (CeD) despite being on a gluten-free diet (GFD). There is scant literature about NRCD in the pediatric population.

Research motivation

Addressing an important knowledge gap, this study examines a large cohort of children with CeD providing data on the characteristics, causes and evolution of pediatric NRCD. By characterizing this sub-population of individuals with CeD, we are better equipped to provide clinical guidance and follow-up in those with persistent symptoms.

Research objectives

Through this retrospective cohort study, we sought to determine the incidence, clinical characteristics, and underlying causes of NRCD in children. Additionally, symptom evolution was detailed and compared to identify any potential predictors for NRCD.

Research methods

Retrospective cohort study performed at Boston Children's Hospital (BCH). Children < 18 years diagnosed with CeD by positive serology and duodenal biopsies compatible with Marsh III histology between 2008 and 2012 were identified in the BCH's Celiac Disease Program database. Medical records were longitudinally reviewed from the time of diagnosis through September 2015. NRCD was defined as persistent symptoms at 6 mo after the initiation of a GFD, and causes of NRCD as well as symptom evolution were detailed and compared to identify any potential predictors for NRCD.

Research results

Six hundred and sixteen children were included in this retrospective study, of which 91 (15%) met criteria for NRCD, and of this, most were female (77%). Abdominal pain [odds ratio (OR) 1.8 95% confidence interval (CI) 1.1-2.9], constipation (OR 3.1 95%CI 1.9-4.9) and absence of abdominal distension (OR for abdominal distension 0.4 95%CI 0.1-0.98) at diagnosis were associated with NRCD. NRCD was attributed to a wide variety of diagnoses with gluten exposure (30%) and constipation (20%) being the most common causes. 64% of children with NRCD improved on follow-up.

Research conclusions

NRCD after ≥ 6 mo of GFD is frequent among children, especially females, and is associated with initial presenting symptoms of constipation and/or abdominal pain. Gluten exposure is the most frequent cause. Our study highlights the importance of performing a diligent search for the etiologies for NRCD in any celiac child with persistent clinical symptoms despite being on GFD and reinforces the need for close follow up in the first year of a CeD diagnosis.

Research perspectives

Although the use of a large pediatric cohort positively contributes to the breadth of knowledge surrounding NRCD, and inclusion of only children who were diagnosed at BCH reduces referral bias, our cohort may differ from populations in other geographic areas. As such, a future direction of note is to extend this project to include pediatric Celiac Disease Programs across the United States, to assess if geographic location is a factor in the manifestation and characterization of NRCD.

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ORIGINAL ARTICLE

Retrospective Study

Endoscopic diagnosis for colorectal sessile serrated lesions

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Author contributions: Nishizawa T analyzed the data, and wrote the manuscript; Yoshida S performed colonoscopy and collected the data; Toyoshima A contributed to the data interpretation; Yamada T reviewed endoscopic images; Sakaguchi Y, Irako T, and Ebinuma H critically revised the manuscript; Kanai T and Koike K supervised the study; Toyoshima O designed the study, and analyzed the data.

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Abstract

BACKGROUND

Hyperplastic polyps are considered non-neoplastic, whereas sessile serrated lesions (SSLs) are precursors of cancer *via* the ''serrated neoplastic pathway''. The clinical features of SSLs are tumor size (> 5 mm), location in the proximal colon, coverage with abundant mucus called the ''mucus cap'', indistinct borders, and a cloud-like surface. The features in magnifying narrow-band imaging are varicose microvascular vessels and expanded crypt openings. However, accurate diagnosis is often difficult.

AIM

To develop a diagnostic score system for SSLs.

METHODS

We retrospectively reviewed consecutive patients who underwent endoscopic resection during colonoscopy at the Toyoshima endoscopy clinic. We collected

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data on serrated polyps diagnosed by endoscopic or pathological examination. The significant factors for the diagnosis of SSLs were assessed using logistic regression analysis. Each item that was significant in multivariate analysis was assigned 1 point, with the sum of these points defined as the endoscopic SSL diagnosis score. The optimal cut-off value of the endoscopic SSL diagnosis score was determined by receiver-operating characteristic curve analysis.

RESULTS
Among 1288 polyps that were endoscopically removed, we analyzed 232 diagnosed as serrated polyps by endoscopic or pathological examination. In the univariate analysis, the location (proximal colon), size (> 5 mm), mucus cap, indistinct borders, cloud-like surface, and varicose microvascular vessels were significantly associated with the diagnosis of SSLs. In the multivariate analysis, size (> 5 mm; $P = 0.033$), mucus cap ($P = 0.005$), and indistinct borders ($P = 0.033$) were independently associated with the diagnosis of SSLs. Size > 5 mm, mucus cap, and indistinct borders were assigned 1 point each and the sum of these points was defined as the endoscopic SSL diagnosis score. The receiver-operating characteristic curve analysis showed an optimal cut-off score of 3, which predicted pathological SSLs with 75% sensitivity, 80% specificity, and 78.4% accuracy. The pathological SSL rate for an endoscopic SSL diagnosis score of 3 was significantly higher than that for an endoscopic SSL diagnosis score of 0, 1, or 2 ($P < 0.001$).

CONCLUSION
Size > 5 mm, mucus cap, and indistinct borders were significant endoscopic features for the diagnosis of SSLs. Serrated polyps with these three features should be removed during colonoscopy.

Key Words: Sessile serrated lesion; Mucus cap; Indistinct borders; Hyperplastic polyp; Endoscopic features; Size

Core Tip: The features of sessile serrated lesions (SSLs) include tumor size > 5 mm, location in the proximal colon, ''mucus cap'', indistinct borders, cloud-like surface, and varicose microvascular vessels. Our multivariate analysis revealed that size > 5 mm, mucus cap, and indistinct borders were independent predictors for SSLs. The combination of these three features in serrated polyps allowed the diagnosis of SSLs with 75% sensitivity, 80% specificity, and 78.4% accuracy.

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INTRODUCTION

Recent molecular biology studies have supported the classification of colorectal serrated lesions into hyperplastic polyps (HPs), sessile serrated lesions (SSLs), and traditional serrated adenomas (TSAs)[1]. HPs are considered non-neoplastic, whereas SSLs and TSAs are both precursors of cancer *via* the "serrated neoplastic pathway", which is responsible for up to 20% of all sporadic colorectal cancers[2]. The features of serrated neoplastic pathways include high microsatellite instability and the potential for rapid conversion to malignancy[3,4]. This implies that all SSLs should be accurately diagnosed and endoscopically removed[5], whereas HPs do not need to be removed. Therefore, it is essential to differentiate SSLs from HPs.

SSLs typically occur in the proximal colon[6]. Endoscopically, SSLs usually measure > 5 mm, are frequently covered by mucus called ''mucus cap'', and have indistinct borders and a cloud-like surface[7]. The features of SSLs under magnifying narrow-

band imaging (NBI) are varicose microvascular vessels and expanded crypt openings[8]. The feature of SSLs on chromoendoscopy is a type II open-shape pit pattern. In contrast, HPs commonly occur in the distal colon or rectum and are often ≤ 5 mm in size. However, differential diagnoses are often difficult[9]. We hypothesized that well-designed combination of endoscopic features might be useful for the diagnosis of SSLs. Therefore, the present study aimed to develop a diagnostic score system for SSLs.

MATERIALS AND METHODS

Ethics

This retrospective study was approved by the Ethical Review Committee of the Hattori Clinic on September 4, 2020 (approval No. S2009-U04). Written informed consent was obtained from all participants. All clinical investigations were conducted according to the ethical guidelines of the Declaration of Helsinki.

Patients

We retrospectively reviewed consecutive patients who underwent polypectomy or endoscopic mucosal resection during colonoscopy between July and September 2019 and between January and March 2020 at the Toyoshima Endoscopy Clinic. Colonoscopy was performed to evaluate symptoms such as hematochezia, rectal bleeding, abdominal symptoms, abnormal bowel habits, anemia, or positive fecal immunochemical test findings or for colorectal polyp surveillance. We used an electronic endoscopy reporting system (T-File System; STS Medic, Japan) that was integrated into the clinic's patient record systems.

Endoscopic procedures

The colonoscopies were performed using an Elite CF290 endoscopy system (CV-290 and CLV-290, Olympus, Japan) with a 290 series colonoscope (CF-HQ290Z, CF-HQ290, or PCF-H290Z, Olympus, Japan) or a 260 series colonoscope (PCF-PQ260)[10]. The patients underwent colonoscopies while under conscious sedation with midazolam and/or pethidine hydrochloride. Pancolonic chromoendoscopy was performed by spraying 0.05% indigo carmine[10,11]. All polyps detected by white light imaging during colonoscopy were washed with water, and also assessed by NBI with and without magnification. The colonoscopies were performed by three expert endoscopists (Nishizawa T, Sakaguchi Y, and Toyoshima O).

Endoscopic diagnosis was performed based on the Workgroup on Serrated Polyps and Polyposis classification[12]. We removed lesions diagnosed as adenomas or clinically significant serrated polyps. A clinically significant serrated polyps was defined as any SSL, TSA, or HP measuring ≥ 1 cm anywhere in the colon or HP measuring ≥ 5 mm located proximal to the sigmoid colon[13-15]. We included polyps measuring 15 mm or more in diameter.

The location, size, morphology, and endoscopic diagnosis for each detected polyp were recorded. The location of the polyps was specified as the cecum; ascending, transverse, descending, or sigmoid colon; or rectum. The distal side from the splenic flexure was defined as the distal colon, while the proximal side to the splenic flexure was defined as the proximal colon. The polyp size was estimated by comparison with a closed snare or forceps. The morphology and presence of a mucus cap, indistinct borders, cloud-like surface, varicose microvascular vessels, and expanded crypt opening were also recorded. The mucus cap was defined as coverage with abundant mucus (Figure 1A). Indistinct borders were defined as vague demarcations of the lesion border (Figure 1B). A cloud-like surface appeared granular, nodular, or bumpy and like the surface of a cumulus cloud. Varicose microvascular vessels were defined as vessels thicker than meshed capillary vessels that meandered similar to varicose veins. Expanded crypt opening was defined as the heterogeneous expansion of nearby crypts.

The pathological findings were evaluated by hematoxylin and eosin staining and histological diagnoses were made by an expert gastrointestinal pathologist (H. W.). Serrated lesions were classified as HP, SSL, and TSA according to the World Health Organization classification[16,17]. We collected data on polyps with endoscopic or pathological diagnosis of serrated polyps.

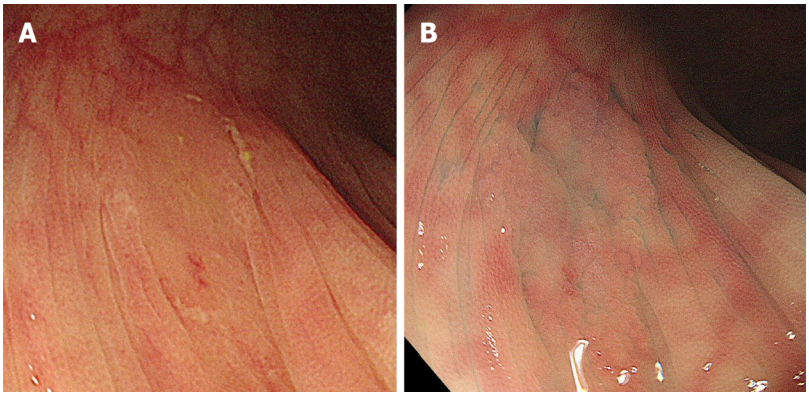

Figure 1 Endoscopic findings regarding sessile serrated lesions. A: "Mucus cap" was defined as coverage with abundant mucus; and B: Indistinct borders were defined as vague demarcations of the lesion border.

Statistical analysis

The significant factors for the diagnosis of SSLs were assessed using logistic regression analysis. The univariate and multivariate analyses included the location (proximal colon) and size (> 5 mm) and presence of mucus cap, indistinct borders, cloud-like surface, varicose microvascular vessels, and expanded crypt opening. Each item that was significant in the multivariate analysis was assigned 1 point and the sum of these points was defined as the endoscopic SSL diagnosis score. The optimal cut-off endoscopic SSL diagnosis score was determined by receiver-operating characteristic (ROC) curve analysis. The calculations were performed using StatMate software, version IV (ATOMS, Tokyo, Japan) and Ekuseru-Toukei 2015 (Social Survey Research Information company, Limited, Tokyo, Japan).

RESULTS

A total of 629 patients underwent colonoscopies with polypectomy or endoscopic mucosal resection. Among 1288 polyps removed from these patients, we identified 232 polyps as endoscopically or pathologically diagnosed serrated polyps. A study flowchart of the included polyps was presented in Figure 2.

The characteristics of the polyps are shown in Table 1. The pathological diagnoses included 72 SSLs, 130 HPs, 1 TSA, 7 adenomas, 20 normal mucosa, and 2 others.

Table 2 shows the results of the univariate and multivariate analyses for the diagnosis of SSL. In the univariate analyses, the location (proximal colon), size > 5 mm, mucus cap, indistinct borders, cloud-like surface, and varicose microvascular vessels were significantly associated with the diagnosis of SSL. On the other hand, expanded crypts opening was not significant in the univariate analysis. In the multivariate analysis, size > 5 mm (P = 0.033), mucus cap (P = 0.005), and indistinct borders (P = 0.033) were independently associated with the diagnosis of SSL. Size > 5 mm, mucus cap, and indistinct borders were each assigned 1 point and the sum of the points was defined as the endoscopic SSL diagnosis score. The minimum and maximum endoscopic SSL diagnosis scores were 0 and 3, respectively. In the ROC curve analysis, the area under the curve was 0.806 and the optimal endoscopic SSL diagnosis score cut-off was 3 (Figure 3A), which corresponded to 75% sensitivity, 80% specificity, and 78.4% accuracy for the prediction of pathological SSL. The ROC curves of the three predictors (size, mucus cap, and indistinct borders) were presented in Figure 3B-D, respectively.

Figure 4 shows the pathological SSL rates for each endoscopic SSL diagnosis score. The pathological SSL rate for an endoscopic SSL diagnosis score of 3 was significantly higher than that for an endoscopic SSL diagnosis score of 0, 1, or 2 (P < 0.001).

DISCUSSION

We found that size > 5 mm, mucus cap, and indistinct borders were independent predictors for SSLs. The combination of these three features in serrated polyps allowed the diagnosis of SSLs with 75% sensitivity, 80% specificity, and 78.4% accuracy.

Table 1 Polyp characteristics

Polyp characteristics	
Size	
Diminutive (≤ 5 mm)	114
Small (6-9 mm)	82
Large (≥ 10 mm)	36
Shape (Paris classification)	
Is	4
IIa	226
IIb	1
IIc	1
Location	
Cecum	33
Ascending colon	61
Transvers colon	71
Descending colon	12
Sigmoid colon	42
Rectum	13
Pathology	
Sessile serrated lesion	72
Hyperplastic polyp	130
Traditional serrated adenoma	1
Adenoma	7
Normal mucosa	20
Others	2

Table 2 Univariate and multivariate analyses on the diagnosis of sessile serrated lesion

Variables	Univariate analysis			Multivariate analysis		
	Partial regression coefficient	95% confidence interval	*P* value	Partial regression coefficient	95% confidence interval	*P* value
Location (proximal colon)	1.094	0.374-1.813	0.003	0.197	-0.685-1.078	0.662
Size (> 5mm)	2.050	1.369-2.731	< 0.001	0.904	0.074-1.733	0.033
Mucus cap	2.548	1.657-3.440	< 0.001	1.520	0.448-2.592	0.005
Indistinct borders	2.067	1.371-2.764	< 0.001	0.926	0.075-1.778	0.033
Varicose microvascular vessel	0.668	0.088-1.248	0.024	-0.133	-0.869-0.602	0.723
Cloud-like surface	1.640	1.015-2.264	< 0.001	0.708	-0.022-1.437	0.057
Expanded crypts opening	0.367	-0.202-0.936	0.206	-0.497	-1.198-0.203	0.164

Various studies have sought to clarify the features of SSLs. Hasegawa *et al*[18] investigated 107 SSLs to discriminate them from other serrated lesions, reporting that the mean lesion size of SSLs was larger than that of HP (14.2 *vs* 6.2 mm; $P < 0.01$). Moreover, SSL was preferentially located in the proximal rather than the distal colon (81.8% *vs* 18.2%), whereas HP was located less often in the proximal colon than in the distal colon.

Hazewinkel *et al*[19] investigated 150 polyps, including 50 SSLs, 50 HPs, and 50

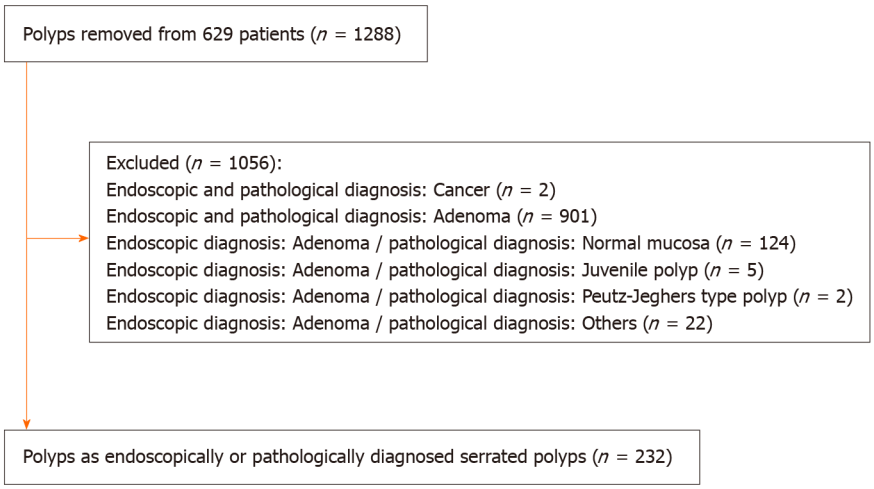

Figure 2 Flowchart of polyp enrollment.

adenomas. Their multivariate analysis demonstrated that indistinct borders [odds ratio (OR): 3.11, P = 0.001] and cloud-like surface (OR: 2.65, P = 0.015) were independent diagnostic factors for SSLs on white-light imaging.

Uraoka *et al*[1] investigated 89 Lesions, including 38 SSLs and 41 HPs. Their multivariate analysis showed that varicose microvascular vessels (OR: 8.2, P = 0.001), size ≥ 10 mm (OR: 7.2, P = 0.002), and proximal location (OR: 6.1, P = 0.004) were independent diagnostic factors for SSLs.

Pereyra *et al*[20] investigated 440 polyps, including 34 SSLs, 135 HPs, and 249 adenomas. Their multivariate analysis showed that flat morphology (OR: 3.81, P = 0.002), red-colored surface (OR: 12.97, P <0.001), right-sided location (OR: 22.21, P <0.001), and mucus cap (OR: 8.77, P < 0.001) were independent diagnostic factors for SSLs.

Murakami *et al*[7] summarized the features of SSLs in their review report, indicating that SSLs were > 5 mm, frequently covered by a mucus cap, and more commonly located in the proximal colon. The features also included small dark spots and varicose microvascular vessels on magnifying NBI and type II open pit patterns on magnifying chromoendoscopy. The small dark spots are nearly synonymous with expanded crypt openings and these are considered to correspond to type II open pit patterns. This phenomenon is likely due to mucin overproduction, which could also lead to the mucus cap[19].

The results of our univariate analysis showed that proximal colon, size > 5 mm, mucus cap, indistinct borders, cloud-like surface, and varicose microvascular vessels were significant factors for the diagnosis of SSLs. Multivariate analysis revealed that size > 5 mm, mucus cap, and indistinct borders were independent predictors for SSLs in our clinical setting.

A combination of endoscopic features has also been previously proposed for the diagnosis of SSL. For instance, Yamada *et al*[21] evaluated the combination of three endoscopic features for SSLs. The combination of dilated and branching vessels (synonymous with varicose microvascular vessels), proximal location, and size (≥ 10 mm) resulted in an area under the curve of 0.7832. The best cut-off point was 2, which corresponded to 79% sensitivity and 81% specificity.

The endoscopic SSL diagnosis score consists of size (> 5 mm), mucus cap, and indistinct borders. The area under the curve in this study was 0.806. The optimal cut-off point was 3, which resulted in 75% sensitivity and 80% specificity. Our score is simple and does not require magnifying NBI. Thus, it may be useful in busy clinical settings or examinations with a scope without a magnifying function.

The present study has several limitations. First, this retrospective study was conducted at a single institution; however, the recording of medical data was well controlled. Second, our study did not perform magnifying chromoendoscopy. A follow-up study is needed to verify the validity of the endoscopic SSL diagnosis score.

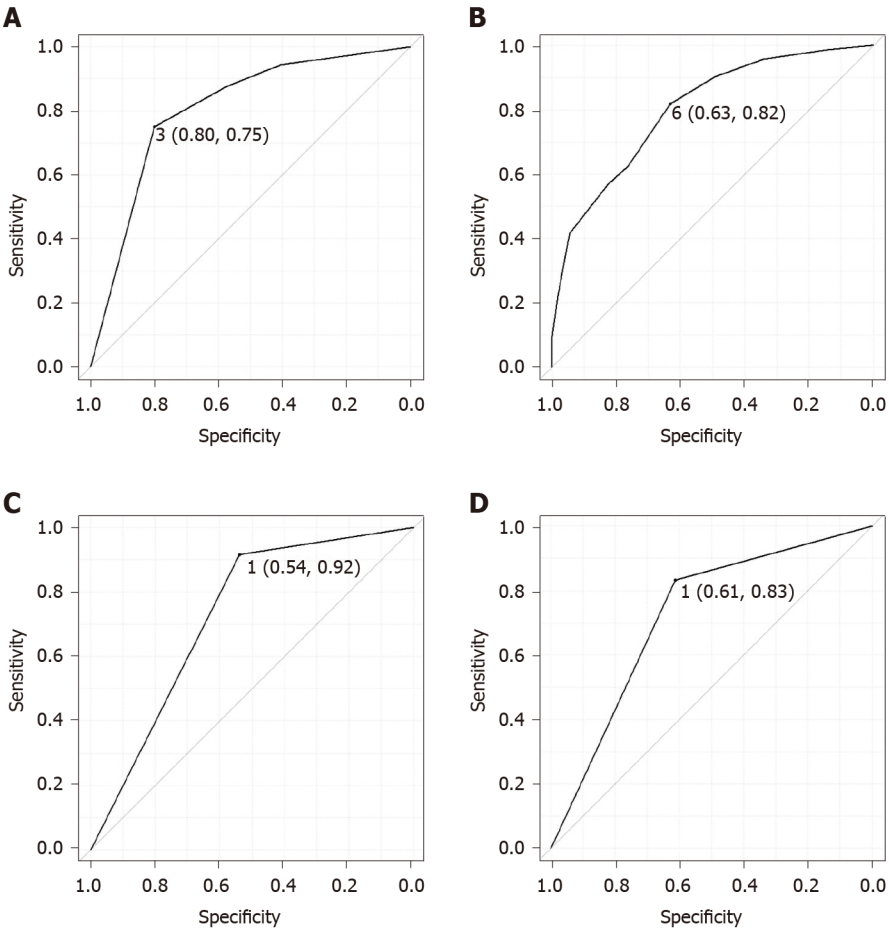

Figure 3 Receiver-operating characteristic curve for predicting sessile serrated lesions. A: Receiver-operating characteristic (ROC) curve for predicting sessile serrated lesion (SSL) based on the endoscopic SSL diagnosis score: The area under the curve (AUC) was 0.806. The optimal cutoff value was 3, for which the endoscopic SSL diagnosis score predicted pathological SSLs with 75% sensitivity and 80% specificity; B: ROC curve for predicting SSL based on polyp size: AUC was 0.801. Size ≥ 6 mm predicted pathological SSLs with 82% sensitivity, and 63% specificity; C: ROC curve for predicting SSL based on mucus cap: AUC was 0.727. The presence of mucus cap predicted pathological SSLs with 92% sensitivity, and 54% specificity; and D: ROC curve for predicting SSL based on indistinct borders: AUC was 0.723. The presence of indistinct borders predicted pathological SSLs with 83% sensitivity, and 61% specificity.

CONCLUSION

In conclusion, size (> 5 mm), mucus cap, and indistinct borders were significant endoscopic features for the diagnosis of SSLs. Serrated polyps with these three features should be removed during colonoscopy.

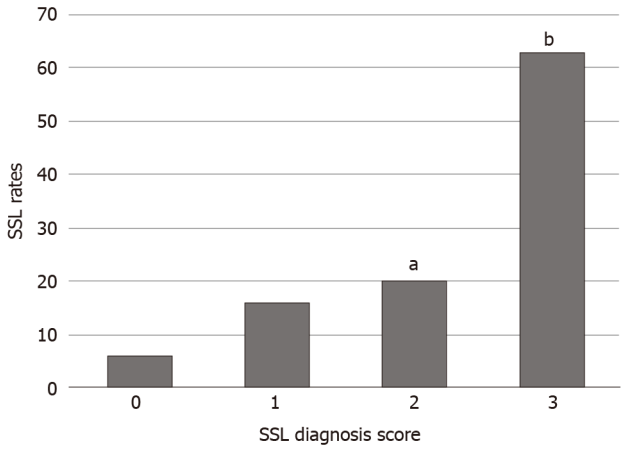

Figure 4 Sessile serrated lesion diagnosis rates based on the endoscopic sessile serrated lesion diagnosis score. [a]*P* < 0.05 compared to an endoscopic Sessile serrated lesion diagnosis score of 0; [b]*P* < 0.001 compared to an endoscopic sessile serrated lesion diagnosis score of 0, 1, or 2. SSL: Sessile serrated lesion.

ARTICLE HIGHLIGHTS

Research background

The serrated neoplastic pathway is responsible for up to 20% of all sporadic colorectal cancers. Sessile serrated lesions (SSLs) should be accurately diagnosed and endoscopically removed.

Research motivation

Various findings have been proposed as features of SSLs. However, accurate diagnosis is often difficult.

Research objectives

This study developed a scoring system to predict the diagnosis of SSLs.

Research methods

We retrospectively reviewed patients who underwent endoscopic resection at the Toyoshima Endoscopy Clinic. We collected data on 232 polyps that were endoscopically or pathologically diagnosed as serrated polyps. The significant factors for the diagnosis of SSLs were assessed using logistic regression analysis.

Research results

In the multivariate analyses, size (> 5 mm; $P = 0.033$), mucus cap ($P = 0.005$), and indistinct borders ($P = 0.033$) were independently associated with a diagnosis of SSL. The endoscopic SSL diagnosis score consisted of three features. An endoscopic SSL diagnosis score of 3 predicted pathological SSLs with 75% sensitivity, 80% specificity, and 78.4% accuracy.

Research conclusions

Size (> 5 mm), mucus cap, and indistinct borders were significant endoscopic features for the diagnosis of SSL. Serrated polyps with these three features should be removed during colonoscopy.

Research perspectives

A follow-up study is needed to verify the validity of the endoscopic SSL diagnosis score.

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ORIGINAL ARTICLE

Retrospective Study

Factors influencing the short-term and long-term survival of hepatocellular carcinoma patients with portal vein tumor thrombosis who underwent chemoembolization

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Abstract

BACKGROUND

The factors affecting the short-term and long-term prognosis of hepatocellular carcinoma (HCC) patients with portal vein tumor thrombosis (PVTT) receiving transarterial chemoembolization (TACE) are still unclear.

AIM

To clarify the predictors correlated with the short-term and long-term survival of HCC patients with PVTT who underwent TACE.

METHODS

The medical records of 181 HCC patients with PVTT who underwent TACE at the Second Affiliated Hospital of Chongqing Medical University from January 2015 to July 2019 were retrospectively analyzed. We explored the short-term and long-term prognostic factors by comparing the preoperative indicators of patients who died and survived within 3 mo and 12 mo after TACE. Multivariate analyses were conducted using logistic regression. The area under the receiver operating characteristic curve (area under curve) was used to evaluate the predictive ability of the factors related to the short-term and long-term prognosis.

RESULTS

The median survival time was 4.8 mo (range: 2.5-8.85 mo). The 3 mo, 6 mo, and 12 mo survival rates were 68.5%, 38.7%, and 15.5%, respectively. In multivariable analysis, total bilirubin, sex, and aspartate aminotransferase (AST) were closely linked to short-term survival. When AST ≥ 87 U/L and total bilirubin ≥ 16.15 μmol/L, the 3-mo survival rate after TACE was reduced significantly ($P < 0.05$). AST had the best predictive ability, followed by total bilirubin, while sex had the worst predictive ability for short-term survival area under curve: 0.763 (AST) *vs*

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0.707 (total bilirubin) *vs* 0.554 (sex)]. The long-term survival outcome was significantly better in patients with a single lesion than in those with ≥ three lesions (P = 0.009). Patients with massive block HCC had a worse long-term survival than patients with nodular and diffuse HCC (P = 0.001).

CONCLUSION
AST, total bilirubin, and sex are independent factors associated with short-term survival. The number of tumors and the gross pathological type of tumor are related to the long-term outcome.

Key Words: Transarterial chemoembolization; Hepatocellular carcinoma; Portal vein tumor thrombosis; Survival; Prognostic factors

Core Tip: It is unclear which factors affect the short-term and long-term prognosis of hepatocellular carcinoma patients with portal vein tumor thrombosis receiving transarterial chemoembolization. In our research, we clarified the predictors correlated with the short-term and long-term survival of hepatocellular carcinoma patients with portal vein tumor thrombosis who underwent transarterial chemoembolization by analyzing preoperative indicators. Results showed that aspartate aminotransferase, sex, and total bilirubin were independent factors associated with short-term survival. The number of lesions and the gross pathological type of tumor were related to the long-term outcome.

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INTRODUCTION

Primary liver cancer is currently one of the most common malignant tumors, and it is among the top five causes of death and morbidity from malignant tumors around the world[1]. Hepatocellular carcinoma (HCC) is the most common pathological type, accounting for approximately 90% of all primary liver cancers. Because of its biological and anatomical characteristics, HCC easily invades the portal vein during the progression of liver cancer and forms portal vein tumor thrombosis (PVTT). Some reports have proposed that the incidence of HCC with PVTT is approximately 44%-62.2%[2]. HCC accompanied by PVTT is closely associated with a poor prognosis and is likely to lead to intrahepatic metastasis, liver function damage, portal hypertension, upper gastrointestinal bleeding, and other complications. The median overall survival time of HCC patients with PVTT who receive no treatment is only 2.7 mo[3].

There is still controversy about the treatment of HCC with PVTT in Eastern and Western countries. Previous studies have suggested that HCC with PVTT should be classified as stage C of Barcelona Clinic Liver Cancer and is no longer suitable for surgical treatment[4]. Some researchers have mentioned that compared with conservative treatment, TACE is a safe and effective therapy for selected HCC patients with PVTT[5-7]. However, some patients die in the short term after TACE, and patients who have expected postoperative survival times of less than 3 mo may not be suitable for TACE.

At present, the factors affecting the short-term (3 mo) and long-term (12 mo) prognosis of HCC patients with PVTT treated with TACE are still unclear. We aimed to identify the preoperative factors related to the short-term and long-term prognosis by comparing the preoperative clinical data of patients who died in the short-term (< 3 mo) with those who survived into the long-term (> 12 mo) after TACE. The area under the curve (AUC) was utilized to assess the predictability of the factors related to short-term and long-term survival to provide some help for doctors when screening HCC

patients with PVTT to identify those who can benefit from TACE.

MATERIALS AND METHODS

Patient

We enrolled a total of 181 HCC patients with PVTT who received TACE in the Second Affiliated Hospital of Chongqing Medical University from January 2015 to July 2019. The inclusion criteria were as follows: (1) Histopathology confirmed as HCC or clinically diagnosed as HCC; (2) Abdominal color Doppler ultrasound, digital subtraction angiography, contrast-enhanced computerized tomography, or magnetic resonance imaging showed signs of PVTT; (3) No treatment, such as surgery, radiotherapy, chemotherapy, liver transplantation, targeted, or biological therapy, was administered before and after TACE; (4) Age ≥ 18 years; and (5) Complete clinical data were available. Patients with other malignancies or severe heart and lung diseases were eliminated. The follow-up endpoint was July 2020 or the date of death, whichever came first. The follow-up was conducted by telephoning or outpatient visits. During our follow-up, 14 patients received other treatments, such as targeted therapy and High Intensity Focused Ultrasound, and one patient was diagnosed with and treated for a hematologic malignancy. Twenty-six patients were lost to follow-up. The above patients were not included in the study. The study complied with the ethical guidelines of the Declaration of Helsinki in 1964 and passed the review of the Review Committee of the Second Affiliated Hospital of Chongqing Medical University.

Data collection

We collected the basic data of the patients, including sex, age, cause of hepatitis, ascites, hepatoportal arteriovenous fistula, liver cirrhosis, cavernous transformation of the portal vein, Child-Pugh grade, the model for end-stage liver disease score, the Eastern Cooperative Oncology Group score, and albumin-bilirubin grade (ALBI grade). The biochemical parameters included the following indicators: Prealbumin, serum albumin (ALB), total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha-fetoprotein, activated partial thromboplastin time, prothrombin time, international normalized ratio, hemoglobin, platelet count, white blood cell count, serum cholinesterase, and gamma-glutamyl transpeptidase. The characteristics of the tumors included the number and size of tumors, the type of PVTT, gross pathological type, invasion of the left and right liver lobes and inferior vena cava tumor thrombus. Cheng's classification of PVTT was applied[8]. The formula for calculating the ALBI score was as follows: ALBI = log10 bilirubin × 0.66 + albumin × (-0.085)[9]. Gross pathological types of liver cancer included the massive block type, the nodular type, and the diffuse type. The clinical diagnostic criteria for patients with HCC are in line with the European Society for Medical Oncology Clinical Practice Guidelines[10].

TACE procedure

The puncture of the artery used Seldinger technology. The right femoral artery was often selected. The catheter was delivered to the celiac trunk and the common hepatic artery, and arteriography was performed to identify the tumor's nutrient artery. The catheter was sent into the tumor-feeding artery and injected with chemotherapy drugs. Chemoembolization drugs commonly included lipiodol, polyvinyl alcohol particles, cisplatin, bleomycin, gelatin sponge particles, *etc*.

Statistical analysis

The mean ± SD or median were used to express the continuous variables. Categorical variables are presented as *n* (%). Two groups of data were compared using independent-samples *t* tests, Mann-Whitney *U* tests, χ^2 tests or Fisher's exact tests. The statistically significant indicators in the univariate analysis ($P < 0.05$) were selected in multivariate analysis in the study. The independent predictors for survival were determined by logistic regression analysis. The predictive ability of the independent predictors for short-term survival was assessed by the AUC. The cutoff value was calculated by the receiver operating characteristic (ROC) curves. The Kaplan-Meier method was used to plot the survival curves. SPSS 25.0 software (Armonk, NY, United States) was used to perform all statistical analyses. When $P < 0.05$, the statistical results were considered significant.

RESULTS

Patient basic characteristics

A total of 181 HCC patients with PVTT who underwent TACE were enrolled in our study. Their baseline data are shown in Table 1. The average age of the patients was 52.16 ± 9.73 years. The 3 mo, 6 mo, and 12 mo survival rates were 68.5%, 38.7%, and 15.5%, respectively. In our study, 159 patients (87.8%) were men and 22 patients (12.2%) were women, hepatitis B patients accounted for 90.1%, and 133 (73.5%) patients had a background of cirrhosis. According to the type of PVTT, 29 (16%) patients, 77 (42.5%) patients, 68 (37.6%) patients, and 7 (3.9%) patients were classified as having type I, II, III and IV PVTT, respectively. For the Child-Pugh grade, 123 (68%) had grade A, 55 (30.4%) had grade B, and 3 (1.6%) had grade C. Patients with greater than or equal to 3 lesions accounted for 48.1%. The median survival time was 4.8 mo.

Short-term survival outcome

Of the 181 patients, 56 patients died within 3 mo after interventional therapy, and 125 patients survived. The clinical data of patients who died and survived within 3 mo after treatment were compared. The results of the univariate analysis are shown in Table 2. Multivariate analysis showed that total bilirubin [odds ratio (OR): 1.027, 95% confidence interval (CI): 1-1.054 P = 0.046), sex (OR: 2.832, 95%CI: 1.025-7.828, P = 0.045] and AST (OR: 1.014, 95%CI: 1.006-1.021, P < 0.01) were significant independent predictors of short-term survival (Table 3).

The AUC was used to evaluate the predictive ability of these indicators for short-term survival. AST (AUC: 0.763, 95%CI: 0.686-0.841) had the best predictive ability, followed by total bilirubin (AUC: 0.707, 95%CI: 0.627-0.788), while sex (AUC: 0.554, 95%CI: 0.461-0.648) had the worst predictive ability (Figure 1).

The cutoff values of the above indicators were calculated by using ROC curves, and the 3-mo cumulative survival rates of patients with AST < 87 U/L and AST ≥ 87 U/L were 84.6% and 40.6% (Figure 2A), respectively. The difference was significant (P < 0.001). The 3-mo cumulative survival rates of the patients with total bilirubin < 16.15 μmol/L and bilirubin ≥ 16.15 μmol/L were 86.9% and 53.6% (Figure 2B), respectively, and the difference was statistically significant (P < 0.001). The 3-mo cumulative survival rates of men and women were 71.1% and 50% (Figure 2C), respectively. The discrepancy was statistically significant (P = 0.019).

Long-term survival outcome

Of the 181 patients, 150 died within 12 mo after interventional therapy, and 31 survived. Univariate analysis showed that the number of tumors, invasion of the left and right liver lobes, pathological type, platelet count, and gamma-glutamyl transpeptidase were correlated with the long-term survival of patients (Table 4), and logistic regression analysis showed that the number of lesions and pathological type were independent predictors of long-term survival (Table 3). The long-term survival outcome was significantly better in patients with a single lesion than in those with ≥ three lesions (OR: 5.809, 95%CI: 1.563-21.594, P = 0.009). Patients with massive block HCC had a worse long-term survival than patients with nodular and diffuse HCC (OR: 0.197, 95%CI: 0.075-0.521, P = 0.001).

We used ROC curves to evaluate the predictability of the number of tumors and pathology for long-term survival. Number of tumors (AUC: 0.665, 95%CI: 0.568-0.763) had the best predictive ability, followed by gross pathology (AUC: 0.620, 95%CI: 0.510-0.730) (Figure 3).

DISCUSSION

In this paper, we found that sex, AST, and total bilirubin were independent influential factors for short-term survival. The incidence and mortality of HCC in men were higher than in women[11]. This was different from the conclusion in our article. The main reason was the imbalance of gender ratio in the article. The AUC of sex was not high (AUC: 0.554, 95%CI: 0.461-0.648, P > 0.05), which showed that although sex was an independent factor influencing short-term prognosis, its predictability was not well.

Total bilirubin was a biochemical indicator of liver metabolic function. Carr *et al*[12] believed that high levels of serum bilirubin can increase the risk of death in HCC patients with PVTT. In our study, it was also found that elevated bilirubin levels were associated with poor short-term survival in HCC patients with PVTT who underwent TACE. According to the AUC of bilirubin, we thought that this indicator had good

Table 1 Characteristic of the 181 patients

Parameters	Patients, *n* = 181
Age in yr	52.16 ± 9.73
Sex, men/women	159/22
Cause of liver disease, hepatitis B/C/B and C/others	163/3/1/14
Tumor size, < 5 cm/≥ 5 cm, < 10 cm/≥ 10 cm	39/86/56
Number of tumors, 1/2/≥ 3	82/12/87
Liver cirrhosis, no/yes	48/133
Ascites, no/small/moderate-massive	91/70/20
CTPV, no/yes	158/23
Invade left and right liver lobes, no/yes	119/62
Type of gross pathology, massive/nodular/diffuse	112/59/10
PVTT type, I/II/III/IV	29/77/68/7
Inferior vena cava tumor thrombus, no/yes	171/10
Arteriovenous fistula, no/yes	125/56
Total bilirubin, μmol/L	17.1 (12.3-24.7)
Prealbumin, mg/L	110 (79-147)
Albumin, g/L	37 ± 4.8
Hemoglobin, g/L	127 (115-142)
WBC, 10^9/L	5.1 (3.925-6.505)
PLT, 10^9/L	116 (81- 179.5)
INR	1.12 (1.055-1.205)
PT, S	14.3 (13.7-15.25)
APTT, S	39.5 (37-42.8)
ALT, U/L	46 (31-71.5)
AST, U/L	65 (46-109.5)
GGT, U/L	211 (124.5-352.5)
Cholinesterase, kU/L	4.5 (3.065-5.78)
Creatinine, μmol/L	68 (57.7-78.1)
AFP, μg/L	1210 (82.905-1210)
Child-Pugh grade, A/B/C	123/55/3
ECOG score, 0/1/2/3	8/142/29/2
ALBI grade, 1/2/3	56/120/5
MELD score	7.67 (6.08-9.54)
Overall survival time, mo	4.8 (2.5-8.85)

AFP: Alpha fetoprotein; ALBI: Albumin-bilirubin; ALT: Alanine aminotransferase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; CTPV: Cavernous transformation of the portal vein; ECOG: Eastern Cooperative Oncology Group; GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio; MELD: Model of end-stage liver disease; PLT: Platelets; PVTT: Portal vein tumor thrombosis; WBC: White blood cell.

predictive power. When total bilirubin was greater than 16.15 μmol/L, the patients' mortality within 3 mo after TACE was significantly increased. This suggested that for patients with preoperative total bilirubin greater than 16.15 μmol/L, the treatment of lowering the bilirubin level may be beneficial in reducing the short-term postoperative mortality.

AST level is elevated in patients with liver disease, reflecting the degree of liver

Table 2 Univariate analysis of survivors *vs* non-survivors at 3 mo after transarterial chemoembolization

Parameters	Survivors, *n* = 125	Non-survivors, *n* = 56	*P* value
Age in yr	52.56 ± 9.966	51.27 ± 9.212	0.18
Sex, men/women	114/11	45/11	0.04
Cause of liver disease, hepatitis B/C/B and C/others	113/1/0/11	50/2/1/3	0.157
Tumor size, < 5 cm/≥ 5 cm, < 10 cm/≥ 10 cm	29/57/39	10/29/17	0.659
Number of tumors, 1/2/≥ 3	62/10/53	20/2/34	0.06
Liver cirrhosis, no/yes	36/89	12/44	0.299
Ascites, no/small/moderate-massive	68/47/10	23/23/10	0.087
CTPV, no/yes	110/15	48/8	0.67
Invade left and right liver lobes, no/yes	85/40	34/22	0.34
Type of gross pathology, massive / nodular and diffuse	78/47	34/22	0.829
PVTT type, I/II/III/IV	24/52/44/5	5/25/24/2	0.354
Inferior vena cava tumor thrombus, no/yes	119/6	52/4	0.775
Arteriovenous fistula, no/yes	89/36	36/20	0.352
Total bilirubin, μmol/L	15 (11.15-21.75)	22.25 (17.175-32)	< 0.01
Prealbumin, mg/L	119 (87-159.5)	91.5 (67.25-120.5)	< 0.01
Albumin, g/L	36.407 ± 4.749	37.205 ± 4.853	0.758
Hemoglobin, g/L	127 (115-142)	127 (113.25-143)	0.89
WBC, 10^9/L	5.06 (4.06-6.27)	5.09 (3.61-6.72)	0.87
PLT, 10^9/L	119.00 (86.00-180.50)	111.00 (69.25-178.25)	0.15
INR	1.11 (1.06-1.19)	1.12 (1.05-1.26)	0.33
PT, S	14.20 (13.70-15.20)	14.35 (13.70-15.78)	0.38
APTT, S	39.60 (37.25-42.80)	39.35 (36.32-42.85)	0.47
ALT, U/L	41.00 (29.50-62.00)	58.00 (41.25-85.75)	< 0.01
AST, U/L	56.00 (41.00-81.00)	109.00 (72.00-161.75)	< 0.01
GGT, U/L	188.00 (109.00-300.50)	256.00 (186.00-415.25)	< 0.01
Cholinesterase, kU/L	4.88 (3.44-5.84)	3.89 (2.69-5.4675)	0.02
Creatinine, μmol/L	68.60 (58.20-77.25)	66.95 (54.98-79.60)	0.63
AFP, μg/L	1210 (47.86-1210)	1210 (270-1210)	0.34
Child-Pugh grade, A/B/C	94/30/1	29/25/2	< 0.01
ECOG score, 0/1/2/3	8/102/14/1	0/40/15/1	0.016
ALBI grade, 1/2/3	43/79/9	13/41/2	0.31
MELD score	7.53 (5.88-9.02)	9.10 (7.08-11.19)	< 0.01

AFP: Alpha fetoprotein; ALBI: Albumin-bilirubin; ALT: Alanine aminotransferase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; CTPV: Cavernous transformation of the portal vein; ECOG: Eastern Cooperative Oncology Group; GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio; MELD: Model of end-stage liver disease; PLT: Platelets; PVTT: Portal vein tumor thrombosis; WBC: White blood cell.

damage[13]. In this study, AST was found to be closely related to short-term survival after TACE, while ALT was not found to be associated with short-term survival after interventional treatment.

Xie *et al*[14] suggested that the total cause mortality rate, liver disease mortality and liver cancer mortality rate of those with elevated AST were higher than those of the correspondingly elevated ALT patients. This may mean that patients with

Table 3 Multivariate analysis of short-term and long-term survival

Variables	Short-term survival		Long-term survival	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Total bilirubin, μmol/L	1.027 (1-1.054)	0.046		
AST, U/L	1.014 (1.006-1.021)	$P < 0.01$		
Sex				
Men	1			
Women	2.832 (1.025-7.828)	0.045		
Type of gross pathology				
Massive			1	
Nodular and diffuse			0.197 (0.075-0.521)	0.001
Number of tumors				
1			1	
2			1.365 (0.283-6.581)	0.698
3			5.809 (1.563-21.594)	0.009

AST: Aspartate aminotransferase; CI: Confidence interval; OR: Odds ratio.

preoperative high levels of AST do not obtain good short-term survival from TACE. When AST ≥ 87 U/L, the 3-mo mortality rate after TACE increased significantly, which indicated that correcting high levels of AST before TACE may be an effective measure to reduce short-term mortality.

In long-term survival analysis, we found that patients with only one lesion had better long-term survival than those with ≥ three lesions. Liu *et al*[15] obtained the same conclusion. Second, patients with massive block liver cancer had a worse long-term outcome than patients with nodular and diffuse liver cancer. This study used the gross pathological type of liver cancer because some patients were clinically diagnosed with liver cancer, and it was not possible to obtain liver tissue biopsy results. The gross pathological type could be judged by imaging, and its clinical applicability was better. However, in this research, the sample size of patients with the diffuse type of liver cancer was small, and this conclusion may need to be further validated in a larger sample size.

In addition, most patients had a background of viral hepatitis in the study, which was different from some areas. This may limit the application of our findings in regions where the cause of liver cancer was non-viral hepatitis. Therefore, research in other countries or regions may be needed to make up for the shortcoming of our study. Because patients with viral hepatitis accounted for the majority, model for end-stage liver disease scores in our study were higher than those of patients whose cause of liver cancer was alcohol or cholestasis.

The following limitations existed in this study. First, this study was a single-center, retrospective study, and it was difficult to avoid selection bias. Second, perhaps because of the small sample size, AUC value was not high, but the AUC value was still statistically significant. The larger and multicenter studies are needed to validate further the results in the future.

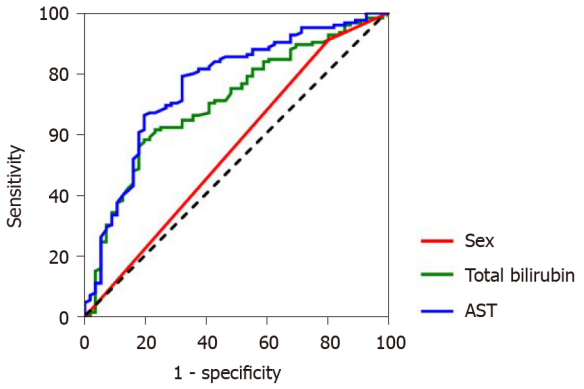
CONCLUSION

In summary, sex, AST, and total bilirubin were associated with the short-term survival outcomes in HCC patients with PVTT who underwent TACE. According to the AUC, AST was the best predictor of short-term survival, followed by total bilirubin. Multiple tumor lesions and massive block types of liver cancer were closely related to long-term adverse survival outcomes in HCC patients with PVTT who underwent TACE. In the future, multi-center, prospective and large sample studies are needed to verify these results.

Table 4 Univariate analysis of survivors *vs* non-survivors at 12 mo after transarterial chemoembolization

Parameters	Survivors, *n* = 31	Non-survivors, *n* = 150	*P* value
Age in yr	54.770 ± 10.724	51.800 ± 9.400	0.225
Sex, men/women	28/3	131/19	0.871
Cause of liver disease, hepatitis B/C/B and C/others	30/0/0/1	133/3/1/13	0.751
Tumor size, < 5 cm/≥ 5 cm, < 10 cm/≥ 10 cm	11/14/6	28/72/50	0.081
Number of tumors, 1/2/≥ 3	21/4/6	61/8/81	0.002
Liver cirrhosis, no/yes	4/27	44/106	0.059
Ascites, no/small/moderate-massive	16/11/4	75/59/16	0.892
CTPV, no/yes	26/5	132/18	0.74
Invade left and right liver lobes, no/yes	26/5	93/57	0.019
Type of gross pathology, massive/nodular and diffuse	13/18	99/51	0.012
PVTT type, I/II, III/IV	7/10/11/3	22/67/57/4	0.155
Inferior vena cava tumor thrombus, no/yes	30/1	141/9	0.854
Arteriovenous fistula, no/yes	21/10	104/46	0.861
Total bilirubin, μmol/L	19.550 (11.200-33.400)	16.600 (12.900-24.000)	0.436
Prealbumin, mg/L	124.500 (86.750-160.500)	105.000 (78.000-142.000)	0.163
Albumin, g/L	37.047 ± 5.635	36.813 ± 4.622	0.669
Hemoglobin, g/L	132.500 (117.500-146.500)	125.000 (115.000-142.000)	0.316
WBC, 10^9/L	4.565 (3.398-5.850)	5.100 (4.050-6.610)	0.284
PLT, 10^9/L	89.000 (63.250-126.000)	119.000 (87.000-182.000)	0.046
INR	1.120 (1.070-1.253)	1.110 (1.050-1.200)	0.281
PT, S	14.400 (13.850-15.875)	14.300 (13.700-15.200)	0.266
APTT, S	39.200 (36.450-42.075)	39.700 (37.100-42.900)	0.541
ALT, U/L	46.500 (38.500-72.000)	45.000 (30.000-72.000)	0.770
AST, U/L	56.500 (41.750-83.750)	71.000 (46.000-115.000)	0.108
GGT, U/L	177.500 (72.250-265.250)	216.000 (132.000-356.000)	0.036
Cholinesterase, kU/L	4.25 (2.775-5.905)	4.51 (3.08-5.79)	0.945
Creatinine, μmol/L	71.300 (61.475-79.500)	67.600 (57.400-78.100)	0.220
AFP, μg/L	568 (15.13~1210)	1210 (97.335-1210)	0.188
Child-Pugh grade, A/B/C	19/11/1	104/44/2	0.373
ECOG score, 0/1/2/3	4/24/3/0	4/118/26/2	0.086
ALBI grade, 1/2/3	13/16/2	43/104/3	0.09
MELD score	7.130 (5.548-9.315)	7.860 (6.070-9.790)	0.348

AFP: Alpha fetoprotein; ALBI: Albumin-bilirubin; ALT: Alanine aminotransferase; APTT: Activated partial thromboplastin time; AST: Aspartate aminotransferase; CTPV: Cavernous transformation of the portal vein; ECOG: Eastern Cooperative Oncology Group; GGT: Gamma-glutamyl transpeptidase; INR: International normalized ratio; MELD: Model of end-stage liver disease; PLT: Platelets; PVTT Portal vein tumor thrombosis; WBC: White blood cell.



	AUC (95%CI)	*P* value
Sex	0.554 (0.461-0.648)	0.244
AST	0.763 (0.686-0.841)	*P* < 0.001
Total bilirubin	0.707 (0.627-0.788)	*P* < 0.001

Figure 1 Comparisons of the area under the receiver operating characteristics curves for short-term survival among aspartate aminotransferase, total bilirubin, and sex. AST: Aspartate aminotransferase; AUC: Area under curve; CI: Confidence interval.

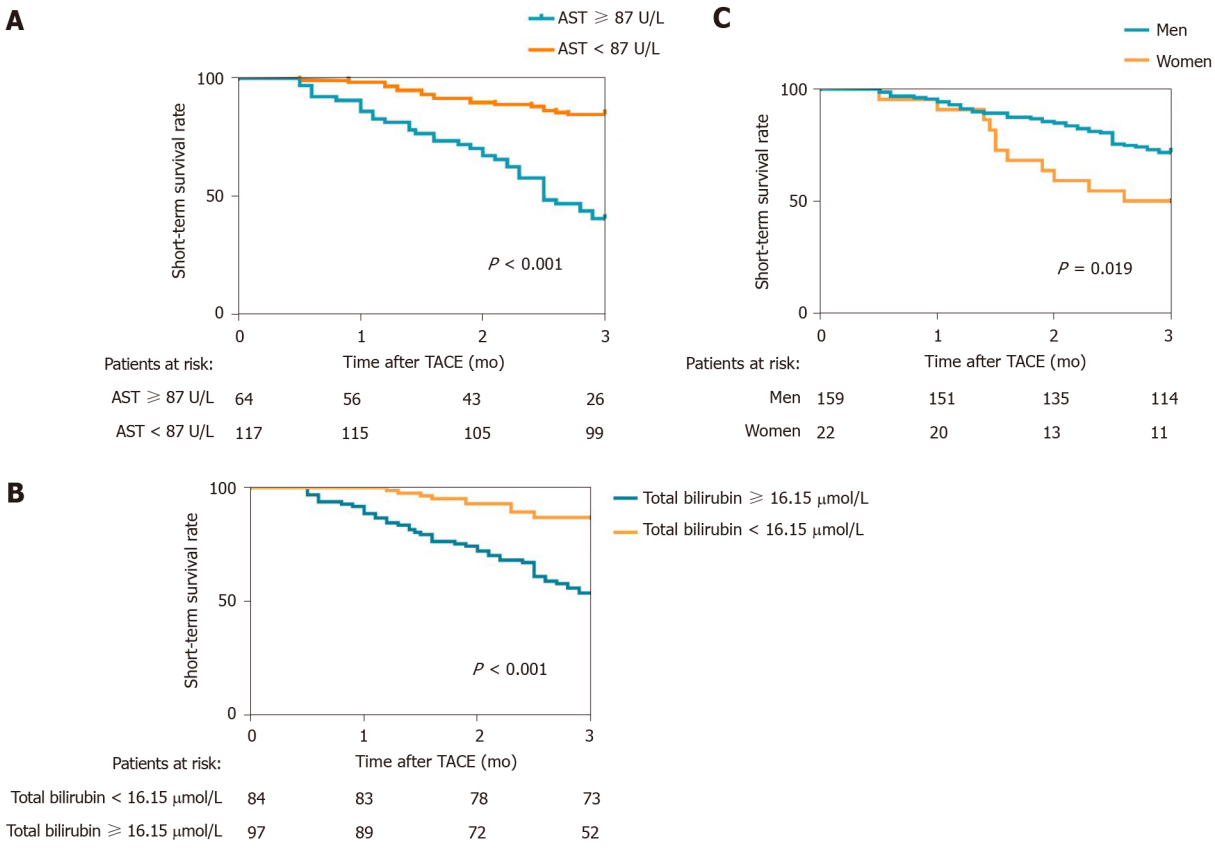
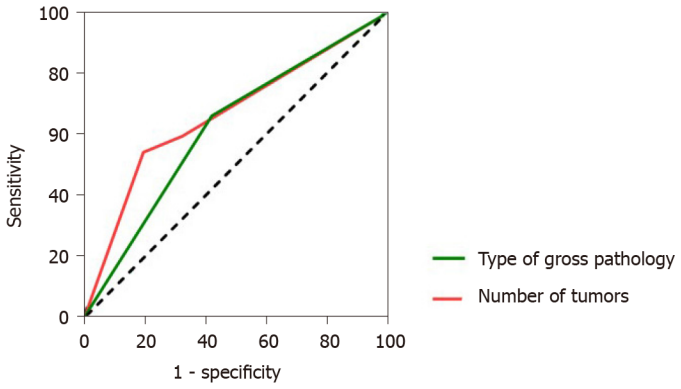


Figure 2 Cutoff values of the indicators were calculated by using receiver operating characteristic curves. A: The 3-mo survival rate curve based on the stratification of aspartate aminotransferase; B: The 3-mo survival rate curve based on the stratification of bilirubin; C: The 3-mo survival rate curve based on the sex. AST: Aspartate aminotransferase; TACE: Transarterial chemoembolization.



Variable	AUC (95%CI)	*P* value
Type of gross pathology	0.620 (0.510-0.730)	0.035
Number of tumors	0.665 (0.568-0.763)	0.004

Figure 3 Comparisons of the area under the receiver operating characteristics curves for long-term survival between pathology and number of tumors. AUC: Area under curve; CI: Confidence interval.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) patients with portal vein tumor thrombosis (PVTT) have poor prognosis. Transarterial chemoembolization (TACE) is an effective treatment for HCC patients with PVTT. The factors affecting the short-term and long-term prognosis of HCC patients with PVTT receiving TACE are still unclear.

Research motivation

The main aim of this study was to clarify the predictors correlated with the short-term and long-term survival of HCC patients with PVTT who underwent TACE.

Research objectives

We can provide some guidance to clinicians for selecting suitable patients for TACE by analyzing preoperative indicators.

Research methods

A total of 181 HCC patients with PVTT who underwent TACE were enrolled in this retrospective study. We explored the short-term and long-term prognostic factors by comparing the preoperative indicators of patients who died and survived within 3 mo and 12 mo after TACE. Multivariate analyses were conducted using logistic regression. The area under the receiver operating characteristic curve was used to evaluate the predictive ability of the factors related to the short-term and long-term prognosis.

Research results

Total bilirubin, sex, and aspartate aminotransferase (AST) were closely linked to short-term survival. When AST ≥ 87 U/L and total bilirubin ≥ 16.15 μmol/L, the 3-mo survival rate after TACE was reduced significantly. In long-term survival analysis, we found that patients with only one lesion had better long-term survival than those with ≥ three lesions. Patients with massive block liver cancer had a worse long-term outcome than patients with nodular and diffuse liver cancer.

Research conclusions

Sex, AST, and total bilirubin were associated with short-term survival outcomes in HCC patients with PVTT who underwent TACE. According to the area under the curve, AST was the best predictor of short-term survival, followed by total bilirubin. Multiple tumor lesions and massive block types of liver cancer were closely related to long-term adverse survival outcomes in HCC patients with PVTT who underwent TACE.

Research perspectives

Larger and multicenter studies are needed to validate further the results in the future.

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SCIENTOMETRICS

Global research trends in the microbiome related to irritable bowel syndrome: A bibliometric and visualized study

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Author contributions: Zyoud SH designed the study, collected the data, analyzed the data, made major contributions to the manuscript's existing literature search and interpretation, and drafted the manuscript; Al-Jabi SW, and Sweileh W participated in the study design; Smale S and Waring WS contributed towards the conception, wrote part of the article; Al-Jabi SW, Sweileh W, Smale S and Waring WS were involved in interpretation of the data, and made revisions to the initial draft; all authors provided critical review and approved the final manuscript before submission.

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Abstract

BACKGROUND

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder. Dysregulation of the gut–brain axis plays a central role in the pathophysiology of IBS. It is increasingly clear that the microbiome plays a key role in the development and normal functioning of the gut–brain axis.

AIM

To facilitate the identification of specific areas of focus that may be of relevance to future research. This study represents a bibliometric analysis of the literature pertaining to the microbiome in IBS to understand the development of this field.

METHODS

The data used in our bibliometric analysis were retrieved from the Scopus database. The terms related to IBS and microbiome were searched in titles or abstracts within the period of 2000–2019. VOSviewer software was used for data

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visualization.

RESULTS
A total of 13055 documents related to IBS were retrieved at the global level. There were 1872 scientific publications focused on the microbiome in IBS. There was a strong positive correlation between publication productivity related to IBS in all fields and productivity related to the microbiome in IBS ($r = 0.951$, $P < 0.001$). The United States was the most prolific country with 449 (24%) publications, followed by the United Kingdom ($n = 176$, 9.4%), China ($n = 154$, 8.2%), and Italy ($n = 151$, 8.1%). The h-index for all retrieved publications related to the microbiome in IBS was 138. The hot topics were stratified into four clusters: (1) The gut–brain axis related to IBS; (2) Clinical trials related to IBS and the microbiome; (3) Drug-mediated manipulation of the gut microbiome; and (4) The role of the altered composition of intestinal microbiota in IBS prevention.

CONCLUSION
This is the first study to evaluate and quantify global research productivity pertaining to the microbiome in IBS. The number of publications regarding the gut microbiota in IBS has continuously grown since 2013. This finding suggests that the future outlook for interventions targeting the gut microbiota in IBS remains promising.

Key Words: Irritable bowel syndrome; Bibliometric; Microbiota; Microbiome; Scopus; Brain gut axis

Core Tip: This is the first study to evaluate and quantify the global research productivity pertaining to the microbiome in irritable bowel syndrome (IBS). We present a holistic picture of this emerging topic and explore future research directions. A number of abnormalities have been described within the microbiome of patients with IBS. The relationships of these abnormalities to the causality of dysfunction and associated symptomatology have not been clearly elucidated. Our finding, while preliminary, suggests that the future outlook for interventions targeting the gut microbiota in IBS remains promising.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a term used to describe a group of symptoms, including abdominal pain and altered bowel habits, that arises because of disturbances of the interactions between the diet, the brain, the gut, and the microbiome. IBS poses a significant disease burden, with 20% of adults estimated to develop IBS symptoms in any given year. Symptoms arise as a consequence of abnormal visceral sensation and or motility as a result of abnormalities within the enteric or central nervous systems, or both[1]. This usually occurs in the absence of 'organic' disease, but the association with other gastrointestinal diseases is common. Many patients experiencing gastrointestinal inflammation due to infection or inflammatory bowel disease develop IBS symptoms[2]. Nearly a quarter of patients with IBS without identifiable 'organic' disease appear to have low-grade intestinal inflammation as judged by faecal inflammatory markers (calprotectin)[3]. Inflammation may lead to sensitization of peripheral nerves. Most inflammation within the gastrointestinal tract settles, but in susceptible individuals sensitization of peripheral afferents may persist. Such sensitization may be driven by ongoing mast cell activation and increased epithelial

permeability associated with low-level inflammation[4].

The onset of symptoms in IBS is often associated with psychological stressors. A high proportion of people with IBS have psychological comorbidities. Chronic stress is associated with stress-induced hyperalgesia. This hyperalgesia is mediated *via* corticotrophin-releasing hormone and the hypothalamic–pituitary axis (HPA)[5]. Dysfunction within these systems is well documented in patients with functional gastrointestinal disorders and other chronic pain syndromes. Furthermore, many patients have histories of traumatic events in early life. These events may result in changes that profoundly affect the microbiome and brain development, predisposing patients to visceral pain syndromes[6-8]. In addition to direct effects on neurons proximal to the bowel, bacteria and their products have a profound effect on the development and activity of the central nervous system and, consequently, upon the psychological state and behaviors[9]. Within the enteric nervous system, certain bacterial strains may directly promote sensitization or downregulation of peripheral afferents. Of note, low-grade inflammation seen in a proportion of patients with IBS may result from the ability of specific bacteria to enhance the production and the effects of proinflammatory cytokines. Bacteria may also be able to transmit signals *via* the enteric nervous and immune systems to the central nervous system[10].

Although several bibliometric studies have been conducted to evaluate research productivity regarding various research aspects in the medical field[11-18], and some bibliometric studies have focused on microbiota science[19-25], there has not been an assessment of the research output regarding the microbiome in IBS. This study undertakes a bibliometric analysis of the literature within this field to understand the development of the literature pertaining to the microbiome in IBS and to facilitate the identification of specific areas of focus that may be of relevance to future research. This study evaluates data that will help to address the research gaps in this field. The current bibliometric study plays a significant role for researchers interested in the relationship between the microbiome and IBS, because it offers a quick reference guide for interdisciplinary researchers to know how this field has been assessed by scientific experts in previous years.

MATERIALS AND METHODS

The data used in our bibliometric analysis were retrieved from Scopus, which is a multidisciplinary database owned by Elsevier and is considered the largest and most widely used database[26-28]. Most of the publications in this field were published between 2000 and 2019. The following term combinations were searched in the title or abstract within this period: Terms related to IBS, *i.e.*, 'adaptive colitis' OR 'colon spasm' OR 'functional bowel disease' OR 'irritable bowel' OR 'irritable colon' OR 'membranous colitis' OR 'mucous colitis' OR 'spastic colitis' OR 'spastic colon' OR 'spastic bowel' OR 'functional colonic disease' OR 'colon irritable' OR 'colon neurosis' OR 'bowel neurosis' OR 'functional colopathy' OR 'functional colonopathy' OR 'chronic catarrhal colitis' OR 'colica mucosa' OR 'colonic enterospasm' OR 'dyskinesia of the colon' OR 'dyssynergia of the colon' OR 'functional enterocolonopathy' OR 'functional diarrhea' OR 'Glarry enteritis' OR 'glutinous diarrhea' OR 'intestinal croup' OR 'irritable gut syndrome' OR 'lienteric diarrhea' OR 'Membranous catarrh of the intestine' OR 'mucomembranous colic' OR 'myxoneurosis' OR 'nervous diarrhea' OR 'neurogenic mucous' OR 'nonspecific diarrhea' OR 'tubular diarrhea' OR 'unhappy colon' OR 'unstable colon', and terms related to microbiome, *i.e.*, 'microbiome' OR 'microflora' OR 'microbiota' OR 'flora' OR 'probiotic' OR 'Saccharomyces' OR 'Lactobacillus' OR 'Bifidobacterium' OR 'Escherichia coli'. The terms were selected from related reviews on IBS[29,30] and the microbiome[19,20,31-33].

Data analysis

Microsoft Excel was used to analyze the retrieved data to calculate frequencies and percentages. The current bibliometric study included quantitative data about document types, language, country of the publications with their h-index and collaboration pattern, authors of the publications, journals that published papers on IBS, microbiome documents, and citation reports. In addition, we used the Statistical Package for the Social Sciences 16.0 software for Windows to assess the correlation between yearly quantitative distributions of publications related to IBS in all fields and publications related to the microbiome in IBS by using the Pearson correlation test. Analysis with $P < 0.05$ is considered statistically significant. In addition, the VOSviewer program[34,35] was used for mapping and clustering terms according to their

occurrence in both titles and abstracts to determine hot topics in this field. Furthermore, network visualization maps for collaboration among the most productive countries and the most prolific authors in this field were prepared using VOSviewer.

RESULTS

A total of 13055 documents related to IBS in titles and abstracts were retrieved over the period of 2000–2019 at the global level. There were 1872 scientific publications focused on the microbiome in IBS. The research findings showed that English was the main research language of documents indexed in Scopus, accounting for 48.8% of the documents, followed by German (3.9%) and Chinese (2.9%). Articles were the main type of document for publications related to the microbiome and IBS, accounting for 51.9% of documents, followed by reviews (35.3%).

The number of publications related to the microbiome in IBS has increased year by year. The quantity of documents has increased each year, from 9 documents in 2000, to 84 documents in 2009, to 219 documents in 2019 (Figure 1). Published papers during the last 5 years (2015 to 2019) accounted for 47.0% of the total publications. There was a strong positive correlation between the publication productivity related to IBS in all fields and the productivity related to the microbiome in IBS ($r = 0.951$, $P < 0.001$).

In total, authors from 105 countries published their work on the microbiome and IBS topics. Table 1 lists the top 10 most prolific countries. The United States was the most prolific country with 449 (24%) publications, followed by the United Kingdom ($n = 176$, 9.4%), China ($n = 154$, 8.2%), and Italy ($n = 151$, 8.1%). The h-index for all retrieved publications related to the microbiome in IBS was 138. Figure 2 shows the network visualization map for the collaboration among the 40 most productive countries. This includes countries that published more than 5 papers on microbiome and IBS research according to the number of publications in the last two decades (2000–2019). The United States and the United Kingdom can be referred to as central countries for this network because they published research in collaboration with 40 and 31 countries, respectively. In total, 5960 authors published their work on the microbiome and IBS topics. Figure 3 shows the network visualization map for the collaboration among the 32 most productive authors (authors who published more than 10 papers) on the microbiome in IBS research according to the number of publications in the last two decades (2000–2019). EMM Quigley (United States), G Barbara (Italy), JF Cryan (Ireland), and TG Dinan (Ireland) can be considered the prolific authors of this network due to the fact that they published 61, 25, 25, and 23 documents, respectively.

Terms were extracted from the titles and abstracts of all retrieved publications related to the microbiome in IBS and analyzed by the VOSviewer software to identify the current state and hot topics in this field. Terms appearing more than 10 times were included in the map (Figure 4) and were stratified into four clusters: IBS related to the gut–brain axis (yellow cluster), clinical trials related to IBS and the microbiome (red cluster), drug-mediated manipulation of the gut microbiome (blue cluster), and the role of the altered composition of intestinal microbiota in IBS prevention (green cluster).

The top 10 journals accounted for 19.98% of all articles (Table 2). The four journals that published the most studies related to microbiome and IBS were *World Journal of Gastroenterology* ($n = 70$), *Alimentary Pharmacology and Therapeutics* ($n = 54$), *Neurogastroenterology and Motility* ($n = 52$), and the *Journal of Clinical Gastroenterology* ($n = 39$).

The sample presents an average citation rate of 42 citations per publication. However, 16.6% of the publications have never been cited. Table 3 lists the information on the top ranking of publications in terms of the highest number of citations[36-55]. The article 'Prebiotic Effects: Metabolic and Health Benefits" is the most cited article with 1147 citations; it was authored by Roberfroid *et al*[49] and published in the *British Journal of Nutrition* in 2010. This study reported that a substantial number of studies about human intervention have shown that dietary intake of food products/ingredients/supplements results in statistically important improvements in the faecal gut microbiota composition. The second most highly cited article is 'Lactobacillus and Bifidobacterium in IBS: Symptom Responses and Relationship to Cytokine Profiles' by O'Mahony *et al*[44] and published in *Gastroenterology* in 2005. This study showed that intake of *Bifidobacterium infantis* over 8 wk was associated with symptomatic improvement in IBS.

Table 1 Top 10 most productive countries on the microbiome in irritable bowel syndrome research, ranked by the total number of publications in the last two decades (2000-2019)

SCR	Country	Number of documents	%
1st	United States	449	24.0
2nd	United Kingdom	176	9.4
3rd	China	154	8.2
4th	Italy	151	8.1
5th	France	116	6.2
6th	Germany	107	5.7
7th	Canada	103	5.5
8th	Ireland	102	5.4
9th	Australia	72	3.8
10th	Spain	69	3.7

SCR: Standard competition ranking.

Table 2 Top 10 most productive journals on the microbiome in irritable bowel syndrome research, ranked by the total number of publications in the last two decades (2000-2019)

SCR[1]	Journal	Frequency	%	IF[2]
1st	*World Journal of Gastroenterology*	70	3.74	3.665
2nd	*Alimentary Pharmacology and Therapeutics*	54	2.88	7.515
3rd	*Neurogastroenterology and Motility*	52	2.78	2.946
4th	*Journal of Clinical Gastroenterology*	39	2.08	2.973
5th	*American Journal of Gastroenterology*	34	1.82	10.171
6th	*Gastroenterology*	33	1.76	17.373
7th	*Current Opinion in Gastroenterology*	27	1.44	3.225
8th	*PLoS One*	24	1.28	2.740
9th	*Nutrients*	21	1.12	4.546
10th	*Gut Microbes*	20	1.07	7.740

[1]Equal journals have the same ranking number, and then a gap is left in the ranking numbers.
[2]Impact factors based on Journal Citation Reports 2019 from Clarivate Analytics.
SCR: Standard competition ranking; IF: Impact factor.

Table 4 presents the top 10 most productive institutions on the microbiome in IBS research, ranked by the total number of publications. The four institutions that have published the most studies related to microbiome and IBS are the University College Cork (n = 87), APC Microbiome Ireland (n = 84), McMaster University (n = 45), the Mayo Clinic (n = 32), and Alma Mater Studiorum Università di Bologna (n = 38) (Table 4).

DISCUSSION

This bibliometric study was carried out using data from Scopus for the period of 2000–2019. It presents a comprehensive review of research progress on the microbiome and IBS, identifies hot topics within this field, and indicates potential directions for future research. It is apparent that the research productivity in this field has progressively increased due to the increasing recognition of the role of the gut microbiota[56,57]. In addition, this increase is probably linked to the growing number of

Table 3 Top 20 most cited articles on the microbiome in irritable bowel syndrome research in the last two decades (2000-2019)

SCR	Ref.	Title	Year	Source title	Cited by
1st	Roberfroid *et al*[49]	"Prebiotic effects: Metabolic and health benefits"	2010	*British Journal of Nutrition*	1147
2nd	O'Mahony *et al*[44]	"Lactobacillus and Bifidobacterium in irritable bowel syndrome: Symptom responses and relationship to cytokine profiles"	2005	*Gastroenterology*	1042
3rd	Claesson *et al*[37]	"Composition, variability, and temporal stability of the intestinal microbiota of the elderly"	2011	*Proceedings of the National Academy of Sciences of the United States of America*	836
4th	Collins *et al*[38]	"The interplay between the intestinal microbiota and the brain"	2012	*Nature Reviews Microbiology*	735
5th	Kassinen *et al*[40]	"The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects"	2007	*Gastroenterology*	684
6th	Pimentel *et al*[46]	"Rifaximin therapy for patients with irritable bowel syndrome without constipation"	2011	*New England Journal of Medicine*	659
7th	Rinttilä *et al*[48]	"Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR"	2004	*Journal of Applied Microbiology*	658
8th	O'Mahony *et al*[45]	"Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses "	2009	*Biological Psychiatry*	634
9th	Rolfe[50]	"The role of probiotic cultures in the control of gastrointestinal health"	2000	*Journal of Nutrition*	584
10h	Carabotti *et al*[36]	"The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems"	2015	*Annals of Gastroenterology*	580
11th	De Vrese and Schrezenmeir[39]	"Probiotics, prebiotics, and synbiotics"	2008	*Advances in Biochemical Engineering/Biotechnology*	575
12th	Rajilić-Stojanović *et al*[47]	"Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome"	2011	*Gastroenterology*	545
13th	Whorwell *et al*[55]	"Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome"	2006	*American Journal of Gastroenterology*	541
13th	Swidsinski *et al*[54]	"Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease"	2005	*Journal of Clinical Microbiology*	541
15th	Malinen *et al*[41]	"Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR"	2005	*American Journal of Gastroenterology*	523
16th	Mayer *et al*[42]	"Gut/brain axis and the microbiota"	2015	*Journal of Clinical Investigation*	519
17th	Simrén *et al*[52]	"Intestinal microbiota in functional bowel disorders: A Rome foundation report"	2013	*Gut*	510
18th	Spiller and Garsed[53]	"Postinfectious irritable bowel syndrome "	2009	*Gastroenterology*	508
19th	Rousseaux *et al*[51]	"Lactobacillus acidophilus modulates intestinal pain and induces opioid and cannabinoid receptors"	2007	*Nature Medicine*	500
20th	Nobaek *et al*[43]	"Alteration of intestinal microflora is associated with reduction in abdominal bloating and pain in patients with irritable bowel syndrome"	2000	*American Journal of Gastroenterology*	498

researchers who are interested in the fields of neurology and gastroenterology [*e.g.*, EMM Quigley (the United States), JF Cryan (Ireland), TG Dinan (Ireland), G Barbara (Italy), Y Ringel (the United States), M Simrén (Sweden), M Camilleri (the United States), A Palva (Finland), M Pimentel (Canada), NJ Talley (Australia), and others]. Over the last two decades, advances in our understanding of the enteric and central nervous systems have led to an evolving understanding of their interactions and how the gastrointestinal microbiome influences their function. Disturbances in the gastrointestinal microbiome have been identified in patients with functional gastrointestinal disease, like IBS, and it is often the case that symptoms may be triggered by events that lead to changes in the microbiome, where the emotional context and the enteric nervous system function in tandem[1].

The results of this study indicate that the United States has been the most prolific country in this field. These findings are consistent with many previous bibliometric studies[12,58]. Research productivity in the United States is likely associated with

Table 4 Top 10 most productive institutions on the microbiome in irritable bowel syndrome research, ranked by the total number of publications in the last two decades (2000-2019)

SCR[1]	Institute	Country	*n*	%
1st	University College Cork	Ireland	87	4.65
2nd	APC Microbiome Ireland	Ireland	84	4.49
3rd	McMaster University	Canada	45	2.40
4th	Alma Mater Studiorum Università di Bologna	Italy	38	2.03
5th	Helsingin Yliopisto	Finland	36	1.92
5th	Mayo Clinic	United States	36	1.92
7th	Inserm (Institut national de la santé et de la recherche médicale)	France	30	1.60
8th	Baylor College of Medicine	United States	27	1.44
9th	Göteborgs Universitet	Sweden	27	1.44
10th	The University of North Carolina at Chapel Hill	United States	27	1.44

[1]Equal institutes have the same ranking number, and then a gap is left in the ranking numbers.
SCR: Standard competition ranking.

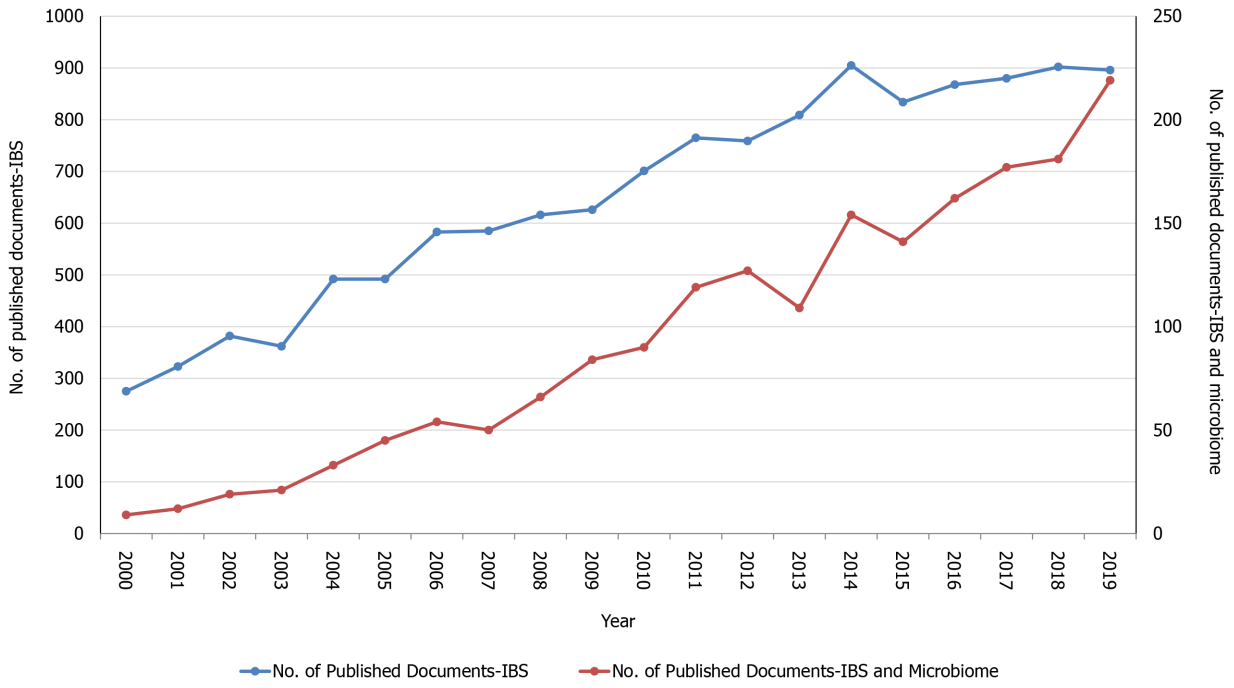

Figure 1 The annual number of publications related to irritable bowel syndrome and the microbiome from 2000 to 2019. IBS: Irritable bowel syndrome.

overwhelming support in terms of research, the wide range of researchers with an interest within this field, several well-resourced research environments, and greater availability of a well-trained workforce. In addition, the economic strength of the United States has led to a substantial amount of financial support for researchers and has enhanced the mobility of researchers[59,60]. Furthermore, in 2013, the United States launched an exceptional research project on the gut microbiota–brain axis[61], thus contributing to the increasing number of publications regarding the microbiome in IBS.

Our study demonstrates that the gut–brain axis has become the most prolific area for research within IBS and the microbiome globally, and several studies in a range of journals are proof of this emerging trend. According to these data, we can infer that central pain amplification occurs through a wide variety of mechanisms within the

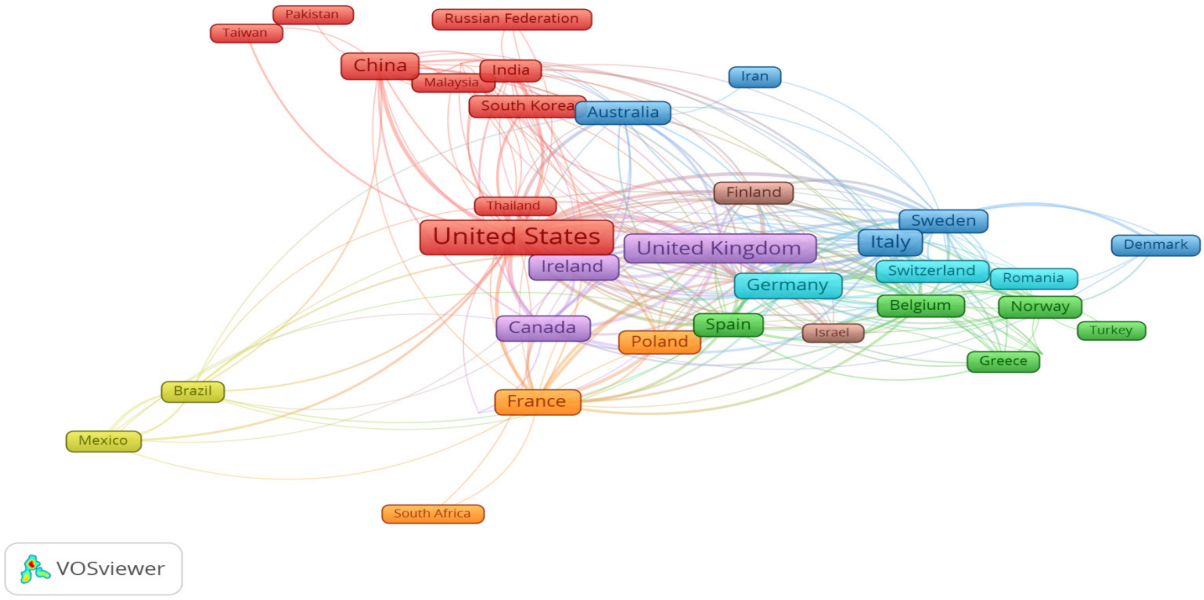

Figure 2 Network visualization map for the collaboration among the 40 most productive countries on the microbiome in irritable bowel syndrome research according to the number of publications in the last two decades (2000–2019).

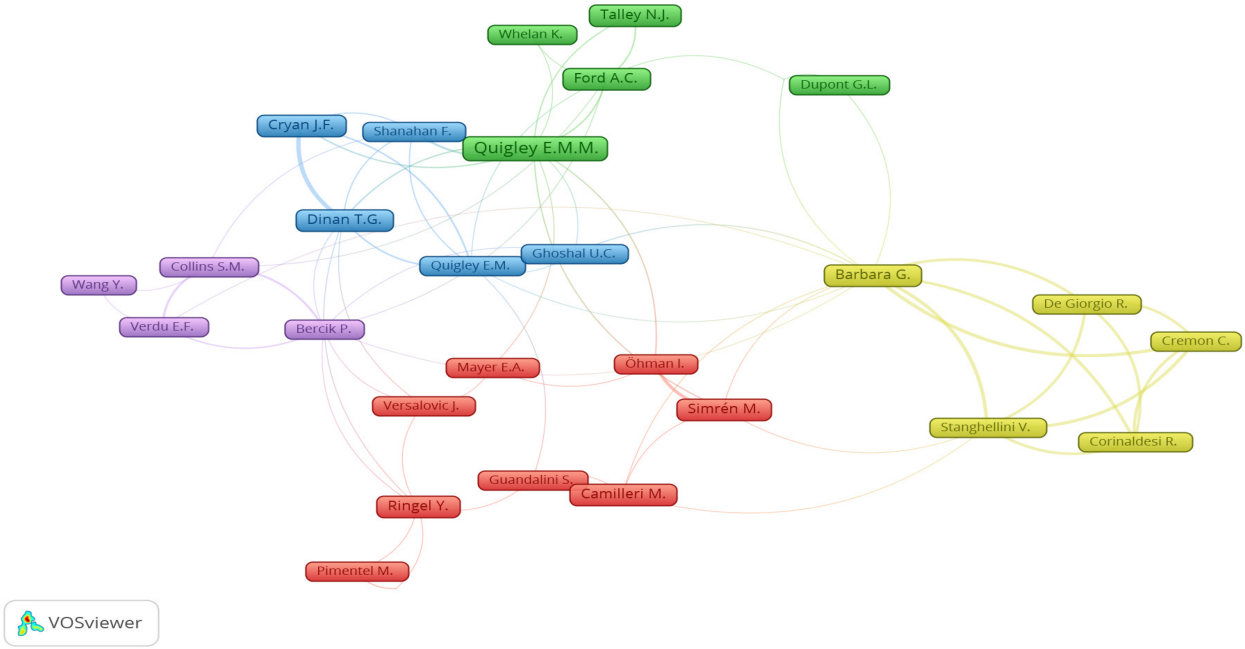

Figure 3 Network visualization map for the collaboration among the 32 most productive authors on the microbiome in irritable bowel syndrome research according to the number of publications in the last two decades (2000–2019).

central nervous system and mediates the effects of mood, emotional context, and environmental circumstances upon our central pain perception. Functional magnetic resonance imaging (fMRI) studies have demonstrated that patients with IBS often display an exaggerated response of arousal circuits associated with the HPA and activation of endogenous pain pathways in response to visceral stimulation. Similarly, studies have shown enhanced anticipation of pain in response to conditioning stimuli in patients with functional gastrointestinal disease[62]. Neuro-immune activation within the central nervous system is associated with altered astrocyte and microglial activity that further enhances central sensitisation.

Early deprivation in patients with IBS can have profound effects on the microbiome, and researchers recognise that these factors may predispose susceptible individuals to more severe IBS[8,63-66]. Changes in central nervous system function arise as a result of

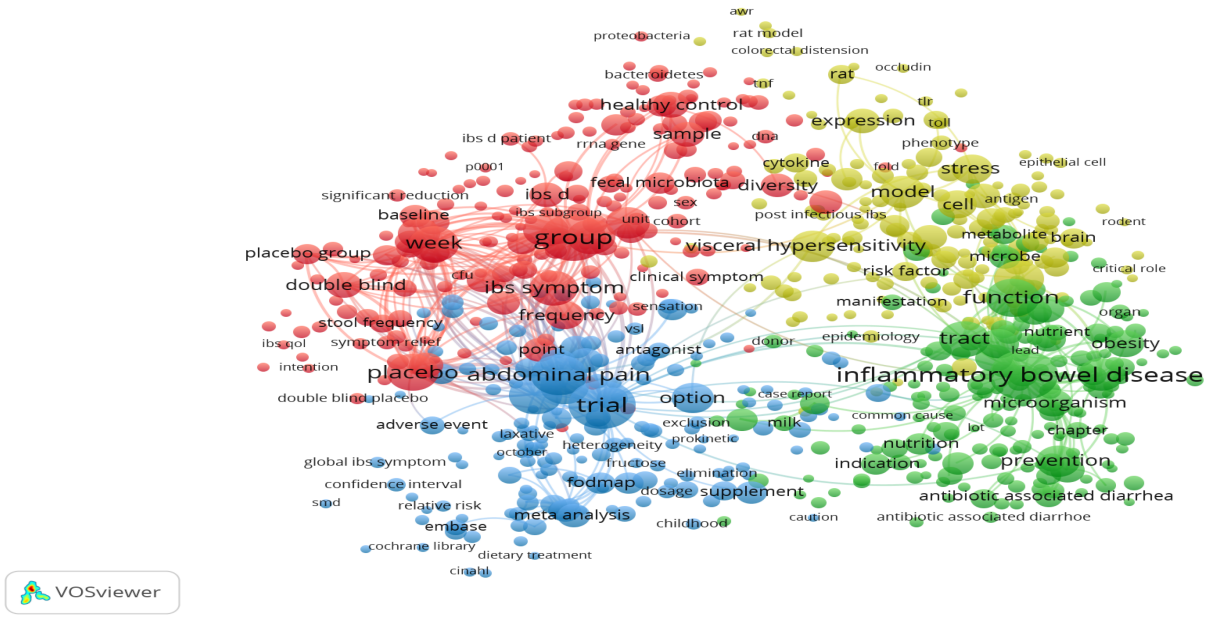

Figure 4 Network visualization map for the terms with high co-occurrence frequencies, based on the titles and abstracts of documents related to the microbiome in irritable bowel syndrome research published in the last two decades (2000–2019).

microbial products, which then influence astrocyte, microglial, and central nervous system inflammatory responses[67]. Certain bacterial strains, possibly as a result of being able to modulate these effects, may influence behaviour. Offspring of mice fed a high-fat diet display disordered behaviour and socialisation. These behavioural changes appear to be manifested by alterations in the microbiome. Correction of 'bacterial deficits' or their metabolic sequelae leads to improvements in behaviour. Specific bacterial strains have been demonstrated to increase resilience in individuals with anxiety, and these behavioural changes have neurological correlates in fMRI studies. Those factors known to provoke IBS have profound effects on the microbiome function; gastroenteritis can deplete the microbiome, and psychological stress can lead to significant changes in the representation of different phyla within the microbial flora. These potentially detrimental changes may facilitate feedback from the gut and, consequently, permit the gut to have effects on mood, behaviour, and cognitive functions.

Limitations

There are some limitations in our study that are similar to previous bibliometric studies. First, although Scopus is the most recognized and the largest database for peer-reviewed literature, our study is restricted to only the Scopus database as a source of data collection. Second, some authors or institutions have different name formats in the Scopus database, and their research count might be scattered; therefore, their names might not show in the active list. Despite these limitations, we still consider that the findings of our analysis were adequate to characterize accurately the state of IBS and microbiome research at the global level.

CONCLUSION

This is the first study to evaluate and quantify the global research productivity pertaining to the microbiome in IBS to present a holistic picture of this emerging topic and explore future research directions. The number of publications regarding the gut microbiota in IBS has continuously grown since 2013. The United States, the United Kingdom, Italy, and Ireland have been the most productive regions. Currently, the main hot topics regarding the gut microbiota in IBS are the gut–brain axis related to IBS, clinical trials related to IBS and the microbiome, drug-mediated manipulation of the gut microbiome, and the role of the altered composition of intestinal microbiota in IBS prevention. A number of abnormalities have been described within the microbiome of patients with IBS. The relationship between these abnormalities and the causality of dysfunction and associated symptomatology have yet to be clearly

elucidated. Our findings, while preliminary, suggest that the future outlook for interventions targeting the gut microbiota in IBS remains promising.

ARTICLE HIGHLIGHTS

Research background

The ability of specific bacteria to boost the development and the effects of proinflammatory cytokines can result in low-grade inflammation seen in a proportion of patients with irritable bowel syndrome (IBS).

Research motivation

The current bibliometric analysis plays an important role for researchers interested in the relationship between the microbiome and IBS. It provides a simple reference guide for interdisciplinary researchers to learn how scientific experts have examined this area in previous years.

Research objectives

This study aimed to carry out a bibliometric review of the IBS and the microbiome literature to explain the growth of this field and assist the identification of unique focus areas that may be important for future research.

Research methods

The information used in our bibliometric research was derived from the Scopus database. Terms related to IBS and the microbiome were searched in titles or abstracts during the period of 2000–2019. For data visualization, VOSviewer software was used.

Research results

Since 2013, the number of publications on gut microbiota in IBS has continuously increased. This result indicates that the future outlook remains optimistic for treatments targeting the gut microbiota in IBS.

Research conclusions

This is the first study to analyze and measure the global research productivity of IBS and microbiome research to provide a holistic view of this evolving subject and explore future research directions. It is evident that, due to the growing understanding of the role of the gut microbiota, research productivity in this area has steadily increased. Currently, the key hot topics are the gut–brain axis related to IBS, clinical trials related to IBS and the microbiome, drug-mediated modulation of the gut microbiome, and the role of the altered composition of the intestinal microbiome in the prevention of IBS.

Research perspectives

Our results indicate that the future outlook for IBS therapies targeting the intestinal microbiota remains promising.

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CASE REPORT

Transperineal core-needle biopsy of a rectal subepithelial lesion guided by endorectal ultrasound after contrast-enhanced ultrasound: A case report

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Abstract

BACKGROUND

Rectal subepithelial lesions (SELs) are commonly seen in endoscopic examination, generally manifested as bumps with a smooth surface. Precise preoperative diagnoses for rectal SELs are difficult because abnormal tissues are not easily to be obtained by regular endoscopic forceps biopsy. Traditional guidance modalities of preoperative biopsy, including endoscopic ultrasound, computed tomography, and transabdominal ultrasound, are often unsatisfactory. An updated, safe, and effective biopsy guidance method is required. We herein report a new biopsy guidance modality—endorectal ultrasound (ERUS) combined with contrast-enhanced ultrasound (CEUS).

CASE SUMMARY

A 32-year-old woman complained of a mass inside the rectovaginal space for 9 years, which became enlarged within 1 year. A rectal SEL detected by endoscopy was suspected to be a gastrointestinal stromal tumor or exophytic uterine fibroid. Pathological diagnosis was difficult because of unsuccessful transabdominal core needle biopsy with insufficient tissues, as well as vaginal hemorrhage. A second biopsy was suggested after multiple disciplinary treatment discussion, which referred to a transperineal core needle biopsy (CNB) guided by ERUS combined with CEUS. Adequate samples were procured and rectal gastrointestinal stromal tumor was proved to be the pathological diagnosis. Imatinib was recommended for first-line therapy by multiple disciplinary treatment discussion. After the

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tumor shrunk, resection of the rectal gastrointestinal stromal tumor was performed through the posterior vaginal wall. Adjuvant therapy was applied and no recurrence or metastasis has been found by the last follow-up on December 13, 2019.

CONCLUSION

Transperineal CNB guided by ERUS and CEUS is a safe and effective preoperative biopsy of rectal SELs yet with some limitations.

Key Words: Transperineal core needle biopsy; Endorectal ultrasound; Contrast-enhanced ultrasound; Rectal subepithelial lesion; Case report

Core Tip: Rectal subepithelial lesions (SELs) often manifest as bumps with a smooth surface on endoscopy. An efficient biopsy strategy is crucial to sampling for differentiation of pathological types. The transperineal core needle biopsy guided by endorectal ultrasound combined with contrast-enhanced ultrasound overcomes the limitations of previous guidance, such as sampling inadequacy, high risk of complications, and exposure to radiation. In our case, unsuccessful transabdominal biopsy led to failure of pathological diagnosis. The patient underwent this new biopsy modality then, and a diagnosis was finally made. No complications occurred. We recommend this new strategy as a promising tool for rectal SEL biopsy.

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INTRODUCTION

Rectal subepithelial lesions (SELs) vary in types and there have been many studies on the diagnostic value of ultrasound (US). Pelvic lipomas, gastrointestinal stromal tumors (GISTs), carcinoid tumors, leiomyomas, schwannomas, and lymphomas often originate from the anorectum[1,2]. These tumors are apt to manifest as rectal SELs on endoscopy. Traditional biopsy guidance methods include endoscopy, endoscopic US (EUS), transabdominal ultrasound, and computed tomography (CT), which have been reported with many disadvantages[1-4] to achieve successful biopsies. By application of endorectal US (ERUS) in anorectal and pelvic diseases in our daily work, we found a novel guidance method for rectal SELs biopsy, which is safer and more effective. Out of several preoperative biopsy approaches for rectal SELs, this case report focuses on transperineal core needle biopsy (CNB)[5] guided by contrast-enhanced US (CEUS) combined with ERUS. We present the following case in accordance with the CARE reporting checklist.

CASE PRESENTATION

Chief complaints

A 32-year-old woman was admitted to our hospital with a progressively enlarged mass in the rectovaginal space for 9 years.

History of present illness

The mass was found during a routine examination 9 years ago and no obvious progression was seen in following annual examinations until a year ago. The maximum diameter of the mass had significantly grown from 4.0 cm to 9.7 cm in a year, with dents in stools, which was caused by tumor compression. The patient had a

submucosal protrusion demonstrated by endoscopy in a local hospital. However, no absolute tumor tissue but only inflammation was found after several times of endoscopic biopsies in the latest 2 years, which was not consistent with clinical estimation. Therefore, she came to our hospital for further diagnosis (Table 1).

History of past illness

The patient had no previous medical history.

Personal and family history

The patient and family had no history of previous similar illness.

Physical examination

The patient's mental status, appetite, sleep, and weight were normal without any obvious symptoms of abdominal distension, tenesmus, prolapse, diarrhea, or constipation. The anterior rectum wall was plump during digital rectal examination without tenderness or blood stain on the fingertip.

Laboratory examinations

Laboratory examinations were normal, including routine blood analysis, carcinoembryonic antigen, α-fetoprotein, carbohydrate antigen (CA) 19-9, and CA125.

Imaging examinations

Contrast-enhanced CT (CECT) showed a mass with liquefaction necrosis (low density area) inside the rectovaginal space, with an obscure margin (Figure 1A). Transabdominal US was performed. A heterogeneous hypoechoic mass with a maximum diameter of 9.7 cm was also seen in the rectovaginal space (Figure 2A), which was suspected to be a rectal GIST or exophytic uterine fibroid.

Further diagnostic work-up

Transabdominal CNB (MG1522 BARD MAGNUM Biopsy Instrument; Tempe, AZ, United States; disposable core tissue biopsy needle, gauge size and needle length: 16 G and 16 cm) was performed to make a definite diagnosis (Figure 2B). Before the operation, written informed consent was obtained from the patient, and her preoperative regular laboratory examinations were normal, including routine blood test, coagulation function, and blood transfusion set. Some hemorrhage occurred from the vagina, causing premature end of the biopsy, and spontaneously relieved several days later. Strips of greyish shattered tissue (Figure 3A) were obtained, with the largest diameter smaller than 0.3 cm. Pathology indicated inadequate biopsy tissue for diagnosis. Another biopsy after ERUS assessment was recommended by multiple disciplinary treatment (MDT).

First, ERUS was performed with the MyLab Twice US system (Esaote, Genoa, Italy) equipped with a biplane endoscopic probe (TRT33, linear frequency of 4–13 MHz, convex frequency of 3-9 MHz), recording the location, size, stratification, adjacence, and echogenicity of the tumor. Then CEUS was performed with a bolus injection through the elbow vein of 2.4 mL SonoVue (Bracco, Milan, Italy) (Figure 4). Because of the heterogeneity of the tumor, it was difficult to recognize the necrotic from non-necrotic part in B mode, which can be clearly depicted by CEUS as nonenhanced *vs* enhanced area. As previous CECT has pointed out necrosis, CEUS was performed right before puncture to confirm the substantial part of the tumor for biopsy guidance for the second time, avoiding the failed samples inside presumptive solid area, which turned to be non-enhanced after CEUS. This guarantees the precision and efficiency of biopsy samples.

The probe was switched to linear mode. The transperineal puncture site and path were decided, and disinfection as well as drape were also completed. After local anesthesia, a freehand biopsy of the lesion was performed with the guidance of in-plane needle. The needle tip and its movements were continuously monitored in real time by ERUS during the whole puncture procedure[6] to procure the tissue of lesion from enhanced area of the tumor on CEUS (Figure 5). Several strips of greyish tissue were obtained with a length of 0.3-1.2 cm (Figure 3B and C). No complications occurred.

Table 1 Timeline of diagnosis and treatment process

Time	Events
January 2018	A mass in the rectovaginal space found 9 years ago had significantly grown in a year, with several times of negative endoscopy biopsy results
January 31, 2018	Transabdominal biopsy failed to provide qualified tissues to make well-defined pathological result
February 8, 2018	The patient underwent transperineal biopsy guided by ERUS and CEUS, diagnosed as rectal GIST
July 23, 2018	The tumor shrunk after 5-mo imatinib treatment, and was completely resected through the posterior vaginal wall
October 13, 2019	Fifteen months after tumor resection, no obvious sign of recurrence was found

ERUS: Endorectal ultrasound; CEUS: Contrast-enhanced ultrasound; GIST: Gastrointestinal stromal tumor.

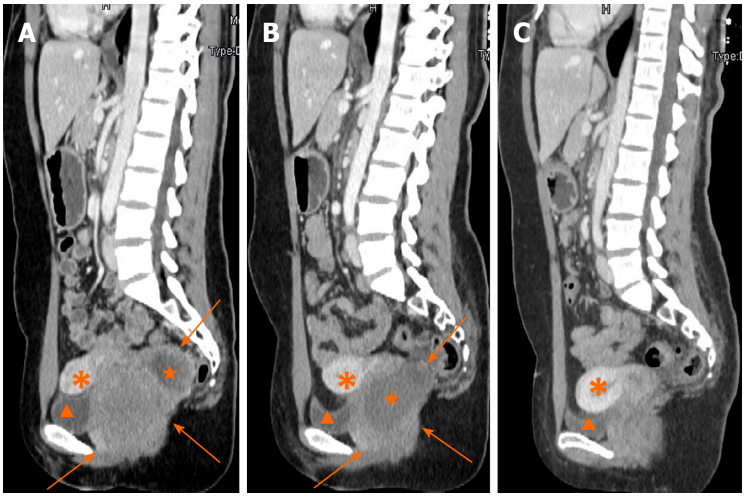

Figure 1 Contrast-enhanced computed tomography in sagittal section. A: A rectovaginal space mass of 8.0 cm in maximum diameter with non-enhancing liquefaction necrosis at the time of admission; B: The mass significantly shrunk to 6.4 cm in maximum diameter after 5 mo of imatinib treatment and non-enhanced liquefaction area enlarged clearly; C: No obvious sign of tumor recurrence in rectovaginal space 15 mo after tumor resection. Arrows: The mass; pentagrams: Liquefaction necrosis area; arrow heads: Bladder; asterisks: Uterus.

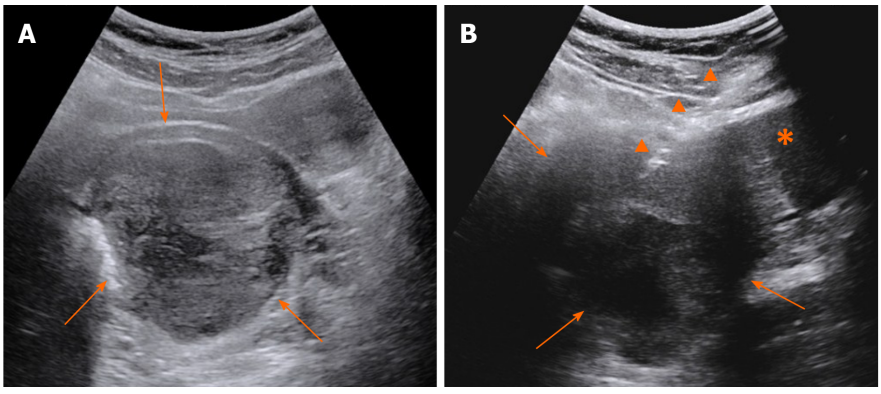

Figure 2 Grey scale ultrasound. A: A mass was demonstrated inside the rectovaginal space in the grey scale images; B: Transabdominal core needle biopsy of the mass. Arrows: The mass; arrow heads: Core needle; asterisk: Uterus cervix.

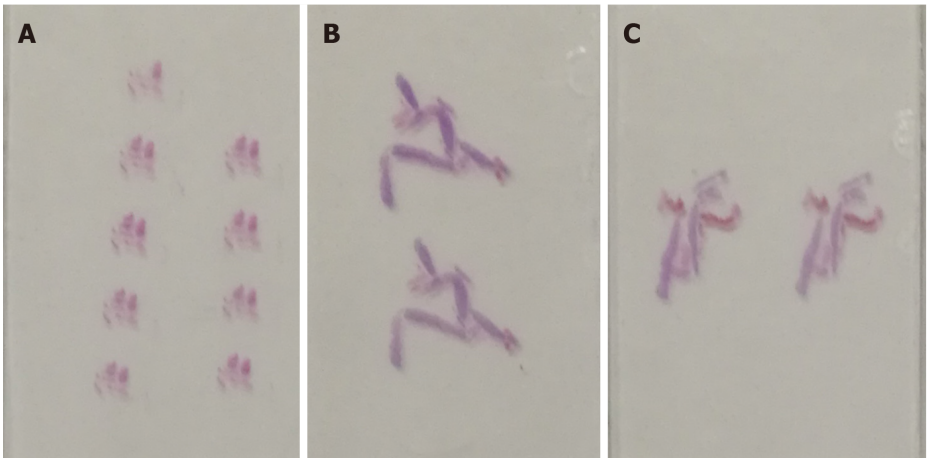

Figure 3 Slide samples. A: The shattered slide samples from transabdominal ultrasound-guided biopsy; B and C: The slide samples from transperineal biopsy guided by endorectal ultrasound, which were abundant and intact.

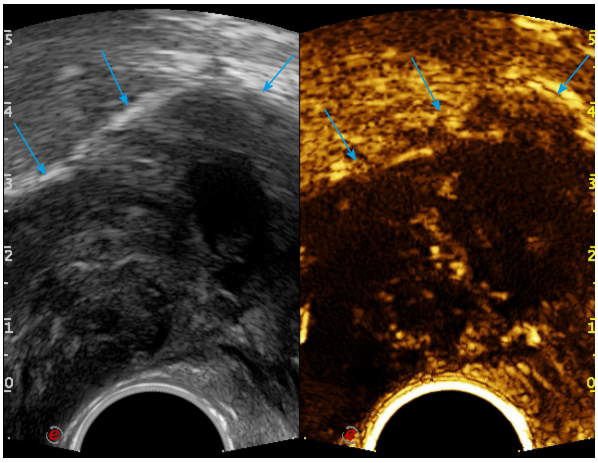

Figure 4 Endorectal ultrasound performed with the convex probe of TRT33. Liquefaction necrosis and cellular appearance were found inside the tumor in transverse sections, and the solid part of the tumor was either hypo- or iso-enhancing. Arrow: Rectal stromal tumor.

FINAL DIAGNOSIS

According to the post-operation immunohistochemical (IHC) staining, CD117, Dog-1, CD34, and succinate dehydrogenase B subunit were positive, and spinal muscular atrophy, Des, and S-100 were negative. The proliferation index of Ki-67 was 2%. The diagnosis was finally settled as GIST with post-therapeutic reaction (Figure 6).

TREATMENT

After the second MDT discussion, imatinib (Gleevec; Novartis, Basel, Switzerland) was decided as first-line therapy. It was suggested to wait for the treatment reaction before making a further therapeutic schedule. After 5 mo, CECT showed that the tumor had significantly shrunk (Figure 1B). The patient underwent complete GIST resection through the posterior vaginal wall. During the surgery, the tumor was found to originate from the rectal muscular layer, close to the posterior vaginal wall, with a smooth capsule, 5.5 cm in maximum diameter. It was greyish white on the cut surface with an interlaced appearance. The postoperative pathology findings were as follows: A suspected spindle cell tumor, interstitial and fibrous tissue proliferation, focal glassy degeneration area accompanied by a few lymphocyte reactions, remote hemorrhage with hemosiderin deposition, and a negative surgical margin.

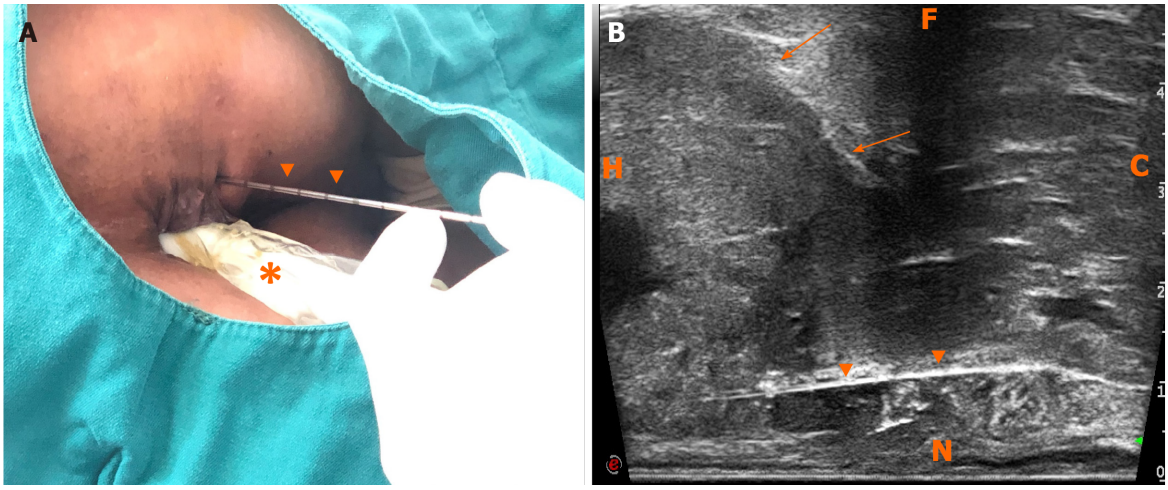

Figure 5 Freehand transperineal biopsy of the rectal subepithelial mass guided by endorectal ultrasound. A: The patient in left lateral decubitus, and the freehand transperineal biopsy was performed, guided by endorectal ultrasound (ERUS); B: Longitudinal sectional image of transperineal core needle biopsy guided by ERUS obtained with the linear probe of TRT33. H: Head; C: Caudal; N: Near field; F: Far field; arrows: Rectal stromal tumor; triangular arrowheads: Core needle; asterisk: TRT33 probe.

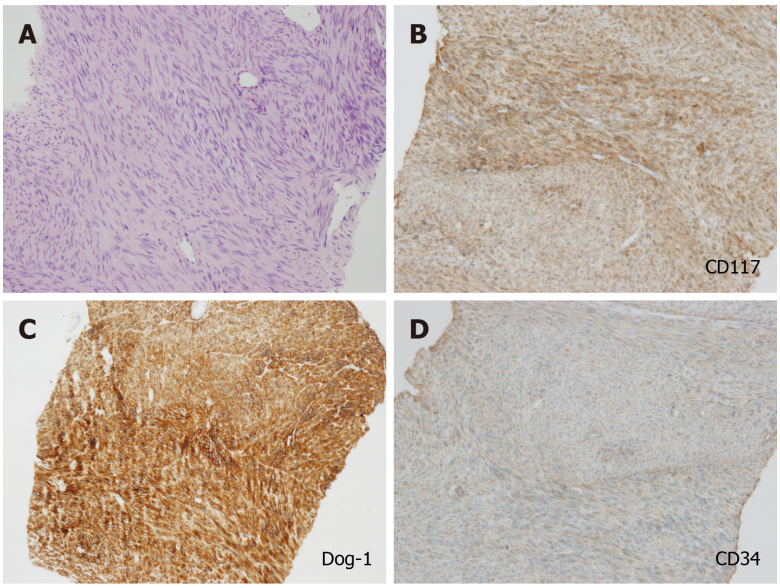

Figure 6 Pathological results. A: A mass consisting of spindle-shaped cells in hematoxylin and eosin staining; B-D: Immunohistochemical staining of the rectal stromal tumor: CD117, Dog-1, and CD34 were strongly positive (low power field).

OUTCOME AND FOLLOW-UP

Fifteen months after resection, CECT of the pelvic cavity on October 26, 2019 (Figure 1C) indicated no obvious sign of tumor recurrence. No recurrence or metastasis has been found either by the last follow-up in December 13, 2019.

DISCUSSION

GISTs are nondirectional differentiated mesenchymal neoplasms that arise anywhere along the digestive tract with an incidence of 3% in the rectum[7]. Approximately 25% of GISTs were found accidently during surgery or by imaging and about 5% at autopsy[8].

Rectal GISTs are often concealed, accompanied by long disease duration, thus symptoms often appear in the late phase of the disease. Common imaging findings include a well-circumscribed tumor with nonannular and exophytic growth pattern, without causing perirectal lymphadenopathy or bowel obstruction in most cases[9].

Rectal GISTs are of increasing concern because of the rare site and post-treatment implications for lifestyle. Targeted therapy has been successful compared with conventional chemotherapy and radiotherapy. Surgery is still the main approach to cure GISTs[10,11]. Preoperative diagnosis is crucial for GISTs, because they differ from other rectal SELs in treatment and prognosis.

GISTs have malignant potential and treatment depends on accurate pathological diagnosis by IHC staining, which requires adequate tissue samples. Endoscopic biopsy only samples mucosal tissue, yet rectal GISTs often present as submucosal growths, which forms a barrier for endoscopic biopsy. EUS-guided fine needle aspiration (EUS-FNA) is considered for preoperative diagnosis of submucosal lesions. In our case, the results of several times of EUS-FNA from the local hospital were negative. Inadequate tissue samples were mentioned by Yegin and Duman[1] in approximately one third of cases. A multicenter retrospective study[3] of EUS-FNA of suspected GISTs gave cytopathological yield, IHC yield, and diagnostic yield of 46%, 41%, and 37%, respectively. In addition, EUS-FNA has been limited in its inability to procure intact and adequate samples that are vital for histopathological and IHC analysis, causing difficulties in pathological diagnosis of GISTs[2,4]. EUS-guided fine needle biopsy (EUS-FNB) is still the commonest biopsy mode for GISTs. However, it requires adequate preoperative bowel preparation, and the risks of infection and hemafecia are higher in transrectal approach than in transperineal approach[12,13]. Also, the technique of EUS-FNB is more difficult, and it requires complex instruments, leading to unavailability in primary hospital.

We performed CNB guided by transabdominal US at first. Post-urination procedure was taken in order to avoid bladder injuries. Compression of the pelvic cavity by the probe was made to protect the surrounding bowel. The needle still went through the vagina and caused vaginal hemorrhage. Eventually, we were not able to obtain a pathological diagnosis because of the conflict between high risk of complications and inadequate sampling.

Then we performed transperineal freehand biopsy under the guidance of ERUS and CEUS, and it succeeded. We consider that ERUS guided transperineal biopsy is a reliable and safe method for preoperative pathological diagnosis of rectal SELs. And CEUS does help in the location of sampling. This method possesses advantages of both high resolution and low risk of infection[14], allowing acquirement of adequate tissue through a short puncture path. And it can be carried out under local anesthesia in outpatient department, with a lower cost of time and money[12]. The needle does not go through the rectal wall, so there is no need for bowel preparation. This method also reduces the application of antibiotics due to a low risk of infection[15].

For patients manifesting as rectal SELs and suspected tumors, to obtain preoperative diagnosis is difficult. ERUS guided transperineal biopsy can be one of the supplementary methods to routine biopsy work-up, especially for the difficult cases after several times of failure for insufficient sampling and risk of puncture complications. In addition, freehand biopsy is more suitable for underdeveloped areas because it does not need needle guide kit and costs less.

There are some limitations of this approach. For patients with stenosis in the rectum or anal tube, or those with high-position lesions beyond the display limit of the probe, the transperineal CNB guided by ERUS combined with CEUS would not be able to carry out.

CONCLUSION

Transperineal CNB guided by ERUS ensures adequate samples with a shorter puncture path, avoiding injuries to the bladder, bowel, uterus, vagina, and prostate. It also protects patients from infection caused by transrectal puncture. CEUS of the lesion can make it clear which points are the most appropriate for sampling. Transperineal CNB guided by CEUS combined with ERUS could be a supplement to traditional biopsy guidance method and it has the potential to be an important tool for preoperative biopsy of rectal SEL. The application could be limited by anorectal stenosis or with high-position lesions.

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