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EDITORIAL

COVID-19-induced transaminitis and hyperbilirubinemia: Presentation and outcomes

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Abstract

The risk of liver injury in patients with coronavirus disease 2019 (COVID-19) infection is quite evident. Furthermore, liver function test abnormalities are still detected in COVID-19 patients despite the development of antivirals and the availability of several types of vaccines. This editorial describes liver involvement during COVID-19 infection in patients with or without preexisting liver injury, such as chronic liver disease, to elucidate COVID-19-induced liver function abnormalities and their severity, pathophysiology, clinical manifestations, and clinical and laboratory outcomes. We also discuss the effect of vaccination against COVID-19 to better understand host factors, such as age, gender, and race, on the incidence and severity of liver dysfunction at initial presentation and during the illness. Finally, we summarize the results of relevant meta-analyses published to date and highlight the importance of adequate liver function monitoring in the current climate of the overwhelming COVID-19 pandemic.

Key Words: COVID-19; SARS-CoV-2; Liver injury; Transaminases; Hyperbilirubinemia; Pathophysiology

Core Tip: Recent evidence confirmed coronavirus disease 2019-induced liver function test abnormalities in patients with or without preexisting liver injury. Understanding the mechanism and recognizing the clinical picture, as well as identifying the risk factors for developing such abnormalities, will pave the way for early diagnosis and better management of such cases.

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INTRODUCTION

The continuing evolution of coronavirus disease 2019 (COVID-19) has led to the identification of a wide spectrum of associated symptoms, ranging from asymptomatic disease to severe manifestations that resulted in acute respiratory distress syndrome, respiratory failure, or multiple organ dysfunctions with risk of thrombosis and death[1,2]. Accumulating evidence indicates an association between COVID-19 infection and liver function test (LFT) abnormalities, and there have been many reports of liver injury, even in those without pre-existing liver disease[3-5]. It was shown that COVID-19 binds to angiotensin-converting enzyme 2 (ACE2) receptors (part of the renin–angiotensin system) to gain entry and damage the target organ[2]. Since ACE2 receptors are found in both bile duct epithelial cells (cholangiocytes) and liver cells (hepatocytes)[4], the liver is a potential target for direct infection. COVID-19 liver infection is related to disease severity and older age[5], and LFTs usually reveal a cholestatic or hepatocellular pattern[6]. Thus, a more detailed understanding of host factors including underlying comorbidities, in addition to adequate monitoring of patients with liver damage, is mandatory in the current overwhelming COVID-19 pandemic[7].

INCIDENCE OF COVID-19-INDUCED LIVER DYSFUNCTION

Sun *et al*[8] defined COVID-19-related liver injury as any liver damage occurring during disease progression and treatment in patients with or without pre-existing liver disease. The reported incidence of LFT abnormalities observed with COVID-19 is variable. A recent meta-analysis of 107 studies consisting of 20874 COVID-19-positive patients reported the pooled incidence of elevated liver enzymes at presentation as 23.1%[9]. A similar frequency was reported in another meta-analysis, where abnormal aminotransferase levels were present in 24% of 17776 patients[10]. Most enzyme elevations associated with COVID-19 infection are transient and self-limited[8]. Notably, elevated aminotransferase levels were reported in 14%–58% of hospitalized patients with COVID-19[4,11]. Additionally, the pooled prevalence of elevated alanine transaminase (ALT), aspartate transaminase (AST), and total bilirubin were evaluated as 21% (14%–29%),18% (13%–25%), and 6% (3%–11%), respectively[12]. Males are at high risk of getting acute liver injury related to COVID-19 than females since it was reported that bilirubin, ALT, alkaline phosphatase (ALP), and gamma-glutamyl transferase values were higher in male patients with COVID-19[13,14]. Regarding age, a meta-analysis by Kulkarni *et al*[10] revealed that the incidence of elevated liver enzymes in children (> 10 years) was 17.8% [95% confidence interval (CI): 9.9–29.8] among 283 patients, meanwhile, in adults it was 24.1% (95%CI: 20–28.8) among 12756 patients. There was also a significant difference in the prevalence of liver injury among different races and ethnicities. On adjusted analyses, white patients were less likely to develop liver injury compared with Asian patients (OR: 1.65, 95%CI: 1.37–2.02) and multiracial patients (OR: 1.65, 95%CI: 1.37–2.02). Those with a non-Hispanic ethnicity had a lower association with sustaining liver injury (OR: 0.77, 95%CI: 0.75–1.03)[15].

PATHOPHYSIOLOGY

The detailed mechanism of liver injury in COVID-19 infection remains unclear. Several possibilities involving a combination of direct viral-mediated effects due to viral replication within hepatocytes[8,9,16] and the viral-induced cytokine storm have been postulated[8,17]. Remarkably, severe acute respiratory syndrome coronavirus-1 RNA was detected in liver tissue from SARS-infected patients, although viral inclusions were not detected under electron microscopy[18]. Viral entry occurs through ACE2 receptors, which are expressed on many cell types, including hepatocytes and cholangiocytes[9,19]. Increased ACE2 expression in cholangiocytes (59.7% of cells) and, to a lesser extent, hepatocytes

(2.6% of cells) confirms that severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection alters liver function by direct cytotoxicity due to continuous viral replication[20]. However, severe SARS-CoV-2 infection results in a clinical state resembling sepsis due to the massive release of cytokines, which may progress to apoptosis and necrosis of infected cells, resulting in multiorgan failure late in the course of the disease[21]. Immune-mediated injury is supported by a marked rise in serum ferritin, lactate dehydrogenase, interleukin (IL)-2, and IL-6[6,19]. Pneumonia-associated hypoxia or ischemic hepatitis due to prolonged hypotension/shock is also speculated[5,6,8,9,16]. Exposure to hepatotoxic agents must be considered since drug-induced hepatotoxicity varies with age, sex, and race [22]. Antiviral agents directed against COVID-19 (*e.g.*, lopinavir or ritonavir), antibiotics used against bacterial infections, antipyretics, anticoagulants, and steroids may also cause liver function deterioration [23,24]. Additionally, underlying liver disease is considered a risk factor.

CLINICAL/LABORATORY MANIFESTATIONS

COVID-19-related liver injury may manifest as hepatobiliary symptoms and elevated liver enzymes. Among patients with COVID-19, liver symptoms are not atypical and may be present without any respiratory symptoms. Furthermore, hepatic symptoms are associated with worse clinical outcomes and an increased risk of mortality[25]. The incidence of a worse clinical outcome is high in hospitalized COVID-19 patients who suffer from jaundice. Additionally, the intensive care unit (ICU) admission rate is approximately 2.5 times higher for patients with hepatic jaundice ($P < 0.001$), mainly due to complicated bacterial sepsis or severe systemic inflammation[26].

Patients with COVID-19, both with and without pre-existing liver disease, may have elevated aminotransferase levels. A mixed pattern of both hepatocellular and cholestatic affection, without significant liver synthetic dysfunction has been reported, although ACE2 receptors are more frequently expressed on cholangiocytes than hepatocytes[4,27]. There is usually a greater elevation of AST than ALT levels, and this pattern has been associated with disease severity. Additionally, both ALT and AST are more usually elevated than bilirubin or ALP[28,29]. Sun *et al*[8] categorized the degree of liver damage as mild if ALT was elevated < 2 × upper limit of normal (ULN), moderate if 2 × < ALT < 5 × ULN, and severe if ALT > 5 × ULN. However, lower AST/ALT ratios may be more specific for hepatic injury[6]. A retrospective study evaluating the levels of hepatic enzymes of 1827 COVID-19 patients at admission and during hospitalization demonstrated abnormal levels of AST (66.9%), ALT (41.6%), and ALP (13.5%) at admission with peaks of AST (83.4%), ALT (61.6%), and ALP (80%) during hospitalization[30]. Another retrospective cohort study on 230 Covid-19 positive patients showed that the prevalence of abnormal liver enzymes among those with severe COVID-19 infection were as follows: AST (77%), ALT (49%), gamma glutamyl transpeptidase (GT) (37%), and ALP (12%). A severe COVID-19 infection was more likely present in patients with abnormal levels of AST ($P = 0.015$), gamma GT ($P = 0.022$), and ALP ($P = 0.03$)[31].

Regarding age, children appear to have a milder illness with significantly less need for inpatient admission or respiratory support and are less likely to have the multiple comorbidities present in older adults. Hepatitis is common in children with multisystem inflammatory syndrome and is associated with a more severe presentation and persistent elevation of LFTs in many patients[32]. Furthermore, older patients are more likely to develop more severe COVID-19 and at greater risk of abnormal liver function. The latter is more common in patients with severe or critical presentations of COVID-19[5]. In pregnant women with COVID-19, observational studies showed an increased prevalence of preeclampsia and hemolysis, elevated liver enzymes and low platelet (HELLP) syndrome. Despite a possible pathophysiology linkage between COVID-19 and HELLP syndrome, the evidence on temporality to prove a causal association between SARS-CoV-2 infection and HELLP syndrome is still insufficient[33].

A cohort study reported that acute liver injury with a hepatocellular pattern was common in patients who tested positive for SARS-CoV-2 but was usually mild[16]. However, 6.4% of patients had a severe liver injury with a severe disease course, where elevated AST levels may be indirect indicators of multiorgan involvement[34]. There is also a recent case report of a young male with COVID-19 who suffered from acute icteric hepatitis with a marked rise in bilirubin and liver transaminase levels without any respiratory symptoms[6]. Another case report found that COVID-19 infection could be a risk factor or comorbidity of acute liver failure, with only isolated hyperbilirubinemia indicating liver involvement[17]. Moreover, severe infections with COVID-19 followed by death were more often associated with hypertransaminasemia and high bilirubin levels compared with mild and moderate infections[14,35]. Patients with severe liver injury were more likely to need ICU-level care, intubation, and renal replacement therapy and showed a greater risk of in-hospital mortality[6,16,36]. A high bilirubin level and liver stiffness (measured using shear wave elastography) have been reported as correlated with more severe outcomes[37-39]. Liver injury and failure are frequently observed in critically ill cases, and their occurrence is associated with high morbidity and mortality[40-42]. Recently a potential link between Omicron variant infection and severe hepatitis of unknown etiology in children was observed, where it was postulated that previous infection or co-infection with SARS-CoV-2

increases the susceptibility to adenovirus infection[43]. Figure 1 summarizes the clinical and laboratory presentation of COVID-19-induced liver dysfunction.

COVID-19 IN- PATIENTS WITH PRE-EXISTING LIVER DISEASE

The impact of COVID-19 on chronic liver disease (CLD) is variable. Several studies have reported that patients with CLD, regardless of its etiology, may be at higher risk for severe illness from COVID-19[44-47]. A systematic review of 40 studies with 908032 participants (most of them from China and United States) showed that COVID-19 cases with CLD had significantly higher chance of having a severe form of COVID-19 (pooled OR: 2.44; 95%CI: 1.89–3.16) and death (pooled OR: 2.35; 95%CI: 1.85–3.00) when compared with COVID-19 cases without CLD[48]. A United States-based multicenter study reported a mortality rate of 12% in COVID-19 patients with pre-existing liver disease compared with 4% in those without[49]. Li *et al*[5] found that patients with underlying chronic hepatitis B virus infection suffered from a higher rate of severe or critical COVID-19 illness than mild/moderate illness ($P < 0.0001$). However, it remains unclear whether COVID-19 infection causes an already susceptible liver to fail or is just a risk factor for fulminant hepatic failure[21]. Acute and chronic liver failure related to COVID-19 infection has been shown in patients with decompensated alcoholic and non-alcoholic liver cirrhosis[50,51]. Whether cases with cirrhosis and COVID-19 are at higher risk of decompensation or development of acute-on-chronic liver failure, as has been reported for influenza infection, remains undetermined [52]. Notably, existence of metabolic-associated fatty liver disease (MAFLD) was considered an independent factor for the severity of COVID-19 in a series of non-diabetic COVID-19 infected cases, indicating an injurious bidirectional relationship between liver disease and COVID-19 infection[53]. Obesity is an essential criterion in MAFLD[54], and it was reported that the severity of COVID-19 showed a six-fold increase in obese patients with MAFLD[28]. Furthermore, patients with MAFLD and a high fibrosis score were more liable to suffer from severe COVID-19 disease, regardless the existing metabolic abnormalities[55]. Large amounts of IL-6 are produced in patients with severe COVID-19, particularly those with obesity, and this is considered a primary factor in triggering a systemic inflammatory response and cytokine storm, as well as multiple organ dysfunctions[53,56]. Moreover, there is altered secretion of inflammatory lipid mediators and a reduction in adiponectin levels in obese patients with MAFLD[57]. In patients with underlying advanced CLD, SARS-CoV-2 infection could lead to hepatorenal syndrome and liver transplantation[50]. Liver transplant recipients and other immunosuppressed patients who have COVID-19 may have a longer duration of viral shedding than non-immunosuppressed patients[58]. Additionally, Center for Disease Control and Prevention considers patients on immunosuppressive therapy for autoimmune liver diseases (AILD) are at high risk for severe COVID-19 disease and have prolonged viral shedding[59]. Meanwhile, patients with well-controlled Wilson disease or with genetic hemochromatosis showed no increased risk of having COVID-19 infection[60]. On the other hand, children with CLD, including those with AILD and post liver transplant, do not have an increased risk for severe COVID-19 disease, with little or no liver dysfunction [61,62]. It was found that the risk of mortality in COVID-19 patients is associated with the severity of the underlying liver diseases[45,57]. A recent meta-analysis based on confounding cofactors-controlled data demonstrated that cirrhosis was an independent risk factor for prediction of mortality associated with SARS-CoV-2 infection[63].

SARS-COV-2 VACCINATION AND LIVER DISEASE

Patients with CLD can receive a COVID-19 vaccination, although the immunogenicity and effectiveness of these vaccines have not been fully evaluated in this group of patients. However, vaccination has been associated with a lower risk of COVID-19-related infection and mortality in patients with cirrhosis[4,64]. Following COVID-19 vaccination, immune-mediated liver injury (ILI) is not well-characterized. A recent meta-analysis of 23 patients (mean age, 55.3 years) showed jaundice as the most common symptom (78.3%). Peak bilirubin, ALT, and ALP levels were 10.8 (6.8–14.8) mg/dL, 1106.5 (757.0–1702.5) U/L, and 229 (174.6–259.6) U/L, respectively. Histological examination showed intense portal lymphoplasmacytic infiltrate with interface hepatitis. The mean duration between receiving the vaccine dose (either first or second) and subsequent development of liver injury was 17.3 (11.2–23.4) d. Steroids were used in 86.9% of cases, and complete response, recovery, and death were reported in 56.5%, 39.1%, and 4.3% of cases, respectively. A temporal course between vaccination and the onset of liver injury was reported. Shroff *et al*[65] noted that most cases of severe liver injury were described after SARS-CoV-2 mRNA vaccines. Most cases occurred following the first vaccination dose, and two developed ILI following the second dose. Interestingly, there was one case of ILI after both doses of vaccine. It is also notable that pre-existing comorbidities (69.6%) were common, including liver disease in 26.1% and thyroid disorders in 13% of patients[66].

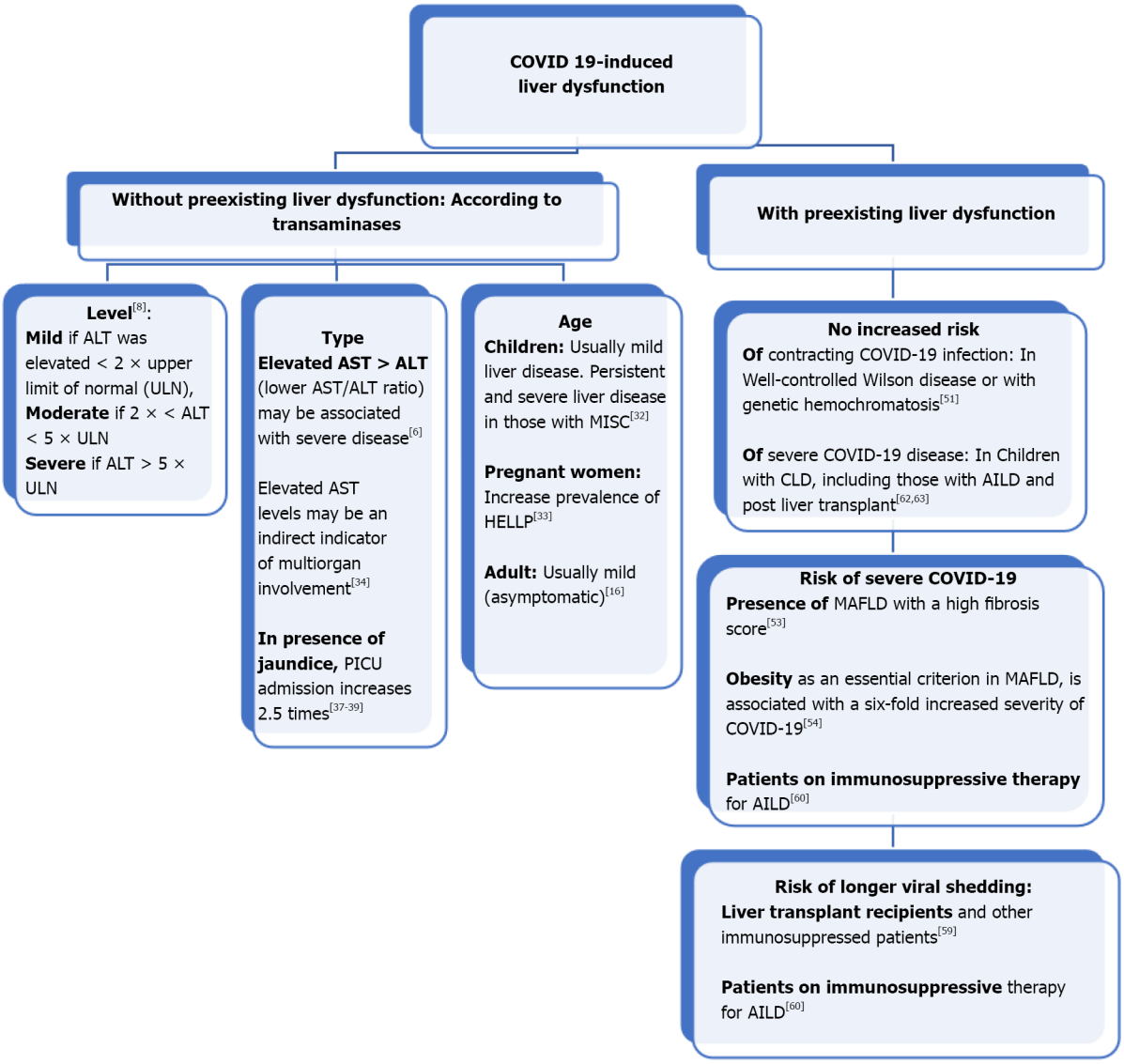

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Figure 1 Summary of coronavirus disease 2019-induced liver dysfunction. MAFLD: Metabolic-associated fatty liver disease; AILD: Autoimmune liver diseases; COVID-19: Coronavirus disease 2019; ALT: Alanine transaminase; ULN: Upper limit of normal; AST: Aspartate transaminase; PICU: Pediatric intensive care; CLD: Chronic liver disease; HELLP: Hemolysis, elevated liver enzymes and low platelet; MISC: Multisystem inflammatory syndrome of children.

CONCLUSION

This editorial sheds light on liver involvement in COVID-19 patients with and without pre-existing liver injury. Further studies are necessary to elucidate the etiology and mechanism(s) of liver dysfunction associated with COVID-19 infection, particularly in patients aged less than 18 years. Liver function should be monitored carefully during COVID-19 infection.

FOOTNOTES

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OPINION REVIEW

Tranexamic acid may be a useful pharmacotherapy for endoscopically resistant small bowel angiodysplasia

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Abstract

Small bowel angiodysplasia (SBAD) is reported to account for nearly 50% of cases of small bowel bleeding. When SBAD occurs frequently, it is difficult to treat all the angiodysplasias endoscopically, and gastrointestinal bleeding often recurs. Hormone therapy, somatostatin analogs, thalidomide and vascular endothelial growth factor (VEGF)-neutralizing antibodies have been reported to reduce gastrointestinal angiodysplasia (GIAD) bleeding. However, there is no strong evidence to recommend them. Also, there are no guidelines for their use. Hereditary hemorrhagic telangiectasia (HHT) is a hereditary disease caused by abnormalities in VEGF, resulting in multiple GIADs. A treatment guideline has been created for GIAD in HHT, and the use of tranexamic acid, an antifibrinolytic agent, is the first recommendation pharmacotherapy for GIAD with gastrointestinal bleeding that is difficult to treat endoscopically. It has been reported that fibrinolysis is accelerated in GIAD patients who are not HHT, similar to HHT patients. The use of tranexamic acid for gastric antral vascular ectasia in GIAD has been reported to be useful. However, there are very few reports of its use for SBAD. There are concerns with tranexamic acid use regarding the development of thrombosis/embolism, but there are few reports of such side effects. Future clinical trials including tranexamic acid for SBAD are desired.

Key Words: Angiodysplasia; Intestine; Hereditary hemorrhagic telangiectasia; Tranexamic acid; Endoscopic treatment; Pharmacotherapy

Core Tip: It is difficult to treat all multiple small bowel angiodysplasias (SBAD) endoscopically. Four main types of drugs, including somatostatin analogs, hormone therapy, thalidomide, and vascular endothelial growth factor-neutralizing antibodies, have been reported for use in gastrointestinal angiodysplasias (GIAD). However, there is no recommended pharmacotherapy for SBAD. Tranexamic acid is recommended for patients with GIAD in hereditary hemorrhagic telangiectasia who are difficult to treat endoscopically. Investigation of the use of tranexamic acid for SBAD is desired.

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INTRODUCTION

Gastrointestinal angiodysplasia (GIAD) is a benign vascular malformation of the GI tract and is a frequent source of GI bleeding. AD has been reported under various names, such as vascularectasia, angioectasa, and angiectasia, but since all of these generally present with the same lesions, they are collectively referred to here as AD. GIAD patients include patients with isolated AD in the stomach, small bowel, and large bowel; gastric antral vascular ectasia (GAVE) secondary to cirrhosis; and Heyde's syndrome secondary to aortic stenosis. GIAD causes 10% of all GI bleeding cases and 50% of small bowel bleeding cases[1]. GIAD has also been reported to cause approximately 4%-7% of upper nonvariceal bleeding, 30%-40% of occult small bowel bleeding, and 3%-40% of colonic bleeding episodes [2].

GIAD diagnosed as a source of GI bleeding often causes rebleeding even after endoscopic treatment, and subsequent treatment is difficult in these cases. After hospitalization and treatment in patients diagnosed with GIAD as the source of GI bleeding, the rate of rebleeding reaches 20% 30 d after discharge, and medical expenses are high[3]. In addition, a systematic review showed that 42.7% (209/490) of small bowel AD (SBAD) patients had rebleeding even after endoscopic treatment, and subsequent treatment is difficult in cases of rebleeding[4].

A major reason for rebleeding is that ADs tend to occur frequently. Even gastric ADs such as GAVE occur so frequently that endoscopic treatment is difficult. In addition, it has been reported that 2 or more ADs in the small bowel were observed in more than 63% of patients[5]. It is difficult to perform enteroscopy frequently for multiple SBADs, and it is difficult to find all the ADs in the long length of the small bowel. In other words, even if one lesion is treated, the other lesions bleed. Some types of pharmacotherapies are desirable for patients with SBAD whose overt bleeding or anemia progresses even after multiple endoscopic treatments. There are no established pharmacological treatments for SBAD. However, several pharmacological treatments for GIAD have been proposed, such as somatostatin analogs, hormone therapy, thalidomide and angiogenesis inhibitors.

Hereditary hemorrhagic telangiectasia (HHT) is a congenital disease in which AD occurs frequently in various organs. In HHT, GIADs occur so frequently that endoscopic treatment is difficult. Therefore, the pharmacotherapy of HHT has been studied for a long time, many reports have been published, and guidelines have been established. In this paper, the GI bleeding section of the HHT guideline is outlined, the pharmacotherapy options for GIAD that have been performed thus far are introduced, and finally, the treatment policy for SBAD is considered based on the HHT guideline.

GUIDELINE FOR HHT

HHT is a multiorgan disease in which AD occurs frequently in the skin, mucous membranes, and GI tract, causing recurrent bleeding and arteriovenous malformations (AVMs) in the brain, spinal cord, lungs, and liver[6,7]. HHT is inherited in an autosomal dominant manner and is caused by abnormalities in vascular endothelial growth factor (VEGF)[8]. The gene associated with HHT type 1 is a transforming growth factor-beta binding protein of endothelial cells[9]. HHT types 1 and 2 arise from mutations in endoglin and activin receptor-like kinase 1, respectively[10].

Epistaxis is a serious problem in HHT patients, but GI bleeding due to multiple GIADs is also a major problem. HHT has international guidelines, updated in 2020[11]. The guidelines recommend endoscopic treatment for GIAD in patients with HHT first. Second, antifibrinolytic therapy is encouraged as pharmacotherapy for endoscopic treatment resistant cases. However, the degree of encouragement is weak. This guideline is due to a recent report that showed that the antifibrinolytic agent tranexamic acid reduced the need for endoscopic management in patients with HHT[12]. The latest report summarizing the main points of the guideline recommends using tranexamic acid as the first step after performing

argon plasma coagulation of actively bleeding lesions at the initial diagnosis. Systemic antiangiogenic agents are recommended as a next step if no improvement is seen with administration of tranexamic acid[13]. The guideline committee states that, where possible, the use of dual antiplatelet therapy and/or a combination of antiplatelet therapy and anticoagulation should be avoided in patients with HHT. If anticoagulation is not tolerated in HHT patients with atrial fibrillation, discontinuation of anticoagulation with alternative approaches, such as left atrial appendage closure, is recommended[14]. Since the bleeding in HHT patients tends to be serious, the use of anticoagulant therapy and antiplatelet therapy is recommended with the minimum amount that is necessary.

Patients with multiple SBADs often have fewer ADs than patients with HHT. However, SBAD is a similar pathology, and HHT is a reference for SBAD treatment. There are no trials of tranexamic acid in the pharmacological treatment of SBAD in non-HHT patients, nor does tranexamic acid appear in various reviews as a treatment for SBAD. The next section considers the drug treatment of GIAD, especially SBAD, with reference to HHT.

PHARMACOLOGICAL TREATMENT OF GIAD

According to an analysis of the MEDLINE, Cochrane, Scopus and Embase databases, there are four main pharmacological therapies for GIAD that have been reported: Somatostatin analogs, hormone therapy, thalidomide and angiogenesis inhibitors[15]. These drugs are discussed first, followed by a discussion of tranexamic acid, which is used in the treatment of HHT.

Hormone therapy

Studies of oral contraceptives have shown that there is a risk of thrombosis with hormonal therapy with estrogens and progestogens[16]. It has been reported that this hormone therapy affects blood clotting ability and shortens bleeding time[17], which leads to its use as a hemostatic agent. Activated protein C resistance was reported to be involved in this coagulation enhancement[18]. On the other hand, postmenopausal hormonal therapy has been reported to increase plasma fibrinolytic activity, plasma levels of D-dimer and tissue plasminogen activator activity[19]. Thus, the therapy does not appear to increase coagulability in postmenopausal women. The increase in plasma fibrinolytic activity due to postmenopausal hormone therapy may be considered a secondary phenomenon of hypercoagulability. Fundamentally, hormone therapy for GIAD is considered to be an attempt to utilize the enhancement of blood coagulability for hemostasis against GI bleeding.

In a double-blind, placebo-controlled, crossover trial using estrogens and progestogens in 10 patients with GIAD requiring frequent blood transfusions, it was reported that hormone therapy reduced the amount of transfusion[20]. Various studies have originated from this Lancet report in 1990. In a prospective observational study with an average follow-up period of 535 d in 43 patients with obscure GI bleeding, all 38 who received concomitant hormonal therapy were bleeding-free. In contrast, all 5 patients treated with estrogen alone had episodes of rebleeding[21]. However, a double-blind randomized control trial (RCT) was conducted in 72 noncirrhotic patients with hemorrhages of GIAD-probable origin; rebleeding occurred in 46% of the 35 patients; and no significant difference was observed[22]. As a result of this study, the number of reports on hormone therapy has decreased, but reports on treatment using hormone therapy have been published recently. For example, when comparing 6 mo before and after hormone therapy for GIAD, anemia improved, and the number of blood transfusions decreased[23]. Hormone therapy has side effects peculiar to hormone drugs, but it is generally safe and has the advantage of being inexpensive. For these reasons, the search for a hormone therapy treatment for difficult-to-control GI bleeding continues.

Somatostatin analogs

Since the 1990s, attempts have been made to use the somatostatin analogs octreotide and lanreotide to treat hemorrhagic GIAD. Somatostatin analogs for GIAD are thought to inhibit angiogenic promoters by affecting chemical factors such as VEGF, basic fibroblast growth factor, and insulin-like growth factor 1 and to stimulate relaxation of intestinal smooth muscle, relieve chronic submucosal vein occlusion, and reduce the intravascular pressure on the arterial side[24]. There are many reports showing that somatostatin analogs are effective in patients with GIAD, but most have a small number of patients and are retrospective studies[1]. The usefulness of somatostatin analogs was reported in an RCT targeting 70 patients in which the actuarial probability of remaining free of rebleeding at 1 and 2 years of follow-up was 77% and 68%, respectively, in the octreotide group and 55% and 36%, respectively, in the placebo group ($P = 0.030$)[25].

Recently, a systematic review of 212 patients from 11 studies investigating the usefulness of somatostatin analogs for patients with GIAD, with a median duration of treatment of 12 mo, was reported. Somatostatin analogs were reported to reduce the number of red blood cell transfusions by an incidence rate ratio of 0.18 ($P < 0.0001$). The most common side effects of somatostatin reported in this study were loose stools (3%), cholelithiasis (2%), flatulence (2%), and administration site erythema (2%)[26]. Another systematic review and meta-analysis suggested that somatostatin analogs are more useful

in patients with GIAD than hormone therapy[27]. Based on the above, somatostatin analogs are thought to be useful for the treatment of patients with GIAD. However, since most somatostatin analogs are a daily subcutaneous injection and are expensive, there is a high possibility that the number of patients that can be treated with them are small.

Thalidomide

Thalidomide was reported to inhibit angiogenesis by suppressing VEGF[28]. Recently, it was reported that EGF-like domain multiple 6 (EGFL6) is overexpressed in patients with SBAD, and *in vitro* and *in vivo* assays reveal that thalidomide can act as an anti-angiogenic agent through the regulation of EGFL6 in a proteasome-dependent manner[29].

One RCT showing the efficacy of thalidomide for patients with GIAD has been published. The study randomized 55 patients with GIAD to receive either thalidomide 100 mg (n = 28) or iron 400 mg (n = 27, controls) daily for 4 mo. The treatment was considered to be effective when patients showed a 50% or greater reduction in bleeding episodes (fecal occult blood) in the first year of follow-up; the response rates in the thalidomide group and the control group were 71.4% and 3.7%, respectively (P < 0.001)[30].

Thalidomide is also considered useful in patients with HHT[31]. Therefore, thalidomide is listed as one of the systemic antiangiogenic therapies in the HHT guidelines[11]. However, thalidomide is highly teratogenic, and since it was developed as a sleeping drug, it has been shown to cause neurological symptoms such as somnolence in a drug-dependent manner. These issues limit its use.

VEGF-neutralizing antibodies

As previously mentioned, HHT is caused by abnormalities in VEGF. First, a VEGF-neutralizing antibody was reported to prevent cutaneous AVM formation and ameliorate the internal bleeding in Alk1-deficient adult HHT model mice[32]. In animal experiments, sorafenib and a pazopanib analog were reported to be effective against a mouse model of HHT, and there have been many case reports of the effectiveness of bevacizumab for HHT[33-39]. However, since these are all case reports, generally, VEGF-neutralizing antibodies cannot be judged to be effective, and further investigation is needed.

On the other hand, patients with GIAD did not show any abnormalities in their VEGF levels. In patients with GIAD, angiopoietin-2 was increased, but VEGF did not increase[40]. Similarly, when the serum from patients with SBAD, portal hypertensive gastropathy, and GAVE was compared with the serum from nonbleeding, nonanemic control patients, angiopoietin-2 was increased, but there was no difference in the blood VEGF concentration[41]. Although the relationship between GIAD and VEGF is not clear, there are reports that VEGF-neutralizing antibodies are effective in patients with GIAD. A study reported that bevacizumab was effective for treating GIAD in two patients with Heyde's syndrome[42]. There is one case report involving Heyde's syndrome[43] and another case report showing that bevacizumab is useful for patients with GIAD[44]. Since the number of reports is still small, VEGF-neutralizing antibodies cannot be judged to be effective, and further examination is necessary. Even if effective, VEGF-neutralizing antibody preparations are extremely expensive and difficult to use in many patients.

Tranexamic acid

Plasma hyperfibrinolysis has been reported to occur in both patients with HHT[45] and patients with hemorrhagic GIAD[46]. The latter report suggests that intrinsic ischemia may be the cause of GIAD, which may result in increased fibrinolytic activity in patients with GIAD. This plasma hyperfibrinolysis may promote GI bleeding in patients with GIAD. Tranexamic acid has been widely used as a hemostatic agent for many bleeding disorders by utilizing its anti-hyperfibrinolysis action. Tranexamic acid is a synthetic derivative of the amino acid lysine and exerts antifibrinolytic effects through reversible blockade of the lysine binding site on the plasminogen molecule[47].

A comparative study published in 1973 showed that tranexamic acid is effective in stopping bleeding in the upper GI tract[48]. Gastric juice has been reported to induce marked fibrinolysis, and tranexamic acid was expected to stop bleeding, especially in gastric lesions[49]. In a meta-analysis, tranexamic acid was shown to reduce mortality in patients with upper GI bleeding by 5%-54% and by 40% compared to placebo and is expected to be effective in a variety of nongastrointestinal bleeding disorders[47]. Tranexamic acid was also used for GAVE in patients with cirrhosis that was unresponsive to propranolol therapy and transjugular intrahepatic portosystemic shunt and was reported to reduce bleeding by 20%-30% and the need for surgery by 30%-40%[50-52].

As mentioned above, although the recommendations for it are weak, tranexamic acid is considered to be the first-choice drug for GI bleeding in patients with HHT that is difficult to treat endoscopically. For GIAD, it was reported for the first time in 1998 that tranexamic acid is effective for treating chronic bleeding from colonic AD in dialysis patients[53]. Jejunal AD with persistent bleeding is called Bernard-Soulier, and it was reported in 2013 that tranexamic acid is an effective treatment[54]. Tranexamic acid was reported to be very effective in a patient with multiple duodenal and jejunal ADs who had a medical history of ineffective hormone therapy; discontinued thalidomide treatment due to side effects such as nausea, dizziness, and severe fatigue; and discontinued octreotide treatment after one dose due to a hypoglycemic episode[55]. However, there are still only a few reports of the use of tranexamic acid

Table 1 Mechanisms of drugs used to treat gastrointestinal angiodysplasia and the main studies

Drug/therapy	Mechanism	Leading clinical trial	No. of cases	Result	Ref.
Hormone therapy	Enhanced coagulability				
		Double-blind, placebo-controlled, crossover trial for GIAD	10	Effective	[20]
		Prospective observational study for OGIB	43	Effective	[21]
		Double-blind RCT for GIAD	72	Not effective	[22]
		Systematic review for GIAD	63	Not effective	[27]
		Comparing before and after therapy for GIAD	12	Effective	[23]
Somatostatin analogs	Inhibition of angiogenic promoters				
	Relaxation of intestinal smooth muscle	RCT for gastrointestinal bleeding due to GIAD	70	Effective	[25]
		Systematic review for GIAD	72	Effective	[27]
		Systematic review for GIAD	212	Effective	[26]
Thalidomide	Anti-angiogenic agent				
		RCT for GIAD	55	Effective	[30]
VEGF-neutralizing antibodies	Angiogenesis inhibition				
		Only case reports			
Tranexamic acid	Antifibrinolytic effects				
		Observational study for GAVE	8	Effective	[51]
		Retrospective study for HHT	42	Effective	[12]

OGIB: Obscure gastrointestinal bleeding; GAVE: Gastric antral vascular ectasia; HHT: Hereditary hemorrhagic telangiectasia; GIAD: Gastrointestinal angiodysplasia; RCT: Randomized control trial; VEGF: Vascular endothelial growth factor.

for patients with SBAD.

Since tranexamic acid is an antifibrinolytic agent, there are concerns about thrombosis and embolism, and the following reports have been published. First, there are reports of ischemic episodes and pulmonary embolisms with the use of tranexamic acid[52]. In addition, there is a case report in which tranexamic acid was used to treat SBAD in dialysis patients for whom endoscopic treatment was difficult, and thrombosis of the arteriovenous fistula occurred[56]. In contrast, a meta-analysis of 4747 patients undergoing cesarean section or vaginal delivery found no association between tranexamic acid and deep vein thrombosis[57]. Additionally, in an RCT conducted in patients undergoing bilateral total knee arthroplasty, adverse effects such as deep vein thrombosis and pulmonary embolism were not significantly different between 245 patients who received tranexamic acid and 271 who did not[58]. Furthermore, a review of short-term tranexamic acid use in postdental surgery patients on anticoagulant therapy found no complications, such as thrombosis, in 125 tranexamic acid-treated patients[59]. Based on these reports, tranexamic acid appears to be relatively safe for short-term use. However, there have been no reports on the long-term use of tranexamic acid, especially during anticoagulant or antiplatelet therapy for myocardial infarction or cerebral infarction, and caution should be exercised when using tranexamic acid in patients with these high-risk diseases. Although there are concerns about the risks described above, tranexamic acid is an inexpensive drug that can be expected to reduce the amount of GI bleeding in patients with SBAD who are difficult to treat by endoscopy. Future reports are needed.

CONCLUSION

Table 1 summarizes the drugs currently being studied for use in patients with GIAD, including SBAD. There are several reports that hormone therapy, somatostatin analogs, thalidomide and VEGF-neutralizing antibodies are useful for SBAD for which endoscopic treatment is difficult. Hormone therapy is a good choice considering its less side effects and costs, but there are negative reports. Due to the small number of reports, it is not possible to decide which drug to strongly recommend. Tranexamic

acid has been adopted as a first-line pharmacological treatment in the guidelines for GIAD in HHT patients who are difficult to treat endoscopically. It is also effective for GAVE in non-HHT patients. Although there are concerns about the risk of thrombosis and embolism, tranexamic acid is expected to reduce the amount of GI bleeding in patients with SBAD in whom endoscopic treatment is difficult. Future reports are expected, as tranexamic acid could be a first-line drug for patients with SBAD.

FOOTNOTES

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REVIEW

Are we ready for telemonitoring inflammatory bowel disease? A review of advances, enablers, and barriers

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Abstract

This review summarizes the evidence about telemonitoring in patients with inflammatory bowel disease (IBD). To give an overview of the advances performed, as well as the enablers and barriers which favoured/hindered telemonitoring implementation. We performed a literature search in PubMed, EMBASE, MEDLINE, Cochrane Database, Web of Science and Conference Proceedings. Titles and abstracts published up to September 2022 were screened for a set of inclusion criteria: telemonitoring intervention, IBD as the main disease, and a primary study performed. Ninety-seven reports were selected for full review. Finally, 20 were included for data extraction and critical appraisal. Most studies used telemonitoring combined with tele-education, and programs evolved from home telemanagement systems towards web portals through mHealth applications. Web systems demonstrated patients' acceptance, improvement in quality of life, disease activity and knowledge, with a good cost-effectiveness profile in the short-term. Initially, telemonitoring was almost restricted to ulcerative colitis, but new patient reported outcome measures, home-based tests and mobile devices favoured its expansion to different patients´ categories. However, technological and knowledge advances led to legal, ethical, economical and logistic issues. Standardization of remote healthcare is necessary, to improve the interoperability of systems as well as to address liability concerns and users´ preferences. Telemonitoring IBD is well accepted and improves clinical outcomes at a lower cost in the short-term. Funders, policymakers, providers, and patients need to align their interests to overcome the emerging barriers for its full implementation.

Key Words: Inflammatory bowel disease; Telemedicine; Telemonitoring; Information and

communication technology; Crohn´s disease; Ulcerative colitis

Core Tip: In this review we focus on the advances performed in telemonitoring of patients with inflammatory bowel disease, taking into consideration the elements which enabled its use and how technological achievements led to other barriers for its full implementation. We detail the impact of telemonitoring on health outcomes and its cost-effectiveness. We also describe the advances on new patient-reported outcome measures, home-based tests and wearables which improve the ability to manage new patients´ profiles remotely. However, during the pandemic, e-mail and telephone still represented the main resources used. Then, we describe the emerging barriers which explained the limited application of mature telemonitoring programs.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of disorders characterized by the chronic and recurrent inflammation of different segments of the gastrointestinal tract, which usually associates extraintestinal manifestations and complications due to sustained activity. Unlike other chronic pathologies, IBD mainly affects young individuals in their optimal period of personal and professional development. As such, IBD is related to high levels of school absenteeism and work disability[1], interference with social activities, and impaired health-related quality of life (QoL)[2]. Therefore, IBD has a significant medical, social, and financial impact, further increased by the global increase in its incidence and prevalence in recent years[3].

It is suggested that the "treat-to-target" strategy leads to better outcomes[4]. However, in the conventional management of IBD, scheduled outpatient visits show difficulties to address the disease evolution in each patient, with frequent discrepancies between medical practice and guideline recommendations. Furthermore, patients have little involvement in decision-making, and nearly 50% of adults[5] and an even higher percentage of adolescents with IBD[6,7] are nonadherent to treatment. All these factors prevent the effectiveness of traditional interventions in disease control and increase health expenses[8], especially considering that patients with IBD use health care resources more often than patients with other conditions[9].

Nowadays, health systems are facing financial problems, and telemedicine has been proposed as an alternative to provide an efficient and equitable use of health resources. Information and communication technologies (ICTs) have the potential advantages of providing better communication between healthcare providers and patients, as well as educational resources adapted to patients´ needs. On the one hand, communication improvements could overcome limitations of health access in remote areas, also developing telementoring systems and contact between different specialists in centres where multidisciplinary teams are not available. On the other hand, educational elements could favour patients' empowerment and treatment optimization throughout the disease course[10,11], also addressing behavioural and psychological factors related to nonadherence[5].

Telemedicine has been successfully used in other chronic diseases such as congestive heart failure[12,13], diabetes mellitus[14,15] or chronic obstructive pulmonary disease[16,17] and showed excellent acceptance by patients, improvement in health related QoL and a reduction in hospitalizations[12,16,18]. Owing to these positive results, telemedicine systems have been evaluated in patients with IBD, especially in mild to moderate ulcerative colitis (UC)[19,20]. Telemedicine in IBD started with the adaptation of telemonitoring programs previously used in other chronic pathologies[21], but these were subsequently replaced by web and m-health systems, which represented more attractive options to maintain patients adherence to remote follow-up.

Telemonitoring is the main form of telemedicine in IBD. It is based on the provision of health services at a distance, related to diagnosis, treatment, follow-up or education. It is characterized by the structured and continuous monitoring of clinical data that is self-reported by patients in their usual environment, and then sent to health providers. The objective is the early detection and intervention on complications related to the disease itself or its treatment. It usually includes tele-education interventions and shares many features with other domains of use of ICTs in the health-care setting (Figure 1). Web telemonitoring in IBD is safe and reduces the duration of disease flares[22]. Moreover,

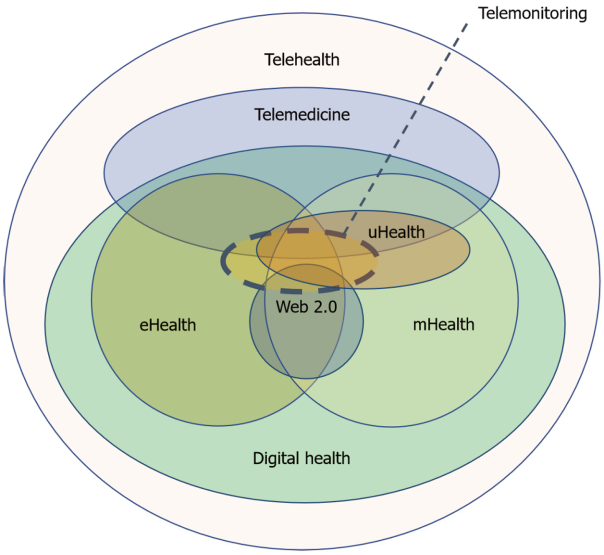

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Figure 1 Telemonitoring in relation to other domains associated with the use of information and communication technologies in the health context.

patients´ empowerment has been related to a reduction in outpatient visits and hospitalizations[23-26], which represent potential cost savings[22,24,27].

The development of more sophisticated telemonitoring programs and point of care (PoC) testing during recent years provided additional value to remote follow-up in the IBD context, improving the ability to cover different patients' profiles more objectively. These advances gained special interest after the advent of coronavirus disease 2019 (COVID-19) outbreak, as distance management offered new elements to overcome healthcare challenges posed during the pandemic[28]. However, telemedicine was represented mainly by telephone and e-mail, previously available in many centres[29,30], but the development of mature telemedicine programs integrated with electronic health records were still the exception, due to a series of remaining barriers.

In this review we focus on the advances performed in telemonitoring of patients with IBD, taking into consideration the elements which enabled its use and how technological achievements led to other barriers for its full implementation. The search strategy is detailed in the Supplementary material.

TELEMEDICINE IN CHRONIC DISEASES

There is a wide heterogeneity of telemedicine programs considering the different types of technological resources used, the different diseases and populations in which they are applied, and the objectives pursued with their use. The variability in the quality and design of the published studies (randomized clinical trials, before-and-after studies, qualitative studies, *etc*.) can partially explain the variable results obtained. Furthermore, in some studies these systems are part of wider interventions, rendering the comparability between programs even more difficult. These factors limit the quality of evidence regarding the efficacy of telemedicine to improve outcomes in chronic diseases.

Despite this, ICTs have been used in a wide range of pathologies, with improvement of patients´ empowerment and with good acceptance[31]. With the aim of giving response to the rise in chronic diseases and multimorbidity worldwide, different projects in Europe have studied the use of telehealth, mainly in diabetes mellitus, cardiovascular diseases, depression, and chronic obstructive pulmonary disease (COPD).

There is moderate evidence about the efficacy of telehealth systems in the improvement of glycaemic control, mainly in terms of HbA1c in patients with type 2 diabetes mellitus. Active telemonitoring including providers´ feedback, as well as tele-education have shown positive results compared with usual practice[14]. In fact, the highest impact was seen in the combination of telemonitoring and tele-education for both patients and providers, allowing for shared decision-making[15].

In patients with heart failure, telemonitoring has been shown to reduce global mortality and hospitalizations compared to usual care[12]. Many telemonitoring systems were part of multidisciplinary programs managed by specialized nurses and incorporated tele-education and action plans before hospital discharge[13]. Interactive monitoring with healthcare providers has also shown to improve blood pressure in hypertensive patients, weight control and lipidic profile[32]. Video consultation and biosensors are especially useful in cardiovascular diseases, with reduced costs compared with other

pathologies[33].

Most digital resources used in the mental health context refer to the application of cognitive behavioural therapy (CBT) in patients with depression. Both traditional and e-Health CBT are effective [34], but its use with telemedicine programs could offer additional advantages, such as better accessibility. However, many studies with psychotherapies show a high rate of nonadherence to follow-up [35]. Similarly, in patients with IBD one clinical trial showed a significant improvement in QoL after 12 wk of self-administered computerized CBT, but this outcome was not maintained at 6 mo, with a high rate of dropouts[36].

In patients with asthma, the use of multiplatform programs combining tele-education, telemonitoring and individualized action plans reduced hospitalizations compared with traditional care, mainly in more severe patients[18]. In patients with COPD, the use of telemedicine also reduces hospitalizations, but without an improvement of global mortality[16]. With the development of mHealth, the use of SMS combined with telephone support is associated with an improvement in respiratory function and QoL in patients with asthma[32], but telemonitoring in COPD has not demonstrated any improvement in these outcomes[17,37]. Telemonitoring of patients with COPD is more expensive due to associated multimorbidity[33].

The use of telemedicine in digestive diseases is more limited, and most studies focused on IBD and irritable bowel syndrome. Unlike other chronic diseases such as diabetes mellitus, IBD implies the consideration of many clinical, biological, endoscopic, and even histologic variables to reach disease control. However, early detection of complications usually requires invasive tests in IBD. The absence of validated tools with adequate cost and accuracy to measure disease activity at a distance have represented an important limitation.

Most studies about telemedicine in IBD have emerged in the last decade. The development of mHealth, the validation of patient reported outcome measures (PROMs), PoC and home-based tests to measure fecal calprotectin (FC) near the patient improved the ability to evaluate more types of patients with IBD at a distance, even in more complex cases.

PROGRAMS FOR TELEMONITORING IBD

The increase in the capacity of data transmission and storage, as well as the evolution of wireless communications provided many resources that are easy to use and adaptable to IBD telemonitoring.

Initially, telemonitoring systems for IBD allowed communication between health centres and patient´s home using computers. Afterwards, the development of web-based systems permitted easy-to-use and cheaper telemonitoring programs. In the last years, mobile devices (Smartphone, Tablet, *etc.*) made it possible to establish the communication process with the patient during his/her daily activities. Furthermore, in other settings (such as cardiovascular diseases) the transmission of continuous physiological data through biotelemetry has evolved with the incorporation of wearables.

In the IBD setting, telemonitoring is a safe, acceptable, and effective option to improve clinical outcomes[38,39], but the results of studies are still variable. In this context, telemonitoring has mainly used programs requiring home installation or web-systems, although e-mail and telephone have supported some of these programs. The main telemonitoring platforms used in IBD are summarized in Table 1.

Home telemanagement systems

The Cross group was the first to apply ICTs in adult patients with IBD, mainly with UC. This research team developed a remote-control system (home automated telemanagement system: HAT system) made up of 3 stations, adapted from a program previously used in self-management of patients with asthma [40]. The Home Unit was made up of a portable computer that collected patients' information (symptoms, adverse effects, medication, *etc.*), and these data were then sent to a decision-support server connected to a provider's PC[21]. The computer created alerts if the values collected in a web portal surpassed pre-established thresholds. Moreover, the HAT system incorporated educative elements.

Different exploratory studies showed good acceptance with the use of this system. In 2 studies with 10 and 23 patients with IBD, all of them considered that the HAT system was simple and increased patients´ knowledge[21,41]. To confirm the acceptability and adherence to follow-up with this program, the authors performed a subsequent study with 25 patients followed-up over 6 mo. Adherence to the weekly questionnaire was 91% and 86% had a prescribed medication adherence over 80%. This good adherence corresponded to a trend towards improvement in disease activity and QoL levels, together with a statistically significant improvement in understanding the disease (P = 0.0015). These good results led to the hypothesis that the HAT system could be feasible for telemonitoring patients with IBD.

Subsequently, the same group designed a randomized clinical trial including 47 patients with mild to moderate UC. Twenty-five patients were controlled with the HAT system and 22 followed usual in-person visits together with educational support and individualized action plans to make groups more comparable[42]. The groups had similar baseline characteristics, except for the use of immunosuppressants in 56% of the study group and 27% of the control group (P = 0.05), which would indicate a

Table 1 Studies of Telemonitoring in inflammatory bowel disease

Ref.	Disease	Type of study	*n*	Application	Outcomes
Cross *et al*[21]	IBD	Noncontrolled, clinical trial	10	Telemonitoring, home unit-server PC provider	Feasible method Excellent patient acceptance
Cross *et al*[113]	IBD	Noncontrolled clinical trial	25	Telemonitoring, home unit-server PC provider	Feasible method Excellent patient acceptance Improvement in QoL, disease activity, and disease knowledge
Cross *et al*[42]	UC	Controlled randomized clinical trial	47	Telemonitoring, home unit-server PC provider	Feasible method Excellent patient acceptance Improvement in QoL
Elkjaer *et al*[115]	UC	Validation study in 2 groups	21	Telemonitoring through the web	Feasible method Excellent patient acceptance
Elkjaer *et al*[22]	UC	Controlled randomized clinical trial	333	Telemonitoring through the web	Feasible method Excellent patient acceptance Improvement in QoL, disease knowledge, and adherence
Pedersen *et al*[10]	CD	Pilot study, controlled	27	Telemonitoring through the web	Feasible and safe method for individualized scheduling of maintenance IFX treatment
Pedersen *et al*[11]	UC	Prospective noncontrolled study	95	Telemonitoring through the web	Feasible and improve adherence to therapy
Torrejón *et al*[56]	IBD	Descriptive, observational, retrospective	1784	Telecare through e-mail, phone calls, fax	Increased telematic contacts and decreased in-person care
Johnson *et al*[27]	IBD	Telemonitoring project	420	A web-guided programme	Effective, safe and cost savings
De Jong *et al*[24]	IBD	Controlled randomized clinical trial	909	Telemonitoring through the web (mHealth)	Reduced outpatient visits and hospitalizations
Carlsen *et al*[43]	IBD	Controlled randomized clinical trial	53	Telemonitoring through the web (mHealth)	Reduced outpatient visits No differences in disease activity, QoL or adherence compared with standard care
Walsh *et al*[112]	UC	Pilot study, non controlled	66	Telemonitoring through the web (mHealth)	Feasible and usable to measure disease activity, QoL and medication use
Del Hoyo *et al*[52]	IBD	Controlled randomized clinical trial	63	Telemonitoring through the web	Higher improvement in disease activity compared to usual care Similar improvement in QoL, social activities and satisfaction between groups
Cross *et al*[25]	IBD	Controlled randomized clinical trial	348	Telemonitoring through the web (mHealth)	Improvement in disease activity and QoL, although not superior to usual care Decrease in hospitalizations and increase in distance contacts
Bilgrami *et al*[48]	IBD	Controlled randomized clinical trial	222	Telemonitoring through the web (mHealth)	No differences in self-efficacy or patient activation compared with standard care
Schliep *et al*[47]	IBD	Controlled randomized clinical trial	217	Telemonitoring through the web (mHealth)	No significant improvement in depressive symptoms or QoL compared with standard care
Heida *et al*[45]	IBD	Controlled randomized clinical trial	170	Telemonitoring through the web, e-mail and telephone	Similar improvement in QoL compared to conventional care Reduction in outpatient visits and societal costs Satisfaction
Linn *et al*[46]	IBD	Controlled randomized clinical trial	160	Telemonitoring through the web or SMS combined with tailored counselling	Improved self-efficacy Satisfaction

Bonnaud *et al* [51]	IBD	Controlled randomized clinical trial	54	Telemonitoring through the web (mHealth)	Significant improvement in QoL A trend to reduce outpatient visits Satisfaction
McCombie *et al*[50]	IBD	Controlled randomized clinical trial	100	Telemonitoring through the web (mHealth) and home-based FC	Non-inferiority of QoL and symptoms Reduced outpatient visits

IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; QoL: Quality of life

higher level of disease activity in the experimental group. There were no statistically significant differences for improvement of disease activity, treatment adherence, and quality-of-life values between both groups at 12 mo. These results could be related to the small sample size as well as a higher dropout rate in the intervention group, possibly due to the platform design, which required installation and eventual repairs at home.

To avoid these problems, Cross and cols developed web telemonitoring using mobile devices[25].

Web-based systems

In the last decade, telemonitoring systems have progressively evolved with web programs and mHealth solutions. Web applications are less expensive, safe, and feasible in the management of IBD not only in adults but also in adolescents[43-45], and they are associated with a reduction in outpatient visits and hospitalizations[22,24,27,43,45].

A Danish group developed telemonitoring through the web under the concept of "Constant-care". The system was developed through the web http://www.constant-care.dk, which also incorporated an educational centre. These investigators designed a randomized controlled trial with 333 UC patients treated with 5-aminosalicylates (5-ASA) from different hospitals in Denmark and Ireland[22]. The intervention group introduced clinical data and analytic results in the web to guide changes in the follow-up schedule and treatment. This intervention was compared to usual care.

After 12 mo of follow-up, in both the Danish and Irish population 88% of patients showed good acceptance with the web telemonitoring. There was a statistically significant improvement in adherence to treatment after 4 wk and a lower duration of disease flares. This was related to the use of high doses of 5-ASA in 100% of patients from the intervention group in Denmark, who also had improved QoL and disease knowledge. However, these outcomes were not reproduced in the Irish population. Moreover, in the Danish population telemonitoring reduced outpatient and emergency department visits, which led to direct cost-savings of 189 euros per patient-year, but also to an increase of e-mails and telephone contacts.

The use of this web-system in paediatric patients also reduced outpatient visits and school absenteeism, without differences in disease activity, QoL and adherence to treatment compared to the control group[43]. In another study developed in the University of California with a telemonitoring program combined with tele-education, patients followed remotely used less corticosteroids and suffered less hospitalizations and emergency department visits, with cost reductions of 16%[23]. In short, these studies show that web-telemonitoring is feasible, safe and could reduce health costs, although there are reproducibility differences depending on the population in which telemonitoring is applied[19].

Moreover, web-systems have been used to individualize the treatment according to the disease course. In a prospective study with 95 patients with mild to moderate UC, web control allowed the adjustment of 5-ASA doses and improved adherence. This was related with a significant improvement in clinical activity and FC values after 3 mo of follow-up[11]. Telemonitoring has even been used to individualize the treatment schedule with infliximab. After 1 year of follow-up, there were no significant changes in disease activity and QoL, although there was an estimated cost-saving of 699 euros/patient, compared with a historic control group[10].

In the same line and to avoid problems generated with the HAT system in the pioneering studies, the Cross group developed a web system for the management of patients with IBD (TELE-IBD) through text messages. In a randomized clinical trial with 3 parallel groups (TELE-IBD weekly, TELE-IBD every other week and control group) in 3 reference centres for IBD, they included 348 patients who had at least one disease flare in the last 2 years. Seventy-five percent completed the study, with an improvement in disease activity and QoL in the 3 groups, but without a higher improvement in these outcomes, depressive symptoms, or self-efficacy in the web control group, although in another study self-efficacy improved when tailored counselling was associated[46]. Moreover, telemonitoring was associated with a change in the profile of health expenses. Less hospitalizations were seen in the telemonitoring group but with higher use of non-invasive tests and telephone or e-mail[25,47,48].

The largest clinical trial with a telemonitoring program to date was performed with the Dutch web myIBDcoach (http://www.mijnibdcoach.nl). This web allows distance monitoring of disease activity, treatment adherence and side effects, as well as nutritional status, smoking habits, QoL, fatigue, stress,

anxiety and depression. As other platforms, it provides educational elements to improve empowerment. Patients showed good acceptance with its use in a pilot study[49]. In a clinical trial including 909 patients with different disease characteristics, the use of this system reduced outpatient visits and hospitalizations compared to standard care after 12 mo of follow-up[24]. Similarly, a reduction in outpatient visits was also obtained in adolescents[43,45] and in adults who used home-based tests to measure FC[50]. In a pilot study performed in France with the EasyMICI-MaMICI® platform, a reduction in outpatient visits was also associated with a significant improvement in QoL and satisfaction[51].

Our study group evaluated the impact on health outcomes of the telemonitoring web platform TECCU (Telemonitorización de la Enfermedad de Crohn y Colitis Ulcerosa or Telemonitoring of Crohn's Disease and Ulcerative Colitis), as compared to standard care and telephone care. In a 3-arm randomized clinical trial, 63 patients (21 per arm) with complex IBD were managed with each follow-up method over 24 wk. At the end of the study, the percentage of patients in remission was higher in the TECCU group (17/21, 81%) compared to nurse assisted telephone care (14/21, 66.7%) and standard care (15/21, 71.4%). The telemonitoring group had more improvement in disease activity, and this was associated with a larger reduction in FC values. All completers adhered to treatment in the TECCU group, while QoL, social activities, and satisfaction improved in all 3 groups[52].

Telephone and e-mail support in web-systems

Telephone and e-mail are resources attended by both medical doctors and specialized nurses in some IBD units, with high capacity to solve problems at less cost[53-55]. These tools have also been used to coordinate action plans in telemonitoring systems.

In Spain, the Crohn´s and Colitis Care Unit model has been used since 1999 as a multidisciplinary model of continuous care for patients with IBD. This model manages health demands with distance management mainly through telephone or e-mail with the support of a web page, which includes educational elements. The number of users has risen over the years, with a reduction of in-person care [56]. In Illinois, the Sonar Project is based on monthly web monitoring of symptoms in patients with IBD. Nurses exert a central role and use telephone contact for those patients who send results out of normal ranges, and together with medical health providers management adjustments are performed. This system also reduced hospitalizations, emergency visits and costs[57].

Therefore, beyond the use of telephone and e-mail in units which work as centres for resource coordination, telemedicine in IBD is expanding through the use of web and mHealth systems. These include telemonitoring, tele-education and videocalls in some cases. Its application allows the development of projects to provide health resources in remote areas[58,59], mainly with the use of mobile apps and the integration of some of these platforms into the electronic medical records, as is the case of the app HealthPROMISE and mynexuzhealth[60,61]. These models promote collaboration and mentoring between specialists, which could reduce variability in medical practice and modify the structure of future health systems if they demonstrate to be cost-effective.

Cost-effectiveness of telemonitoring IBD

Although many data about cost-savings have been published, they refer almost exclusively to direct costs[22,27], without considering costs of installation and maintenance of platforms or indirect costs.

In the IBD setting, our research group published the first cost-effectiveness and cost-utility analysis of a telemonitoring program compared to telephone and standard care[62,63]. The differences between groups and statistical uncertainty in disease activity, quality-adjusted life-years, and costs were calculated using nonparametric bootstrap estimations. Even though our trial only included 63 patients, we imputed the original dataset 5 times, and the bootstrapping estimations allowed us to extract 1000 random samples (of 21 patients per arm) from each of the 5 imputations, thus generating 5000 bootstrap replications.

We concluded that there is a high probability (79.96%) that the use of the TECCU web platform for telemonitoring complex IBD patients produces a greater improvement in disease activity at a lower societal cost, compared with standard care. Telemonitoring through the TECCU platform saved €2250 per additional patient in remission (95%CI: €–15363 to 11086) *vs* telephone care, and telephone care saved €538 compared with standard care (95%CI: €–6475 to 5303). Moreover, the use of the TECCU platform and telephone care showed an 84% and 67% probability, respectively, of producing a cost saving per additional quality-adjusted life-year (QALY) compared with usual care, even considering the simulations that involved negative incremental QALYs.

With a similar methodology, our results were reproduced by de Jong *et al*[64] who concluded that telemedicine with myIBDcoach is cost-saving and has a high probability of being cost-effective, without a decline in QoL. In this study, telemedicine resulted in lower mean annual costs of €547/patient (95%CI: €1029-2143) without changing quality adjusted life years. At the Dutch threshold of €80000 per quality adjusted life year, the intervention had an increased incremental cost-effectiveness over standard care in 83% of replications.

The authors included all subtypes of IBD, whereas our study with the TECCU platform recruited complex patients with IBD who needed to start immunosuppressants and/or biologic agents. According to our conclusions, the big sample size recruited in this article is useful to confirm our prior results, and

the reproducibility of the favourable cost-effectiveness profile of telemedicine applied in IBD across countries and patients' characteristics. In fact, during the COVID-19 pandemic, similar cost-savings with a higher gain of QALYs have been observed with the use of telemonitoring for IBD in Hong Kong[65].

ENABLERS AND BARRIERS FOR THE IMPLEMENTATION OF TELEMONITORING IN IBD

Unlike the use of ICTs in other fields (streaming entertainment services, grocery delivery, e-banking, *etc.*), telemonitoring interventions deal with a series of barriers which hinder their definitive implementation to reorganize health systems, despite other associated advantages (Table 2)[66-71]. The factors which favoured and limited these changes can be classified in 5 groups: technological, organizational, legal, acceptability and costs[72].

MODELS OF TELEMONITORING IN IBD

Telemonitoring theoretically include three different diagnostic models: patient self-diagnosis, remote providers´ diagnosis and computer-assisted diagnosis. They usually work as triage systems but, beyond diagnostic capabilities, telemonitoring platforms allow for remote management of other aspects such as disease treatment or education. In the IBD setting, they usually combine self-management, remote providers´ management and computer-assisted telemanagement[10,11,21,22,24,25,42,43,45,47,48,50-52].

Patient self-management

Self-management refers to a dynamic, interactive, and daily process in which individuals engage to manage a chronic illness[73]. This process includes the ability to deal with their own symptoms, treatment, physical and social consequences, and lifestyle changes to maintain a satisfactory QoL[74]. In this sense, telemonitoring platforms for IBD have incorporated PROMS and home-based tests that allowed patients to self-report their health status. This information has been even used to guide treatment adjustments by themselves[22].

Resources for patients´ self-management: PROMs, home-based tests and wearable devices

Considering the "treat-to-target" strategy, the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) project recognized different evidence and consensus-based recommendations to optimize outcomes in patients with IBD. Among the different targets proposed, clinical remission and endoscopic healing were confirmed in the STRIDE-II actualization, while normalization of serum and fecal markers of inflammation have been determined as short-term targets[4]. There was agreement to evaluate disease remission with clinical indexes, PROMs and endoscopic criteria [or also radiologic criteria in Crohn's disease (CD)].

Usually, endoscopic disease activity has been considered the gold standard to measure inflammation and to consider mucosal healing, but endoscopy is invasive and expensive.

In this sense, with the aim of measuring inflammation non-invasively, different PROMs and PoC tests have been developed over last years. Moreover, some of these tools have been specifically validated for their use in telemedicine programs.

PROMs: A PROM is a measurement of any aspect of a patient's health status that comes directly from the patient, without the interpretation of the patient's responses by healthcare providers and without the need of laboratory tests[75]. PROMs are designed for screening of disease activity, and then they need to be sensitive enough, especially if it implies more false positive results.

The Simple Clinical Colitis Activity Index (SCCAI) has shown a high correlation and good agreement between patient and clinician reported versions[76]. Compared to UC, PROMs used in the context of CD have shown worse correlation with other markers of clinical or endoscopic activity. The Harvey-Bradshaw index (HBI) had high correlation but only moderate agreement between versions registered by the patient or the clinician[77], although a recent version of the HBI self-administered by the patient through a mobile app showed a high percentage of agreement with in-clinic physician assessment, with a remarkably high PPV for remission[78]. Both SCCAI and HBI show good agreement between paper and online versions[78-80], and represent attractive tools for telemonitoring IBD.

Few PROMs have had their correlation with endoscopic activity evaluated. The global assessment of the patient, based on an analogic visual scale about how they felt regarding their UC during the previous 2 d, only showed moderate correlation with endoscopic activity[81]. The subscore of the global medical assessment and the 6-point Mayo index (which includes stool frequency and rectal bleeding) have a high correlation with the whole Mayo index[82]. Moreover, the 6-point Mayo index has an AUC of 0.80 compared to the endoscopic subscore[83].

Recently, the mobile Health Index was validated to monitor IBD activity through mHealth systems. In patients with CD, it showed high correlation and agreement with the Crohn´s Disease Activity Index and the HBI, as well as in patients with UC when it was compared to the partial Mayo index[84]. The

Table 2 Enablers and barriers for the implementation of telemonitoring in inflammatory bowel disease

	Enablers	Barriers
Technological	Adequate support	Lack of EMR integration
	Sufficient training	System maintenance required to avoid malfunction
	Fast internet connections	
	5G network	
Organizational	Continuous monitoring	Multidimensional nature: complex comparability between programmes
	Overcome geographic barriers	
	Safe assistance during COVID-19 pandemic	Lack of robust data: small studies, short-term follow up periods
	Structured data collection	
	Favours experimental studies and epidemiological surveillance	Lack of standardized remote medical practice (Interstate Mecial Licensure compact in the United States)[66]
	Multicentric access to data	Reimbursement limitations
	Telementoring: professional support and education	
Legal		Lack of legal framework[67,68]
		Data security
Acceptability/accessibility	Patient empowerment	Technological knowledge[69-71]; Some demographic factors increase the likelihood of a telematic encounter failure
	Wide use of smartphones	High drop-out rate in some clinical trials
	Wide use of wearable devices	
	Cheap internet plans	
Costs	Potential decrease of direct and indirect costs	High initial investment
		Limited cost-effectiveness data

intraclass correlation coefficient for test-retest reliability was high for CD and for UC. Nevertheless, its agreement with endoscopic scores was poor in CD and moderate in UC.

QoL and absence of disability are other targets of the STRIDE-II initiative. The Inflammatory Bowel Disease Questionnaire (IBDQ) was specifically validated in patients with IBD and has a moderate to high correlation with treatment response and the endoscopic Mayo index. However, their 32 and 36 items versions require a lot of time for their interpretation, so the reduced versions of 9 and 10 questions were subsequently validated[85,86]. On the other hand, the IBD disability index predicts active disease, nonadherence, and treatment with corticosteroids when high disability values are obtained[87]. Finally, health-related fatigue was incorporated in the Monitor IBD At Home index, but it is still not considered a specific target.

Probably the accuracy of PROMs increases when used in combination with FC. Thus, the Monitor IBD At Home index was developed to predict the endoscopic activity in patients with IBD. The association of FC to both the CD and UC versions showed high sensibility and NPV to rule out endoscopic activity[88]. The development of home-based FC tests that can be measured by the patient represent a potential option to measure disease activity in telemonitoring programs.

PoC tests and home-based tests: The use of PoC tests refers to patient specimens assayed at or near the patient with the assumption that test results will be available instantly or in a very short timeframe to assist caregivers with immediate diagnosis and/or clinical intervention[89]. In the IBD setting, the interest has centered on FC, and even though lactoferrin tests have been developed with adequate accuracy[90], FC offers better sensibility at certain cutoffs[90,91].

FC has good correlation with endoscopic activity in both UC[92,93] and CD[94-96]. FC helps to differentiate between functional and inflammatory diseases in patients with digestive symptoms. Moreover, in patients already diagnosed of IBD it allows the evaluation of disease activity, response to treatment, post-surgical recurrence and it predicts relapses after the withdrawal of anti-TNF agents[97,98]. These features, its non-invasiveness and a relative low cost makes FC tests in a useful tool in the diagnosis, monitoring and treatment adjustment in IBD.

Furthermore, the diagnostic accuracy of FC in different clinical scenarios has increased the interest in its use in telemonitoring IBD programs. In line with a patient-centered care and to favour empowerment, during the last years and the COVID-19 pandemic, different home-based FC tests have

been developed as an additional tool for a home-based follow-up[99]. These tests are based on kits that analysed faecal samples through immunochromatography. Then, the results are read with a smartphone camera, and they are sent through a specific app to a server accessible by providers (Figure 2).

Comparison between different home-based FC tests: Three main FC tests have been developed for its use by patients at home: CalproSmart, IBDoc and QuantonCal.

In a recent study, these 3 tests were compared with the ELISA method of the same manufacturer [100]. Considering the importance of obtaining good agreement in the low range of FC values (ruling-out disease activity), IBDoc, QuantonCal and CalproSmart have an 87%, 82% and 76% agreement, respectively, compared with their corresponding ELISA readings.

However, and similarly to its validation study[101], CalproSmart showed a trend to overestimate FC values with a mean bias of +141 μg/g (95%CI: -316 to 598 μg/g), while IBDoc tended to underestimate FC values with a mean bias of -105 μg/g (95%CI: -576 to 366 μg/g). This could generate more false positive results when using CalproSmart, but misclassification in the low (< 250 μg/g), medium (250-500 μg/g) or high (> 500 μg/g) range of FC values with this test showed large differences (*i.e.*, to classify as > 500 with CalproSmart and < 250 with ELISA, and viceversa) in only 2% of measures, compared to 5% in IBDoc and 8% in QuantonCal.

In any case, the error range between FC measured with home-based tests and their corresponding ELISA method was high. This happens especially when FC values are > 500 μg/g. With values ≤ 500 μg/g, differences were also over the acceptable range of 200 μg/g (+/-100 μg/g), but they were not wide enough to induce errors in the interpretation of inflammatory activity. Therefore, home-based tests are considered useful to rule-out inflammation at a distance when FC values are < 500 μg/g, but when values are > 500 μg/g disease activity should be evaluated with other methods.

Home-drug monitoring: Home therapeutic drug monitoring of monoclonal antibodies appears to be an innovative possibility to improve and simplify IBD management. To date, these tests are performed only in some hospital laboratories and results are not immediate. This delay, of months in some cases, impairs drug monitoring and dose adjustment, compromising its utility.

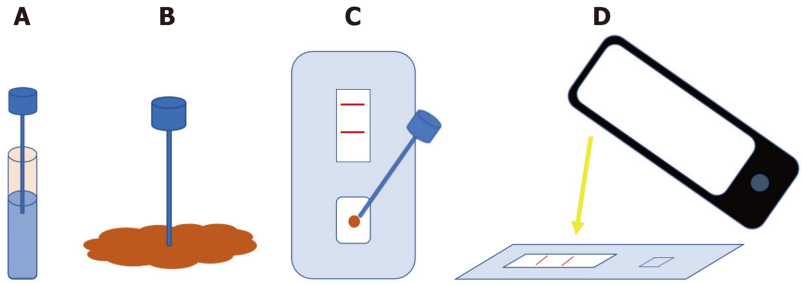
To solve this issue, home drug monitoring using dried blood samples is being evaluated in different inflammatory diseases. The first data were published by Kneepkens *et al*[102] in patients with rheumatic inflammatory diseases. Adalimumab and anti-adalimumab antibodies concentration measurements in finger prick dried blood spots were compared with simultaneous serum measurements. They found that both drug levels and antibody concentrations from the finger prick method correlated well with serum measurements (correlation coefficient > 0.87). However, some disadvantages should be considered, such as loss in precision, workload and elevated costs.

Berends and colleagues did a similar study with 40 IBD patients, comparing infliximab concentrations in dried blood samples and serum. Home-based test infliximab concentrations showed a good correlation (correlation coefficient: 0.671) with serum measurements[103]. This author also published data with adalimumab treatment one year later. A high correlation was found (Pearson's correlation: ≥ 0.96) between dried blood test and venipuncture results when performed at the same time during the outpatient clinic. Moderate correlation (Pearson coefficient = 0.51) was reported between home self-performed finger test and estimated adalimumab concentrations[104]. Larger studies are needed to confirm the reliability, accuracy, and cost effectiveness of home-based testing as a new telemonitoring tool in IBD.

Wearable devices: Wearable devices are electronic gadgets that consumers wear to track health-relevant physiological data to monitor and improve health[105]. IBD patient preferences and interest in wearable technology were evaluated by Hirten and colleagues using a 28-question survey. Four hundred patients completed the survey. Of these, 42.7% reported prior or current use of wearable devices, mainly smart watches (34.5%) and wrist band devices (29.1%). Almost 90% of subjects believed these gadgets could provide important information about their health and 93.8% reported that they would use them if it could help doctors manage their disease[106].

In the last few years, several studies have tried to demonstrate the utility of wearable devices in IBD telemonitoring[107]. The first available data was published by Yvellez *et al*[108] in 2018. They prospectively assessed daily health-related QoL, pain and sleep data using validated indexes through a mobile application and a Fitbit® device. Fitbit® compliance was almost 80%, suggesting this technology is feasible[108]. The Fitbit® device has also been used to predict disease activity. In one study a significant reduction in daily steps has been shown over the week before CRP or FC elevation, but without differences in daily resting heart rate[109]. Two years later, however, Hirten *et al*[110] demonstrated that significant changes in heart rate variability measured using VitalPatch® were observed before the development of symptomatic or inflammatory flare in ulcerative colitis patients.

Step count and sleep monitoring have also been used, not to predict flares but the intent to determine post-operative length of stay. Overall, step count and sleep duration/efficiency did not predict length of stay. However, in a multivariable linear regression model, significant interaction was found between postoperative complications and step count, suggesting that increased physical activity was associated with a reduction in duration of hospital stay[111].



DOI: 10.3748/wjg.v29.i7.1139

Figure 2 Procedure to measure fecal calprotectin at home. A: Extraction buffer; B: Collection of faecal sample; C: Control (C) and test (T) lines appear after application of extracted calprotectin to the sample window; D: A smartphone camera is used to read the result obtained.

Remote providers´ management

All the telemonitoring interventions in IBD reported in this review comprise healthcare providers´ advice. Most systems employ store and forward programs, where nurses acquire a central role in tracking the information received and making contact between patients and specialists to set up healthcare plans. Communication is established through websites, usually with the support of telephone and e-mail, as reported above.

Computer-assisted telemanagement

Computerized systems have been used for telemonitoring IBD and they usually work as a triage system to identify which patients might require further evaluation. Many of them can generate automatic action plans through the integration of different monitorable indicators in decision-making algorithms. In most telemonitoring programs tested thus far, these tools are combined with self-management and the remote providers´ management models seen above.

Telemonitoring systems in IBD are integrated by personal computers or mobile devices used by patients, a decision support server and a website for staff and providers. The website provides an interface to collect data from testing sessions, and these platforms usually generate automated reminders to favour adherence to follow-up. The structure of most eHealth tools in IBD are based on a traffic light system[10,11,22,25,27,43,45,52,112]. Patients usually enter their symptoms in scheduled online controls, mainly in a structured manner through PROMS, but many apps also include a comment box to freely express anything outside the questionnaires. Then, the patient´s status appears as red, yellow or green when disease is highly, moderately active, or quiescent, respectively.

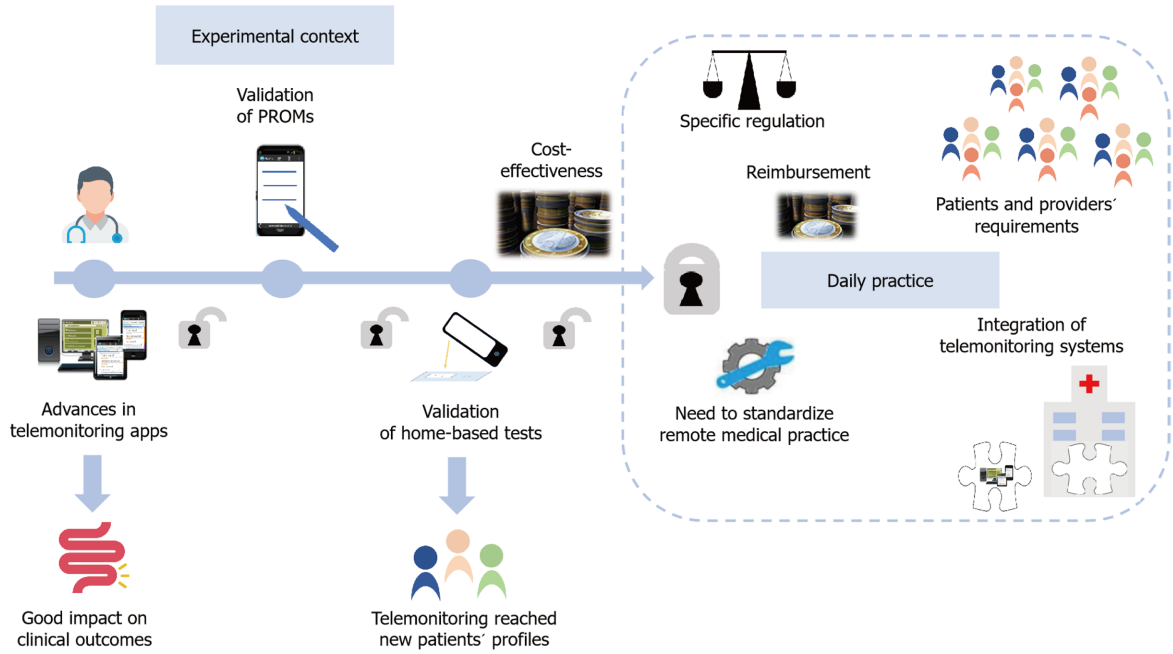
Some systems combine self-reported symptoms with the level of FC in a total inflammation burden score. In fact, recent clinical trials have incorporated FC tests performed by patients at home[45,50,112]. This status is sometimes supplemented with disease activity and QoL graphs[10,11,22]. Depending on the level of alert, simultaneous action plans and email alerts are sent to the participant and providers, who review the information to decide if further management changes are necessary.

Altogether, these new models and resources for patients´ self-management represent advances to reach the implementation of telemonitoring in daily practice, but still some legal, ethical, economical and logistic barriers need to be solved (Figure 3).

DISCUSSION

Telemonitoring is about communication, and the development of faster and wireless systems at a lower cost has supported the use of proactive remote monitoring. As well, the increase in data storage also favoured the incorporation of tele-education in most telemonitoring programs in the IBD setting. However, during the pandemic, e-mail and telephone still represented the main resources used[29,30], while the application of mature telemonitoring programs was the exception. As different enablers encouraged advances of telemonitoring in IBD, many other barriers emerged and hindered its full implementation in daily practice.

Pioneering studies evaluated telemonitoring programs previously used in other chronic diseases[21,42,113,114]. Feasibility and patients´ acceptance of these applications was excellent. Yet, they were not able to clearly demonstrate an improvement in QoL, disease activity, and treatment adherence. These systems were based on remote monitoring through computers, they needed to be adapted to the IBD context and required eventual repairs at home, which were time expensive. Telemonitoring subsequently evolved towards the use of web-based systems, which were cheaper and easy to use. Remote monitoring through the web demonstrated its feasibility and excellent patients' acceptance, with an improvement in QoL, disease activity, and disease knowledge[22,115].



DOI: 10.3748/wjg.v29.i7.1139

Figure 3 Advances in telemonitoring of inflammatory bowel disease related to the enablers and barriers for its implementation in daily practice. PROMs: Patient reported outcome measures.

In the last few years, telemonitoring prioritized the use of mHealth resources[24,25,116]. Beyond the improvement in clinical outcomes, mHealth telemonitoring associated cost-savings in outpatient visits and hospitalizations[23-26]. Almost parallel to the mHealth evolution, many PROMs have been validated to self-report disease activity. In line with patient-centered care, empowered patients can use new home-based calprotectin tests, which are accurate enough and useful to rule-out disease activity at low FC values[100,101]. In fact, new home drug monitoring is being developed to measure levels of monoclonal antibodies near the patient[103,104]. Furthermore, the development of mobile devices even enabled the increasing use of wearables to monitor physiological predictors of disease activity and postoperative length of stay[110,111]. These new tools could represent one of the first steps towards ubiquitous Health in IBD, and in a near future machine learning may allow the integration of large data sets in personalized algorithms.

Despite the technological and knowledge advances reached, the effect of telemonitoring on health outcomes is not consistent in different populations and health systems[22,24,25,42,44,45,52]. Initially, remote monitoring was mostly restricted to UC patients, while the design of new apps, PROMs and home-based tests allowed to progressively expand its use to a broad range of patients´ profiles. However, telemonitoring has not been demonstrated to improve QoL or clinical/endoscopic remission in the long-term. In addition, biomarkers in IBD are less accurate compared to other chronic diseases such as diabetes mellitus, and the early recognition of complications in IBD still require invasive tests in many cases.

On the other hand, although telemedicine has been traditionally considered cost-effective, cost-saving data previously published referred almost exclusively to direct costs[22,27]. The implementation of telemonitoring services represents a short-term high initial cost, not only from a technological point of view, but also by changes in the organization of the IBD units. Thus, decision-makers have had difficulties to support the implementation and investment in telemedicine due to a lack of solid evidence so far. In addition, these decisions become even more complicated in areas where reimbursement is an important factor in the setup of clinical activity. In this regard, recent studies suggested a good cost-effectiveness profile of telemonitoring[62-65], even after considering the costs of installation and maintenance of platforms, as well as indirect costs.

The availability of more powerful and cheaper communication tools turned technical challenges into legal, ethical, economical, and logistic issues[28]. To standardize remote medical practice, in the US the Interstate Medical Licensure Compact was created to increase efficiency in multistate licensing of physicians[66] but such a proposal is lacking in Europe. Besides, only a few examples of full integration of telemonitoring programs into electronic medical records are available to date[60,61]. In this sense, interoperability of systems while maintaining the confidentiality of data cannot be guaranteed in many centres. Moreover, the provision of remote health safely also requires a specific European regulation to protect remote medical practice and to lift some existing legal barriers. Finally, to keep adherence to follow-up, it is essential to adapt telemedicine programs according to patients' and providers´ character-

istics, because some demographic factors such as increasing age, commercial insurance status and racial differences increase the likelihood of a telematic encounter failure in some contexts[117].

CONCLUSION

Therefore, telemonitoring IBD is well accepted and improves clinical outcomes at a lower cost in the short-term. The advances performed on new PROMS, home-based tests and wearables improved the ability to manage new patients´ profiles remotely. However, it is still necessary to overcome many legal, ethical, economical and logistic barriers. Funders, policymakers, providers and patients need to align their interests to successfully implement telemonitoring, and further collaborative efforts based on teamwork between centres are essential to help reorganize health systems.

FOOTNOTES

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REVIEW

Mucosal healing and inflammatory bowel disease: Therapeutic implications and new targets

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Abstract

Mucosal healing (MH) is vital in maintaining homeostasis within the gut and protecting against injury and infections. Multiple factors and signaling pathways contribute in a dynamic and coordinated manner to maintain intestinal homeostasis and mucosal regeneration/repair. However, when intestinal homeostasis becomes chronically disturbed and an inflammatory immune response is constitutively active due to impairment of the intestinal epithelial barrier autoimmune disease results, particularly inflammatory bowel disease (IBD). Many proteins and signaling pathways become dysregulated or impaired during these pathological conditions, with the mechanisms of regulation just beginning to be understood. Consequently, there remains a relative lack of broadly effective therapeutics that can restore MH due to the complexity of both the disease and healing processes, so tissue damage in the gastrointestinal tract of patients, even those in clinical remission, persists. With increased understanding of the molecular mechanisms of IBD and MH, tissue damage from autoimmune disease may in the future be ameliorated by developing therapeutics that enhance the body's own healing response. In this review, we introduce the concept of mucosal healing and its relevance in IBD as well as discuss the mechanisms of IBD and potential strategies for altering these processes and inducing MH.

Key Words: Inflammation; Injury/repair; Mucosal healing; Mucosal barrier; Therapeutics; Colitis

Core Tip: Mucosal healing (MH) is vital in maintaining intestinal homeostasis and protecting against infection and injury. MH has emerged as an important clinical criterion in effective treatment of inflammatory bowel disease (IBD). However, there remains a relative lack of therapeutics that can restore MH due to the complexity of the disease and healing processes. Through increased understanding of the molecular mechanisms of MH, tissue damage from IBD may be ameliorated by developing novel therapeutics. Here, we introduce the concept of MH and its relevance in IBD, and discuss the mechanisms of IBD and potential strategies for altering these processes for inducing MH.

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INTRODUCTION

Mucosal healing (MH) is a process of wound repair that restores the integrity of damaged epithelial barrier and homeostatic function after an injury compromises barrier integrity[1]. It is a complex process regulated by multiple cell types, through distinct mechanisms in response to highly specific stimuli within multiple signaling and cytokine pathways[1]. For simplification, the MH process is considered to have three phases: Epithelial restitution, proliferation, and differentiation and maturation[1]. Restitution consists of epithelial cells migrating into a wound within hours, followed by proliferation of epithelial cells in hours to days, and finally differentiation of intestinal stem cells into all mature intestinal cell types[1-3]. Each phase is induced and regulated by multiple cytokines, growth factors, and cell types reviewed in[1] and can be influenced by many factors that enhance or prevent wound healing as well as by the source of barrier injury. When the homeostatic process of wound healing is slowed or delayed by external or genetic factors, chronic inflammation may develop because repair of the intestinal epithelial barrier (IEB) and subsequent reduction of inflammation will not occur unless wound healing mechanisms are present[3]. Due to inflammation and chronic immune response, the consequences of impaired MH are chronicity of autoimmune diseases, including Inflammatory Bowel Disease, (IBD) and its progression to colorectal cancer.

INFLAMMATORY BOWEL DISEASE OVERVIEW AND PATHOGENESIS

Inflammatory Bowel Disease (IBD) is a term that represents autoimmune inflammatory diseases of the gut, with ulcerative colitis (UC) and Crohn's Disease (CD) being the major disease types; however, the etiology of IBD remains unclear. IBD is known to have genetic and environmental risk factors, but the mechanisms by which these factors induce IBD are not well-understood[4]. Common symptoms include abdominal pain, diarrhea, weight loss, malnutrition, and particularly in CD, nausea, vomiting, intestinal blockages, fistulae, and abscesses[4,5]. IBD is an increasingly common and often debilitating disease, affecting up to 200 individuals *per* 100000 people in the United States[4]. Onset of IBD often occurs before the age of 30, and patients experience poor quality of life along with high risk of developing colorectal cancer due to the chronic and progressive symptoms and persistent inflammatory state[1,4]. Though multiple treatment mechanisms exist, including corticosteroids, anti-inflammatory medications, monoclonal antibodies, stem cell treatments, and surgery, IBD cannot currently be cured[4]. Therefore, continued research into the mechanisms of pathogenesis and development of new pharmaceuticals and treatment methods is vital to decrease mortality and improve quality of life for IBD patients.

Autoimmune disease pathogenesis is difficult to study due to the multifactorial causes of disease and complex molecular mechanisms, as well as the predicament of patients not presenting to the clinic until late in the disease development process. Although the disease complexity and difficulty in identifying the initial molecular instigators has thus far precluded full understanding of the specific molecular mechanisms of IBD pathogenesis, the general overarching processes have been elucidated. During IBD pathogenesis, the IEB and mucosal layers become damaged and inflamed due to injury and/or infection, which can develop into a state of chronic inflammation and reduced IEB integrity. Genetic factors can also play a role in pathogenesis, particularly in CD through genes such as a variant that impairs autophagy and dysregulates the IEB and gut microbiota[6,7]. The damage to the IEB results in microbial and antigen exposure in the intestinal lumen, leading to an inflammatory cascade and disturbed homeostasis[8,9]. Multiple cytokines and immune cell types are also thought to contribute to the pathogenesis of or protection against IBD; therefore, further study of the complex interactions governing the maintenance or breakdown of gut barrier homeostasis is imperative to deeper

understanding of IBD pathogenesis and the development of effective treatments.

MUCOSAL HEALING AND PATHOLOGICAL RELEVANCE IN IBD

The main goals of IBD treatment are two-pronged: Reducing symptoms and preventing new inflammation and intestinal injury through traditional treatment methods, and recently a new, ambitious goal of inducing wound healing of existing inflammation and damage[8]. Some current treatments may contribute to both treatment goals; however, many existing clinical treatments are more targeted toward the traditional goal of preventing inflammation and damage. Even with the advent of some newer MH-focused treatments, additional avenues of further enhancing MH should be explored. Although clinical remission or preventing new inflammation is possible, patients may still have residual disease symptoms during remission due to the defective wound healing process leaving previous intestinal damage unrepaired. Additionally, up to half of IBD patients experience non-response or loss of response to standard therapeutics, leading to relapse[10]. Hence, MH induction presents an attractive goal in effective long-term treatment of IBD and prevention of relapse, and thus also prevention of progression to colitis-associated colon cancer. In this review, we will summarize the main current and prospective treatments of IBD, as described in Table 1, and their benefits and limitations towards the goal of reducing inflammation and achieving MH. More detailed analysis of the mechanisms of action, safety, and efficacy profiles of current IBD therapies and clinical trials can be found in Neurath *et al*[11]. However, in this review, we will emphasize areas that have not yet been as extensively clinically explored, including the temporal control of gut immune function, which presents novel potential for fine-tuning of the immune system in MH and restoration of gut homeostasis. We will examine three major factors contributing to the pathogenesis and tissue damage of IBD, as shown in Figure 1: Gut barrier dysfunction, gut dysbiosis, and inflammatory cytokine responses. Specifically, we will focus on the prospect of altering these factors and associated pathways summarized in Table 2 for both the reduction of inflammation and induction of MH.

CURRENT STATUS OF IBD THERAPIES AND FOCUS ON ENHANCED MUCOSAL HEALING

Traditional Therapies

Historically, there has been a disconnect between the expectation of IBD treatment promoting MH and real treatment outcomes, as many therapies for UC and CD primarily target symptom relief and reduction of chronic inflammation[12]. Corticosteroids, Methotrexate, and surgery are typically utilized to achieve these goals, but they do not promote MH as the primary therapeutic endpoint[13,14]. Because UC and CD are progressive diseases, patients may still experience intestinal damage even during periods without physical symptoms, and disease progression is typically only slowed by these treatments, not stopped[12,15]. However, achieving MH may help stop disease progression as well as decrease symptom severity. New treatment plans broaden the therapeutic focus to include inducing MH through a variety of mechanisms, such as by altering the gut microbiome and altering inflammatory and anti-inflammatory cytokines with antibodies or exogenous cytokine therapies.

Inflammation Reduction and Immune Modulation Therapies

One such mechanism for emphasizing MH includes suppressing specific parts of the patient's immune system to decrease the main contributors of chronic inflammation. When new inflammation is reduced, it may then be possible for the wound healing process to begin to keep up with the rate of tissue damage. Methods of achieving this goal include enteral nutrition (EN), partial EN (PEN), 5-aminosalicylates (5-ASA), and Azathioprine treatments, which focus on decreasing IBD-associated inflammation by suppressing the host immune system in a less global manner than corticosteroids or preventing the production of inflammatory molecules. EN is a dietary treatment for IBD patients that can either totally (with EN) or partially (with PEN) replace solid food intake with specialized formula[16]. Although the mechanism by which EN induces MH is currently unknown, microbiome changes are implicated and are hypothesized to aid in decreasing chronic inflammation[17]. While typically a pediatric treatment, a pilot study shows MH in adults following EN and PEN treatment as well[16]. Currently, the largest drawback of EN treatment is low patient compliance, especially in adults[16]. 5-ASA drugs, which are used to induce remission in early IBD, are shown to induce MH in 43.7% of patients[18]. Azathioprine is a purine analog inhibiting purine metabolism and blocking T cell activation and co-stimulation, therefore functioning by suppressing the immune system and decreasing inflammation in IBD[11,19-21]. It is shown to achieve MH independently in some cases (30.1%) alone but is more successful when used in conjunction with anti-TNF-α antibodies such as Infliximab (44%)[22]. Other immunomodulating drugs shown to induce MH when given with monoclonal antibodies include Cylosporine and Tacrolimus[23].

Table 1 Summary of inflammatory bowel disease therapeutics

Treatment type	Available therapeutics	Mucosal healing relevance/ Success
Corticosteroids	Prednisone/ Prednisolone/ Methylprednisone	Prednisone treatment for 14 d (20 mg/day) decreased mucosal inflammation indicating a possible role in developing short-term MH[139]. 29% of patients in one study displayed endoscopic remission after steroid treatment[140].
Nutritional therapy	Enteral nutrition (EN)/ Partial enternal nutrition (PEN)	EN/PEN induce MH in both adults and children[14].
Aminosalicylates (5-ASA)	Sulfasalazine/ Mesalamine/ Olsalazine/ Balsalazide	On average induce MH in 43.7% of patients[141].
Immunomodulators	Azathioprine/ 6-mercaptopurine	Azathioprine alone has achieved MH in 16.5% of cases and in 43.9% when used in combination with antibody therapies[18]. After 16 wk of mercaptopurine treatment, patients in remission showed a 47.1% rate of MH[142].
	Cyclosporine	Shown to induce MH when used in conjunction with Vendolizumab[143].
	Tacrolimus	Shown to induce MH when used in conjunction with Vendolizumab[143].
	Methotrexate	After 36 wk, methotrexate treatment had a MH rate of 47.4%[142].
Monoclonal antibody/ Biologic therapies	Adalimumab	Induced MH in 24% of patients treated[24].
	Certolizumab	Clinical response rate at weeks 2 and 12 was 29.7% and 52.8% (respectively) in CD[25].
	Infliximab	Treatment induced MH in up to 60.3% of patients in phase 2 clinical trials[23].
	Natalizumab	MH achieved by 42.3% of patients after 14.1 mo of treatment[144].
	Risankizumab-rzaa	Endoscopic response and deep remission observed in 55% and 29% of patients (respectively), indicating MH[27].
	Ustekinumab	Treatment of individuals with moderate to severe CD showed MH *via* a reduced disease score after 8 wk[19].
	Vedolizumab	Has shown to induce MH in up to 50% of UC patients and 29% of CD patients in clinical trials[26,27].

MH: Mucosal healing; EN: Enteral nutrition; PEN: Partial enteral nutrition; 5-ASA: Aminosalicylates; CD: Chron's Disease; UC: Ulcerative colitis.

Monoclonal Antibody Therapies

Monoclonal antibodies targeting inflammatory cytokines are an emerging class of IBD therapies that more directly focus on permitting or inducing MH and are an attractive method of IBD treatment. Monoclonal antibody drugs targeting multiple cytokines are approved or in trials, and function by removing inflammatory cytokines. Anti-tumor necrosis factor-alpha (TNF-α) antibody-based drugs such as Infliximab, Adalimumab, and Certolizumab are biologic therapies that have achieved significant clinical success and are now widely used as front-line treatments for IBD, with the goal of reducing inflammation and promoting MH. The target of anti-TNF-α therapies is a pro-inflammatory cytokine contributing to the chronic and severe inflammatory response observed in UC and CD, and the monoclonal antibody functions by downregulating pro-inflammatory molecules and restoring IEB integrity, allowing the body to begin to heal[8,24-26]. Infliximab is an Immunoglobulin G1 anti-TNF-α antibody that is shown to induce MH. Active phase 1 and 2 clinical trials of Infliximab in UC patients find that, respectively, 45.5% of patients and 60.3% of patients exhibit MH[27]. Adalimumab is another anti-TNF-α antibody presenting some indication of MH effects. In the double blind, randomized, placebo controlled clinical trial, 24% of patients display MH at week 52 compared to 0% in the placebo group[28]. Certolizumab pegol is an anti-TNF-α antibody fragment targeting soluble and trans-membrane TNF. Its role in promoting MH is not yet well-investigated, but it demonstrates symptom relief and remission at week 8[29]. In some patients, anti-TNF-α treatments are ineffective or lose efficacy over time. In these patients, biologics targeting other inflammatory cytokines are employed as IBD treatment options. For example, Ustekinumab is a monoclonal antibody that targets interleukin (IL)-12 and IL-23 pro-inflammatory cytokines. Ustekinumab significantly reduces SES-CD (Simplified Endoscopic Activity Score for Crohn's Disease) scores in patients over 44 wk, implying its ability to enhance MH[30]. Similarly, Risankizumab, an IL-23 antibody, induced remission in 45% of patients, with an endoscopic response rate of 29%[31]. Additionally, Natalizumab, an anti-α4-integrin antibody, induces MH in 42.3% of patients[32]. Vedolizumab, on the other hand, is an $\alpha_4\beta_7$ integrin receptor antagonist and biologic medication, with potential immunosuppressive effects localized to the colon. In a clinical trial, 50% of UC patients and 29% of CD patients display MH following long-term use[33,34].

Although inflammation reduction and monoclonal antibody treatments have greatly enhanced the quality of IBD treatment in recent years, lack or loss of response occurs with concerning frequency, and even in patients who respond well to treatments, complete resolution of the disease has not been

Table 2 Summary of molecular pathways involved in mucosal healing

Pathways/ Mechanism of action	Associated models studied	Ref.
EGFR signaling	*In vitro*, colorectal cancer mice, EGFR mutant mice	[43,116]
Hippo/YAP signaling	*In vitro*, YAP-1 transgenic mice	[36,59]
Notch signaling	Villin-Claudin-1 transgenic mice	[41,42]
Wnt/β-catenin signaling	*In vitro* and *In vivo models of injury/repair*	[44,60,61]
Vitamin D receptor (VDR) signaling	*In vitro*, VDR knockout mice	[45]
Src/focal adhesion kinase	*In vitro*, Mechanical colonic wound in mice, Nox1 and AnxA1 knockout mice, oral gavage in mice	[76-78]
Autophagy/ATG16L1	Patient biopsies; ATG16L1 T300A knock-in mice; Atg5-manipulated mice	[6,7,104]
SCFA-mediated signaling [acetate, propionate, butyrate, *etc.*]	*In vitro*, Patient biopsies, oral gavage in mice. T-cell induced colitis, trinitrobenzenesulphonic acid (TNBS) colitis	[83-85,91,93,100,101,114]
TLR-mediated signaling	DSS colitis	[109,110,112]
MyD88 mediated bacterial sensing	Mechanical colonic wound, MyD88 knockout mice	[111]
Prostaglandin-endoperoxidase synthase 2 enzyme (PGE2)	*In vitro*, mechanical colonic wound, Ptgs2 knockout mice, Ptger4 knockout mice	[111,112]
Mucin 2 signaling	*In vitro*, DSS colitis, EGFR mutant mice	[80,116]
IL-6/IL-22/IL-23/STAT3 signaling	DSS colitis, Th2-mediated colitis, cytokine deficient mice, bone marrow transplant mice, T-cell induced colitis, human and mouse intestinal organoid culture	[94,97,98,136-138]
TGF-β signaling	*In vitro*, DSS colitis, TGF-β transgenic mice	[50,130,131]
IL-10 signaling	*In vitro*, mechanical colonic wound in mice, IL-10-deficient mice	[132,133]

EGFR: Epidermal growth factor receptor; YAP: Yes-associated protein 1; ATG16L1: Autophagy related 16 like 1 protein; Atg5: Autophagy related 5; SCFA: Short chain fatty acid; TNBS: Trinitrobenzenesulphonic acid; TLR: Toll-like receptor; DSS: Dextran sodium sulfate; PGE2: Prostaglandin-endoperoxidase synthase 2 enzyme; IL: Interleukin; STAT3: Signal transducer and activator of transcription 3; TGF-β: Transforming growth factor-β.

achieved[10]. This is due to the complex nature of disease development through many aberrant proteins and signaling pathways, as well as the multi-faceted process of MH that must be approached from many angles to restore complete IEB homeostasis. Overall, while current therapies offer evidence of permitting MH in IBD, some of these therapies do not directly promote MH through enhancing the processes of restitution, proliferation, or differentiation, but rather by simply inducing immune suppression to decrease inflammation and associated injury. Subsequently, there is a critical need to better understand the processes of inflammation and MH in IBD to aid in the development of actively MH-inducing IBD therapies.

IBD TISSUE DAMAGE MECHANISMS AND IMPLICATIONS FOR MH INDUCTION

Gut Barrier Functions and Mucosal Healing

A major role of the intestinal epithelium is its function as a barrier against the luminal environment and antigens; a role that is critical in maintaining normal mammalian homeostasis[35]. Accordingly, IEB dysregulation is a major factor in gut inflammation; thus, reinforcement of IEB integrity is a key consideration when developing more effective treatments for gut inflammatory diseases, including IBD. The MH process has been shown to help reinforce barrier integrity[36]. A complex and dynamic coordination between epithelial and immune factors facilitates MH, however, the 'paradoxical' role of the barrier-integral proteins in promoting MH remains ill-understood. Here, we summarize the key components of IEB regulation to illustrate the dynamic causal relationship between MH and the proteins comprising the structural and functional units of the gut barrier.

The physical component of the IEB consists of a single layer of epithelium, with cells linked by junctional complexes along the apicobasal axis. Excellent reviews have described the types and roles of junctional complex proteins in barrier integrity, thus here we focus on mechanisms by which regulation of these proteins influences inflammation and MH[35-39]. Tight junctions, the most apical cell-cell adhesions are considered the "gate" of the IEB and consist of multiple proteins, including the Claudin family of proteins, Occludin, junctional adhesion molecule (JAM) and the zonula occludens (ZO)-proteins[40]. Studies in cell and mouse models demonstrate through genetic manipulation that tight

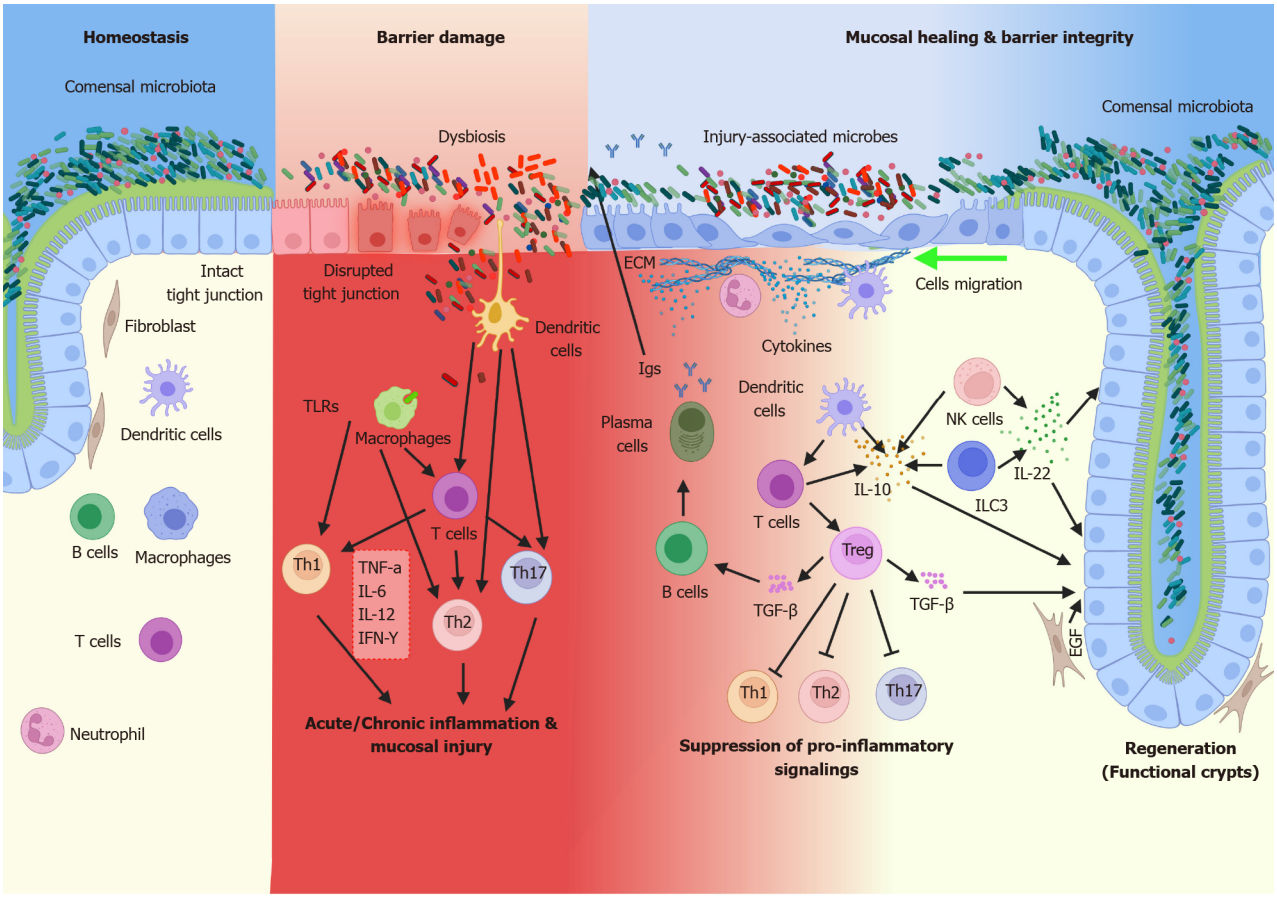

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Figure 1 Pictorial depiction of inter-connection between the immune system, inflammation, and microbiota in mucosal inflammation, associated injury, and healing. Left: normal mucosal homeostasis; Middle: Inflammatory lesions damage the mucosal barrier between the gut lumen and the rest of the body. Barrier damage leads to immune cell activation, cytokine release, and feedback cycles of deteriorating inflammation driven by microbes crossing the damaged barrier; Right: Migration of circulating restitutive immune cells to the wound area, the release of repairing cytokines; crosstalk among extracellular matrix and epithelial cells for proliferation and migration; switching of microbiota and cytokines for mucosal healing and functional crypt regeneration.

junction proteins are not merely static structural entities of the IEB; they perform additional non-canonical roles of regulating epithelial cell proliferation, survival, differentiation, and migration, which are the same processes integral to MH. In this regard, ZO-1 regulates expression and nuclear localization of the transcription factor ZO-1-associated nucleic-acid-binding protein to influence cell proliferation in a density dependent manner[41,42]. Recent studies also show that the ZO-proteins modulate Hippo/Yes-associated protein 1 signaling, a critical regulator of crypt growth and MH[43]. A similar role of JAM in regulating intestinal epithelial cell (IEC) proliferation has been reported[44,45]. Occludin, on the other hand, is shown to regulate IEC apoptosis and survival[46,47]. Recent studies demonstrate that the Claudin family of proteins is integral to tight junction structure and function and plays a complex role in gut inflammation and regenerative processes. For example, Claudin-1 overexpression in the intestinal epithelium of mouse models of IBD induces significant dysregulation of Notch/Wnt signaling and severe colitis, resulting in delayed recovery from colitis-associated injury[48,49]. Claudin-2 is unique among the Claudin proteins expressed in the intestine, as it is primarily expressed at the crypt base among proliferative undifferentiated cells and associates inversely with the differentiation state of IECs[48]. Of note, Claudin-2 is a direct target of Epidermal Growth Factor Receptor (EGFR), Wnt/β-catenin, and Vitamin D receptor signaling, all of which promote intestinal MH [50-52]. Claudin-3 on the other hand, which functions as a receptor for *Clostridium perfringens*, is sharply downregulated in the biopsies of the IBD patients, and loss of its function in the mouse gut promotes colitis-associated cancer[53-55]. We have also previously reported that Claudin-3 Loss enhances gp130/IL-6/STAT3 (signal transducer and activator of transcription 3) signaling, which promotes colitis-associated injury/repair[50]. Interestingly, the loss of intestinal Claudin-7 expression results in spontaneous inflammation due to dysregulation of epithelial-extracellular matrix interactions[56,57]. Claudin-7 also regulates intestinal stem cell function in association with the Epithelial Cellular Adhesion Molecule protein[58,59]. Conversely, Claudin-15 Loss in the IEB results in a mega-intestine[60]. Hence, dysregulation of tight junction protein expression in the IEB results in IBD, indicating that restoration of tight junction homeostasis is a vital component of MH and a promising target for treatment

development.

Like tight junction component proteins, adherens junction proteins contribute significantly to gut inflammation and MH. Here, E-cadherin, a protein whose expression indicates an epithelial phenotype, contributes to inflammation-associated epithelial repair by regulating the epithelial-to-mesenchymal transition, a process associated with cell proliferation and migration[58,61,62]. Specifically, E-cadherin expression inhibits migration of IECs and wound healing[63]. Contrastingly, complete E-cadherin loss causes a severe inflammatory phenotype characterized by villus blunting, which is a marker of premature epithelial death, and incomplete brush border development[64]. The stabilization of E-cadherin expression is facilitated partly by binding with the cytoplasmic domain of p120-cadherin. Accordingly, p120 knockout mice displayed disrupted intestinal integrity and early death from intestinal injury[65]. Other heterodimeric adherens junction proteins, α- and β-catenin, are key players in the regulation of Hippo and Wnt signaling[66,67]. Although α- and β-catenin expression dictates IEC proliferation and differentiation during injury repair, their expression counter-acts each other. Moreover, ubiquitination of β-catenin by α-catenin aids the degradation of β-catenin, balancing the Wnt signaling pathway[68]. Taken together, these findings support a complex and dynamic interdependence between gut barrier regulation and MH, which should be considered for therapeutic potential.

Gut Dysbiosis, IBD, and Mucosal Healing

Aberrant microbiome-immune interactions can lead to improper immune activation and are potentially responsible for the clinical and endoscopic observations in IBD patients. Mechanisms of microbial involvement in IBD include production of short-chain fatty acids (SCFAs), interaction with autophagy pathways, activation of immune cells, TLR signaling, and prostaglandin pathways[69-73]. Excellent reviews have covered the details of such interactions and the dynamic association with gut inflammatory processes[71-75]. Thus, here we focus primarily on how gut microbiota may contribute to MH processes under normal and/or inflammatory conditions.

Gut dysbiosis is mediated by pathogenic microbes harboring genes encoding toxin proteins, which disrupt the IEB *via* disassembly or redistribution of tight junction proteins. For example, human epithelial cells treated with *Escherichia coli* or *Salmonella typhimurium* demonstrate downregulation of ZO-1 and Occludin proteins while by contrast, Claudin-2 is upregulated[76-78]. *Shigella flexneri* and *Campylobacter jejuni* are involved in deregulating E-cadherin, as well as activating IL-8 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), thereby inducing barrier dysfunction and inflammation[79,80]. Overall, studies suggest that pathogenic gut bacteria promote chronic mucosal inflammation by dysregulating IEB integrity.

On the other hand, commensal gut bacteria seem to promote the initial stage of epithelial restitution, as studies in germ-free mice show impaired rates of epithelial cell migration, which is dependent on the formation of focal adhesions[81,82]. In this context, a commensal bacterium activates the focal adhesion kinase, thereby enhancing epithelial restitution and promoting mucosal wound repair in a redox-dependent manner[83-85]. In a mouse colonoscopy-based wound healing model, an abundance of anaerobic bacteria such as *Akkermansia spp* augmented early stages of MH[86]. The selection of mucin-producing bacteria from the mucin layer also helps close mucosal wounds[87,88]. These microbes help generate SCFAs such as acetate, propionate, and butyrate, which are considered the primary energy sources of gut colonocytes and are therefore critical supporters of IEB restoration and integrity following tissue damage[89-92]. The major producers of SCFAs include the genus *Bacteroides*, *Clostridium* clusters IV and XIVa, and *Bifidobacterium*, though they use diverse mechanisms to achieve homeostatic outcomes[89,93,94]. For example, *Bacteroides ovatus* decreases lipopolysaccharide-induced inflammation and produces indole-3-acetic acid that likely promotes IL-22 production by immune cells, yielding beneficial effects in epithelial regeneration[95,96]. SCFAs produced by fiber-fermenting commensal microbes are also linked to upregulation of Foxp3+ T regulatory (Tregs) cell development, which have a widely documented role in protection against epithelial injury and colitis [97]. Inhibition of histone deacetylases and/or activation of the latent form of transforming growth factor-β (TGF-β) to act as a potent inducer of Tregs are potential mechanisms of SCFAs[98,99]. SCFAs also mediate activation of STAT3 which plays a vital role in mucosal homeostasis[100,101]. *Clostridia*-related segmented filamentous bacteria promote IL-23 production by antigen-presenting cells, which activate type 3 innate lymphoid cells (ILCs) to initiate an IL-23R/IL-22/STAT3 loop, thereby producing serum amyloid A which promotes IL-17 production by Th17 cells[102-105]. The importance of the SCFA propionate is the augmentation of dendritic cell and macrophage hematopoiesis precursors that impact intestinal immunity to control the growth of invading mucosal pathogens[106,107]. A breach in this regulation is a central mechanism in triggering, maintaining, and exacerbating IBD. Supplementation with another important SCFA, butyrate, rescues deficiencies in mitochondrial respiration and increases autophagy in the colonocytes of germ-free mice compared to conventionally raised mice[108]. Due to the critical role in repair of gut dysbiosis, the regulation of specific gut bacteria and SCFAs may therefore possess significant potential for clinical treatment of IBD.

As introduced above, accumulating evidence suggests a causal interdependence between autophagic flux in the intestinal epithelium and gut microbiota colonization. In this regard, CD patients who are homozygous for the ATG16L1^{T300A} gene variant exhibit higher abundance of Enterobacteriaceae, Bacteroidaceae and Fusobacteriaceae in the inflamed ileum compared with patients homozygous for the wild

type ATG16L1 allele[6]. Similar findings have been obtained from mice where expression of the autophagy gene Atg7 is genetically knocked out in the gut epithelium, as these mice display altered microbial composition with enrichment in *Clostridium septum*, *Eubacterium cylindroides*, and *Bacteroides fragilis* compared to wild type mice[109]. ATG16L1^{T300A} variant mice also show changes in fecal microbiota composition compared to wild type mice, displaying an increase in the order Bacteroidales, which is associated with increased Th17 and Th1 cells in the colon and ileum lamina propria without the development of intestinal inflammation[7]. However, Atg5 deficient mice display reduced bacterial diversity, as observed in IBD patients, and contain a low number of the Lachnospiraceae, Ruminococcaceae, and Akkermansia families that control inflammatory responses[110]. Of note, a role of autophagy in regulating intestinal stem cell function and mucosal injury/repair has been demonstrated by several recent studies[111,112]. Taken together, these studies highlight a complex causal integration between host cell autophagy processes and intestinal microbial communities in regulating intestinal homeostasis and injury/repair.

Additionally, infiltrating immune cells such as macrophages and neutrophils responding to gut dysbiosis comprise essential components of intestinal wound healing by altering aberrant physiological parameters of the local microenvironment, such as microbe-associated molecular patterns (MAMPs) and decreased oxygen levels from the formation of reactive oxygen species[86,113-115]. Of note, Toll-like receptors (TLRs) expressed on multiple immune cell lineages induce signaling pathways upon binding by MAMPs and improve outcomes in experimental mouse colitis models *via* the promotion of wound healing[116,117]. Specific microbes in proximity to the wound bed also activate host epithelial proliferative signaling through a formyl peptide receptor pathway[83,84]. Studies employing a mechanical colonic wound method further disclose a protective role for prostaglandin E2 (PGE2) in re-establishment of the IEB through a TLR2/MyD88-dependent manner[118,119]. A follow-up study from the same group shows that in the early repair phase, a TLR2/PGE2 axis is required for barrier establishment; however, PGE2 must then decrease to allow for epithelial proliferation and regeneration [118]. In this context, Jain *et al*[120] elegantly demonstrates that temporal regulation of the bacterial metabolite PGE2/deoxycholate during colonic repair is critical for crypt regeneration[120]. The highly specific and time-dependent switching of microbial colonization and signaling pathways can therefore act to promote MH in a localized manner.

Several therapeutic approaches have been examined by administration of prebiotics or probiotics to regulate the microbiota. For example, butyrate enemas are effective in treating experimental colitis and UC patients[121,122]. Also, p40, a protein produced by *Lactobacillus rhamnosus GG* (LGG), activates host epithelial EGFR signaling and mediates wound healing[123]. Of note, the mechanism of MH promotion by LGG is *via* a positive effect on epithelial barrier maturation by upregulation of Claudin-3[124]. Recently, genetically modified probiotic bacteria–based precision delivery of human EGF also appears to be a promising intervention against mucosal inflammation through crypt-derived MH and barrier restoration[125,126]. Firmicutes, such as *Faecalibacterium prausnitzii* play an essential role in mucosal barrier homeostasis by regulating NF-κB activation and IL-8 production[125,126]. In another study, oral gavage with *Faecalibacterium prausnitzii* during dextran sodium sulfate (DSS) colitis improves outcomes compared to mice treated with DSS alone, likely due to participation of Claudin-1 and Claudin-2[125]. The probiotic mixture known as VSL #3, containing 4 strains of *Lactobacilli*, 3 strains of *Bifidobacteria*, and one strain of *Streptococcus* is effective in preventing pouchitis and in treating UC flareups[127]. This probiotic functions by partially upregulating mucin production and restoring the IEB by stimulating ZO-1 and Occludin expression while decreasing Claudin-2[127]. Taken together, a complex interdependence exists between gut microbiota and MH processes in the promotion of barrier integrity that should be fully explored for its intriguing potential in improving clinical outcomes.

Inflammatory Cytokines in IBD and Mucosal Healing

Due to dysregulation of many cytokines and growth factors in IBD and the regulatory importance of many of these same molecules in MH, we discuss the potential of altering immune signaling and inflammatory cascades in restoring proper intestinal homeostatic balance. Increasing knowledge of the coordination of these pathways will contribute to the development of more effective and targeted therapies to ameliorate disease while preserving essential immune system function.

Major cytokines and growth factors that are considered pro-inflammatory in IBD include IL-1β, interferon-gamma (IFN-γ), TNF-α, and IL-6. During an infection in the gut, IL-1β, TNF-α, and IFN-γ are shown to be produced by inflammatory monocytes, among which IL-1β and TNF-α are associated with increased IEB permeability[2]. TNF-α and IFN-γ are also produced by ILCs and serve to recruit and activate additional inflammatory cells[3]. TNF-α is particularly well-studied and often targeted in the treatment of autoimmune diseases as detailed in our discussion of monoclonal antibody therapies above. Although necessary for innate immune responses against acute pathogens and acute DSS colitis, when produced chronically by T cells TNF-α can be a major contributing factor to the loss of epithelial barrier and development of autoimmune disease[128-130]. IFN-γ has been shown to be regulated during wound healing of skin epithelium by Tregs, where lack of Tregs resulted in increased IFN-γ, accumulation of pro-inflammatory macrophages, and hindered wound healing[131]. Gut microbiota can also impact immune system activation through cytokine signaling. Kuhn *et al*.[132] demonstrates that intraepithelial lymphocytes (IELs) must interact with commensal Bacteroidales order microbes to produce IL-

6 in response to acute *C. rodentium* colitis infection, aiding in repair of the IEB *via* increased Claudin-1 expression[132]. Despite the necessary effects of pro-inflammatory cytokines for immune system response and homeostasis, each also possesses drawbacks. When the location, amount, or duration of cytokine production becomes dysregulated, chronic inflammation and disease can result. IFN-γ, TNF-α, and IL-6 are upregulated after secretion by immune cells in the chronic inflammatory state associated with IBD[3,130,132]. These cytokines are known to increase gut permeability by altering tight junction protein expression[132-134]. In particular, increased IL-6 in the IECs and lamina propria mononuclear cells increases Claudin-2, which promotes intestinal permeability and is known to be upregulated in IBD[134,135]. Interestingly, it is IELs that produce IL-6 in a protective manner during acute infection through the c-Jun N-terminal Kinase pathway, rather than the Mitogen-activated protein kinase/extracellular signal-regulated kinase pathway, suggesting both duration and cell type-specific layers of complexity in the role of IL-6[132,134]. It is likely that similar multi-layer and highly context-specific pathways exist for other inflammatory cytokines as well. Therein lies the challenge of harnessing pro-inflammatory cytokines and signaling pathways in the immune system for MH: Targeting modifications toward the decrease of detrimental chronic effects without impairing their beneficial and homeostatic functions. Continuing in the discussion of IL-6, global reduction of IL-6 expression can decrease chronic inflammation in IBD and enhance MH, but may also increase susceptibility to infection[132,136]. It would therefore be ideal to develop a method of reducing IL-6 production in IECs while leaving expression in IELs intact to avoid increasing the risk of dangerous infections in patients. The potential in fine-tuning inflammatory cytokine expression to promote MH is great, but much work remains to ensure it can be accomplished safely without severe detrimental effects to other functions of the immune system.

Conversely, several other cytokines, growth factors, and cell types function primarily in an anti-inflammatory role in IBD and intestinal homeostasis and offer additional mechanisms to enhance MH by resolving chronic inflammation. Growth factors and cytokines considered to be anti-inflammatory include TGF-β, IL-10, IL-22, and IL-17. TGF-β is a well-studied growth factor that has been demonstrated by Beck *et al*[137]. to play a role in the restitution, or IEC migration, phase of MH, evidenced by the lack of IEC migration and impaired wound healing after TGF-β inhibition in DSS colitis[8,137]. Additionally, TGF-β when produced by a macrophage secretome called SuperMApo aids in removal of apoptotic cells and resolution of inflammation in IBD models[57,138]. Importantly, since this secretome produces TGF-β from a singular cell type, rather than globally, its administration can provide the context- and location-dependent production of beneficial TGF-β while avoiding potential opposing or off-target effects. Macrophages and Th2 cells also produce IL-10, which enhances the proliferation phase of MH, maintains immune tolerance to the many antigens encountered by the IEB, and promotes barrier integrity[139,140]. Similarly, the production of IL-17 by anti-inflammatory Tregs helps with IEC proliferation and blockage of detrimental microbiota in colitis models, providing protection against IBD[141]. One of the major players in protection against IBD is IL-22, which is produced by multiple cell types and promotes MH by more than one mechanism, as reviewed in[142]. Most importantly in IBD, IL-22 is produced by ILCs, CD4+ T cells, and NK cells, demonstrating activation of both the innate and adaptive immune system[143,144]. IL-22 primarily acts on intestinal stem cells (ISCs) and IECs and functions by activating STAT3 signaling, which induces IEC proliferation and therefore MH[101,142,143,145]. Regarding ISCs, IL-22 both protects them from depletion during intestinal inflammation and induces regeneration[101,143,145]. Though some of these cytokines and growth factors can be inflammatory under certain circumstances, in the context of IBD they function in an anti-inflammatory manner and are beneficial in promoting MH. Some cytokines even demonstrate potential to be produced or administered selectively in only beneficial locations or cell types. Therefore, anti-inflammatory cytokines and growth factors present promising options for treatment of IBD through selectively reducing inflammatory signals and inducing or enhancing the MH process.

CONCLUSION

In recent years, IBD has become increasingly common in the United States and abroad. As progressive and debilitating diseases, UC and CD have long-term negative implications on health. In the past, treatments have focused on reducing the clinical manifestations of the disease, often leaving underlying disease mechanisms active in the gut[12,15]. With increased understanding of the mechanisms and complex pathogenesis of IBD, further innovation in treatment approaches must occur to improve patients' long-term outcomes. Subsequently, instead of merely hoping to reduce symptoms, doctors now desire therapies that actively aid in healing and regeneration of damaged tissue. MH has therefore emerged as the main goal for research and treatment end points to ensure long-term remission, survival, and a good quality of life for patients. Going forward, understanding the interactions regulating the breakdown and regeneration of the IEB, as well as overarching gut homeostasis processes, will be paramount to treating and curing patients with IBD. Monoclonal antibody therapies offer a promising start to revolutionizing treatment, aiming not only to reduce clinical manifestations, but also to interrupt disease activity on a cellular and molecular level. However, even newly developed antibody therapies

cannot by themselves completely resolve IBD and restore total gut homeostasis. Therefore, the three approaches to targeting the molecular machinery governing IBD of restoring the IEB, regulating the gut microbiota, and altering the cytokine signaling-mediated immune response are all being studied as potential mechanisms for achieving MH. Optimal future treatment protocols for IBD will ideally include a combination of these approaches, with the intent of restoring intestinal homeostasis by balancing expression of multiple proteins and repairing several of the many dysregulated pathways involved in IBD pathogenesis.

FOOTNOTES

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MINIREVIEWS

Choosing the best endoscopic approach for post-bariatric surgical leaks and fistulas: Basic principles and recommendations

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Abstract

Post-surgical leaks and fistulas are the most feared complication of bariatric surgery. They have become more common in clinical practice given the increasing number of these procedures and can be very difficult to treat. These two related conditions must be distinguished and characterized to guide the appropriate treatment. Leak is defined as a transmural defect with communication between the intra and extraluminal compartments, while fistula is defined as an abnormal communication between two epithelialized surfaces. Traditionally, surgical treatment was the preferred approach for leaks and fistulas and was associated with high morbidity with significant mortality rates. However, with the development of novel devices and techniques, endoscopic therapy plays an increasingly essential role in managing these conditions. Early diagnosis and endoscopic therapy initiation after clinical stabilization are crucial to success since clinical success rates are higher for acute leaks and fistulas when compared to late and chronic leaks and fistulas. Several endoscopic techniques are available with different mechanisms of action, including direct closure, covering/diverting or draining. The treatment should be individualized by considering the characteristics of both the patient and the defect. Although there is a lack of high-quality studies to provide standardized treatment algorithms, this narrative review aims to provide a summary of the current scientific evidence and, based on this data and our extensive experience, make recommendations to help choose the best endoscopic approach for the management of post-bariatric surgical leaks and fistulas.

Key Words: Endoscopy; Surgery; Bariatric; Gastrointestinal; Fistulas; Leaks

Core Tip: Post-surgical leaks and fistulas are the most feared complications of bariatric surgery. Endoscopic therapy is essential for effective management of these conditions. Several endoscopic techniques are available, and this review aims to clarify their mechanisms of action, basic principles, and optimal approach for each situation based on a detailed literature review as well as the authors' personal experience.

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INTRODUCTION

Obesity is now a pandemic, and the prevalence of people living with obesity continues to increase. As a chronic and multifactorial disease with several associated comorbidities, a multidisciplinary approach is required to prevent, treat, and reverse obesity-related complications and to improve quality and length of life for people with obesity[1].

Bariatric and metabolic surgery (BMS) remains the most effective and durable therapy for weight loss and improvement of associated comorbidities. Unsurprisingly, the number of bariatric surgeries performed has grown progressively worldwide. In 2019, about 256000 BMS were performed in the United States. The most performed procedure is laparoscopic sleeve gastrectomy (LSG) followed by Roux-en-Y gastric bypass (RYGB), and revisional surgery[2]. With the increasing number of BMS, familiarity with management of procedure related complications is increasingly important for endoscopists.

Although rare, especially in referral centers, complications can occur, such as leaks and fistulas[3]. Traditionally, leaks and fistulas were treated with surgery. However, surgical management of these defects is usually challenging and has been associated with high morbidity and mortality rates[4]. Less invasive approaches are preferred, when possible, to reduce associated morbidity. Endoscopic therapies play an essential role in the management of leaks and fistulas. Several endoscopic devices and techniques, with different mechanisms of action have been developed, with considerable clinical success for what is often morbid and refractory disease[3,4].

In this narrative review we discuss the pathophysiology, characteristics, diagnosis, and management of post-bariatric surgical leaks and fistulas, focusing on endoscopic therapies. We include descriptions of the mechanism of action, indications, contraindications, tips, tricks, and outcomes for different techniques, in order to facilitate choosing the best approach for each unique case.

DEFINITIONS

Leak is defined as a transmural defect with communication between the intra and extraluminal compartments (Figure 1). A fistula is defined as an abnormal communication between two epithelialized surfaces. Fistulas can be divided into internal and external. An internal fistula occurs between two internal epithelialized organs, whereas an external fistula is a communication between an internal organ and the skin surface (Figure 2)[5].

EPIDEMIOLOGY

Despite the increasing number of BMS, the rate of mortality and adverse events (AEs), including leaks and fistulas, has decreased over the past two decades[6] due to improvement in surgical techniques, such as laparoscopy and robotic surgery, improved materials, and surgeons' expertise.

Leaks and fistulas occurred with similar incidence among the most common bariatric surgeries, ranging from 0.4% to 5.6% in RYGB and 1.9% to 5.3% in LSG, with higher rates after revisional surgeries

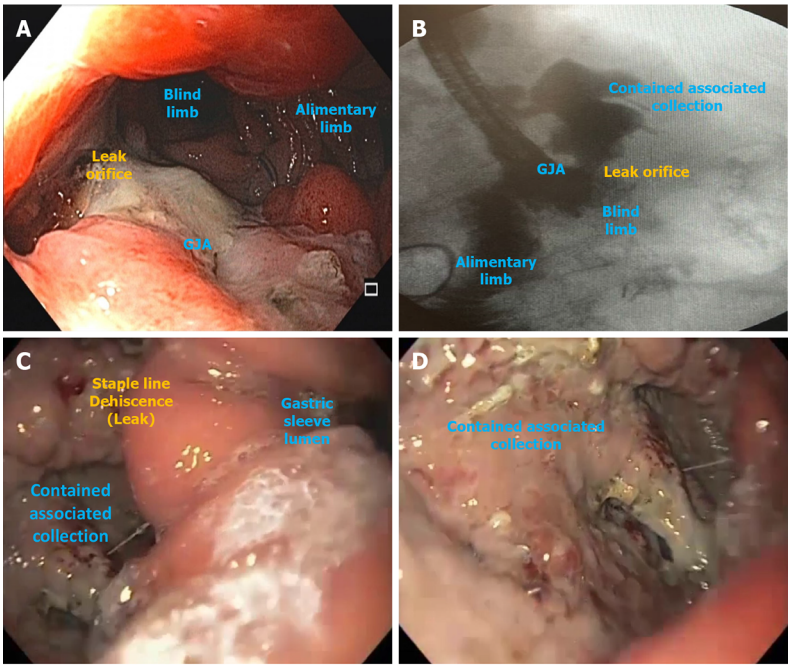

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Figure 1 Post-bariatric surgical leaks illustrations. A and B: Gastrojejunal anastomotic leak with an associated contained collection after Roux-en-Y gastric bypass; C and D: Leak with a contained associated collection due to staple line dehiscence after laparoscopic sleeve gastrectomy. GJA: Gastrojejunal anastomosis.

[7-10]. This incidence is considerably lower in high-volume specialized centers; as low as 0.5%[11]. Post-BMS mortality is very low, ranging from 0.2% to 0.4%, and is mainly related to late diagnosis and management of complications[7-10].

CAUSES AND PREVENTION

Leaks usually occur after surgery and are often located at the suture (staple) line and/or anastomosis, while fistulas are mainly caused by untreated leaks[5].

The causes of transmural defects after BMS are related to several factors, including the patient related risk factors and technical risk factors. Patient related risk factors for transmural defects include high body mass index, age, smoking, alcohol use, malnutrition, and other related comorbidities [*e.g.*, type 2 diabetes mellitus (T2DM)][12]. Technical risk factors include mechanical or ischemic insults, both of which contribute to a mechanism of high intraluminal pressure that overcomes the integrity of the tissue. Meticulous surgical technique, with careful attention to tissue handling and inadvertent excessive tension on anastomoses, luminal narrowing, suturing/stapling ischemic areas, and torsion of blood vessels or organs is mandatory to avoid undesirable outcomes such as downstream stenosis, leaks, and fistulas. Additionally, stapling failure is also a cause of BMS leaks[13,14].

The pathophysiology of leaks and fistulas after RYGB and LSG differ; given distinct technical and anatomic particularities of each surgery. Most commonly in LSG, the creation of a long and narrow conduit combined with pylorus preservation creates a high-pressure region proximally leading to stress on the proximal staple line. In RYGB, the gastric pouch is a low-pressure system with low resistance to gastric emptying through the gastrojejunal anastomosis (GJA) into the small bowel, though high-pressure areas can develop in cases of GJA stenosis or excluded stomach gastroparesis. The anatomy of LSG favors leak and fistula formation at the proximal portion of the staple line, especially near the angle of His. Here the thin gastric wall, high pressure, and borderline vascularization secondary to take down of the short gastric arteries all contribute to comprised wall integrity[14-16].

Of note, for both LSG and RYGB, it has been shown that laparoscopic surgery reduces the incidence of leaks and fistulas as well as mortality rates compared to open surgery[6,17]. Intraoperative leak assessment using methylene blue, endoscopy, or air insufflation allows for immediate repair of defects though this practice has never been proven to reduce the incidence of leak or fistula formation after surgery[14,18].

Stapling technology has evolved in recent years with the development of different staple heights capable of accommodating variable gastric wall thickness according to the anatomic portions of the stomach. To take advantage of this technology, the surgeon must be aware of which one is the most appropriate in each case to avoid mismatch[14]. Moreover, several techniques of staple line reinforcement have been studied, from oversewing the staple line with different suture techniques to

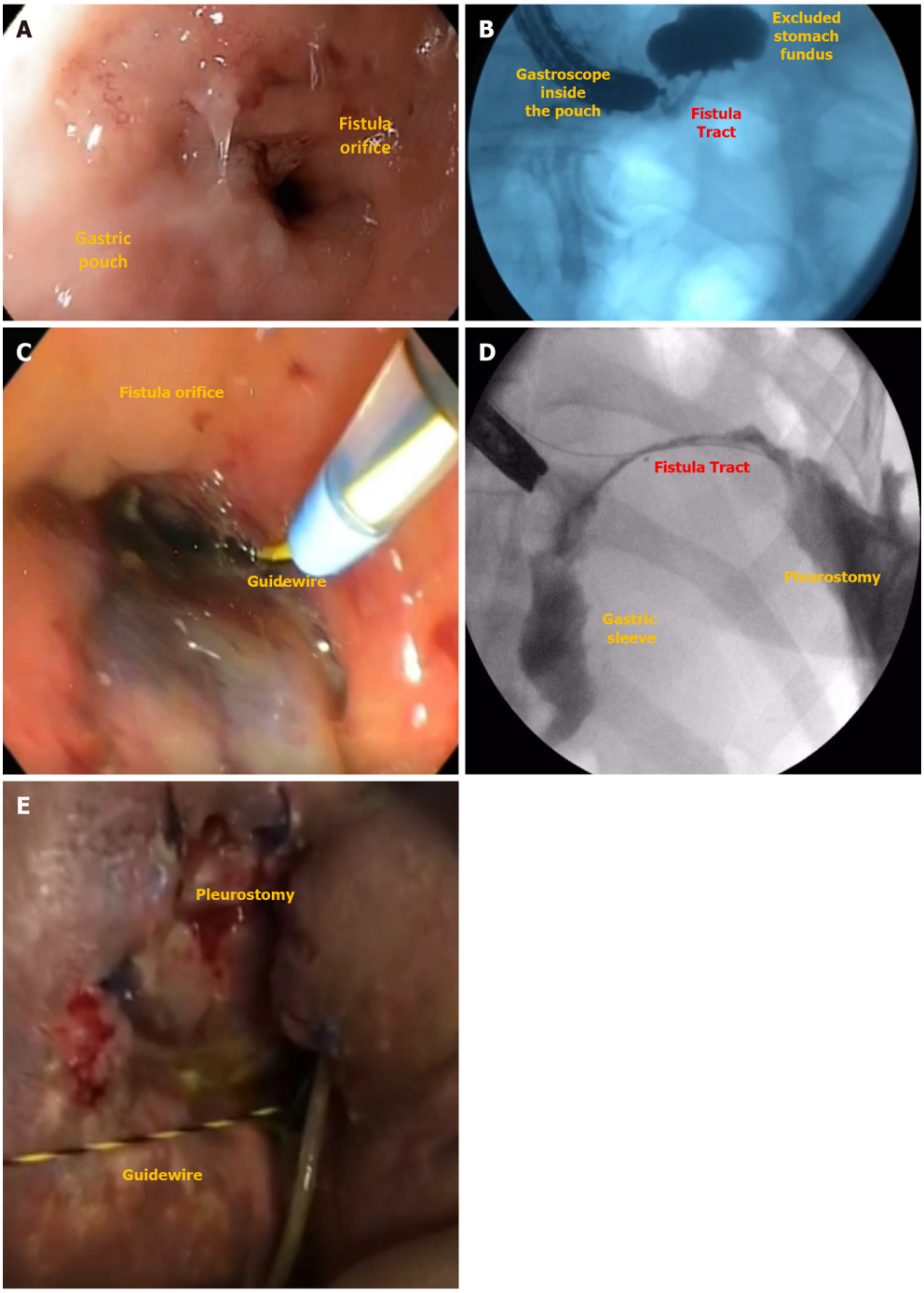

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Figure 2 Post-bariatric surgical fistulas illustrations. A and B: Gastrogastric fistula after Roux-en-Y gastric bypass; C, D, and E: Gastropleural fistula after laparoscopic sleeve gastrectomy.

the use of biologic or synthetic materials, such as glues and tissue sealants, but none of these interventions have been proven to reduce the incidence of post-surgical leaks and fistulas[19-22].

The routine placement of drains near the anastomosis is controversial and there is no consensus in the literature and between surgeons on whether this intervention prevents the development of uncontrolled leakage or increases the risk of developing leaks[23,24].

CLASSIFICATION

Leaks and fistulas can be classified based on several parameters, including time of onset and location.

The definition of acute *vs* chronic leaks and fistulas varies between 30 and 45 d in the literature (acute < 30-45 d and chronic > 30-45 d)[25,26]. In our practice, we use the classification described in the

international LSG consensus, which classified the defects into acute (< 7 d), early (between 7 d and 6 wk or 45 d), late (between 6 wk or 45 d and 3 mo or 90 d), and chronic (> 3 mo or 90 d)[27]. Up to 20% of acute and early leaks and fistulas become late and chronic leaks and fistulas, mainly when the defect is untreated[26,28].

Based on the location, post-RYGB leaks are classified into Type I to VI. The locations include: The gastric pouch (Type I), GJA (Type II), blind jejunal stump (Type III), jejunojejunal anastomosis (Type IV), excluded stomach (Type V), and the blind stump of the biliopancreatic limb (Type VI). The first three topographies are easily accessible by endoscopy and can be treated with endoscopic techniques, unlike the others[29].

LSG leaks mostly occur at the level of the angle of His due to poor vascularization (more than 90% of the cases) but can also occur in the distal body and antrum. Both locations can be treated with an endoscopic approach[27].

DIAGNOSIS

The diagnosis of leaks and fistulas is based on clinical history and physical examination. Complementary studies, including laboratory tests and imaging are usually required to establish a precise diagnosis.

Clinical manifestations vary according to individual factors related to the level of inflammatory response as well as particularities of the leak or fistula, such as the onset time, defect location, and the placement of a post-surgical drain. If an external drain is placed, the leak can be identified early due to increased drainage[5]. On the other hand, patients without external drainage usually present with infectious manifestations, from mild signs and symptoms, such as low-grade tachycardia, fever, and abdominal pain, to sepsis[30].

Clinical manifestations of fistulas depend on the location of the defect. Respiratory symptoms should be an alarm to consider gastrointestinal (GI)-respiratory fistulas, although patients with peritonitis can also present with pleural effusion[31]. GI-cutaneous fistulas are easily diagnosed if secretions are observed coming out through the skin[32]. Gastrogastric fistula is usually a late complication of RYGB and should be investigated in patients with weight regain and/or gastroesophageal reflux[33].

Laboratory tests are important in acute infection and include leukocytosis, elevated C-reactive protein, and changes indicating organ dysfunction[14]. Additionally, it may be important in patients with long-term fistulas to evaluate nutritional state or electrolyte depletion, for example, in chronic gastrocolic fistulas.

Once suspicion is established, diagnosis is usually confirmed with imaging exams, including radiography of the abdomen, upper gastro-intestinal transit (upper GI series) and computed tomography (CT) scan with administration of water-soluble oral contrast, esophagogastroduodenoscopy (EGD), and/or fistulogram[34] (Figure 3).

Radiography of the abdomen is frequently requested as it can rapidly evaluate for pneumoperitoneum (Figure 3A).

Upper GI transit should be performed with water-soluble contrast if leak or fistula are suspected (Figure 3B). It is important to locate the fistulous orifice and to understand the post-surgical anatomy, including the presence of downstream stenosis for successful treatment. The use of barium is not recommended as it may delay endoscopic treatment. When GI transit findings are doubtful or clinically discordant, CT scan should be used to clarify the results due to its lower sensitivity[35,36].

CT scan allows the evaluation of a fistulous path as well as indirect signs of the source of a leak or fistula, presence of associated collections, pneumoperitoneum, and free fluid. Additionally, it provides a broad evaluation of the intracavitary organs for procedural planning (Figure 3C)[4,37].

EGD is needed to evaluate the fistulous orifice (Figure 3D) surrounding tissue, presence of foreign bodies, and downstream stenosis. Fluoroscopy can be critically useful, especially for orifices smaller than the diameter of the gastroscope. In these cases, a fistulogram can be performed by injecting water-soluble contrast through a catheter or the gastroscope working channel (Figure 3E). Additionally, injection of water-soluble contrast, methylene blue, or a bubble test can be performed if there is an external drain (Figure 3F)[5]. In patients with LSG and suspicion of a leak or fistula without an evident defect, an important reminder is to follow the staple line superiorly looking for small orifices as these can be difficult to locate. A fistulogram can also be performed percutaneously in cases of GI-cutaneous fistulas allowing for a better understanding (size and location) of the fistula tract.

PRINCIPLES OF TREATMENT

The treatment of leaks and fistulas is based on four pivotal principles: (1) Clinical management; (2) Drainage; (3) Treatment of associated factors; and (4) Closure of the transmural defect.

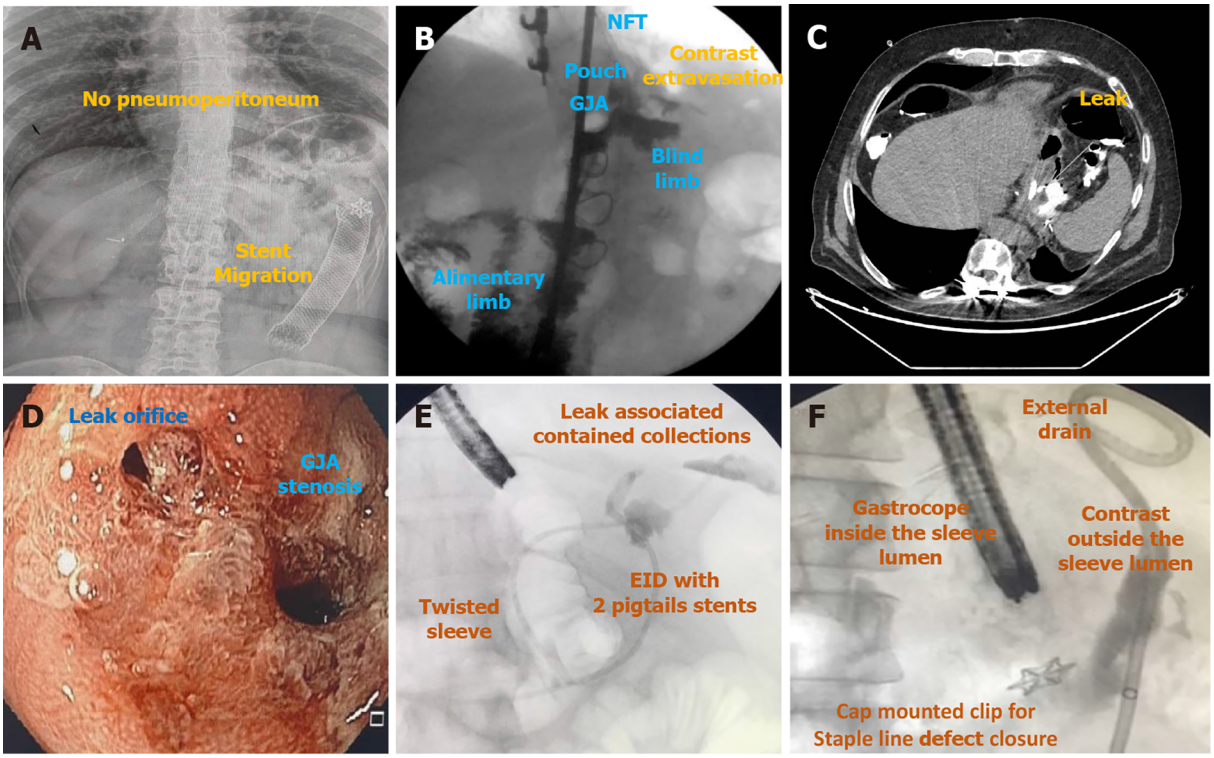

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Figure 3 Imaging exams for diagnosis of leaks and fistulas after bariatric surgery. A: Abdominal radiograph showing an esophageal fully covered metal stent fixed with a cap mounted clip at its proximal edge used for the treatment of a gastrojejunal anastomosis (GJA) leak after Roux-en-Y gastric bypass (RYGB) migrated to distal jejunum with no signs of pneuperitoneum; B: Upper gastrointestinal (GI) transit for suspicious leak after RYGB showing contrast extravasation; C: Computed tomography scan confirming the GJA leak suspected in the upper GI series (Figure 3B); D: Endoscopic visualization of a GJA stenosis associated with a leak; E: Fluoroscopy image during esophagogastroduodenoscopy (EGD) with injection of water soluble contrast through a catheter showing two leaks in the sleeve staple line with associated collections treated with endoscopic internal drainage with pigtail stents; F: Fluoroscopy image during EGD with injection of water soluble contrast through the external drain showing no extravasation for the intraluminal compartment, confirming the successful defect closure. GJA: Gastrojejunal anastomosis; EID: Endoscopic internal drainage; NFT: Nasoenteral feeding tube.

First, unstable patients must be clinically stabilized, including fluid resuscitation, packed blood cells transfusion when indicated, and administration of vasoactive drugs if necessary.

Infectious source control including intravenous antibiotic therapy and drainage must be performed to control sepsis. Drainage can be performed surgically, percutaneously by image guidance, or endoscopically. For unstable patients with peritonitis and free infected fluid in the cavity, surgical lavage and drainage, with or without defect repair, is the preferred approach.

Nutrition is also key for defect healing and is often neglected as most patients are kept nil *per* os initially. Therefore, enteral or parenteral nutrition should be introduced as early as feasible. Enteral nutrition is the preferred option, through a nasoenteral feeding tube placed distal to the defect[5].

Defect related factors must be treated to achieve successful closure, such as dilation of downstream stenosis, and foreign body removal (staples, suture, and drains, *etc.*)[38,39].

After clinical stabilization, endoscopic evaluation is recommended. The endoscopist must communicate with other specialty teams and review the operative report thoroughly before performing the initial endoscopic evaluation. The role of endoscopy in the management of leaks and fistulas includes both diagnosis and treatment. Early diagnosis and endoscopic management are key for success, with closure efficacy as high as 90% when defects are treated within 3 wk of surgery and about 70% after this period[25,26]. Endoscopic treatment of leaks is associated with higher rates of successful closure when compared to fistulas[40]. Despite the lower efficacy of endoscopic treatment for chronic leaks and fistulas compared to acute and early transmural defects, it should be attempted exhaustively before referral for definitive surgical treatment as surgery is often challenging and associated with more morbidity[41]. In this setting, the most commonly proposed revision surgeries are fistulojejunostomy, conversion of LSG to RYGB (without gastrectomy), and total/near total gastrectomy with esophagojejunal anastomosis. Direct surgical repair of the fistula site is not effective and not advised.

ENDOSCOPIC MANAGEMENT

Endoscopic evaluation of leaks must be performed under carbon dioxide insufflation or underwater to reduce the risk of pneumoperitoneum or pneumomediastinum, especially when there is no external drainage. Careful endoscopic assessment is essential to avoid wall rupture of a contained collection[37].

The procedure can be performed in the operating room, endoscopy suite, or at the bedside[4]. The endoscopy suite is preferable due to its lower costs when compared to the operating room and greater resources when compared to the bedside. However, for unstable patients, the operating room or at bedside in an intensive care unit may be preferred.

General anesthesia with orotracheal intubation to reduce the risk of aspiration during fluoroscopy is advised, especially in the initial evaluation. General anesthesia often allows a more detailed inspection of the transmural defect, including defect size, fistula path, and presence of extraluminal collection[4]. The need for general anesthesia and fluoroscopy is not mandatory but can be tailored to the case at hand.

ENDOSCOPIC TECHNIQUES

Endoscopic therapies utilize several mechanisms of action and can be classified into closure, covering, and draining techniques[5].

Closure techniques include clips [through-the-scope clips (TTSC) and cap mounted clips], endoloop, endoscopic suture, and tissue sealants/glues. Despite some studies[42,43] reporting successful closure of leaks and fistulas using the widely available and simple-to-place TTSC, sometimes combined with endoloop, this technique requires robust and healthy tissue around the defect for successful primary closure. Therefore, this approach is not effective in closing leaks and fistulas and should not be recommended[5]. Hence, this closure technique will not be discussed in this review. Common closure techniques are summarized in Table 1.

Covering techniques utilize cardiac septal defect occluders (CSDO) or self-expandable metal stents (SEMS), including conventional (esophageal) stents and custom (bariatric) stents (Table 2). Closure and covering techniques do not allow internal drainage; thus, external drainage is required when an associated collection or intracavitary infected fluid is identified.

Endoscopic drainage techniques include endoscopic vacuum therapy (EVT), endoscopic internal drainage (EID) with double pigtail stent (DPS), and septotomy (Table 3).

All endoscopic techniques are summarized in Tables 1, 2, and 3 discussed in detail below.

Cap-mounted clips

Cap-mounted clips (Figure 4) are usually referred to as over the scope clips (OTSC®, Ovesco Endoscopy GmbH, Tübingen, Germany) as this is the commercial name of the first cap mounted clip. However, it is important to state that the name OTSC® is trademarked and other devices are available.

Cap mounted clip use is increasing due to its benefits compared to the TTSC, including the ability to close leaks and fistulas as it can approximate a larger volume of tissue.

The device is placed into the distal tip of the endoscope and deployment is similar to band ligation, a procedure widely performed by endoscopists, helping to shorten the learning curve[44]. It can only be used in defects smaller than 20 mm.

A meta-analysis including 73 patients from 9 studies evaluating cap-mounted clips for treatment of leaks and fistulas after LSG, including both acute and chronic defects showed a clinical success rate of 63.5% when used as a single therapy and 86.3% when combined with other therapies[45].

In our experience, cap-mounted clips can be used in patients with small orifices without undrained associated collection and always combine with other therapy (Figure 4A). Additionally, cap-mounted clips can be used for stent fixation (Figure 4B and C).

Tissue sealants/Glues

Tissue sealants (Figure 5A) and glues (Figure 5B) include fibrin glue, acellular matrix biomaterial (SurgiSIS® – Cook Medical, Winston-Salem, North Carolina, United States), and cyanoacrylate. These materials are primarily used to close fistulous tracts and are usually used as an adjunctive therapy.

Despite the closure effect of both glues and tissue sealants, the last group also play a role in tissue healing. Fibrin glue induces a cellular response, extracellular matrix formation and neovascularization, while acellular matrix biomaterial induces fibroblast proliferation[46,47].

Although these techniques have a well-established safety profile, reported clinical success is variable and multiple applications are usually required. In a recent meta-analysis evaluating 10 case series involving 63 patients, the number of sessions needed for treatment ranged from 1 to 9, with a pooled successful closure rate of 96.8%[48]. Regardless of the excellent result shown in this meta-analysis, our experience does not reflect this high efficacy. It is important to analyze this data with care given significant limitations and risk of bias for retrospective case series. Furthermore, clinical success is related to the characteristics of the leak or fistula, including defect site, chronicity, and size. Therefore,

Table 1 Endoscopic closure techniques

Endoscopic techniques	Indications/advantages	Not indicated/disadvantages	Authors experience
Cap-mounted clips	(1) Acute/ early/ late/ chronic; (2) Small orifices (< 20 mm); and (3) Safe	(1) Orifices > 20 mm; (2) Need for external drainage; and (3) Variable efficacy	(1) Acute/ early/ late/ chronic; (2) Safe; (3) < 10 mm: > efficacy; (4) > 10mm: very low efficacy; (5) Combined therapy improves its efficacy; and (6) Can be removed when fails to close the defect (not easy to remove)
Glues/ tissue sealants	(1) Acute/ early/ late/ chronic; (2) Diameter < 10 mm; (3) Low drainage (< 200 ml/24 h); and (4) Safe	(1) Multiple sessions are usually required; (2) Need for external drainage; and (3) Variable efficacy	(1) Late/ chronic; (2) Low efficacy; (3) Safe; (4) Helpful as an adjunctive therapy; (5) Never use it as a single therapy; (6) Multiple sessions; (7) Cytology brushing or APC is useful to loosen granulation tissue before application; (8) Delivery *via* endoscopic or percutaneous access; and (9) High cost (tissue sealants)
Endoscopic suturing	(1) Acute/ early/ late/ chronic; (2) High technical success; and (3) Safe	(1) Need for external drainage; (2) Low efficacy (need for robust and healthy tissue for primary closure); (3) Challenging: Previous experience with the device is needed; and (4) High costs (most countries)	(1) Very poor long-term clinical success; (2) Helpful for other devices fixation; (3) Not recommended for chronic defects; and (4) High cost

APC: Argon plasma coagulation.

Table 2 Endoscopic covering techniques

Endoscopic techniques	Indications/advantages	Not indicated/disadvantages	Authors experience
Conventional (esophageal) stents	(1) Acute/ early; (2) Satisfactory efficacy; (3) Very popular; (4) Widely available; (5) International guidelines support; (6) Easy placement; (7) Early oral intake; and (8) Low number of repeated procedures	(1) Late/ chronic; (2) High migration rates; (3) Need for external drainage; (4) Mild symptoms related to the stent; and (5) Possible "surprise" when removing it	(1) Acute/ early; (2) Satisfactory efficacy; (3) Easy placement/ not expensive; (4) Helpful for complete dehiscence; (5) PCSEMS > FCSEMS (challenging removal – do not keep it for > 3 wk); and (6) High migration rates (FCSEMS)
Bariatric stents	(1) Acute/Early; (2) Satisfactory efficacy; (3) "Perfect" shape for LSG leaks; (4) Low number of repeated procedures ; and (5) Easy placement	(1) Late/ chronic; (2) High migration rates; (3) Need for external drainage; (4) Severe symptoms related to the stent; and (5) Possible "surprise" when removing it	(1) Acute/ early; (2) Similar efficacy to the conventional stent; (3) More expensive than the conventional stent; (4) Helpful for downstream stenosis and complete dehiscence; (5) Pre-pyloric position: more symptoms; (6) Post-pyloric position: more migration; (7) High rates of adverse events (ulcers and perforations); and (8) Intolerance due to symptoms related to the stent (GERD, pain, and emesis)
Cardiac septal defect occluder	(1) Late/ chronic; (2) High efficacy; (3) Safe; and (4) Epithelialized surface is required for device fixation	(1) Need for external drainage; (2) Off-label use; (3) High cost; and (4) Acute and early: enlargement and migration if no epithelialized surface	(1) Very high efficacy for late/ chronic defects with epithelialized tract without associated collection; (2) Safe; (3) High cost; (4) Off-label; (5) Indicated after conventional techniques failure; and (6) Size selection based on defect size (2:1)

FCSEMS: Fully covered self-expandable metal stents; GERD: Gastroesophageal reflux disease; LSG: Laparoscopic sleeve gastrectomy; PCSEMS: Partially covered self-expandable metal stents.

all these variables should be considered in a treatment plan.

In our experience, the best indication for tissue sealants and glues are GI-cutaneous fistulas, with thin tract and low output and always combined with additional therapies[31]. Tissue sealants and glues can be delivered *via* endoscopic or percutaneous tract, facilitating the procedure[49]. Cytology brushing or ablation of the epithelialized (chronic) tract to induce granulation is recommended.

Endoscopic suturing

Endoscopic suturing (Figure 6) allows for full-thickness closure. However, similar to TTSC, any type of suturing (surgical or endoscopic) needs robust and healthy tissue around the defect for primary closure. Therefore, this technique is not effective in closing most leaks and fistulas and should not be recommended[5]. In a multicenter study evaluating fistula closure by endoscopic suturing, 56 patients underwent the procedure with 100% technical success. Despite the high technical success, only 22.4% of patients achieved clinical success at one year follow-up[50]. Additionally, endoscopic suturing is expensive in most countries and requires specialized training.

Table 3 Endoscopic draining techniques

Endoscopic techniques	Indications/advantages	Not indicated/disadvantages	Authors experience
Endoscopic vacuum therapy	(1) Acute/ early/ late/ chronic; (2) High efficacy in leaks and fistulas with or without associated collection; (3) No need for external drainage; (4) Superior to stent in upper GI tract; and (5) Unique mechanism of action: macro-deformation/ micro-deformation, changes in perfusion/ angiogenesis/exudate control/bacterial clearance	(1) Inability to achieve negative pressure; (2) No endoscopic access; (3) Patient discomfort related to nasogastric tube; (4) Usually repeated procedures are needed (especially when traditional sponge is used); and (5) Longer hospital stay/ high costs (?)	(1) Acute/ early/ late/ chronic; (2) Very high clinical success rates; (3) You must place the EVT system in intracavitary position when an associated collection is identified; (4) Placement of both intracavitary and intraluminal EVT appears to be the best approach; (5) Traditional sponge: challenging placement and removal (mouth), prolonged procedures, and need for multiple exchanges (6) Low-cost modified EVT: easy placement and removal, reduction in procedure time, longer interval between EVT system exchanges, low cost, and low AEs rates; and (7) Modified triple-lumen EVT: drainage and nutrition with one tube through the nares
Endoscopic internal drainage with double pigtail stent	(1) Acute/early/late/chronic; (2) High efficacy; (3) No need for external drainage; (4) Need of an associated collection; (4) Easy placement (7fr – gastroscope); (5) Small or large orifices; and (6) Short hospital stay	(1) Defects without an associated collection; (2) No place to accommodate the DPS (small collection: < 2 cm); (3) Long period for complete healing; (4) Risk of migration, perforation and bleeding; and (5) Usually, fluoroscopy is needed	(1) Acute/ early/ late/ chronic; (2) High clinical success rates; (3) Small orifices with associated collection; (4) Easy placement; (5) Shorter hospital stay/ electives procedures for DPS exchanges; (6) Faster oral intake (start with clear liquids); (7) Better patient acceptance – no symptoms; (8) Long period for complete healing; and (9) Ureteral stents appear to be safer with similar efficacy
Septotomy	(1) Early/late/chronic (> 15 d); (2) High efficacy; (3) Safe; (4) Septum between the orifice/ collection and the gastric lumen; and (5) Must do it when a septum is identified	It is only performed when a septum is identified	(1) Early/late/chronic (> 15 d); (2) Very high clinical success; (3) Usually more than 1 session is required; (4) Cut until the staple line; (5) APC or Knife (APC < bleeding); (6) Always dilate after septotomy; (7) Outpatient procedure; and (8) Septum is the cause of most late/chronic refractory defects treated in a center without experience

APC: Argon plasma coagulation; DPS: Double-pigtail stents; EVT: Endoscopic vacuum therapy; GI: Gastrointestinal.

In our experience, we only use endoscopic suturing as an adjunctive therapy, for example to fix a stent aiming to reduce the risk of migration.

Self-expandable metal stents

SEMS (Figure 7) are the most used technique for the treatment of post-bariatric surgical leaks and fistulas worldwide and is recommended by the American Society for Metabolic and Bariatric Surgery in a position statement from 2015[14]. SEMS are widely available, easy to place and usually with a low cost.

SEMS are indicated for acute and early leaks and should be avoided in late and chronic defects and late stenosis due to reduced efficacy and increased AE rates, especially migration[51]. The American Gastroenterology Association suggests the use of SEMS for leaks and fistulas earlier than 6 wk, preferably in defects < 10 mm[15].

Despite satisfactory efficacy in acute and early leaks as shown in a recent meta-analysis (RYGB: 76.1% and LSG: 72.8%), high migration rates (RYGB: 30.5% and LSG: 28.2%) must be considered, as an urgent surgery due to device migration may be catastrophic[52]. It is important to remember that SEMS placement requires drainage of any associated collections before or promptly after stent placement.

Choosing the correct SEMS facilitates successful therapy. Fully-covered SEMS (FCSEMS) are easy to remove but have a high rate of migration (Figure 7A). Fixation of the FCSEMS with suturing, cap mounted clips or the nasal bridle technique is recommended[53]. On the other hand, partially-covered SEMS (PCSEMS) are associated with low rates of migration, with challenging removal, particularly after 3 wk due to tissue ingrowth (Figure 7B). Nevertheless, some groups argue that the PCSEMS may be better than the FCSEMS as tissue ingrowth prevents leakage between the esophageal wall and the stent. In cases of stent removal failure, several rescue techniques may be used such as the stent in stent technique, ablation and/or endoscopic resection of the tissue ingrowth[15].

Recently, due to the non-negligible migration rate of the esophageal SEMS (Figure 7C), novel models of stents (longer and with a larger diameter) have been developed, focused on the gastric sleeve anatomy. These are customized bariatric stents (Figure 7D). In a retrospective multicenter study[26], 37 patients with early and acute post-LSG leaks were treated with the 24-cm length customized bariatric stent showing similar efficacy and migration rates reported with conventional esophageal SEMS[52]. However, more severe AEs were reported, including two urgent surgeries due to stent migration[54], esophageal perforation[55], contained gastric perforation, and symptoms such as pain and gastroesophageal reflux. In this study, post-pyloric position was associated with high rates of migration and pre-

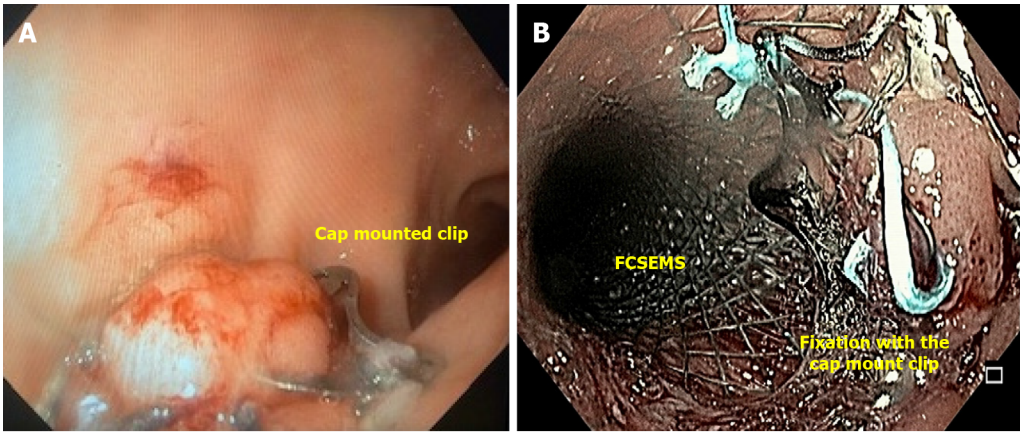

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Figure 4 Cap-mounted clip. A: Cap-mounted clip for an acute leak (orifice < 2 cm) in the staple line after laparoscopic sleeve gastrectomy in a stable patient with external drainage; B: Cap-mounted clip used for stent fixation; C: Enteroscopy to remove stent after migration due to cap mounted clip fixation failure. FCSEMS: Fully covered self-expandable metal stents.

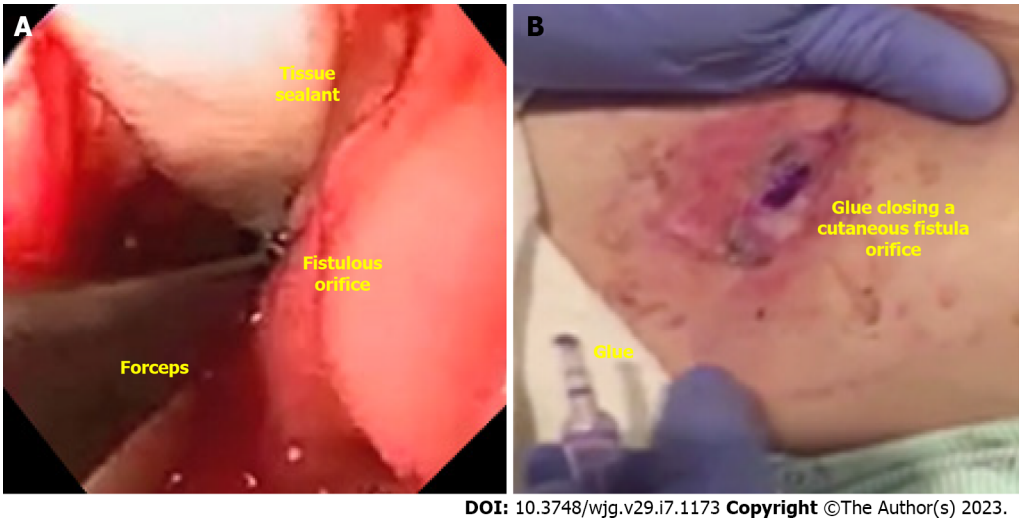

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Figure 5 Tissue sealants/Glues. A: Endoscopic placement of acellular matrix biomaterial in a late gastrocutaneous fistula after laparoscopic sleeve gastrectomy (LSG); B: Percutaneous application of glue (cyanoacrylate) in a chronic gastrocutaneous fistula tract after LSG.

pyloric position with more stent related symptoms[26]. In a meta-analysis comparing conventional esophageal SEMS and the customized bariatric stents, there were no statistical difference in terms of efficacy (93% *vs* 82%) and migration (15% *vs* 32%) rates[56]. Therefore, this novel customized bariatric stent should be used with caution, preferably in specialized centers with close follow up[57].

In our experience, SEMS are best applied in acute and early leaks with complete dehiscence and/or with associated downstream stenosis, always in conjunction with external drainage. Moreover, we

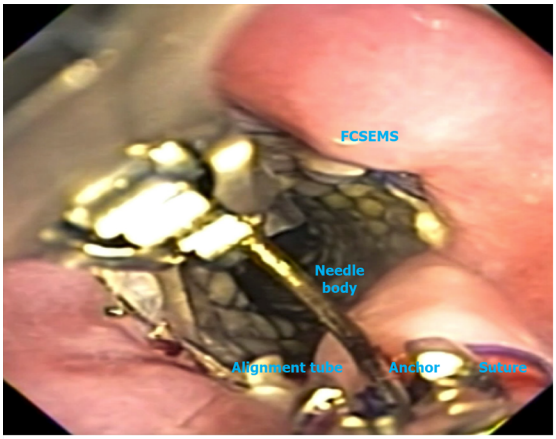

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Figure 6 Endoscopic suturing for stent fixation placed in the distal esophagus of a patient with an early leak after Roux-en-Y gastric bypass. FCSEMS: Fully covered self-expandable metal stents.

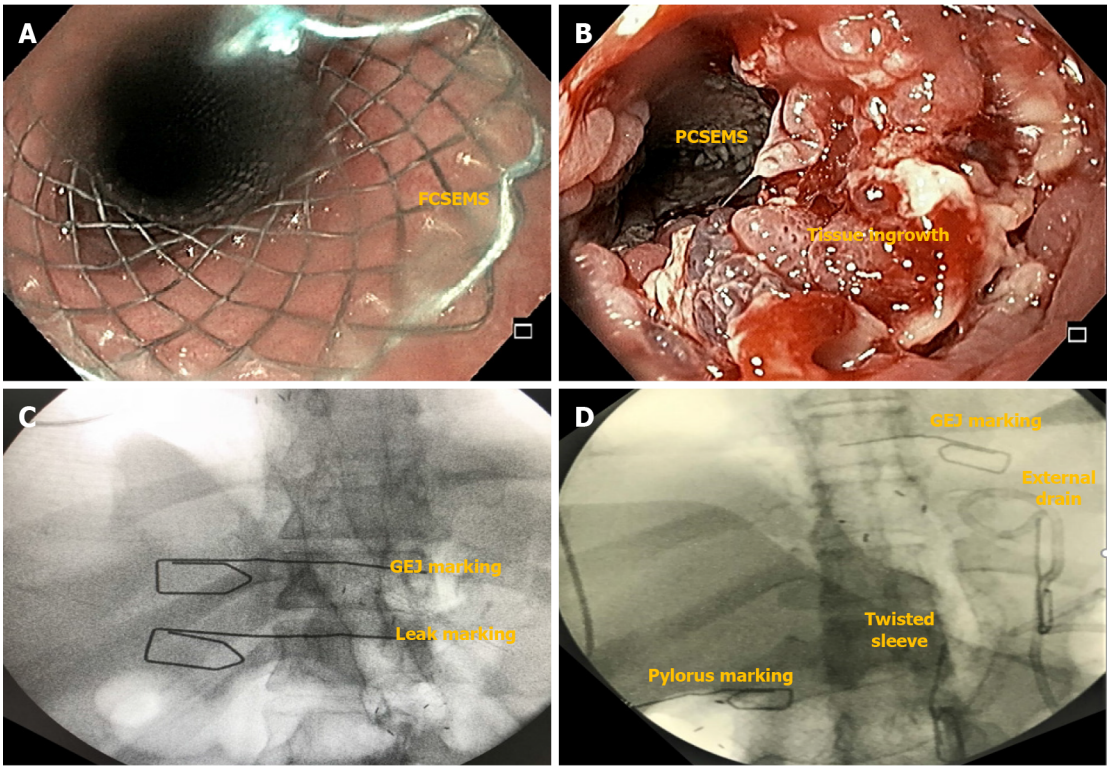

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Figure 7 Stents for the treatment of post bariatric and metabolic surgery leaks. A: Esophageal fully covered self-expandable metal stent; B: Tissue ingrowth in an esophageal partially covered self-expandable metal stent; C: Esophageal stent for the treatment of an acute leak after Roux-en-Y gastric bypass; D: Customized bariatric stent for the management of an acute leak in the angle of His after laparoscopic sleeve gastrectomy showing a stenosis at the level of the incisura angularis. FCSEMS: Fully covered self-expandable metal stents; GEJ: Gastroesophageal junction; PCSEMS: Partially covered self-expandable metal stents.

prefer the esophageal PCSEMS with dwell time of no more than 3 wk, over customized bariatric stents.

Cardiac septal defect occluder

The non-surgical closure of a cardiac septal defect was first described in 1976 and was considered a revolution in the treatment of cardiac defects[58]. Although developed for the closure of cardiac defects, the off-label use of the CSDO has been reported for the management of bronchopleural and GI fistulas [59].

The CSDO is a self-expanding, double-disc closure device composed of nitinol and polyester, with shape-memory and impressive expansion force (Figure 8)[28,59]. The thick waist portion serves to self-center the device during deployment to close the defect. The disc diameter varies from 9 mm to 54 mm, and the waist size varies from 4 mm to 38 mm.

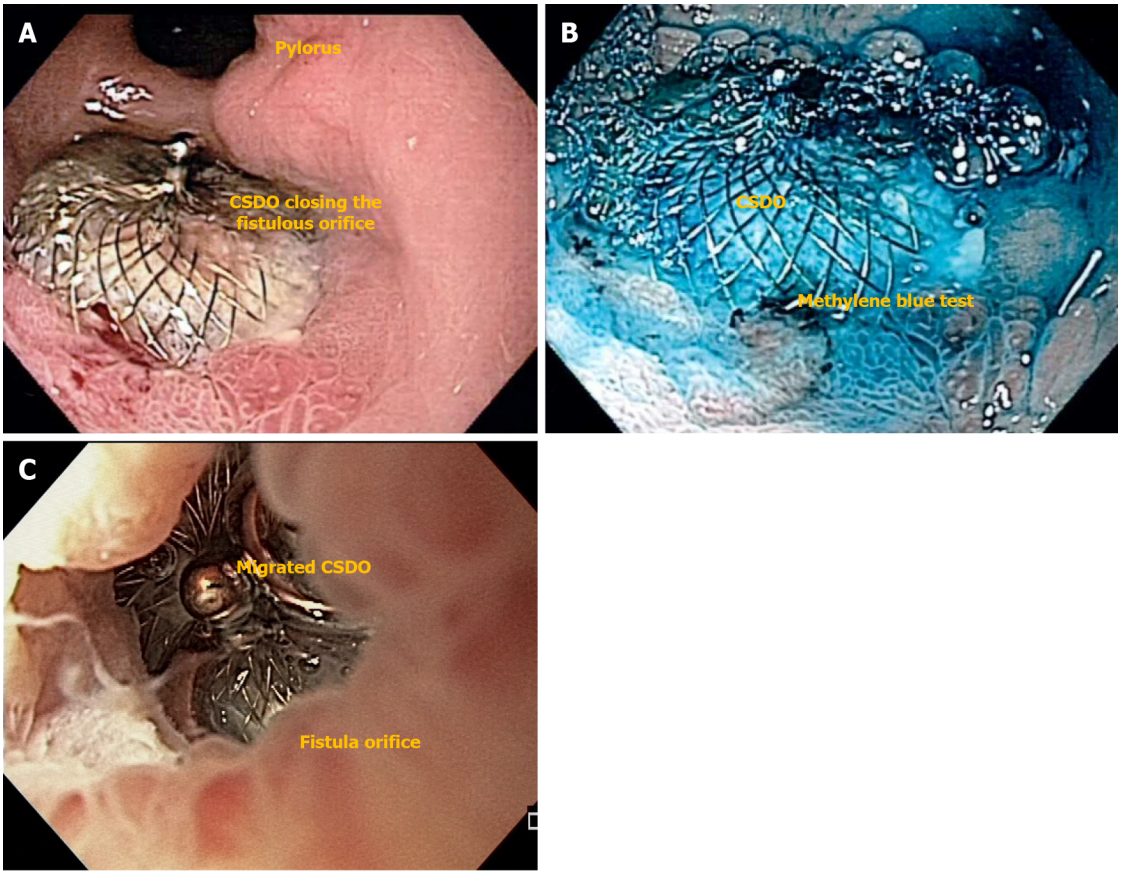

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Figure 8 Cardiac septal defect occluder for the treatment of chronic fistulas after bariatric and metabolic surgery. A: Endoscopic imaging of a successful closure of a gastrocutaneous fistula after cardiac septal defect occluder (CSDO) placement in a distal staple line leak after laparoscopic sleeve gastrectomy; B: Methylene blue test showing no leakage to the skin; C: CSDO migration after placement in an early leak after Roux-en-Y gastric bypass surgery. CSDO: Cardiac septal defect occluder.

The appropriate device size ("waist diameter") should be 50% larger than the defect orifice. Regarding placement, first, the distal flange is released either into the GI lumen (percutaneous placement) or the fistula tract (endoscopic placement), then, after adequate position is confirmed, the proximal flange is deployed[28].

In a multicenter study, clinical success rate for late and chronic defects after BMS was 97.1% *vs* 62.5% in acute and early scenarios[28]. Due to its expansion force, immediate contrast study or methylene blue test after the procedure usually confirms complete occlusion of the fistulous tract allowing hospital discharge after anesthesia recovery (Figure 8A and B)[32]. If immediate, complete closure fails, adjunctive therapies such as glues/tissue sealants can be performed[31]. Therefore, CSDO is recommended for late and chronic defects as an epithelialized tract is needed for device accommodation. The device should not be used in acute and early leaks or fistulas as it can increases the size of the defect due to its significant expansion force (Figure 8C)[3].

In our experience, the CSDO is the best approach for epithelialized fistulas (Figure 5B) tracts but as an off-label high-cost device, we only use it after conventional technique failure. Regarding long-term follow-up, some devices stay in place and other migrate after complete healing of the defect[28,60].

Endoscopic vacuum therapy

EVT is indicated for GI transmural defects (Figure 9). Its high efficacy is associated with its mechanism of action that involves micro-deformation, macro-deformation, changes in perfusion (angiogenesis), exudate control, and bacterial clearance[4]. The traditional technique involves the use of a polyurethane sponge connected to a nasogastric tube placed into the extraluminal compartment (intracavitary position) or into the GI lumen (intraluminal position). The nasogastric tube is then connected to a vacuum machine with continuous negative pressure (between -125 and -175 mmHg). It is important to understand that, when an associated collection is identified (Figure 9A), the EVT system must be placed inside the collection (intracavitary) (Figure 9B). Despite the high efficacy, the traditional system (Figure 9C) is associated with challenging placement and removal (through the mouth) leading to a prolonged procedure, and the need for frequent exchanges due to tissue ingrowth.

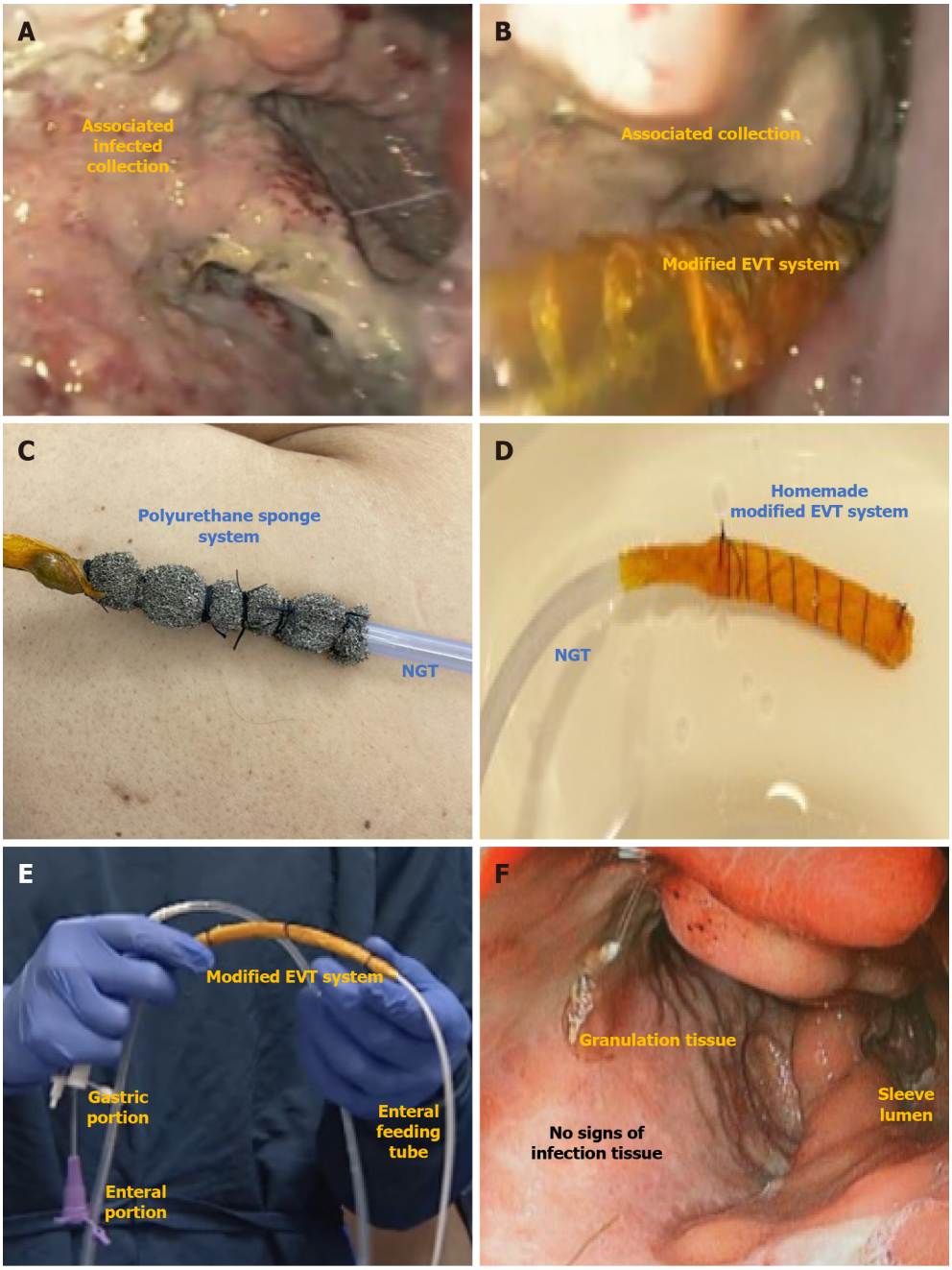

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Figure 9 Endoscopic vacuum therapy for the treatment of a post bariatric and metabolic surgery leak. A: Leak associated collection after laparoscopic sleeve gastrectomy; B: Intracavitary modified endoscopic vacuum therapy (EVT) placement; C: Traditional (polyurethane) sponge system; D: Homemade modified EVT; E: Triple lumen tube used for intraluminal EVT allowing for drainage and nutrition with one tube through the nares; F: Complete tissue healing showing granulation tissue and no signs of infection after EVT treatment. EVT: Endoscopic vacuum therapy; NGT: Nasogastric tube.

To overcome these limitations, the open pore film system was developed showing several benefits compared to the traditional sponge system, maintaining a high efficacy and safety profile. These advantages include easy placement (through the nares), reduction in procedure time, longer interval between EVT system exchanges, and a lower rate of AEs[61]. Despite these improvements, the high cost and limited availability of the open pore film remain barriers to its use and widespread adoption. Recently, a cost-effective modified-EVT manufactured from widely available materials and utilizing wall suction was described with promising results (Figure 9D)[62-65]. Furthermore, the use of intraluminal EVT *via* a triple-lumen tube was reported that allowed both drainage (gastric fenestrations) and nutrition (enteral portion) using a single tube through the nares (Figure 9E)[64,66].

Previous studies had already demonstrated very positive outcomes for EVT in the management of general upper GI leaks and fistulas, including with higher success rates and lower AEs rates when compared to SEMS[67]. A recent meta-analysis evaluated the use of the EVT in the management of leaks and fistulas after BMS demonstrating a pooled clinical success rate of 87.2% with 6% AE rate. In this

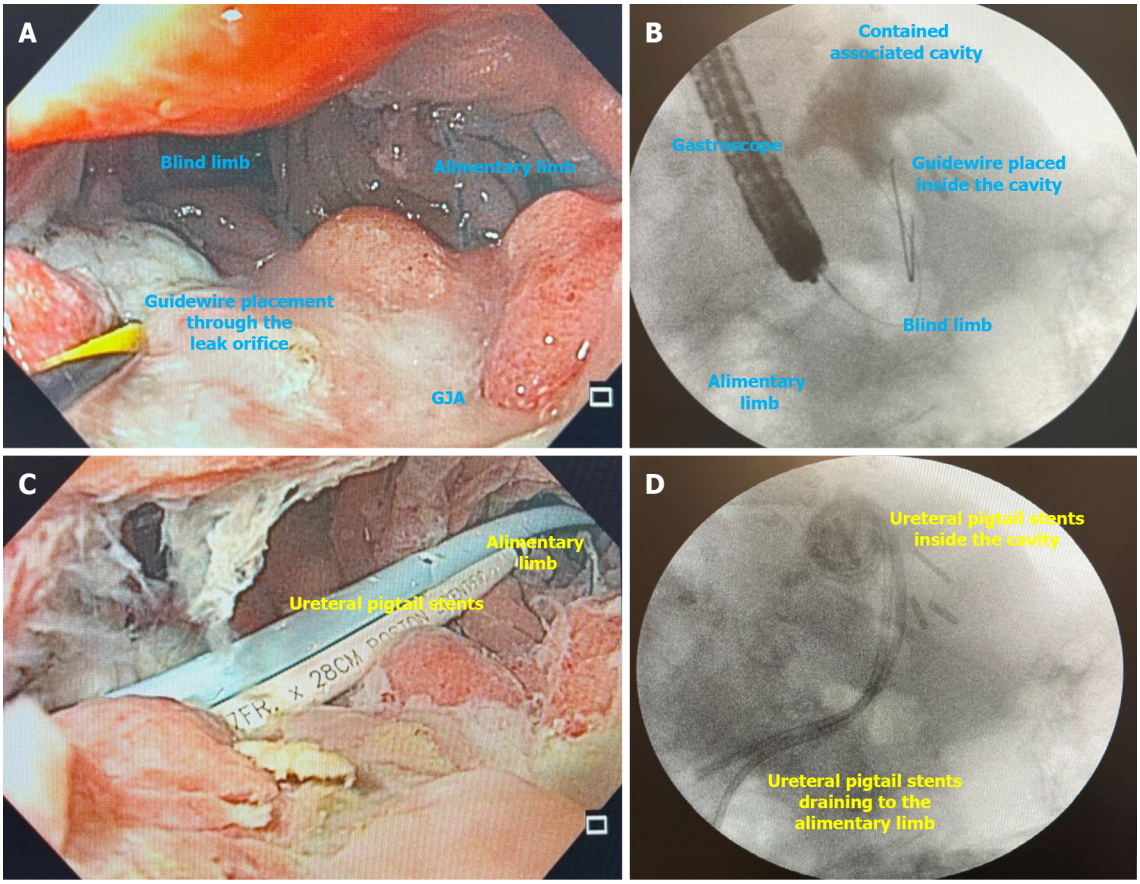

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Figure 10 Endoscopic internal drainage with double pigtail stents. A: Leak orifice at the gastrojejunal anastomosis suture line; after Roux-en-Y gastric bypass surgery; B: Fluoroscopy showing a contained associated collection and guidewire placement; C: Endoscopic internal drainage with ureteral pigtail stents; D: Fluoroscopy showing adequate placement and drainage of the leak associated collection. GJA: Gastrojejunal anastomosis.

study, the mean number of EVT system exchanges was 6.47 with a mean interval of 4.39 d[68]. Despite the satisfactory results, there is a concern regarding patients' complaints due to the nasogastric tube and a possible prolonged hospital stay. Additionally, the risk of major bleeding related to the development of a fistula between the wound cavity and major blood vessels, including the aorta or its branches is a reported concern from some experts[4,69,70].

In our practice, the cost-effective modified EVT is the preferred approach due to its high efficacy, satisfactory patient acceptance, and safety benefit of avoiding sponge dislodgement and exchange related bleeding[71]. Based on our experience, the best indication for EVT is acute and early leaks with associated infected collections. In these cases, after clinical improvement and collection clearance associated with granulation tissue (Figure 9F), we do change the intracavitary EVT for EID with DPS allowing earlier hospital discharge or when the associated cavity is smaller than 3 cm, we change the intracavitary EVT for intraluminal EVT.

Endoscopic internal drainage with double pigtail stent

EID with DPS (Figure 10) is indicated for leaks and fistulas with associated fluid collections after BMS. The principle for internal drainage is based on the concept that when the pressure within the gastric lumen is lower than that of the perigastric collection, flow will be directed into the GI tract. Additionally, oral contents will preferentially flow through the gastric lumen.

For EID with DPS to be effective as a sole strategy, the following conditions should be absent: (1) Uncontained perigastric collections; (2) High intragastric pressure secondary to downstream stenosis; and (3) Existence of a gastropleural fistula. With drainage, the perigastric cavity, will typically contract until it is obliterated, resolving the leak. When evaluating the response to therapy, the ability to tolerate diet as well as a decrease in the size of the perigastric cavity are indicative of clinical success[72,73].

EID has been widely adopted, especially in Europe, with high clinical success rates for both acute and chronic leaks and fistulas and a low rate of AEs, allowing a shorter hospital stay.

The largest study evaluating EID with DPS for the treatment of complications after BMS included 617 patients, with a clinical success rate of 89.5% for leaks and 78.5%, for fistulas[74].

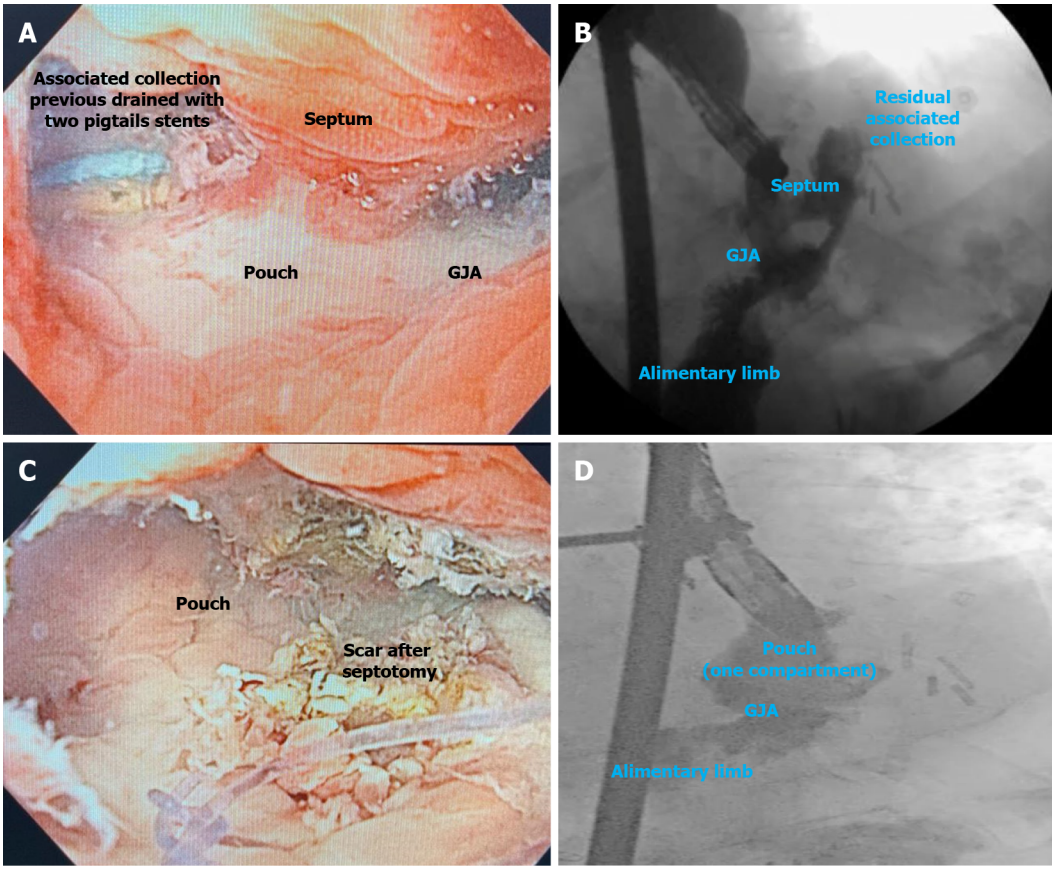

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Figure 11 Septotomy. A: Septum between the associated collection and the leak orifice at the gastrojejunal anastomosis staple line after Roux-en-y gastric bypass surgery; B: Fluoroscopy showing a septum between the leak associated collection and the gastric pouch; C: Endoscopic image after septotomy; D: Fluoroscopy showing a gastric pouch without associated collections as the septotomy transformed the pouch and the associated collection in one compartment. GJA: Gastrojejunal anastomosis.

Placement of DPS is easy to learn (Figure 10A and B), demonstrated by high clinical success when performed by an endoscopist with (84.71%) or without (83.41%) experience in EID as demonstrated by a recent systematic review[72]. Additionally, it also presents satisfactory clinical success as a rescue therapy (78.05%)[72].

Defect closure is usually achieved after a long period of treatment. However, as most patients are not hospitalized, present no symptoms, receive oral diet, and return to their daily activities, the long treatment time is not considered an issue when compared to other techniques which are associated with symptoms related to the therapy, such as bariatric stents, or need for hospital stay, such as EVT[72].

AEs are reported in about 13.8% of patients, mostly due to stent migration, but also including perforation and bleeding[72].

In our experience, EID with DPS is the best approach for defects with associated collections in patients with no signs of sepsis. As most patients are not hospitalized, close follow-up is needed to avoid complications. Additionally, to minimize the risk of AEs related to the DPS, we prefer to use ureteral stents as they are more flexible and softer than biliary DPS, avoiding damage to tissue and vessels (Figure 10C and D)[75].

Septotomy

Septotomy (Figure 11) must be performed when a septum between the defect orifice/associated collection and the GI lumen is identified (Figure 11A and B). The principle of this therapy is similar to the Zenker´s diverticulotomy. The septum is sectioned to match the pressure of the leak or fistula orifice within the gastric chamber, providing better drainage (Figure 11C and D). The septotomy can be performed with endoscopic electrosurgical knives, through the scope scissors or argon plasma coagulation (APC), until the depth of the suture line without exceeding this limit (the cut must not extend beyond the base of the perigastric cavity) to avoid perforation[3,76]. It is important to state that in most cases, several sessions are needed.

Septotomy is associated with high clinical success rates and with low AEs in expert hands. In a study involving 27 patients with leaks and fistulas after BMS, including RYGB, LSG, and duodenal switch, the clinical success rate after one to six septotomies was 100%, with an average treatment time of 18.11 d

Table 4 Suggested recommendations on management of post bariatric surgical leaks and fistulas

Leak or fistula characteristics + patient clinical condition	Recommended management (first line approach)	Possible therapy (second line approach)	Possible endoscopic therapies based on defect characteristics
Acute and early leaks with undrained uncontained collection in unstable patients	Surgical lavage + external drainage (surgical placement) ± surgical repair ± endoscopic therapy (see column 4)	Image-guided external drainage + endoscopic therapy (see column 4) OR Intracavitary EVT	Defect < 2 cm: Cap mounted clips OR stents OR intraluminal EVT; Defect > 2 cm: Stents OR intraluminal EVT; If a septum is diagnosed (early): Septotomy must be performed
Acute and early leaks with undrained uncontained collections in stable patients (rare condition as most patients with undrained uncontained collections presents with peritonitis/sepsis)	Image-guided external drainage + endoscopic therapy (see column 4)	Surgical lavage + external drainage (surgical placement) ± surgical repair ± endoscopic therapy (see column 4) OR Intracavitary EVT	Defect < 2 cm: Cap mounted clip OR stents (prefer stents if associated with downstream stenosis) OR intraluminal EVT; Defect > 2 cm: Stents OR intraluminal EVT; If a septum is diagnosed (early): Septotomy must be performed
Acute and early leaks with undrained contained collections (both unstable or stable patients - most of these patients are stable due to the contained collection)	Endoscopic drainage techniques: Intracavitary EVT OR EID with DPS; If a septum is identified, septotomy must be performed	Image-guided external drainage + endoscopic therapy (see column 4)	Defect < 2 cm: Cap mounted clips OR stents (prefer stents if associate with downstream stenosis) OR intraluminal EVT; Defect > 2 cm: Stents OR intraluminal EVT; If a septum is diagnosed (early): Septotomy must be performed
Late and chronic leaks (both unstable or stable patients - most of these patients are stable as uncontained collection are extremely rare in late and chronic leaks)	Endoscopic drainage techniques: EID with DPS OREVT (intracavitary if associated collection > 3 cm); If a septum is identified, septotomy must be performed	Image-guided external drainage + endoscopic therapy (see column 4) OR Surgical approach	Defect < 2 cm: Cap mounted clips OR CSDO OR tissue sealants/glues (as an adjunctive therapy); Defect > 2 cm: CSDO OR tissue sealants/glues (as an adjunctive therapy)
Late and chronic fistulas (both unstable or stable patients - most of these patients are stable)	Endoscopic therapy (see column 4); Cytology brushing or APC to loosen granulation tissue before endoscopic therapy is helpful; If a septum is identified, septotomy must be performed	Surgical approach	Defect < 2 cm: CSDO ± tissue sealants/glues OR tissue sealants/glues ± cap mounted clips OR tissue sealants/glues + intraluminal EVT; -Defect > 2 cm: CSDO ± tissue sealants/glues OR tissue sealants/glues + intraluminal EVT,
Late and chronic gastro-gastric fistula	Defect < 10 mm: Endoscopic therapy (see column 4); Defect > 10 mm: Surgical approach	Surgical approach after endoscopic management failure	APC ± CSDO ORAPC + suturing OR APC + cap mounted clip

APC: argon plasma coagulation; CSDO: Cardiac septal defect occluder; DPS: Double pigtail stents; EID: Endoscopic internal drainage; EVT: Endoscopic vacuum therapy.

[77]. Our experience reflects the results of this study. We do prefer to use APC as it is associated with a low rate of bleeding.

In our practice, the presence of a septum is the most common cause of late and chronic leaks and fistulas refractory to endoscopic treatment in non-specialized centers referred to our institution. Therefore, when treating a leak or fistula after BMS, attention to the presence of a septum and need for septotomy is required to achieve clinical success.

CHOOSING THE BEST APPROACH

Based on the pathophysiology of leaks and fistulas after BMS, the appropriate therapy should be individualized, considering the patient´s clinical condition, defect characteristics (size, chronicity, associated collections, and health of the surrounding tissue), device availability, cost, patient preference, mechanism of action of each endoscopic therapy, one's personal experience and comfort and local availability of experienced physicians. Although there is a paucity of high-quality studies to provide standardized treatment algorithms, we summarize our recommendations on the management of these challenging conditions in Table 4 and Figure 12 based on our large referral volume, and review of the available literature.

DISCUSSION

The management of post bariatric surgery complications is challenging as patients have obesity-mediated chronic pro-inflammatory state and are in a catabolic state related to weight loss. Additionally, most patients have comorbidities, such as T2DM, arterial hypertension, among others. The most feared complication is leak, as an infection in these patients can be life-threatening or severely

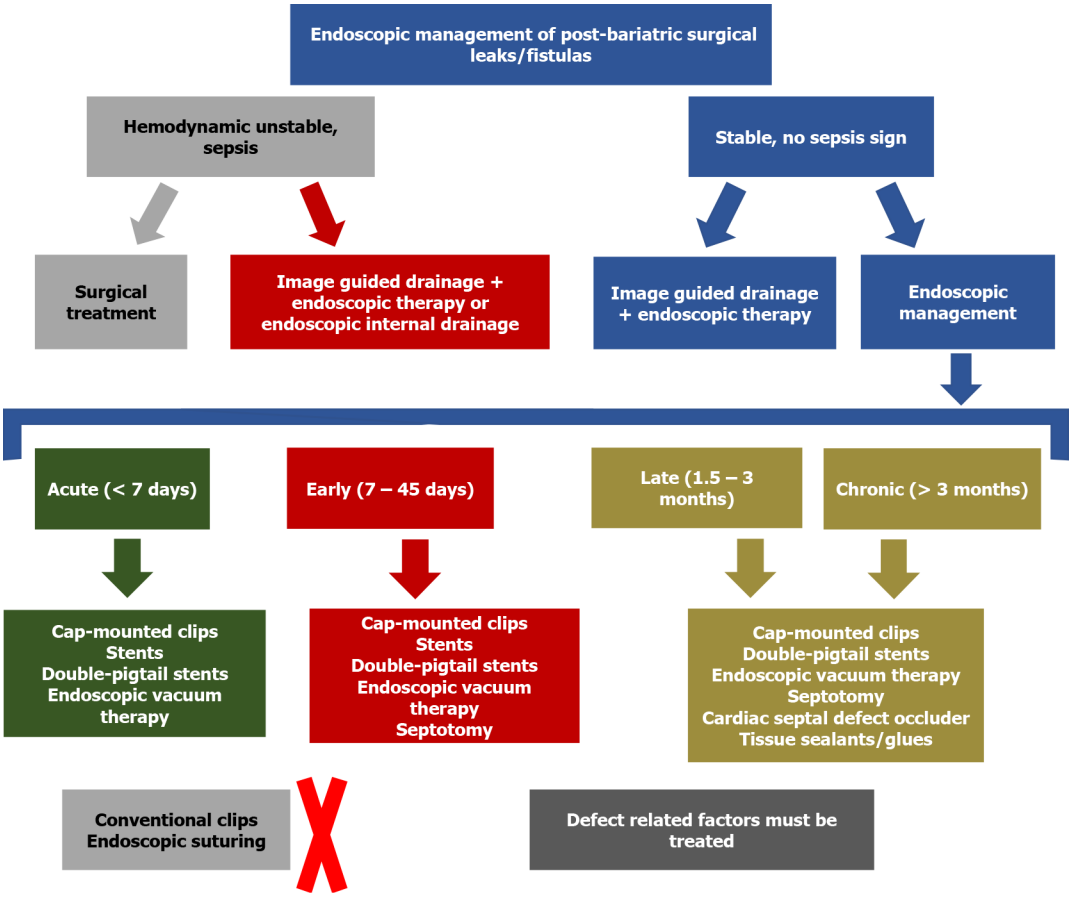

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Figure 12 Suggested algorithm for the management of post bariatric surgical leaks and fistulas.

morbid, especially when early identification and management is not achieved. Although laboratory tests and imaging are important for diagnosis, patient clinical conditions must be the primary consideration. If a patient presents with tachycardia, fever, and intense abdominal pain, a surgical revision should not be postponed.

In cases of acute and early leaks associated with infection, there can be a need for admission to an intensive care unit and long-term hospital stay is often needed. Additionally, some patients cannot take an oral diet and there is a need for enteral nutrition or parenteral nutrition. Unfortunately, long-term inpatient related diseases such as pneumonia, deep venous thrombosis, healthcare-associated infections, and others, can affect these patients. Therefore, multidisciplinary care is required, including surgeons, endoscopists, radiologists, intensivists, infectious disease experts, nutrition experts, nurses, physiotherapists, and other specialists contributing to successful outcomes. Ideally, these patients should be treated in a tertiary referral center given the complexity and relative rarity of this disease state.

For late and chronic leaks and fistulas, almost all patients present in a stable condition, most of them need several procedures and longer treatment time to achieve defect closure. Despite the need for more sessions, as these patients are stable, they do not need to be hospitalized in most cases and can be discharged to attend subsequent elective endoscopic procedures while they carry on with their normal activities.

Endoscopy has evolved to become first line therapy for the treatment of post-bariatric leaks and fistulas, except in unstable patients with undrained, uncontained collections (peritonitis) where a surgical approach is preferred. There are several available endoscopic devices and techniques with different mechanisms of actions, including closing, covering, and draining therapies. When determining the appropriate endoscopic technique, fundamental principles must be considered, such as draining of associated collections and treatment of related factors such as dilation of downstream stenosis and foreign body removal. It is mandatory that endoscopists incorporating management of BMS complications into their clinical practice have expertise in several advanced therapeutic endoscopic techniques as well as knowledge in using fluoroscopy, managing percutaneous drains, and also understanding of post-surgical anatomy and pathophysiology concepts for origin and maintenance of leaks and fistulas. It is also important to understand that there is no gold standard approach for these patients and usually more than one endoscopic intervention is required, especially in a chronic scenario. Furthermore, combined therapies may be required for some patients to achieve clinical success.

CONCLUSION

In summary, a multidisciplinary team and individualized evaluation considering patient and defect characteristics, available resources, local and personal experience, and knowledge of fundamental principles and mechanisms of action of each technique is essential to choosing the best approach for the management of post-bariatric surgical leaks and fistulas. With a committed approach, high rates of leak and fistula closure can be achieved.

FOOTNOTES

Author contributions: de Oliveira VL, Bestetti AM, Trasolini RP, de Moura EGH, de Moura DTH performed the conception and design of the work; de Oliveira VL, de Moura DTH, Bestetti AM drafted the manuscript; de Oliveira VL, Bestetti AM, Trasolini RP, de Moura EGH, de Moura DTH contributed to the critical review of the manuscript for important intellectual contents; de Moura DTH, Trasolini RP, de Moura EGH contributed to the manuscript supervision; Trasolini RP revised the manuscript for English language polishing requirements; de Oliveira VL, Bestetti AM, Trasolini RP, de Moura EGH, de Moura DTH contributed to the approval of the version to be published, have participated in conceptualizing the research or content of the manuscript, in writing or critically editing the manuscript, and/or in analysis of data presented in the manuscript; Consent to submit has been received from all co-authors.

Conflict-of-interest statement: Dr. Eduardo Guimarães Hourneaux de Moura reports personal fees from Boston Scientific, personal fees from Olympus, outside the submitted work; Dr. Diogo Turiani Hourneaux de Moura reports personal fees from Bariatek, outside the submitted work; The other authors reported no potential conflict of interest.

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MINIREVIEWS

Advances in acute and chronic pancreatitis

Williamson B Strum, Clement Richard Boland

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Abstract

Acute pancreatitis (AP) and chronic pancreatitis are the third leading gastrointestinal causes for admissions and readmissions to hospitals in the United States. This review of articles published between 2019-2022 (December) from international sources identified four categories of crucial new findings: The report includes (1) New genetic pathogenic mutations (*TRPV6*); expected genetic outcomes in a Northern European population; (2) a new serum diagnostic marker for AP-fatty acid ethyl esters-distinguishing acute pancreatitis associated with alcohol; explanations of the impact of monocytes/macrophages on the inflammatory process that defines their future in diagnosis, staging, and treatment; (3) innovations in timing of per os low-fat, solid food intake immediately on admission; resolution of concepts of aggressive parenteral fluid intake; dramatic shifts to nonoperative from operative treatment of infected pancreatic necrosis. Each modification reduced interventions, complications, and lengths-of-stay; and (4) authoritarian recommendations for medical treatment of chronic pain. These advances offer opportunities to initiate newly proven treatments to enhance outcomes, alter the natural history, and envision the future of two diseases that have no known cure.

Key Words: Genetics; New diagnostics; Macrophage regulation; New approaches to early feeding; Fluid management

Core Tip: Acute and chronic pancreatitis are leading causes for admissions to hospitals. This review identified four categories of crucial new findings including: (1) New genetic mutations (*TRPV6*); expected genetic outcomes; (2) new serum diagnostic markers-distinguishing pancreatitis associated with alcohol, and defining the impact of monocytes/macrophages on the inflammatory process; (3) critical innovations: In timing of PO low-fat, solid food intake immediately on admission; resolved concepts on fluid intake; non-operative treatment of infected necrosis; and (4) authoritarian recommendations for treatment of chronic pain. These advances offer opportunities to initiate newly-proven treatments to enhance outcomes and alter the natural history.

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INTRODUCTION

Acute pancreatitis (AP) and chronic pancreatitis (CP) are two of the most common causes for gastrointestinal-related office visits and admissions to hospital[1-3]. AP and CP are multifactorial diseases in which the former may progress to the latter as chronic inflammation and autodigestion lead to fibrosis and atrophy. Information that improves understanding of the diseases is critical to advancing therapy. The objective of this review was to identify pivotal studies that directly impart usable information relative to pathophysiology, diagnosis, preventative and other treatments.

METHODS

The information in this review was obtained between 2019 through December 2022 from PubMed, Ovid, International Guidelines, expert opinion, and personal contact with experts. We looked specifically for well-designed, randomized, controlled original research, systematic reviews, meta-analyses, expert guidelines, and professional association guidelines addressing changes favorably affecting outcomes. Case reports, case series and others considered irrelevant to the study were excluded as were most articles outside the inclusive dates, although some were included for supporting information. Over 400 articles were reviewed for possible relevance, with primary selections based on new information considered important and usable for health care workers.

OBSERVATIONS AND DISCUSSION

Pathophysiology: Genetic risks and clinical characterization

The landmark discovery of mutations in the pancreatic digestive genes coding for trypsinogen (*PRSS1* and *PRSS2*[4] catalyzed a search for additional pathologic variants in pancreatic digestive genes. The search has been rewarded with the discovery of a sufficient number of genetic variants to permit a mechanistic classification suggesting their pathogenic effect[5-7]. These include genetic variants in: (1) The trypsin-dependent pathway, including *PRSS1*, *PRSS2*, *SPINK1*, and chymotrypsin C (*CTRC*) genes; (2) the ductal physiology pathway including the cystic fibrosis transmembrane conductance receptor (*CFTR*), *claudin* 2 (*CLDN2*), possibly the *calcium sensing receptor* (*CASR*); proposed, and offered on many testing panels, but still controversial), plus newly discovered variants in the calcium channel coding genes (*TRPV6*, not yet on most testing panels)[8]; and (3) the misfolding-dependent pathway including a distinct subset of *PRSS1* variants, carboxypeptidase A1 (*CPA1*), and rare variants of carboxyl ester lipase (*CEL*) (Figure 1). The *TRPV6* variants are important new additions to the ductal-pathway genes, which alter calcium concentration in ductal fluid. The *TRPV6* variant is identified in 4.3% of nonalcoholic CP patients in Japan and 2.0% in Europe, indicating a substantial global risk gene for CP. Most of the carriers were heterozygous for the variant, implicating the potent pathogenic effect of certain *TRVP6* variants (*i.e.*, haploinsufficiency). Furthermore, 20% with defective *TRPV6* variants were transheterozygous for *SPINK1* variants, an unusually high association[8,9]. More than half of the *TRPV6* variants cause subtle functional defects with loss-of-function for removing calcium from the ductal fluid, leading to increased ductal calcium concentrations. This discovery offers a possible novel target for therapeutic intervention since *TRPV6* expression is regulated by 1-alpha,25-dihydroxyvitamin D3, and partial correction of *TRPV6* dysfunction in heterozygous carriers might be feasible with vitamin D supplements[9]. Collateral laboratory studies demonstrate that much of the injury in acute and chronic

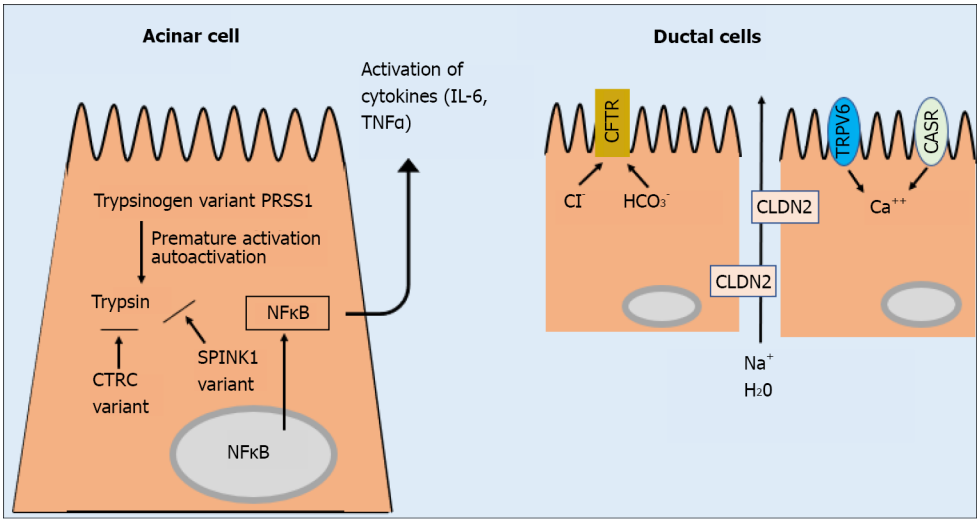

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Figure 1 Diagrammatic depiction of fundamental events contributing to the complex molecular pathways resulting in pancreatitis. Although a unifying concept is not available for all events, recent literature has provided a clearer description of the complexity of pathologic events which is briefly summarized. A proposed first step is injury to cytoplasmic organelles from external stressors, including prolonged, high intake of ethanol and smoking. These injuries result in endoplasmic reticulum stress, mitochondrial depolarization, inadequate ATP production, vacuole accumulation, protein misfolding, and disordered autophagy leading to activation of trypsinogen and inflammatory pathways. Disruption of the normal pathways may be augmented by genetic mutations at key functional sites responsible for trypsinogen activation, trypsin inhibition, Ca^{++} concentrations, and $HCO3^-$ and H_2O movement[4-9,20,21]. CTRC: Chymotrypsin C; CFTR: Cystic fibrosis transmembrane conductance receptor; NF-Kb: Noncanonical nuclear factor-kappaB; TNF-α: Tumour necrosis factor alpha; IL: Interleukin.

pancreatitis evolves from alterations of acinar and ductal digestive function by these pancreatitis-susceptibility mutants, culminating in disruption of the protease-antiprotease interaction in the serine protease trypsinogen pathway within pancreatic acinar and ductal cells[5-9].

Recent studies by Lewis *et al*[10] provide clinical characterization of genetic risks for CP in a North American population of European ancestry. Patients with early-onset (< 35 years) idiopathic CP and no alcohol exposure had significantly more pathogenic variants in 49% of cases compared to no more than 26% in those with late-onset idiopathic CP or CP associated with light-to-moderate or heavy-to-very heavy drinking. Likewise, the early-onset group was significantly more likely to have pathogenic variants in *SPINK1* (24%) or *CFTR* (36%) than patients with late-onset idiopathic CP (9% and 15%) or CP associated with light-to-moderate drinking (7% and 19%) or heavy-to-very heavy drinking (9% and 13%). Evidence supporting pathogenicity of the mutants included a younger age of onset of symptoms in which the *SPINK1* mutation decreased the age at onset to 12 years from 24 years in early-onset idiopathic CP ($P = 0.004$) and to 24 years from 50 years in the light-to-moderate drinkers ($P < 0.001$). The *CFTR* mutation dropped the age at onset to 41 years from 50 years in the light-to-moderate drinkers ($P = 0.030$). Sixty-one cases of early-onset ICP were characterized by persons with an average age of 20 years, incessant pain, pancreatic calcifications, and genetic variants in the *SPINK1*, *CTRC*, or *CFTR* genes in almost half the cases. These collective findings reclassified as many as 23%-49% of cases of ICP to those with potential genetic causes.

CFTR was the most common gene with pathogenic variants in 13%-36%. It is important to recognize that interactions between incompletely penetrant genetic variants-including several working synergistically (*i.e.*, epistasis) and a pronounced exposure to alcohol, smoking, or other toxins are frequently involved in the final pathways leading to acute and chronic pancreatitis. More often than not, multiple factors are responsible. The pathologic progression then gradually leads to well-recognized complications of chronic inflammation with fibrosis, ductular calcifications, pain, steatorrhea, insulin insufficiency, fluid collections, disability, and premature death.

Along with appropriate counseling, testing for the six (or seven including the newly described *TRPV6* variants) commercially evaluable genetic causes of acute and chronic pancreatitis is recommended in cases that have age of onset < 35 years, relapsing pancreatitis, or are idiopathic. The results offer valuable information regarding diagnostic probability of a genetic predisposition, a prediction as to probable cofactors, how to initiate available treatment as early in the process as possible, and do not require repetitive testing. Persons who have extensive comorbidities or are elderly may not benefit from genetic investigation.

Acute pancreatitis: New tools for diagnosis

AP, a disease with a variety of etiologies and risk factors is diagnosed by blood levels of amylase and lipase, abdominal pain, and imaging abnormalities of the pancreas[11-13]. A new diagnostic test has produced intriguing results. Vela and colleagues conducted a prospective, blinded trial comparing

blood levels of fatty acid ethyl esters (FAEEs), nonesterified fatty acids, and ethanol in 175 patients at the time of hospitalization with alcoholic pancreatitis, alcoholic intoxication, nonalcoholic pancreatitis, and normal controls[14]. Distinguishing differences were that median FAEEs were similarly elevated in alcoholic pancreatitis (103 nmol/L) and alcoholic intoxication (205 nmol/L), whereas there was no significant elevation in nonalcoholic causes of AP (8 nmol/L) or controls (1.7 nmol/L). By its unique ability to demonstrate diagnostic elevations of FAEEs in association with alcohol intake, this test could become an efficient tool for identifying alcohol as the cause in alcohol-associated pancreatitis when present and eliminating alcohol as the culprit when caused by other factors. That alcohol intoxication without AP raises the level of FAEEs is explained by a well-known effect of excessive ethanol ingestion on cell membranes. The study did not distinguish between AP and alcohol-induced AP superimposed on CP, although the latter would be suspected in most of the latter cases.

In a similar vein, Manohar *et al*[15] have presented data defining the complex role of immune responses in monocytes and macrophages using single-cell mass Cytometry by Time Of Flight in two experimental models and in patients with acute, recurrent, and severe AP. As inflammatory cells determine disease severity and pancreatic damage is increased by tissue-derived neutrophils, macrophages are the predominant immune cell in the early phases of AP. Their report explains the role of the immune response in AP, provides insight toward the biological significance of novel Ly6Gc+/CD206+ monocytes and macrophages, and shows how these cellular components may be used to diagnose AP, define its severity, and target potential treatment[15,16].

Chronic pancreatitis: Alcohol and smoking

CP is diagnosed by imaging scans showing glandular atrophy, ductal distortion, and calcifications, or by biopsy demonstrating widespread fibrosis, and is, like AP, characterized by a variety of similar etiologies and risk factors, excepting gallstones and medications[2,3,13]. The relationship of ethanol and smoking to CP is indisputable. Both the amount and duration of exposure matter, and the exposures may vary with time and gender. A contemporary analysis of daily ethanol consumption in persons with alcoholic CP revealed a median of 5.1 drinks/d, in which 12.0 g/drink = 61.5 g/d of ethanol[17]. Consumption of up to 110-277 g/d over 5-25 years may transpire by the time of diagnosis of alcoholic CP[2,3,17-19]. A lower intake of < 50 g/d is implicated where the disease progresses more slowly, and persons are older at the time of diagnosis with less pain and fewer complications[10]. Evidence substantiating the role of ethanol and tobacco intake in perpetuating the fibro-inflammatory phase is exemplified by unabated progression even if ethanol and tobacco intake are discontinued, although at a slower pace when compared with continued usage of both[2,3,10], implicating ethanol and tobacco activating subclinical pancreatic stellate cell fibrosis for years before becoming clinically apparent[19-21].

Recently, models ranging from necroptosis to pyroptosis show how a pathogenic metabolic pathway can provoke inflammation, often initiated by premature intrapancreatic activation of trypsinogen, and be perpetuated by synergy between genetic, epigenetic, immune and environmental factors[5-9] as clinical and post-mortem studies of chronic alcoholics without a sentinel AP event show pathologically recognizable CP in the form of fibrosis and/or ductal calcifications in 47%-68%, indicating extensive damage present before diagnosis[19,22,23].

NEW TREATMENTS

Early feedings in acute pancreatitis

Initiating early feeding per os or *via* nasogastric/nasojejunal routes beginning after the onset of AP is supported by multiple randomized controlled trials and has replaced prior practices of resting the gastrointestinal tract until pain subsides. This approach increases bowel motility and reduces rates of organ failure, infection, length of stay and mortality, substantiating the suspicion that an idle intestine leads to bacterial overgrowth, increased permeability and bacterial translocation[2,3,11,24]. Questions regarding preferred timing had remained but new results from a randomized controlled trial of 131 patients with mild-moderate AP provide clarity. The study compared those fed a low-fat, solid diet immediately upon decision for admission, regardless of symptoms or laboratory parameters, to those receiving conventional feeding with progressive diet as clinical and laboratory parameters improved. The results indicate that immediate feeding is safe and feasible with significantly decreased complications (4.2% *vs* 18.3%) and shorter length of stay (3.4 d *vs* 8.8 d) at one-half the costs[25].

Fluid resuscitation in acute pancreatitis

A major question addressing the appropriateness of early, aggressive hydration was just answered in an important multicenter, randomized, controlled trial from India, Italy, Mexico, and Spain that assessed the efficacy and safety of aggressive fluid resuscitation as compared with moderate resuscitation using lactated Ringer's solution in patients with AP; patients with moderate to severe disease or heart failure at baseline were excluded[26]. The primary outcome was the development of moderately severe or severe acute pancreatitis and secondary outcomes included organ failure and local complications

occurring after randomization and during the hospitalization. The aggressive-group received a bolus of 20 mL/kg body weight over 2 h, followed by an infusion rate of 3 mL/kg/h, and the moderate-group a dose of 1.5 mL/kg/h, with a bolus of 10 mL/kg/h if hypovolemic. Oral feeding was started at 12 h if the intensity of abdominal pain permitted. The first interim analysis included 249 of the planned sample size of 744 patients and uncovered significant fluid overload in the aggressive-resuscitation group at 20.5% *vs* 6.3% (P = 0.004), and the trial was stopped[26]. Significant differences were not reached for the primary or secondary endpoints, although the results tended to favor the moderate resuscitation group. An editorial analysis concluded that the trial results were stunning and irrefutable and that clinicians should follow an infusion rate of the moderate group with careful monitoring of volemic status, adding fluid if underhydrated or diuresis if overhydrated[27].

Pancreatic fluid collections

New data have created a decisive shift in treatment of infected necrotizing pancreatitis, in which antibiotics without drainage are replacing the long-held concept of antibiotics and immediate drainage of infection developing after AP[28]. The crucial substantiation came from a randomized, superiority trial in the Netherlands that compared immediate drainage of infected necrotizing pancreatitis *vs* postponement of drainage in patients with infected tissue occurring within 35 d from onset of initial symptoms of AP. Infection was documented by gram stain, culture, or clinical deterioration and both groups received antibiotics. The Dutch study enrolled 104 qualifying patients; complications and mortality were similar for both groups. However, the mean number of interventions was 4.4 for the immediately drained group compared to only 2.6 (40% less) for the deferred group; 37% (19/52) of patients in the deferred group did not require drainage, and the number of hospital days was shorter by 15% (51 d *vs* 59 d). The rationale for the study had been supported by retrospective studies that showed apparent safety in postponing intervention with drainage, although none have demonstrated superiority[28-30].

Current concepts for pain management in chronic pancreatitis

Pain affects most persons with CP and is the most significant obstacle to successful treatment.

Pain is the major cause of morbidity, disability, and impaired quality of life. Pain syndromes are complex, widely variable and range from severe and chronic to absent[31,32]. An up-to-date study of pain patterns in 1131 persons in 30 Dutch hospitals revealed continuous pain in 52%, intermittent pain in 20%, and no pain in 28%[33]. Patients with continuous pattern had more severe pain, used more opioids and neuropathic medications, and had lower quality of life.

Current concepts emphasize that pain may arise from origins other than the pancreas. Persistent, severe pain can transfer centrally in association with cerebral cortical thinning, central sensitization, central pain processing, or other neuropathology, and becomes less likely to respond to conventional measures. Pancreatic quantitative sensory testing that interrogates nociception and sensory response could help unmask irreversible central neuropathic changes that make interventional treatment of pancreatic pain less likely to be successful. During the course of disease, pain severity may increase or decrease as disease progresses over 10-25 years[31-33]. The initial treatment of choice for those who drink and/or smoke is complete abstinence to reduce the pain, prevent further episodes of AP, forestall progressive damage to the pancreas and lessen cancer risk to the pancreas and other organs[2,3,18,19,28-32]. Early diagnosis of the cause represents the first chance to initiate treatment to retard the progress of a disease that no one can cure. In cases of mild or intermittent pain, non-narcotic oral analgesics including NSAIDS, acetaminophen, tricyclic antidepressants, mirtazapine, and cognitive behavioral therapy may be helpful. Treatment with antioxidants may produce a small improvement, but pancreatic enzyme supplementation does not. Opioids are not recommended, due to their frequent and rising association with addiction and hyperalgesia, but may be considered after other reasonable options are exhausted[2,3,31-34].

In recognition that the longer pain is present, the more difficult it is to treat, a recent randomized surgical study of 88 patients (ESCAPE trial) compared early pancreatic ductal drainage by surgery for those with strong to weak pain of less than 2-6 mo *vs* conventional medical and endoscopic treatment. The initial results indicated a lower pain score in the surgical group of 37 *vs* 49 (P = 0.02)[35], similar to data from Cahen[35]. Additional endoscopic and surgical approaches have been addressed[36-41] but are beyond the scope of this review.

CONCLUSION

This narrative review captures the highlights of a number of significant articles published between January 2019 and December 2022 bearing new concepts in pathophysiology, diagnosis, and treatment of AP and CP. The major findings include clinical identification and application of new genetic information, newly identified serum biomarkers-FAEEs, for diagnosis of alcohol-related AP, novel concepts regarding monocyte/macrophage participation in the immune reaction, important acceleration in timing of early per os feedings in AP, answers to major questions concerning the appropriateness of

early, aggressive parenteral hydration, decisive shifts in non-operative management of infected pancreatic fluid, and updated, expert approaches to medical management of pain in CP. These advances are evaluated in the context of enhancing outcomes for these two acute and chronic inflammatory diseases.

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ORIGINAL ARTICLE

Case Control Study

Comparison of genomic and transcriptional microbiome analysis in gastric cancer patients and healthy individuals

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Abstract

BACKGROUND

Helicobacter pylori and the stomach microbiome play a crucial role in gastric carcinogenesis, and detailed characterization of the microbiome is necessary for a better understanding of the pathophysiology of the disease. There are two common modalities for microbiome analysis: DNA (16S rRNA gene) and RNA (16S rRNA transcript) sequencing. The implications from the use of one or another sequencing approach on the characterization and comparability of the mucosal microbiome in gastric cancer (GC) are poorly studied.

AIM

To characterize the microbiota of GC using 16S rRNA gene and its transcript and determine difference in the bacterial composition.

METHODS

In this study, 316 DNA and RNA samples extracted from 105 individual stomach biopsies were included. The study cohort consisted of 29 healthy control individuals and 76 patients with GC. Gastric tissue biopsy samples were collected from damaged mucosa and healthy mucosa at least 5 cm from the tumor tissue. From the controls, healthy stomach mucosa biopsies were collected. From all biopsies RNA and DNA were extracted. RNA was reverse transcribed into cDNA. V1-V2 region of bacterial 16S rRNA gene from all samples were amplified and sequenced on an Illumina MiSeq platform. Bray-Curtis algorithm was used to

construct sample-similarity matrices abundances of taxonomic ranks in each sample type. For significant differences between groups permutational multivariate analysis of variance and Mann-Whitney test followed by false-discovery rate test were used.

RESULTS

Microbial analysis revealed that only a portion of phylotypes (18%-30%) overlapped between microbial profiles obtained from DNA and RNA samples. Detailed analysis revealed differences between GC and controls depending on the chosen modality, identifying 17 genera at the DNA level and 27 genera at the RNA level. Ten of those bacteria were found to be different from the control group at both levels. The key taxa showed congruent results in various tests used; however, differences in 7 bacteria taxa were found uniquely only at the DNA level, and 17 uniquely only at the RNA level. Furthermore, RNA sequencing was more sensitive for detecting differences in bacterial richness, as well as differences in the relative abundance of *Reyranella* and *Sediminibacterium* according to the type of GC. In each study group (control, tumor, and tumor adjacent) were found differences between DNA and RNA bacterial profiles.

CONCLUSION

Comprehensive microbial study provides evidence for the effect of choice of sequencing modality on the microbiota profile, as well as on the identified differences between case and control.

Key Words: Gastric cancer; Microbiome; *Helicobacter pylori*; 16S rRNA gene; 16S rRNA transcript; 16S rDNA

Core Tip: In this study, we aimed to characterize the microbiota of gastric cancer (GC) on two levels: 16S rRNA gene and its transcript. Our study showed that only a small portion of bacterial sequences overlapped using those two approaches. Moreover, our study revealed that obtained results comparing the case group with the controls depend on the chosen modality. We also showed that *Reyranella* and *Sediminibacterium* was associated with the Lauren classification and RNA level was more sensitive to detect low abundant bacteria. This study provides novel insights into microbiome study as well as new founding related to complex GC pathogenesis.

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INTRODUCTION

Microbiota analyses are becoming increasingly relevant in scientific and clinical studies. Most modern microbiome studies use 16S rRNA gene sequence analysis at the DNA level, thereby enabling the identification of bacteria at all stages of their existence (active, dead, and inactive bacteria in the form of endospores) simultaneously. However, some of the more recent studies use RNA samples, which are subsequently reverse transcribed into cDNA for sequencing, giving us knowledge about the metabolic state of the microbial community[1]. RNA has a shorter half-life than DNA and turns over in cells more rapidly, providing a deeper look at bacterial activity[2].

The stomach has long been considered an almost sterile organ due to its acidic environment and enzymatic effects[3]. Since its identification, it is known that *Helicobacter pylori* (*H. pylori*) is perfectly adapted not only to survive in the acidic environment of the stomach, but also to colonize this part of the gastrointestinal tract[4]. *H. pylori* is the major cause of peptic ulcer disease and the most significant risk factor for gastric cancer (GC). GC remains one of the most common cancers in the world and the fourth leading cause of cancer-related death[5]. However, only a minority of people infected with *H. pylori* develop GC, which may be linked to non-*H. pylori* microbiota-associated alterations in the stomach[6]. Studies in insulin-gastrin (INS-GAS) mice and in humans indicated the importance of other members of the stomach bacterial community in the development of gastric carcinogenesis[7-10].

There is only one study that has compared DNA and RNA profiles of the stomach microbiota[11]. However, the profiles of the active and standing microbiota in GC have not been studied. In this study,

we systematically characterized the microbiota of GC on both levels using the 16S rRNA gene (DNA level) and its transcript (RNA level). GC tumor and tumor adjacent tissue samples, as well as healthy mucosa samples from the young control group, were used for the comparison. We obtained detailed data on bacterial composition within groups depending on study modality (DNA or RNA) and performed association analysis with clinical characteristics to question the potential impact of approach on the outcome.

MATERIALS AND METHODS

Study cohort

In total, 316 DNA and RNA samples from a group of 105 individuals were included in the study (Figure 1). The study cohort consisted of 29 healthy control individuals and 76 patients with GC. Participants did not report any antibiotic intake at least a month before endoscopy. Gastric tissue biopsy samples from damaged mucosa and healthy mucosa at least 5 cm from the tumor tissue were collected from GC patients using single-bite biopsy forceps. From the controls, healthy stomach mucosa biopsies were collected. Tissue samples were placed in sterile cryotubes (Thermo Fisher Scientific, United States), snap-frozen in liquid nitrogen, and stored at -86 °C until further study. Clinical data obtained from histological examination, such as tumor size, number of lymph nodes damaged by tumor cells, presence of metastases (TNM classification), cell differentiation (grading), type of GC (Lauren classification) and stage of GC, were included in the analysis. An overview of the demographic and clinical characteristics of the study cohort is given in Supplementary Table 1.

Study individuals were recruited at the Department of Gastroenterology at the Hospital of Lithuanian University of Health Sciences Kaunas Clinics during the years 2012-2018. This study was approved by the local ethics committee (BE-2-10), and all participants gave their written informed consent.

DNA, RNA extraction, cDNA synthesis and amplicon library preparation

Total DNA and RNA were extracted from gastric biopsy samples using an AllPrep DNA/RNA Mini kit (Qiagen, Germany) according to the manufacturer's recommendations. RNA was reverse transcribed into cDNA using the Superscript IV First-Strand Synthesis System Purification Kit (Invitrogen, Carlsbad, CA) and random hexamer primers, following the manufacturer's instructions. Amplicon libraries were generated as described previously[12,13]. The bacterial 16S rRNA gene V1-V2 region was amplified using the 27F and 338R polymerase chain reaction primers and sequenced on a MiSeq (2 × 250 bp; Illumina, Hayward, CA).

Bioinformatic and statistical analysis

Bioinformatic processing was performed as described previously[14]. FastQ files were analyzed using the dada 2 package[15], version 1.10.1, in R. In total, 7735281 paired-end reads were received, with an average of 22953 per sample. Samples that did not reach 5000 reads were discarded from the analysis (21 samples out of initial 337). All samples were rarefied to an equal sequencing depth of 5047 reads using the phyloseq package[16], with returning 10496 phylotypes (Supplementary Table 2). Phylotypes were annotated to a taxonomic affiliation based on the naive Bayesian classification[17] with a pseudo-bootstrap threshold of 80%. The relative abundances (expressed as percentages) of different microbial communities' phylogenetic ranks (from phylum to class, order, family, genus and phylotype) were used for downstream analyses.

The phylogenetic tree was built using the online tool iTOL[18], after hierarchical clustering using the Bray-Curtis algorithm[19] at the phylotype level in Past 3[20]. Bacterial richness and Shannon diversity indices were calculated using the vegan[21] package from R. The data matrices comprising the percentage of abundances of each of the abovementioned taxa were used to construct sample-similarity matrices by the Bray-Curtis algorithm, where samples were ordinated by principal coordinate analysis (PCoA) at the phylotype level using the patchwork[22] package from R.

Differences in relative abundance of detected bacteria (at all taxonomic ranks) between study groups were evaluated by PERMANOVA and ANOSIM statistical tests, using 9999 permutations. Groups were considered significantly different if the P value was < 0.05, considering an estimate effect-size F values for PERMANOVA and R values for ANOSIM tests. Calculation was made by Past 3 program. The distributions of taxa abundance values were compared by Mann-Whitney test followed by Benjamini-Hochberg correction for multiple comparisons, named as false discovery rate value. Differences were considered significant when the corrected p value (q value) was < 0.05.

The bacterial networks were visualized using Cytoscape 3.8.0[23], after the Spearman correlation test performed with the psych[24] package from R, with threshold of 0.2 in absolute value and P value < 0.05. Phylotypes that accounted for at least 1% of the total number of phylotypes and at least 10% of the samples in each group were used for correlation analysis.

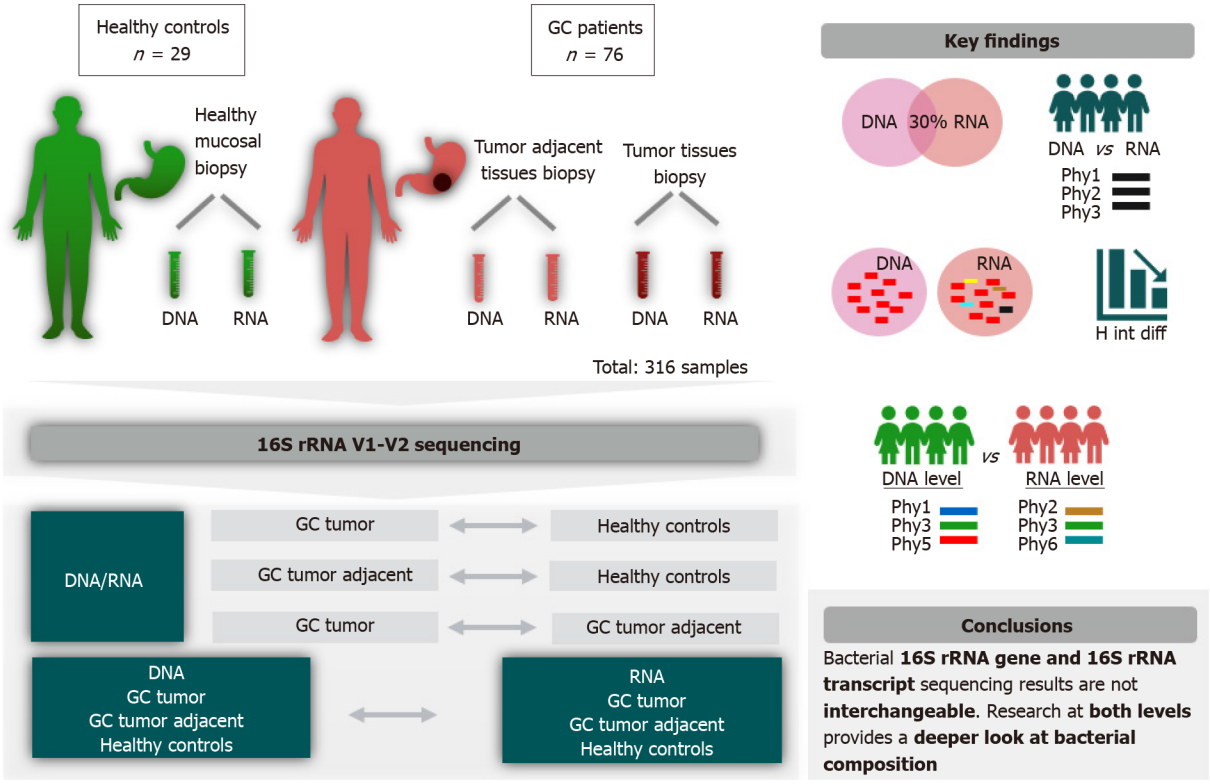

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Figure 1 Study design illustration. GC: Gastric cancer.

RESULTS

General cohort

The bacterial contents of 180 biopsy samples taken from 105 individuals were characterized as described above (Supplementary Table 1). After sequencing and rarefying library size to the minimum sequencing depth, 10496 different phylotypes belonging to 23 phyla, 40 classes, 82 orders, 169 families, and 463 genera were retrieved and taxonomically annotated.

The global bacterial profiles were grouped into two clusters based on their Bray-Curtis similarities as percentages (Figure 2A). Analyzing all samples together, the main factor for clustering was bacterial heterogeneity. The first cluster consisted of samples with a more heterogeneous microbiome profile, where the most abundant bacteria accounted for less than 30%. All control samples (except T10_2) were located in this cluster. The second cluster - where the most abundant bacteria accounted for more than 30% of GC patient samples - was shaped by the most abundant bacteria *Helicobacter*, the abundance of which reached 98%-100% in some samples (Figure 2A, Supplementary Table 2).

Distinct profile of the gastric tissue microbiome at the RNA and DNA levels

Further PERMANOVA and ANOSIM analyses showed that DNA and RNA groups of the same study individuals were significantly different in all taxonomic ranks (Figures 3A-C; Supplementary Table 3). Differences between DNA and RNA samples were noticeable even at the phylum level. Although *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Fusobacteria* were the main bacterial phyla in all groups, *Bacteroides* and *Fusobacteria* were significantly more abundant at the DNA level. However, in the control group, *Firmicutes* and *Proteobacteria* were more abundant at the RNA level. Bacterial profile analysis indicated that only a portion of phylotypes (18%-30%) were common between bacterial profiles obtained from DNA and RNA samples (Figure 3D). PCoA supported the distinction of bacterial communities at the DNA and RNA levels, especially in the control group (Figure 3E). *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Fusobacteria* were major phyla determinants for sample differentiation.

More detailed analysis revealed that DNA and RNA samples differed from each other by 12, 10, and 30 phylotypes and by 18, 17, and 35 genera in the tumor, tumor adjacent, and control groups, respectively (Table 1, Supplementary Table 4). In all study groups, bacteria such as *Neisseria*, *Peptostreptococcus*, *Prevotella*, *Veillonella*, and *Oribacterium* were significantly more abundant at the DNA level, while *Staphylococcus*, *Methyloversatilis*, *Pseudomonas*, *Reyranella*, *Corynebacterium*, and *Sediminibacterium* were significantly enriched at the RNA level. Interestingly, most of these bacteria founded in the RNA samples were not observed in the DNA samples at all, or their relative abundance was low. Some

Table 1 List of significantly (*P* value < 0.05) different genera between DNA and RNA samples in the control, tumor, and tumor adjacent groups

	Increased or decreased at DNA level		
	H	T	Tadj
H & T & Tadj			
Haemophilus	Increased	Decreased	Increased
Methyloversatilis	Decreased	Decreased	Decreased
Neisseria	Increased	Increased	Increased
Peptostreptococcus	Increased	Increased	Increased
Prevotella	Increased	Increased	Increased
Pseudomonas	Decreased	Decreased	Decreased
Reyranella	Decreased	Decreased	Decreased
Sediminibacterium	Decreased	Decreased	Decreased
Staphylococcus	Decreased	Decreased	Decreased
Veillonella	Increased	Increased	Increased
H			
Actinomyces	Increased		
Gemella	Decreased		
Helicobacter	Decreased		
Streptococcus	Decreased		
T & Tadj			
Fusobacterium		Increased	Increased
Granulicatella		Increased	Increased
Porphyromonas		Increased	Increased
Solobacterium		Increased	Increased

The table presents only those bacteria genera, the mean relative abundance of which at least in one of the compared groups exceeded 1%.

changes in the relative abundance of bacteria between DNA and RNA samples were specific for the study group. For instance, in the control group, *Helicobacter*, *Gemella*, and *Streptococcus* were enriched at the RNA level, while *Actinomyces* and *Alloprevotella* were enriched at the DNA level. In the GC groups (tumor and tumor adjacent), *Fusobacterium*, *Granulicatella*, *Solobacterium*, and *Porphyromonas* were enriched at the DNA level. No bacteria were enriched at the RNA level in this group.

Nevertheless, despite the found differences between DNA and RNA, samples of the same origin tended to cluster together in each of the study groups (Figure 2). Paired samples, 46 pairs out of 64 (72%) in the tumor group and 38 pairs out of 58 (66%) in the tumor adjacent tissue group, clustered next to each other, indicating their global similarity (Supplementary Figure 1). Paired samples from the control group were not added to this analysis due to the small number of paired samples.

Revealed microbiome alterations in GC depend on the chosen sequencing modality

The GC samples had lower bacterial richness and diversity compared to control samples (Figures 3F and 3G). While differences in diversity were found both at the DNA and RNA levels, differences in bacterial richness were found only at the RNA level. Group-average agglomerative hierarchical clustering analysis showed that it was possible to distinguish patients with GC from controls by their bacterial profile, as samples tended to cluster based on clinical status (both at the DNA and RNA levels) (Figures 2B and 2C). These results were supported by the phylogenetic analysis of global stomach bacteria, which revealed significant differences between the GC group and control groups at all taxonomic ranks (Figures 3A-C, Supplementary Table 3).

Bacterial abundance differential analysis revealed 15 phylotypes and 17 genera that differed between the GC and control groups at the DNA level (Table 2, Supplementary Figure 2, Supplementary Table 4). Meanwhile, at the RNA level, there were twice as many differences: 40 at the phylotype level and 27 at the genus level. Half of the differences detected at the DNA level were also found at the RNA level (58%

Table 2 List of significantly (*P* value < 0.05) different genera between the control and gastric cancer groups, depending on the selected study material

T *vs* H	Increased or decreased in T		Tadj *vs* H	Increased or decreased in Tadj	
	DNA	RNA		DNA	RNA
Lactobacillus	Increased	Increased	*Lactobacillus*	Increased	Increased
Actinomyces	Decreased	Decreased	*Actinomyces*	Decreased	Decreased
Atopobium	Decreased	Decreased	*Atopobium*	Decreased	Decreased
Granulicatella	Decreased	Decreased	*Granulicatella*	Decreased	Decreased
Propionibacterium	Decreased	Decreased	*Propionibacterium*	Decreased	Decreased
Streptococcus	Decreased	Decreased	*Streptococcus*	Decreased	Decreased
Veillonella	Decreased	Decreased	*Veillonella*	Decreased	Decreased
Rothia	Decreased	Increased	*Rothia*	Decreased	Decreased
Clostridium sensu stricto	Increased		*Leptotrichia*	Decreased	
Prevotella	Decreased		*Prevotella*	Decreased	
Pseudomonas		Increased	*Pseudomonas*		Increased
Staphylococcus		Increased	*Staphylococcus*		Decreased
Gemella		Decreased	*Gemella*		Decreased
Methyloversatilis		Decreased	*Methyloversatilis*		Decreased
Parvimonas		Decreased	*Parvimonas*		Decreased
Reyranella		Decreased	*Reyranella*		Decreased
Sediminibacterium		Decreased	*Sediminibacterium*		Decreased

The table presents only those bacteria genera, the mean relative abundance of which at least in one of the compared groups exceeded 1%.

of genera and 46% of phylotypes). These bacteria include previously described bacteria, such as *Lactobacillus, Propionibacterium, Streptococcus,* and *Veillonella,* among others[25-29].

Although fewer unique bacteria were identified only at the DNA level (8 phylotypes and 7 genera), they were more studied and more frequently discussed in the literature as being associated with various human health conditions (Supplementary Table 4). These bacteria include *Campylobacter*, *Clostridium sensu stricto*, *Prevotella*, and *Saccharibacteria,* among others. Of the listed bacteria, *Clostridium sensu stricto* was enriched, and others were decreased in GC patients. Uniquely, only at the RNA level were 33 and 17 differences at the phylotype and genus levels, respectively, found between the GC and control groups (Supplementary Table 4). Essentially, this group included such bacteria that were not established or their abundance at the DNA level was negligible, for example, *Limnohabitans*, *Methylobacterium*, *Methyloversatilis*, *Pseudomonas*, *Reyranella*, *Rhodoluna*, *Sediminibacterium*, and *Staphylococcus*. Of the listed bacteria, only *Pseudomonas* and *Staphylococcus* were more abundant in GC samples, while all the others were more abundant in healthy individuals.

Bacterial diversity and profile comparison analysis between tumor and tumor adjacent tissues did not reveal significant differences at either the DNA or RNA level (Figures 3A-C, 3F and 3G). Moreover, assemblages of approach from each individual typically clustered together irrespective of tissue type (tumor or tumor adjacent tissue) (Figure 2A).

Bacterial networks in GC patients have fewer components and integrated connections

Analysis of the bacterial network similarity revealed that the main network holding bacteria with the highest betweenness centrality score was different between DNA and RNA levels in all study groups (Supplementary Figure 3). In the bacterial network of the control group at the DNA level, phylotypes depending on the *Streptococcus, Prevotella,* and *Actinomyces* genera accounted for 68% (58 out of 85) of the total number of bacteria and formed the core network keeping bacteria, while at the RNA level, core bacteria were *Streptococcus* and *Gemella*, making up to 62% (47 out of 75) (Supplementary Figures 3A and 3B). The GC groups showed different DNA/RNA networks as well: The main network forming bacteria in the tumor adjacent tissue at the DNA level was *Prevotella*, *Gemella*, and *Granulicatella*, while the RNA network was shaped by *Streptococcus, Reyranella,* and *Fusobacterium* (Supplementary Figures 3C and 3D). The most critical network-forming bacteria in tumor tissue were: *Granulicatella*, *Veillonella*, and *Neisseria* at the DNA level and *Reyranella*, *Acinetobacter*, and *Prevotellaceae* at the RNA level (

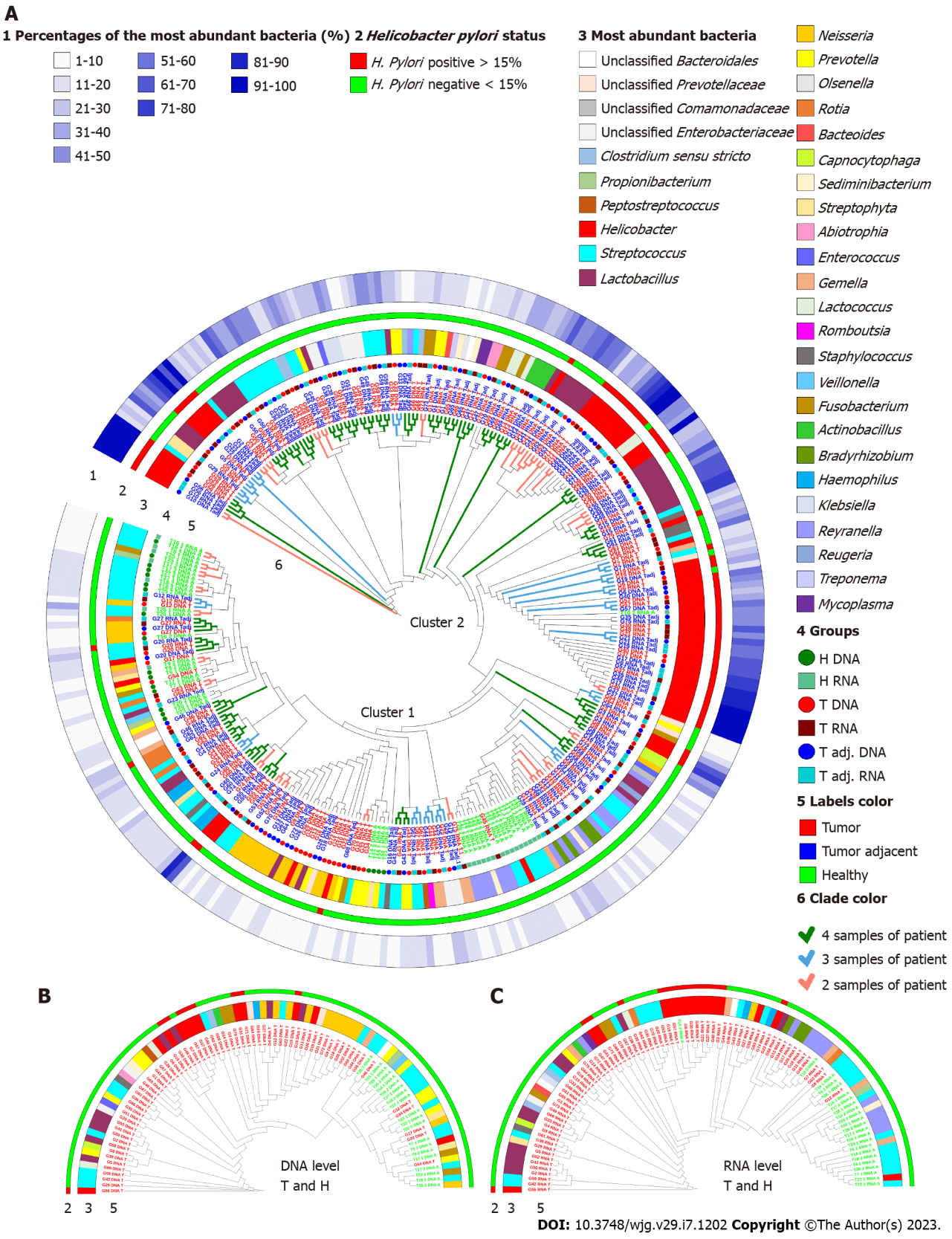

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Figure 2 Group-average agglomerative hierarchical clustering at the phylotype level based on global bacterial profiles. A: Clustering of 139 gastric cancer (GC) tumor, 134 tumors adjacent and 43 control group samples according to individual and general bacterial profiles; B and C: Clustering of GC tumor tissues and the control group at the DNA and RNA levels. The first circle (1) depicts the part of the most abundant bacteria; the second circle (2) represents the status of *Helicobacter pylori*; the third circle (3) shows the most abundant bacteria at the genus level; fourth and fifth circles (4 and 5) display the samples belonging to a certain study group; clade colors (6) represent the abundance of each patient sample clustered together. *H. pylori*: *Helicobacter pylori*.

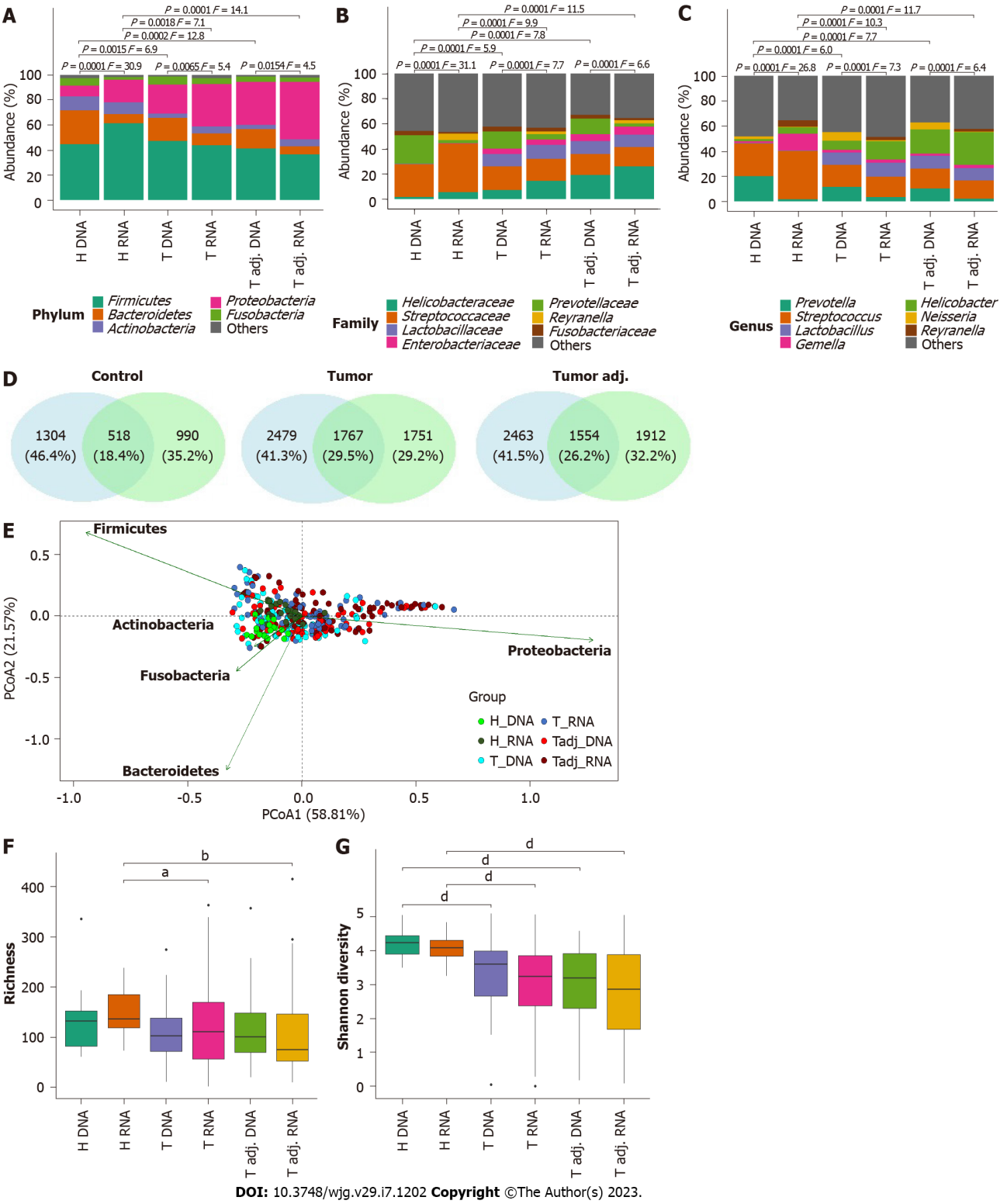

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Figure 3 Shift and diversity of stomach microbiota of healthy controls, gastric cancer patient's tumor and tumor adjacent tissues depending on microbial source (DNA and RNA). A: Relative mean abundance of the top 5 microbiota at the phylum; B: Top 7 microbiota at the family; C: Top 7 microbiota at the genus level. Formal comparisons between groups were evaluated by analysis of similarity (PERMANOVA); D: Venn diagrams show the number of common and group-specific phylotypes comparing each study group at the DNA and RNA levels; E: Principal coordinate analysis of bacterial beta diversity of all study groups at the phylum level; F and G: Comparison of bacterial alpha diversity: Bacterial richness and bacterial diversity. $^{a}q < 0.05$, $^{b}q < 0.01$, $^{d}q < 0.0001$.

Supplementary Figures 3E and 3F).

Two common bacterial clusters (one at the DNA level and another at the RNA level) with strong positive correlations for tumor and tumor adjacent tissues were discovered (Supplementary Figures 3C-F), which confirms the absence of significant differences between tumor and tumor adjacent tissue microbiome profiles. At the DNA level, the common cluster consisted of Phy6 (*Neisseria*), Phy15 (unclas-

sified *Prevotellaceae*), Phy23 (*Neisseria perflava*), Phy29 (*Prevotella melaninogenica*), Phy87 (*Solobacterium*), and Phy98 (*Prevotella*). The common cluster at the RNA level included phylotypes such as Phy7 (*Reyranella*), Phy33 (*Sediminibacterium*), Phy46 (*Propionibacterium acnes*), Phy94 (*Methyloversatilis*), Phy107 (*Pseudomonas aeruginosa*), and Phy108 (*Sphingomonas echinoides*). Detected clusters were not found in the control group.

Generally, under the same analysis conditions, GC patients displayed a simpler bacterial network at both the DNA and RNA levels. At the DNA level, control, the tumor, and tumor adjacent groups had 85, 25, and 23 bacteria, respectively; at the RNA level, they had 75, 21, and 18, respectively. Moreover, analysis of bacterial interactions in controls had not only positive but also negative correlations, while GC analysis showed mostly positive correlations.

GC microbiota alterations and clinical parameters

At the DNA level, according to clinical parameters, statistically significant differences were found only in the decrease in bacterial richness between smaller tumors (T1-T2) and extended tumors (T4). The RNA level turned out to be more sensitive and allowed us to detect richness differences between grade II and grade III (Figures 4A and 4B). Moreover, at the RNA level, the relative abundance of the Phy7 (*Reyranella*) and Phy33 (*Sediminibacterium*) phylotypes was lower in the diffuse type of GC than in the intestinal type (Figures 4C and 4D). No differences were found between subgroups at the DNA level.

The effect of H. pylori infection on stomach microbiota

PCoA showed *Helicobacter* to be the major determinant for differentiating samples based on their bacterial composition in the stomach (Supplementary Figure 4). Overall, *H. pylori* was detected in 115 and 117 DNA and RNA samples, respectively. In the control group, *H. pylori* was lower than that in the GC groups at both the DNA and RNA levels (Figure 5A). The tumor adjacent sample group showed the highest number of samples with high *H. pylori* abundance (Figure 5B). Both in tumor and tumor adjacent groups the mean abundance of *H. pylori* was increased at the RNA level, although no significant differences between DNA and RNA samples were found.

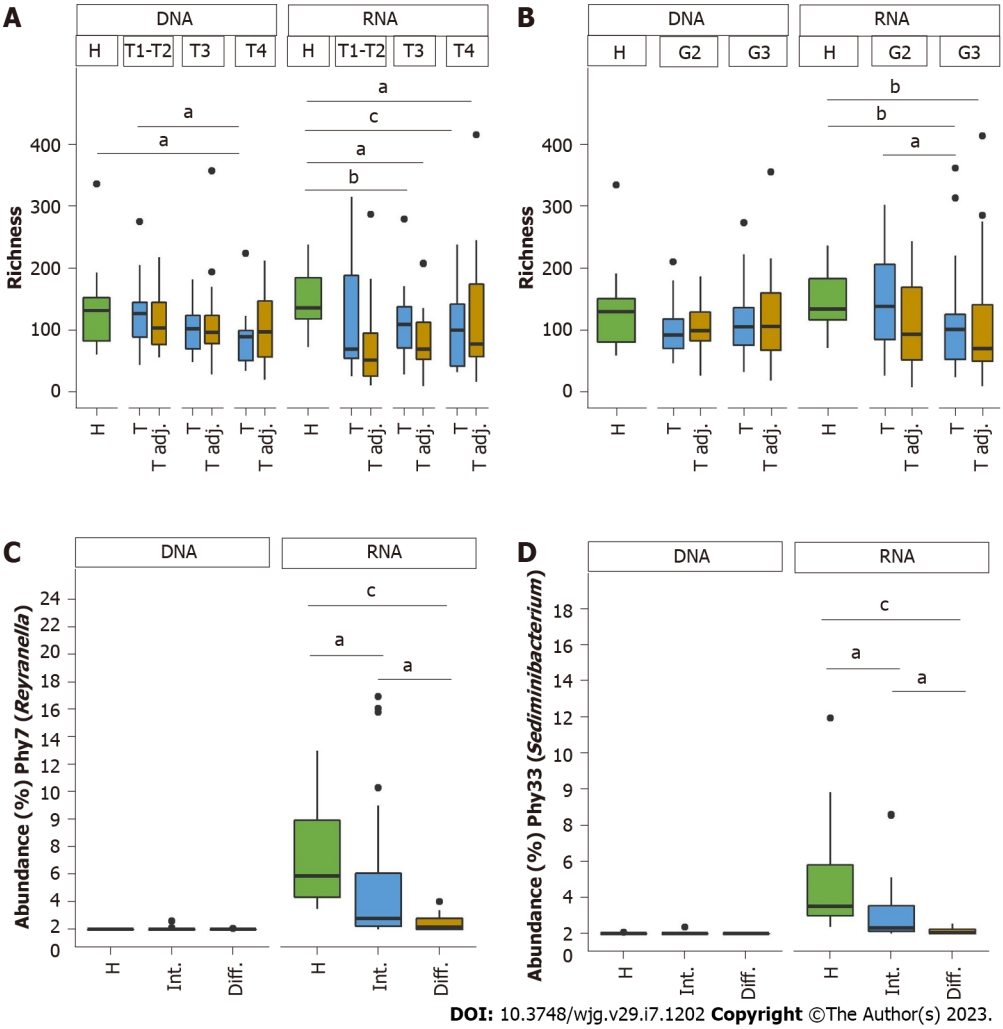
High *H. pylori* relative abundance (> 15%) led to an increase in the relative abundance of *Proteobacteria* and a decrease in other major bacterial phyla, such as *Firmicutes*, *Bacteroidetes*, and *Fusobacteria* (Figure 5C). In tumor tissues analyzed at the DNA and RNA level, *Helicobacter* was only one genus which changed significantly between samples with high and low *H. pylori* abundance (Figure 5D, Supplementary Table 5). On the other hand, in tumor adjacent tissues, more bacteria were found, the number of which changed together with *Helicobacter*. At the DNA level, as the relative abundance of *H. pylori* increased, the abundance of *Porphyromonas* and *Prevotella* significantly decreased. In line with our previous results, more significant differences were found at the RNA level: *Staphylococcus* significantly increased and seven bacteria (*Campylobacter*, *Fusobacterium*, *Prevotella*, *Pseudomonas*, *Reyranella*, *Sediminibacterium*, *Streptococcus*) decreased (Figure 5C, Supplementary Table 5). *Porphyromonas* tended to decrease in tumor adjacent RNA samples as well, although it did not reach a statistically significant level (Supplementary Table 5).

DISCUSSION

Despite growing interest in the study of microbiota, there is still limited agreement on the most appropriate standard for such studies, especially using 16S rRNA sequencing. Here, we performed systematic analysis of bacterial communities at both the 16S rRNA gene and 16S rRNA transcript levels. To estimate the impact of the different approaches, we used the GC model and considered not only healthy gastric tissues but also GC tumor and adjacent tissues.

The analysis of the study results showed that there were significant differences in the relative abundance of the gastric tissue microbiome between 16S rRNA gene transcript and 16S rRNA gene levels in all study groups (control, tumor, and tumor adjacent). This is the first GC study indicating that active and standing gastric microbiomes are distinct even at the largest taxonomic levels.

Differences in bacterial communities at the DNA and RNA levels could be explained by several possibilities. Using DNA as a research material summed up all bacteria, both biologically active passive in the form of endospores, and DNA sequences of already destroyed and dead bacteria[2,30]. The presence and number of ribosomes in bacteria reflects their metabolic activity; thus, the analysis at the RNA level shows the metabolic activity of live and active bacteria in the community[31-33]. For instance, previously GC-associated bacteria such as *Prevotella*, *Veillonella*, and *Neisseria* in our study were present in high abundance in all analyzed groups at the DNA level but were greatly reduced at the RNA level. In contrast, *Pseudomonas*, *Reyranella*, and *Staphylococcus* were present in higher abundance at the RNA level in all groups. However, it is erroneous to assume that only active bacteria can influence host responses. Many studies have shown that inactivated bacteria or parts of their cells can also influence inflammatory processes or other responses in host tissues. For example, Rabie *et al*[34] showed that thermally inactivated *Salmonella*, *Staphylococcus*, *Escherichia*, and *Pseudomonas* strains with unchangeable surface proteins cause colon and breast cancer cell proliferation. In Suprewicz *et al*[35]'s



DOI: 10.3748/wjg.v29.i7.1202

Figure 4 Comparison of bacterial abundance and richness in relation to clinical data at the DNA and RNA levels. A: Bacterial richness according to tumor size; B: Bacterial richness according to grading; C and D: Comparison of the relative mean abundance of Phy7 (*Reyranella*) and Phy33 (*Sediminibacterium*). Statistically significant differences between study groups are indicated, $^{a}q < 0.05$, $^{b}q < 0.01$, $^{c}q < 0.001$.

study, heat-inactivated *Enterococcus faecalis*, *Actinomyces odontolyticus*, and *Propionibacterium acnes* caused cell proliferation changes in lung, breast, and ovarian carcinoma. Postbiotics work based on the same principle. To avoid possible bacterial infection during therapy, instead of active bacteria, their metabolites, which are involved in anti-inflammatory and anticancer mechanisms, are used[36].

It is also cannot be excluded that the shift in bacterial abundance between DNA and RNA levels might stem from varying numbers of copies of the 16S rRNA gene[37] or target sequence quantity inequality[38]. Bacterial rRNAs (16S rRNA, 23S rRNA and 5S rRNA) are typically organized into one operon, and their transcription occurs together, with the number of such operons varying from 1 to 15 [38]. In the case of active bacteria, an increase in 16S rRNA gene copies proportionally increases the pool of 16S rRNA transcripts. However, in the case of inactive bacteria, a larger number of 16S gene copies enables the detection of some bacteria, which could not be detected at the RNA level.

The amount of target sequences using the 16S rRNA gene and its transcript are not the same. Of all types of RNA molecules present in the cell, the most common (80%-90%) are included in the ribosome structure rRNAs[39]. 16S rRNAs make up one-third of the total rRNAs. On the other hand, when analyzing the microbiota using the 16S rRNA gene, only one gene is amplified out of the total number of genes, which in different bacteria varies from 1500 to 7000[37]. Thus, the initial larger amount of the bacterial target sequence at the RNA level makes it possible to increase the depth of sequencing and detect more rare bacteria that would be lost during DNA-level analysis. In addition, a shift toward DNA or RNA levels can also be caused by ingestion of bacterial parts from the higher parts of the digestive tract.

Our analysis revealed that the profile of differences found between GC and control tissue depended on the chosen modality: At the DNA level, 17 bacterial genera were detected, and at the RNA level, 27 bacterial genera were detected. Ten of those bacteria (*Actinomyces*, *Alloprevotella*, *Atopobium*, *Granulicatella*, *Lactobacillus*, *Megasphaera*, *Propionibacterium*, *Rothia*, *Streptococcus*, *Veillonella*) were found to be different from the control group at both levels of sequencing; seven bacterial taxa (*Campylobacter*,

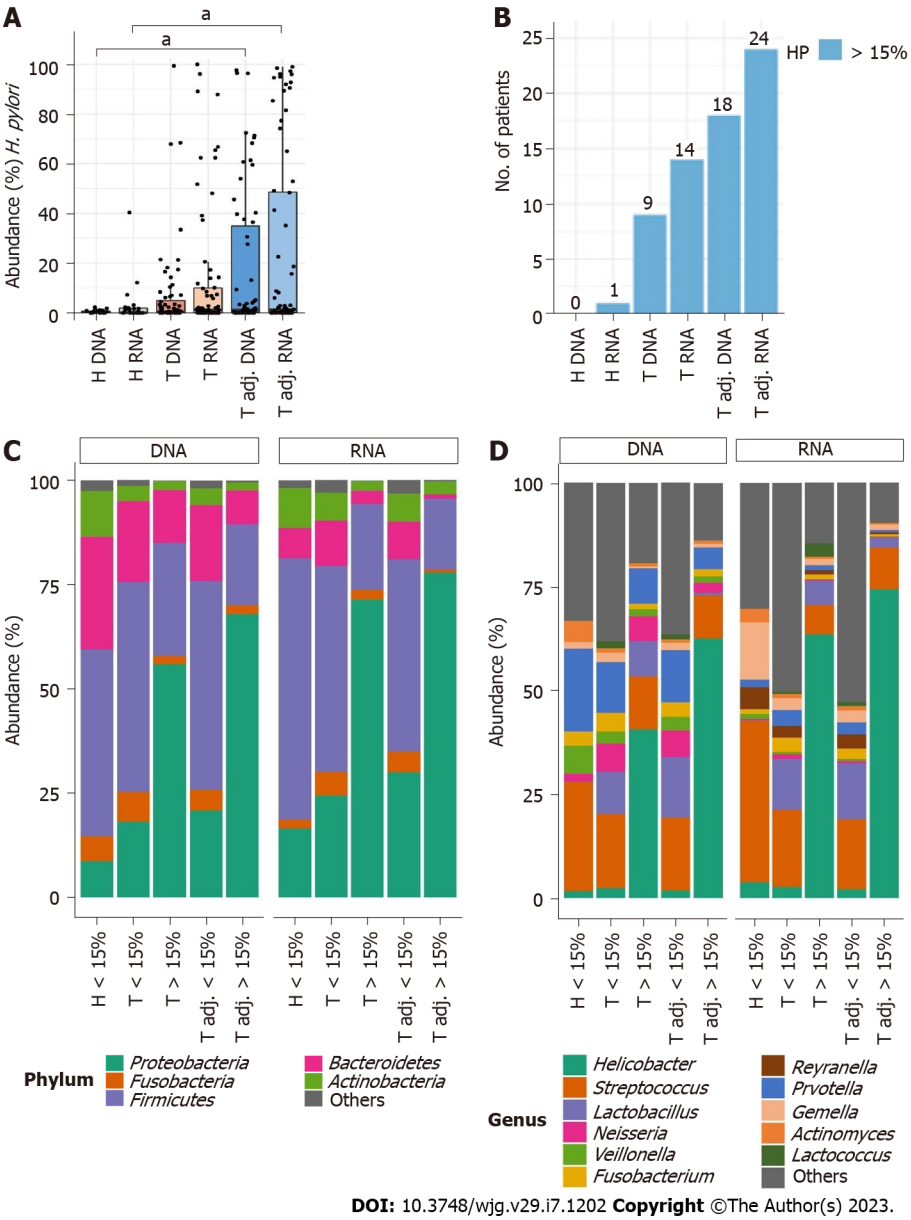


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Figure 5 Comparison of gastric microbiota alterations at the DNA and RNA levels depending on high *Helicobacter pylori* abundance (> 15%). A and B: Relative mean abundance of *Helicobacter pylori* (*H. pylori*) in all study groups and the number of patients with high *H. pylori* abundance; C and D: Comparison of the relative abundance of the top 5 microbiota at the phylum and top 11 microbiota at the genus levels. [a]$q < 0.05$.

Clostridium sensu stricto, *Leptotrichia*, *Oribacterium*, *Prevotella*, *Saccharibacteria genera incertae sedis*, *Stomatobaculum*) were found uniquely only at the DNA level; and 17 (*Anaerococcus*, *Corynebacterium*, *Eubacterium* , *Flavobacterium*, *Gemella*, *Legionella*, *Limnohabitans*, *Massilia*, *Methylobacterium*, *Methyloversatilis*, *Parvimonas*, *Pseudomonas*, *Reyranella*, *Rhodoluna*, *Sediminibacterium*, *Solobacterium*, *Staphylococcus*) were found uniquely only at the RNA level. These results confirm the importance of unifying the procedures for studying the microbiota.

Although our study focused on differences in methodology, it did reveal several important findings for the GC study as well. Fourteen bacteria genera were identified to be decreased in patients with GC. Eleven of these bacteria (*Actinomyces*, *Atopobium*, *Propionibacterium*, *Streptococcus*, *Granulicatella*, *Veillonella*, *Rothia*, *Parvimonas*, *Gemella*, *Prevotella*, *Leptotrichia*) were previously established in the stomach of healthy people in the absence of gastrointestinal diseases[40]. Most of them are common members of the upper gastrointestinal tract and have strong enzymatic activities. Our study also found four bacteria genera, which were significantly increased in GC patients' stomach biopsy: *Lactobacillus*, *Clostridium sensu stricto*, *Staphylococcus*, and *Pseudomonas*.

Lactobacillus is commonly used as a probiotic; however, it has been verified in multiple studies to be enriched in GC[41]. *Lactobacillus* strains, as well as *Clostridium* and *Staphylococcus*, can reduce nitrate to nitrite[42,43]. During the nitrate-reducing process, many N-nitroso compounds are formed that inhibit cell apoptosis and promote mutagenesis and protooncogene expression[44-47]. *Clostridium* is part of the

normal gastrointestinal tract; however, in several previous studies, as in ours, an increase in the number of *Clostridium sensu stricto* was found[48-50]. Interestingly, Lertpiriyapong *et al*[9] showed earlier onset and faster progression of GC in INS-GAS mice with restricted microbiota (including *Clostridium*, *Lactobacillus,* and *Bacteroides*), highlighting a possible role of these bacteria in GC. Additionally, several studies have detected increased levels of *Staphylococcus* in patients with upper gastrointestinal diseases[51-53]. One of the reasons for this may be that stains of *Staphylococcus* have the enzyme urease, and are able to catalyze the hydrolysis of urea to carbon dioxide and ammonia, which can neutralize gastric hydrochloric acid, thus promoting bacterial existence. Although we found an increased number of *Pseudomonas*, this bacterial infection affects people with weakened immune systems (including patients with cancer), and thus, it is more likely that this finding is the result of already developed pathological processes.

We did not detect significant bacterial abundance, richness, or diversity alterations at either the DNA or RNA level between tumor and tumor adjacent tissues. This result is consistent with two previous studies[54,55] but contradicts recent GC studies where significant differences between tumor-affected and nearby healthy tissues were found[27,56]. Moreover, we found the same clusters of bacterial networks in tumor and tumor adjacent tissues at both the DNA and RNA levels. These results may suggest that with the onset and development of carcinogenic processes, local changes in stomach tissues lead not only to a change in the bacterial composition but are also precise uniformity between cancer-affected and still healthy tissues (at least within a radius of 5 cm from the tumor area).

Studying GC samples at the RNA level, we managed to identify microbiome associations with clinical data. Analysis revealed two phylotypes (Phy7 and Phy33) related to *Reyranella* and *Sediminibacterium*, respectively. The relative number of those phylotypes gradually decreased from healthy to GC patients through intestinal growth type (considered as less aggressive cell growing type) to GC patients with diffuse growth type of cancer cells with worse outcome prognosis. To our knowledge, this is the first mention of these bacteria associated with the GC cell growth type. *Reyranella* is part of *Proteobacteria* and has previously been associated with the main chemokine expression, which is involved in T-cell attraction during cancerogenesis[57,58]. In another study, it was shown that there are significantly lower amounts of circulating natural killer and Treg cells in patients with diffuse/mixed-type GC compared to intestinal-type GC[59]. Taken together, these results suggest that *Reyranella* may be involved in the decrease in T-cell number and thus stimulation of cell growth of diffuse-type GC. *Sediminibacterium* was reported to be associated with GC, but there is no knowledge about the possible role of this bacteria in the pathophysiological processes[60,61]. Therefore, more detailed research on the effects of *Reyranella* and *Sediminibacterium* on GC cells is needed to be able to use these bacterial phylotypes as potential biomarkers.

H. pylori is the most common bacterial infection worldwide, as well as the main risk factor for GC [40]. It has been shown that during the transition from *H. pylori*-induced inflammation to the growth and development of carcinogenic cells, *H. pylori* is no longer detected in the affected areas in such large abundance[62]. Our results, showing that more *H. pylori* were found in tumor adjacent tissue than in tumor tissue, both at the DNA and RNA levels, confirm this. According to some previous reports, infection with *H. pylori* promotes the proliferation of non-*Helicobacter* bacteria from *Proteobacteria*, *Spirochetes*, and *Acidobacteria* and limits the spread of bacteria such as *Actinobacteria*, *Bacteroidetes* and *Firmicutes*[63,64]. Although most of the bacteria we found with altered numbers in GC were not associated with *H. pylori*, changes in the number of bacteria, such as *Granulicatella*, *Lactobacillus*, *Rothia*, *Pseudomonas*, *Gemella*, *Prevotella*, *Leptotrichia*, *Clostridium sensu stricto*, and *Fusobacterium,* were associated with high *H. pylori* abundance.

The question regarding the causality in the gastric microbiome is still partially unanswered. On the one hand, alterations in gastric microbiota have a causal role in the progression of carcinogenesis (*e.g.*, *H. pylori*). On the other hand, the role of other bacteria is less understood. However, there are new studies that strongly suggest the impact of the gastric microbiome on inflammation and carcinogenesis. For instance, a recent study by Kwon *et al*[65] showed that intestinal metaplasia or GC patient gastric microbiome transplantation contributes to changes in the phenotype of premalignant lesions. In this regard, a detailed understanding of the output of different sequencing technologies and comparability between RNA/DNA-based analyses is critical.

Since systematic analysis to assess the differences with respect to GC has not been performed before, we would like to point out some limitations of this work. While the primary focus of the work was related to technical differences, thus food preferences, sex, and aging can be potential contributing factors that have not been thoroughly considered in this study. Overall, the focus was on providing a truly confirmed healthy cohort for the most precise comparison to strengthen the differences. Nevertheless, PERMANOVA and Mann-Whitney analyses performed in each of the study groups (tumor DNA, tumor RNA, tumor adjacent DNA, tumor adjacent RNA, control DNA, control RNA) did not reveal significant differences between the sexes and age (divided by median) (Supplementary Tables 6 and 7). Furthermore, due to the sample size, we did not consider the newly proposed TCGA classification for subsequent analysis nor to assess the impact of 4 subtypes on bacterial composition in tumors.

CONCLUSION

In conclusion, our study provides evidence that the tumor microbiome of GC patients has a distinct pattern compared to healthy controls, while the difference analyzed from adjacent tissue was rather low. Despite some overlap between the data obtained from the 16S rRNA transcript and 16S rRNA gene, our results showed the critical importance of the chosen study material on the resulting bacterial profile. Thus, researchers comparing their results with previous studies might take into consideration which initial material was used, either the 16S rRNA gene or 16S rRNA transcript. Our results showed that the RNA level was more sensitive for detecting low abundance bacteria and allowed us to detect differences according to GC clinical data.

ARTICLE HIGHLIGHTS

Research background

There is currently no gold standard for analyzing the microbiome in 16S rRNA studies. Two common modalities are: Sequencing of DNA (16S rRNA gene) and sequencing of RNA (16S rRNA transcript). Gastric cancer (GC) remains one of the most common cancers in the world and microbiome takes important place in its carcinogenesis.

Research motivation

Microbiota studies are becoming more relevant and widespread. Comparison of different approaches for microbiome studying is necessary for correct interpretation of other studies results, as well as for a deeper understanding of bacterial composition.

Research objectives

To investigate how the choice of sequencing modality affects the bacterial profile of differences between case and controls as well as to characterize the microbiota of GC tissues using 16S rRNA gene and its transcript.

Research methods

The study included healthy tissues from the control group, as well as tumor and tumor adjacent tissues from GC patients. From all biopsies RNA and DNA were extracted. 16S rRNA V1-V2 region was sequenced for all samples. For significant differences between groups permutational multivariate analysis of variance and Mann-Whitney test followed by false-discovery rate test were used.

Research results

Only a small portion of bacterial sequences overlapped on DNA and RNA levels in all groups. Differences between GC and control groups also only partially overlayed on DNA and RNA levels. RNA sequencing was more sensitive for detecting differences in bacterial richness, low abundance bacteria, and changes in the relative abundance of *Reyranella* and *Sediminibacterium* according to the type of GC. In each study group differences between DNA and RNA bacterial profiles were identified.

Research conclusions

Chosen study material (16S rRNA transcript or 16S rRNA gene) greatly affects detectable microbiome profile as well as the differences between cases and controls.

Research perspectives

This study provides microbiome analysis applying two different methodologies using GC gastric tissues as example and could serve as a reference for future research.

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FOOTNOTES

Author contributions: Vilchez-Vargas R, Link A, Lehr K, Kupcinskas J, Skieceviciene J and Nikitina D contributed to the study design, analysis, and interpretation of the data; Nikitina D, Link A, and Skieceviciene J drafted the manuscript; Skieceviciene J, Kupcinskas J, Vilchez-Vargas R and Link A supervised the study procedures and revised the manuscript; Nikitina D, Lehr K, and Vilchez-Vargas R performed bioinformatic and statistical analyses; Urba M and Jonaitis LV obtained participant's samples and provided clinical data; Skieceviciene J and Link A have contributed equally to this work and share last authorship; and all authors read and approved the final version of the manuscript.

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

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at jurgita.skieceviciene@lsmuni.lt.

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SYSTEMATIC REVIEWS

Influence of methyl donor nutrients as epigenetic regulators in colorectal cancer: A systematic review of observational studies

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Abstract

BACKGROUND

Dietary methyl donors might influence DNA methylation during carcinogenesis of colorectal cancer (CRC). However, whether the influence of methyl donor intake is modified by polymorphisms in such epigenetic regulators is still unclear.

AIM

To improve the current understanding of the molecular basis of CRC.

METHODS

A literature search in the Medline database, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/), and manual reference screening were performed to identify observational studies published from inception to May 2022.

RESULTS

A total of fourteen case-control studies and five cohort studies were identified. These studies included information on dietary methyl donors, dietary components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl metabolizing enzymes, and/or markers of CpG island methylator phenotype and/or microsatellite instability, and their possible interactions on CRC risk.

CONCLUSION
Several studies have suggested interactions between methylenetetrahydrofolate reductase polymorphisms, methyl donor nutrients (such as folate) and alcohol on CRC risk. Moreover, vitamin B_6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation. Identification of specific mechanisms in these interactions associated with CRC may assist in developing targeted prevention strategies for individuals at the highest risk of developing CRC.

Key Words: Colorectal cancer; DNA methylation; Epigenetics; Methyl donors; Microsatellite instability; Nutrients

Core Tip: Dietary methyl donors might influence DNA methylation during the carcinogenesis of colorectal cancer (CRC). However, whether the influence of methyl donor intake is modified by polymorphisms in such epigenetic regulators is still unclear. We conducted a systematic review on this topic to improve the current understanding of the molecular basis of CRC. Several studies have suggested interactions between methylenetetrahydrofolate reductase polymorphisms, methyl donor nutrients (such as folate) and alcohol on CRC risk. Moreover, vitamin B_6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation.

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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent type of cancer and is responsible for the second highest mortality rate in cancer patients worldwide[1]. Although screening for early detection of CRC is effective to help decrease the trends in mortality rates, understanding daily life factors is also important to prevent this type of cancer[2]. The main factors which may help prevent CRC are those associated with diet, lifestyle, and prevention of metabolic diseases[3].

With regards to the dietary component, nutrients associated with one carbon (1C) metabolism [including folate, other B vitamins, methionine (Met), and choline] have been recognized as anticarcinogenic and chemotherapeutic agents in the 1C metabolic network[4]. Folate has shown to play a preventive role in CRC, probably because of its involvement in the processes of DNA methylation and synthesis[5]. Other nutrients, such as Met and vitamins B_6 and B_{12}, which interact metabolically with folate in this process, may also influence the risk of CRC[6]. Moreover, in some of those studies, the observed inverse association between folate status and CRC risk was further modified by genetic polymorphisms of the enzymes involved in folate metabolism, most notably methylenetetrahydrofolate reductase (*MTHFR*).

A common C677T substitution in the *MTHFR* gene results in a protein with valine instead of alanine, yielding a more thermolabile enzyme with decreased activity[7]. Numerous studies have shown that this variant (TT) is associated with a decreased risk of CRC, but only when folate status is normal or high. *MTHFR* polymorphism is possibly the best-known gene polymorphism that switches from being a risk factor to a protective one depending on nutrient status. In any case, it is also important to evaluate the joint influence that other polymorphisms in genes involved in folate metabolism might exert. Thus, for example, Met synthase requires vitamin B_{12}, as methylcobalamin, as a cofactor. A variant in this gene, A2756G [in methionine synthase (*MTR*) gene], has been described and results in the substitution of aspartate by glycine. Some studies have shown that the MTR 2756 GG genotype is associated with a decreased risk of CRC; however, the association with diet is still unclear[8,9].

Another relevant example of a mutation in a polymorphism associated with CRC risk, but not consistent with nutrients, is the case of serine hydroxymethyltransferase, a pyridoxal phosphate (B_6)-dependent enzyme, in particular the polymorphism C1420T[10]. An additional relationship between 1C metabolism-related nutrients and CRC risk is related to its influence on DNA methylation. Thus, low folate status or intake is related to a decreasing methylation level[11,12], whereas colonic mucosal DNA methylation increased globally as a result of folate supplementation[13]. A sufficient intake of methyl

donors may also prevent aberrant CpG island promoter hypermethylation. The promoter CpG island hypermethylation that characterizes the CpG island methylator phenotype (CIMP) in CRC is common [14]. Moreover, polymorphisms in enzymes involved in folate metabolism may change the potential impact of methyl donor consumption on DNA methylation[15].

According to the molecular subtype, chromosomal instability, and microsatellite instability (MSI) represent the major pathways for CRC[16]. The inactivation of DNA mismatch repair (MMR) genes, which are responsible for correcting mismatched bases during DNA replication, results in MSI[17]. Microsatellites are short sequences that are dispersed across the genome and are likely to undergo MMR machinery-induced deletion or insertion. Defects in the MMR machinery are associated with CRC[18] and can be affected by epigenetic alterations and deregulation of methylation[19]. In this sense, the associations between methyl donor nutrient intake and CRC risk have been extensively studied, although evidence on their effect is limited[20]. However, whether they act as effect modifiers against a background of deficient DNA repair capacity is unknown.

Until now, there have been few studies on the influence of methyl group donors as epigenetic regulators in CRC[21]. In the present work, we reviewed previous studies that have investigated this matter to improve the current understanding of the molecular basis of CRC, which could contribute to a better design of future research and to better preventive nutritional management in this type of cancer.

MATERIALS AND METHODS

Search strategy

The search terms and search strategy were developed by two researchers. To guide this research, we formulated the following question as the starting point: What is the influence of methyl donor nutrients as epigenetic regulators in CRC? For this purpose, a systematic search in the Medline (through PubMed) database, *Reference Citation Analysis* (https://www.referencecitationanalysis.com/, an artificial intelligence technology-based open multidisciplinary citation analysis database), and a manual reference screening were performed to identify observational studies published from inception to May 20, 2022. A search for relevant keywords and medical subject heading terms related to dietary methyl donors, dietary components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl-metabolizing enzymes, markers of CIMP and/or MSI, in combination with keywords related to CRC events was conducted.

The search was amplified through citation chaining (forward and backward) of the included studies. Reference lists of all identified articles and other related review articles, systematic reviews, and meta-analyses were hand-searched for additional articles. The present search was developed according to the "PRISMA Statement" guidelines (see the PRISMA checklist) (www.prisma-statement.org). For this review, a protocol was not prepared or registered. The search strategy is detailed as follows: (1) Colorectal neoplasms/exp or [(colorectal* or rect* or anal* or anus or colon* or sigmoid) adj3 (cancer* or carcinoma or tumour* or tumor* or neoplas* or adenoma or adenocarcinoma)], abstract (ab), keyword hearing word (kf), original title (ot), title (ti), text word (tw); (2) (observational or case-control or cohort), ab, kf, ot, ti, tw; (3) (incidence or prevalence or risk or odds ratio or hazard ratio), ab, kf, ot, ti, tw; (4) (one-carbon metabolism-related nutrient* or dietary methyl donor* or alcohol), ab, kf, ot, ti, tw; and (5) [gen* or single-nucleotide polymorphism (SNP)* or polymorphism* or methyl metabolizing enzyme* or diet–gene interaction* or hypermethylation* or microsatellite instability*], ab, kf, ot, ti, tw.

Review process and selection criteria

Two researchers independently screened the titles and abstracts of the articles to identify potentially relevant studies. Studies that passed the title/abstract review were retrieved for full-text review. The inclusion and exclusion criteria and the quality of the study were assessed by two researchers with the use of a data extraction form especially designed for this study.

The inclusion criteria consisted of studies: (1) With an observational design (case-control or cohort); (2) that evaluated the exposure to at least one of the following dietary components (dietary nutrient intake and/or plasma levels): Folate, other B vitamins, Met, choline, betaine, and/or alcohol; (3) that included genotyping analyses of methyl-metabolizing enzymes (*MTHFR, MTR,* Met synthase reductase), DN methyltransferase 3 b, euchromatin histone methyltransferase 1 and 2, PR domain zinc finger protein 2; (4) CIMP defined by promotor hypermethylation (calcium voltage-gated channel subunit a1 G, insulin-like growth factor 2, neurogenin1, runt-related transcription factor 3, suppressor of cytokine signalling 1), human *MutL* homolog 1 (*hMLH1*) hypermethylation; (5) MSI using markers [such as mononucleotide microsatellites with quasi-monomorphic allele length distribution in healthy controls but unstable (Bat-26 and/or Bat-25), NR-21, NR-22, NR-24)]; (6) in which the outcome of interest was CRC, colon, or rectal cancer (studies investigating benign adenomas or polyps were excluded); (7) that provided estimates of the adjusted odds ratios or relative risks or hazard ratios with 95% confidence intervals (95%CI); (8) conducted in humans (≥ 18 years old); and (9) written in English or Spanish.

The following types of publications were excluded: (1) Nonoriginal papers (reviews, commentaries, editorials, or letters); (2) meta-analysis studies; (3) off-topic studies; (4) studies lacking specific CRC data; (5) nonhuman research; (6) studies conducted in children, adolescents, or pregnant women; (7) duplicate publications; and (8) low-quality studies (Newcastle-Ottawa scale (NOS)[22] < 4 indicating insufficient study quality).

Study quality assessment

To evaluate the validity of the individual studies, two reviewers worked independently to determine the quality of the included studies based on the use of the NOS for case-control or cohort studies[22]. The maximum score was 9, and a high score (≥ 6) indicated high methodological quality; however, given the lack of studies on the subject under study, it was agreed to select those that had a score equal to or greater than 4. A consensus was reached between the reviewers if there were any discrepancies.

Data extraction

The data extracted for each individual study included the following: Name of the first author, study design, characteristics of the study population (age range or mean age, sex, country), dietary exposure, dietary assessment instrument used, outcomes (including cancer site), comparison, odds ratio or relative risk or hazard ratio (95%CI), adjusted variables, and NOS. For case-control studies, the following additional information was extracted: Number of cases and number of controls. For cohort studies, the following additional information was extracted: Number of participants at baseline, number of CRC cases, and length of follow-up. These variables were judged to be most relevant to the outcome studied. Where multiple estimates for the association of the same outcome were used, the one with the highest number of adjusted variables was extracted. Template data collection forms and data extracted from the included studies will be made available upon request from the corresponding author.

RESULTS

Figure 1 shows the PRISMA flow diagram summarizing the identification and selection of the relevant publications. A total of nineteen studies were included in this systematic review: Fourteen case-control studies[23-36] and five cohort studies[9,37-40]. In total, the case-control studies included 7055 cases and 9032 controls. The cohort studies included 256914 participants, with 1109 cases recorded during follow-up periods that ranged from 7.3 to 22 years. Amongst the case-control studies, nine articles were conducted in the United States, two in the United Kingdom, two in Korea and one in Portugal. With regard to the cohort studies, three were conducted in the Netherlands and two in the United States.

Tables 1 and 2 summarize the main characteristics and findings of the case-control and cohort studies, respectively, on the interactive effect between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes, dietary methyl donors and dietary components that potentially modulate the bioavailability of methyl groups on CRC risk. Tables 3 and 4 show the effects of dietary methyl donors and dietary components that potentially modulate the bioavailability of methyl groups on CRC risk, according to SNPs in genes encoding methyl-metabolizing enzymes and/or mutations in oncogenes, CIMP and/or MSI, in the case-control studies and cohort studies, respectively. Table 5 provides a summary of the results of the studies included in the systematic review.

DISCUSSION

This review summarizes previous studies that have investigated the influence of methyl donor nutrients as epigenetic regulators in CRC. The dietary components that showed a higher association with CRC risk were folate and alcohol. Thus, high folate intake was considered a protective factor, while high alcohol consumption proved to be a risk factor. Several studies have investigated the association between methyl donor nutrients and/or methyl antagonists (*e.g.*, alcohol) and *MTHFR* polymorphisms and have reported significant interactions[23,24,26,29,36]. In one of those case-control studies, those with the *MTHFR* 677 TT genotype, who consume low folate diets, had a greater chance of developing CRC than people with the CC or CT genotype[24]. Two other case-control studies reported that *MTHFR* 677 TT carriers with high (above mean) or adequate folate intake had a low risk of CRC[26,29].

Two case-control studies found that alcohol consumption increased CRC risk among *MTHFR* 677 TT carriers[23,26]. The decreased *MTHFR* enzyme activity among those who carried the T allele, and consumed low methyl donor nutrients and large amounts of alcohol can be utilised to explain the increased CRC risk[25,30]. These dietary habits may alter folate metabolism, especially in people with folate deficiencies[41]. In contrast, several studies have not found an association between *MTHFR* polymorphisms and either folate intake or alcohol intake and CRC risk[25,27]. It has been hypothesized that the differences in folate status among various populations may have influenced the contradictory results on the contribution of *MTHFR* genetic variants in CRC[25]. In addition to *MTHFR* poly-

Table 1 Characteristics of the eight case-control studies included in this systematic review examining the interactive effects between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes and one-carbon metabolism-related dietary compounds on colorectal cancer risk

Ref.	Country	Age (yr)	No. cases (M/W), endpoint	No. controls, type	Gene (SNP)	Nutrient/alcohol	Method for measuring nutrition intake	Outcome (OR, 95%CI)			Adjustments to OR	NOS
								SNP	Nutrient/alcohol	Interaction		
Chen *et al* [23]	United States	40-75	144 M, CRC	627 C	*MTHFR* (677C>T)	Dietary folate, Met, and alcohol	Validated FFQ (self-reported)	No assoc	Alcohol (≥ 5 *vs* ≤ 1 drinks/wk): 1.61 (1.01-2.58)	677TT (*vs* CC/CT)-low alcohol consumption (≤ 1 drinks/wk): 0.11 (0.01-0.85) (*P-interac* = 0.02)	Age, CRC family history	7
Guerreiro *et al*[24]	Portugal	Cases (64.2 ± 11.3), controls (62.2 ± 12.1)	104/92 CRC	200 C	*MTHFR* (677C>T), *MS* (2756A>G), *SHMT* (1420C>T)	Dietary folate, vitamins B_6 and B_{12}, glycine, Met, serine, and alcohol	Validated FFQ (by interview)	677TT (*vs* CC/CT): 3.01, (1.3-6.7); 1420TT (*vs* CC/CT): 2.6, (1.1-5.9)	Folate (> 406.7 mcg/d *vs* < 406.7 mcg/d): 0.67 (0.45-0.99)	677TT (*vs* CC/CT)– folate (< 406.7 mcg/d): 14.0, 1.8-108.5 (*P* = 0.05)	Age, CRC history, and sex	5
Kim *et al* [25]	Korea	30-79	465/322 CRC (363 CCa, 330 RCa)	656 H	*MTHFR* (677C>T)	Dietary folate and alcohol	Validated FFQ	677TT (*vs* CC/CT): 0.60 (0.46-0.78)	Folate (high *vs* low intake): 0.64 (0.49-0.84); alcohol (high *vs* low intake): 1.76 (1.26-2.46)	677CC/CT–Low-methyl diet (folate < 209.69 mcg/d and alcohol ≥ 30 g/d): 2.32 (1.18-4.56) (*P-interac* = no assoc)	Age, BMI, CRC family history, energy intake, multivitamin use, sex, smoking status	7
Ma *et al* [26]	United States	40-84	202 M, CRC	326 C	*MTHFR* (677C>T)	Plasma folate, and alcohol consumption	FFQ (self-reported)	677TT (*vs* CC): 0.45 (0.24-0.86)	Folate (plasma deficiency *vs* adequate levels): No assoc	677TT (*vs* TC/CC)–folate (adequate levels): 0.32 (0.15-0.68)	Age, alcohol consumption, aspirin use, BMI, exercise, multivitamin use, and smoking status	7
									Alcohol: Unk	677TT (*vs* CC)–alcohol (0-0.14 drinks/d): 0.12 (0.03-0.57)	Age	
Murtaugh *et al*[27]	United States	30-79	446/305 RCa	979 C	*MTHFR* (677C>T, 1298A>C)	Dietary folate, riboflavin, vitamins B_6 and B_{12}, Met, and alcohol	FFQ (by interview)	W, 677TT (*vs* CC): 0.54 (0.30-0.98). M&W, 1298CC (*vs* AA): 0.67 (0.46-0.98)	Dietary folate (> 475 mcg/d *vs* < = 322 mcg/d): 0.66 (0.48-0.92). High methyl donor status (*vs* low status): 0.79 (0.66-0.95)	No assoc	Age, BMI, ibuprofen use, intake of energy, fibre and calcium, PA, sex, and smoking status	6
Pufulete *et al*[28]	United Kingdom	38-90	13/15 CRC	76 C	*MTHFR* (677C>T, 1298A>C), *MS* (2756A>G), *CBS* (844ins68)	Plasma folate, vitamin B_{12}, and homocysteine, alcohol and folate intake	Validated FFQ (by interview)	677TT (*vs* CC): 5.98 (0.92-38.66), *P* = 0.06; 1298CC (*vs* AA): 12.6 (1.12-143.70), *P* = 0.04	Folate status score (T_3 *vs* T_1): 0.09 (0.01-0.57), *P-trend* = 0.01	Unk	Age, alcohol consumption, BMI, sex, and smoking status	7
Sharp *et al* [29]	United Kingdom		150/114 (189 CCa, 75 RCa)	408C	*MTHFR* (677C>T,	Dietary folate, riboflavin, vitamins B_6	Validated FFQ (self-reported)	No assoc	No assoc	677CT/TT (*vs* CC)–folate (> mean): *P-interac* =	Age, CRC family history, energy	6

					1298A>C)	and B_{12}, and alcohol				0.029. 677CT/TT (*vs* CC)–vitamin B_6 (> mean): *P-interac* = 0.016	intake, NSAID use, PA, and sex	
Slattery *et al*[30]	United States	30-79	824/849 CC (DCCa 405/303; PCCa 395/327)	1816 C	*MTHFR* (677C>T)	Dietary folate, Met, vitamins B_6 and B_{12}, and alcohol	Validated CARDIA diet questionnaire	677TT: No assoc	Unk	TT-low risk diet (high in folate and Met and without alcohol): 0.4 (0.1-0.9)	Age, BMI, intake of energy and fibre, PA, and smoking intensity	7

Asso: Significant association; BMI: Body mass index; C: Community controls; CBS: Cystathionine synthase; CCa: Colon cancer; CI: Confidence interval; CRC: Colorectal cancer; DCCa: Distal colon cancer; FFQ: Food frequency questionnaire; H: Hospital controls; interac: Interaction; M: Men; Met: Methionine; *MS*: Methionine synthase; *MTHFR*: Methylenetetrahydrofolate reductase; NSAID: Nonsteroidal anti-inflammatory drugs; NOS: Quality Newcastle-Ottawa Scale; OR: Odds ratio; PA: Physical activity; PCCa: Proximal colon cancer; RCa: Rectal cancer; *SHMT*: Serine hydroxymethyltransferase; SNP: Single-nucleotide polymorphism; unk: Unknown; W: Women.

Table 2 Characteristics of the cohort study included in this systematic review examining the interactive effects between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes and one-carbon metabolism-related dietary compounds on colorectal cancer risk

Ref.	Country	Study cohort (age, yr)	No. participants (M/W)	No. incident cases	Follow-up length, y	Gene (SNP)	Nutrient/alcohol	Method for measuring nutrition intake	Outcome (RR, 95%CI)			Adjustments to RR	NOS
									SNP	Nutrient/alcohol	Interaction		
de Vogel *et al*[9]	Netherlands	Netherlands Cohort Study on diet and cancer (55-69)	58279/62573	734 CRC	7.3	*MTHFR* (rs1801133, rs1801131), *MTR* (rs1805087), *MTRR* (rs1801394), *DNMT3B* (rs2424913, rs406193), *EHMT1* (rs4634736), *EHMT2* (rs535586), *PRDM2* (rs2235515)	Dietary folate, Met, vitamins B_2 and B_6, alcohol	Validated FFQ (self-reported)	Unk	Unk	≤ 1 rare allele in folate metabolizing enzymes–vitamin B_2 (T_3 *vs* T_1): 0.30, (0.11-0.81), *P-trend* = 0.005. Rare allele of *DNMT3B* C>T (rs406193)–vitamin B_6 (T_3 *vs* T_1): 1.90 (1.00-3.60), *P-trend* = 0.04. Common allele of *PRDM2* G>A (rs2235515)–vitamin B_6 (T_3 *vs* T_1): 1.49 (1.00-2.22), *P-trend* = 0.03. No assoc	Age, alcohol consumption, BMI, CRC family history, intake of energy and alcohol, sex, and smoking status	9

Asso: Significant association; BMI: Body mass index; CI: Confidence interval; CRC: Colorectal cancer; *DNMT3B*: ADN (cytosine-5-)-methyltransferase 3 ; *EHMT1*: Euchromatin histone methyltransferase 1; FFQ: Food frequency questionnaire; M: Men; *MTHFR*: Methylenetetrahydrofolate reductase; *MTR*: Methionine synthase; *MTRR*: Methionine synthase reductase; NOS: Quality Newcastle-Ottawa Scale; *PRDM2*: PR domain zinc finger protein 2; RR: Relative risk; SNP: Single-nucleotide polymorphism; T: Tertile; unk: Unknown; W: Women.

morphisms, de Vogel *et al*[9] also investigated the modifying effects of polymorphisms of Met synthase reductase, *MTR*, DN methyltransferase 3 b, euchromatin histone methyltransferase 1 and 2, and PR domain zinc finger protein 2 but found no interactions, although some *P-trends* were significant.

Regarding the methylation abnormalities of genes, de Vogel *et al*[37] found that high vitamin B_6 intake was associated with an increased CRC risk caused by hypermethylation of the *hMLH1* promoter among men. Therefore, these authors suggest that vitamin B_6 may have had a tumour-promoting effect by increasing promoter methylation. However, the intake of folate, vitamin B_2, Met and alcohol was not associated with the risk of tumours showing *hMLH1* hypermethylation. In any case, other studies

Table 3 Characteristics of the six case-control studies included in this systematic review examining the interactive effects between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes and/or mutations in oncogenes, CpG island methylator phenotype and/or microsatellite instability, and one-carbon metabolism-related dietary compounds on colorectal cancer risk

Ref.	Country	Age (yr)	No. cases (M/W), endpoint	No. controls and type	Gene (SNP)	CIMP markers	MSI	Nutrient/alcohol	Method for measuring nutrition intake	Outcome (OR, 95%CI), interaction		Adjustments to OR	NOS
										CIMP markers/MSI–nutrient/alcohol	CIMP markers-/MSI–SNP–nutrient/alcohol		
Busch *et al*[31]	United States	40-80 (AAs *vs* EAs)	244/241 CRC	Analyses were only performed in tumour tissue		*CACNA1G, hMLH1, NEUROG1, RUNX3, SOCS1*	Unk	Dietary folate and alcohol	Unk	EAs: High *CACNA1G* methylation tumour (cut point of 5%)–high folate intake: 0.3 (0.14-0.66); high *SOCS1* methylation tumour (cut point of 3%)–high folate intake: 0.3 (0.11-0.80)	Unk	-	4
Curtin *et al*[32]	United States	30-79	518/398 CCa	1972 C	*MTHFR* (677C>T, 1298A>C), *TS* variants (*TSER, TTAAAG* in 3´-UTRs 1494), *MTR* (919D>G), *RFC* (80G>A), *MTHFD1* (R134K, R653Q), *ADH3* (1045A>G)	*MINT1, MINT2, MINT31, p16, hMLH1*	Unk	Dietary folate, Met, vitamin B_{12}, and alcohol	Adaptation of the CARDIA diet history	Unk	*MTHFR* 1298AA–alcohol (high *vs* none): CIMP+, 0.5 (0.3-0.97), $P < 0.01$; *ADH3* (1 or 2 variant, slow catabolizing*2 *vs* homozygous for the common allele)–folate (low): CIMP+, 1.6 (1.03-2.6), $P = 0.02$. *MTHFR* 1298AC or CC-high-risk dietary pattern (low in folate or Met intake, high in alcohol): CIMP+, 2.1 (1.3-3.4), $P = 0.03$	Age, centre, other SNPs, sex, smoking intensity, and race	9
Curtin *et al*[33]	United States	30-79	559/392	1205 C	*MTHFR (* 1298A>C), *TP53, KRAS2,*	*CDKN2A, hMLH1, MINT 1, 2* and *31*		Folate, riboflavin, vitamins B_6, B_{12}, and Met	Adaptation of the CARDIA diet history (by interview)	M: Folate (T_3 *vs* T_1)–CIMP+, 3.2 (1.5-6.7), $P < 0.01$	1298 AC/CC (*vs* AA)–folate (T_3 *vs* T_1): 0.4 (0.2-1.0), $P = 0.04$, for CIMP+	Age, centre, intake of energy and fibre, NSAID use, oestrogen use (W), PA, race, referent year, sex, screening, and smoking	8
Kim *et al*[34]	Korea	30-79	465/322 CRC (363 CCa, 330 RCa)	656 H	*MTHFR* (677C>T)	Unk	2 mononuc-leotide markers (Bat25 and Bat26) and 3 dinucleotide markers (D2S123, D5S346, and D17S250)	Folate, vitamins B_2, B_6, B_{12}, niacin, Met, and choline	Validated FFQ	Unk	DCCa: *hMSH3* (rs41097) AG/GG (*vs* AA)–niacin (> 14.00 mg/d *vs* < 14.00 mg/d)–MSI–MMR status: 0.49 (0.28-0.84), *P-interac* = 0.008	Age, intake of energy and alcohol, BMI, CRC family history, educational level, occupation, income, PA,	7

											sex, and smoking status	
Slattery *et al*[35]	United States	30-79	821/689 CRC	2410 C	Unk	Unk	10 tetranucleotide repeats, 3 Bat-26 and TGFbRII	Dietary folate, and alcohol	Validated CARDIA diet questionnaire	Alcohol–MSI+ (*vs* MSI-): 1.6 (1.0-2.5), *P-trend* = 0.03; liquor–MSI+ (*vs* MSI-): 1.6 (1.1-2.4), *P-trend* = 0.02		Age, BMI, intake of energy, fibre and calcium, intake, PA, sex	7
Slattery *et al*[36]	United States	30-79	638/516 CRC	2410 C	*BRAF* (V600E)	*MINT1*, *MINT2*, *MINT31*, *p16* and *hMLH1*	Unk	Folate, vitamins B_6 and B_{12}, Met, and alcohol	Diet history questionnaire	No assoc	MSI tumour–alcohol (high *vs* none): 1.6 (0.9-2.9), *P-trend* = 0.04, for *p16* unmethylated; 1.7 (0.7-4.3), *P-trend* = 0.06, for $CIMP_{low}$ (< 2 markers); 2.2 (1.2-3.7), *P-trend* = 0.01, for *BRAF* wildtype	Age, alcohol intake, BMI, intake of energy and folate, density of calcium and fibre, NSAIDs use, PA, sex, smoking intensity	7

AA: African Americans; *ADH3*: Alcohol dehydrogenase 3; asso: Significant association; Bat: Mononucleotide microsatellite with quasi-monomorphic allele length distribution in healthy controls but unstable; BMI: Body mass index; *BRAF*: B-Raf proto-oncogene; C: community controls; *CACNA1G*: Calcium voltage-gated channel subunit a1 G; CCa: Colon cancer; *CDKN2A*: Cyclin-dependent kinase inhibitor 2A; CI: Confidence interval; CIMP: CpG island methylator phenotype; CRC: Colorectal cancer; EA: European Americans; FFQ: Food frequency questionnaire; H: Hospital controls; *hMLH1*: Human *MutL* homolog 1; *hMSH3*: Human *MutS* homolog 3; interact: Interaction; *KRAS:* Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; M: Men; Met: Methionine; MINT: Methylated in tumours; MMR: Mismatch repair; MSI: Microsatellite instability; *MTHFD:* Metilentetrahidrofolate dehydrogenase; *MTHFR:* Methylenetetrahydrofolate reductase; *MTR:* Methionine synthase; *NEUROG1*: Neurogenin1; NOS: Quality Newcastle-Ottawa Scale; NSAID: Nonsteroidal anti-inflammatory drugs; OR: Odds ratio; PA: Physical activity; RCa: Rectal cancer; *RFC*: Reduced folate carrier; *RUNX3:* Runt-related transcription factor 3; SNP: Single-nucleotide polymorphism; *SOCS1*: Suppressor of cytokine signalling 1; T: Tertile; *TGFbRII*: Transforming growth factor β receptor type II; TS: Thymidylate synthase; *TSER:* Thymidylate synthase enhancer region; TP53: Tumour protein p53; unk: Unknown; UTR: Untranslated region; W: Women.

showed inverse associations between vitamin B_6 intake and CRC risk, both when the intake level was higher[6] and when it was similar[42] to that in the study of de Vogel *et al*[37]. Therefore, further attention should be given to this association in future studies.

Another interesting finding in this review is the inverse association between dietary folate and Met and B-Raf proto-oncogene mutations among men[37]. A previous study showed that folate may increase the risk of tumours harbouring truncating adenomatous polyposis coli mutations in men[43]. Apparently, relatively high folate intake may confer a growth advantage to mutated tumours independent of the type of mutation. Nevertheless, the occurrence of MSI does not seem to be sensitive to methyl donor intake or to that of alcohol[36-37,44].

In one of the case-control studies that we analysed, an interaction was observed between a high- or low-risk diet and *MTHFR* 1298A>C (but not 677C>T) with regard to CIMP status[32]. This result suggests that a genetic polymorphism at *MTHFR* 1298A>C interacts with the diet (with a low folate and Met intake and a high alcohol consumption) increasing the risk of highly CpG-methylated colon tumours. S-adenosylmethionine binds as an allosteric inhibitor in the *MTHFR* regulatory region, which is where the 1298A>C variant is found. This gives some justification for the stronger associations between *MTHFR* 1298A>C and CIMP rather than the 677C>T SNP, which has an opposite effect on the stability of the enzyme. Due to their high linkage disequilibrium, the *MTHFR* 677C>T and 1298A>C polymorphisms should not be viewed separately. van Engeland *et al*[40] also discovered that people

Table 4 Characteristics of the five cohort studies included in this systematic review examining the interactive effects between single-nucleotide polymorphisms in genes encoding methyl-metabolizing enzymes and/or mutations in oncogenes, CpG island methylator phenotype and/or microsatellite instability, and one-carbon metabolism-related dietary compounds on colorectal cancer risk

Ref.	Country	Study cohort (age, yr)	No. participants (M/W)	No. incident cases	Follow-up length, yr	Gene (SNP)	CIMP markers	MSI	Nutrient/alcohol	Method for measuring nutrition intake	Outcome (RR, 95%CI) interaction			NOS
											CIMP markers/MSI–nutrient/alcohol	CIMP markers–/MSI–SNP–nutrient/alcohol	Adjustments to RR	
de Vogel *et al* [9]	Netherlands	The Netherlands Cohort Study on diet and cancer (55-69)	58279/62573	734 CRC	7.3	*MTHFR* (rs1801133, rs1801131), *MTR* (rs1805087), *MTRR* (rs1801394), *DNMT3B* (rs2424913, rs406193), *EHMT1* (rs4634736), *EHMT2* (rs535586), *PRDM2* (rs2235515)	*CACNA1G*, *IGF2*, *NEUROG1*, *RUNX3*, *SOCS1*	Bat-26, Bat-25, NR-21, NR-22, NR-24	Dietary folate, Met, vitamins B_2 and B_6, alcohol	Validated FFQ (self-reported)	No assoc	Unk	BMI, CRC family history, intake of energy and alcohol, sex, and smoking status	9
de Vogel *et al* [37]	Netherlands	The Netherlands Cohort Study on diet and cancer (55-69)	58279/62573	734 CRC	7.3	*BRAF* (V600E)			Dietary folate, Met, vitamins B_2 and B_6, alcohol	Validated FFQ (self-reported)	M: *BRAF* mut–folate (T_3 *vs* T_1): 3.04 (1.13-8.20), *P-trend* = 0.03; *BRAF* mut–Met (T_3 *vs* T_1): 0.28 (0.09-0.86), *P-trend* = 0.02); *hMLH1* hypermethylation–vitamin B_6 (T_3 *vs* T_1): 3.23 (1.15-9.06), *P- trend* = 0.03	Unk	Age, BMI, CRC family history, intake of energy, meat, total fat, fibre, vitamin C, total iron and calcium, smoking status	9
Schernhammer *et al*[38]	United States	The Nurses' Health Study (W) (30-55) and the Health Professional Follow-up Study (M) (40–75)	47371/88691	669 CCa	22	*KRAS*	Unk	D2S123, D5S346, D17S250, Bat25, Bat26 (14), Bat40, D18S55, D18S56, D18S67, D18S487	Folate, vitamins B_6 and B_{12}, Met, and alcohol	Validated FFQ (self-reported)	Unk	MSI/*KRAS*–folate: No assoc for CCa. MSI/*KRAS*–vitamins B_6 or B_{12}: No assoc for CCa	Age, aspirin use, smoking, BMI, colon polyps, CRC family history, intake of alcohol, energy, beef, calcium, vitamins B_6 and B_{12}, and Met, multivitamin use, PA, sex, screening sigmoidoscopy	9

Schernhammer *et al*[39]	United States	The Nurses' Health Study (30-55)	88691 W	375 CCa		*BRAF*	*CHFR, MGMT, p14, WRN, HTC1, MINT1, MINT31, IGFBP3*	Unk			Folate (Q_4 *vs* Q_1: No assoc with CIMP–high tumour risk; and no assoc with *BRAF* status	Unk			
van Engeland *et al*[40]	Netherlands	Netherlands Cohort Study on Diet and Cancer (55-69)	58279/62573	122 CRC	7.3	Unk	*APC-1A, p14ARF, p16INK4A, hMLH1, O 6-MGMT,* and *RASSF1A*	Unk	Dietary folate and alcohol	Validated FFQ (self-reported)	Low *vs* high-methyl donor intake–promoter methylation (> 1 gene methylated): No assoc	Unk		Age, CRC family history, intake of energy, fibre, vitamin C, and iron, sex	9

APC: Adenomatous polyposis coli; asso: Significant association; Bat: Mononucleotide microsatellite with quasi-monomorphic allele length distribution in healthy controls but unstable; BMI: Body mass index; *BRAF*: B-Raf proto-oncogene; *CACNA1G:* Calcium voltage-gated channel subunit a1 G; CCa: Colon cancer; *CHFR*: RING finger domain protein; CI: Confidence interval; CIMP: CpG island methylator phenotype; CRC: Colorectal cancer; *DNMT3B:* DNA methyltransferase 3 *EHMT:* Euchromatin histone methyltransferase; FFQ: Food frequency questionnaire; *hMLH1*: Human *MutL* homolog 1; *HTC*: Histidine triad with channel; IGF2: Insulin-like growth factor 2; *IGFBP*: Insulin-like growth factor-binding protein; *KRAS:* Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; M: Men; Met: Methionine; *MGMT*: DNA repair enzyme O(6)-methylguanine-DNA methyltransferase; MINT: Methylated in tumours; MSI: Microsatellite instability; *MTHFR:* Methylenetetrahydrofolate reductase; *MTR:* Methionine synthase; *MTRR*: Methionine synthase reductase; mut: Mutation; *NEUROG1*: Neurogenin1; NOS: Quality Newcastle-Ottawa Scale; PA: Physical activity; *PRDM2*: PR domain zinc finger protein 2; Q: Quartile; RASSF1A: Ras association domain family 1 isoform A; RR: Relative risk; *RUNX3:* Runt-related transcription factor 3; SNP: Single-nucleotide polymorphism; *SOCS1*: Suppressor of cytokine signalling 1; T: Tertile; unk: Unknown; W: Women; *WRN*: Werner syndrome gene.

diagnosed with CRC, with low intake of folate and high consumption of alcohol, had a greater prevalence of promoter hypermethylation; however, the difference was not statistically significant due to limited power. Therefore, it was proposed that stratification for functionally significant SNPs in the genes encoding folate metabolism enzymes could strengthen the observed effect of folate deficiency on promoter methylation[40].

Additionally, Curtin *et al*´s study[32] found an interaction between alcohol consumption and *MTHFR* 1298A>C in association with CIMP status. Relative to the AA genotype in non-drinkers, the *MTHFR* 1298 AA genotype was linked to a higher risk of CIMP+ in drinkers. In a previous study by Slattery *et al* [35], in which associations between CIMP status and alcohol use were assessed without taking into regard to genotype, no association was observed. These results imply that the activity of the 1C-metabolism enzyme may alter the risk associated with alcohol in determining the CIMP status of colon cancer. In any case, to date, few published studies have evaluated, CIMP in CRC for possible relationships with 1C-metabolism SNPs[32,45,46]. Moreover, these data raise the possibility that more investigation is required to clarify the function of genetic SNPs in relation to CIMP status and the promoter hypermethylation of particular genes.

In one case-control study[36], an association between long-term alcohol consumption and increased likelihood of having a CIMP-low or B-Raf proto-oncogene-mutated tumour was observed. Among those with unstable tumours, they observed that alcohol was more likely to be associated with CIMP-low rather than CIMP-high tumours. A previous study of these same authors showed that alcohol increased the risk for MSI+ tumours in general[35]. Therefore, these findings suggest that the increased risk of MSI associated with alcohol is limited to those tumours that are unmethylated rather than methylated.

Table 5 Summary of results of the studies included in this systematic review

Gen, SNP/CIMP/MSI	Nutrients/alcohol	CRC risk/CIMP+	Ref.
MTHFR 677 TT	Folate; Adequate folate	CRC risk; CRC risk	Guerreiro *et al*[24]; Sharp *et al*[29]; Ma *et al*[26]
	Alcohol	CRC risk	Chen *et al*[23]; Ma *et al*[26]
	Folate/Met, and without alcohol	CRC risk	Slattery *et al*[30]
	Vitamin B_6	CRC risk	Sharp *et al*[29]
BRAF mutation	Folate	CRC risk (M)	de Vogel *et al*[37]
	Met	CRC risk (M)	
hMLH1 hypermethylation	Vitamin B_6	CRC risk (M)	
MTHFR 1298 AC/CC	Folate/Met, and alcohol	CIMP+	Curtin *et al*[32]
MTHFR 1298 AA	Alcohol	CIMP+ (CCa)	
p16 unmethylated, $CIMP_{low}$ or *BRAF* mut	Alcohol	CRC risk	Slattery *et al*[36]
hMSH3, MSI or MMR status	Niacin	CRC risk (DCCa)	Kim *et al*[34]

BRAF: B-Raf proto-oncogene; CCa: Colon cancer; CRC: Colorectal cancer; CIMP: CpG island methylator phenotype; DCCa: Distal colon cancer; h*MLH1*: Human *MutL* homolog 1; *hMSH3:* Human *MutS* homolog 3; M: Men; Met: Methionine; MMR: Mismatch repair; MSI: Microsatellite instability; *MTHFR:* Methylenetetrahydrofolate reductase; mut: Mutation; SNP: Single-nucleotide polymorphism.
↑: High intake or increased risk or high probability.
↓: Low intake or increased risk.

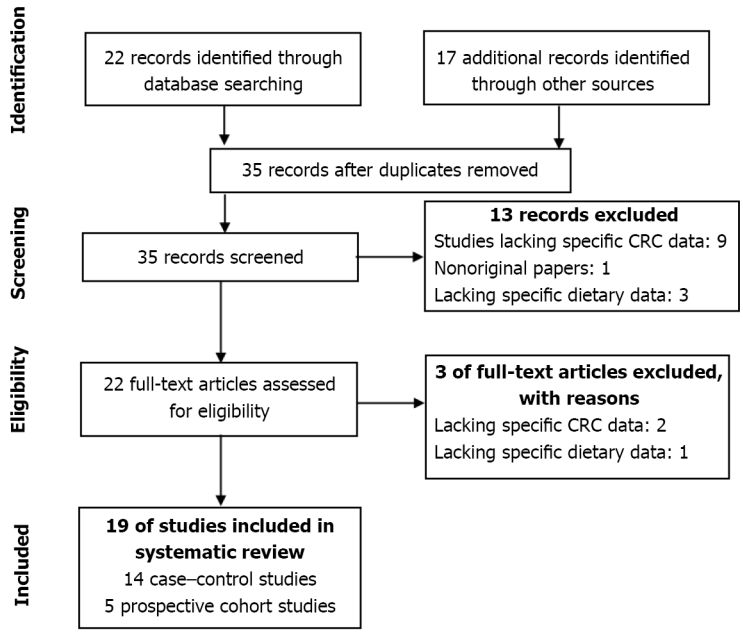

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Figure 1 PRISMA flow diagram summarizing the identification and selection of the relevant publications assessing the influence of methyl donor nutrients as epigenetic regulators in colorectal cancer. CRC: Colorectal cancer.

Finally, regarding the interaction between MMR SNPs and methyl donor nutrient intake on CRC based on MSI status, in a case-control study[34], a strong inverse association was observed for *hMSH3* AG or GG carriers with a high intake of niacin, particularly among patients with CC and microsatellite stability or proficient MMR status. However, to ascertain the processes behind this association with CRC risk, the precise roles of this SNP must be identified.

The importance of interactions between modifiable factors, such as methyl donor nutrients and CRC, is suggested by evidence that colorectal carcinogenesis is generated by numerous molecular pathways in parallel with MSI status, which is caused by a defect in the MMR machinery. In this sense, it is worth

remembering the article mentioned by de Vogel *et al*[37], in which it was reported that high consumption of vitamin B_6 was linked to an increased risk of sporadic CRC with *hMLH1* hypermethylation, indicating that vitamin B_6 affects CRC risk through both genetic and epigenetic mechanisms. Based on the findings of Kim *et al*[34], it may be possible to explain the interactions between dietary methyl donor nutrients, such as niacin, and *hMSH3* genetic variants as predictors of CRC risk.

DNA methylation and microRNA expression levels may be regulated by the MMR machinery's epigenetic relationships with methyl groups[47]. Through the methylation of CpG islands in the promoter region, 1C metabolism mediated by methyl donor nutrients can change how the DNA MMR system is activated. A adequate DNA MMR system with methyl groups may control the equilibrium between the repair and accumulation of short repeat sequences, preventing extensive DNA damage that supports colorectal carcinogenesis.

This systematic review has several strengths: (1) Cohort and case-control studies were identified through a systematic search; (2) a quantitative NOS scale was used to assess the quality of the studies; and (3) most studies (twelve of nineteen) used a validated questionnaire to assess dietary intake. To our knowledge, this is the first systematic review regarding the influence of methyl donor nutrients as epigenetic regulators in CRC.

The limitations of this review include the following: (1) Case-control studies, which are susceptible to recall and selection bias, made up the bulk of the research. However, most studies were based on community controls; thus, they might be a good representation of the frequency of genetic variants or of dietary habits of the overall population; (2) the heterogeneous nature of studies, including the study population characteristics, sample size, study design, and follow-up periods; (3) potential residual confounding because of the observational nature of the studies included or the possibility that not all the studies were adjusted for important nutrient variables; (4) some of the dietary assessments were self-reported, which may affect the reliability of the reported intakes, although the use of validated questionnaires in most studies could reduce this bias; (5) some studies had a relatively limited sample size or effect size, which made it difficult for them to detect the interactions between genetic and nutritional information, which could explain, in part, the lack of results with a statistically significant level; and (6) despite the fact that some research have found differential interactions based on the CRC subtype[34], the majority of studies lacked stratified analysis. Considering these limitations, the conclusions from these researchers should be taken carefully. Consequently, it is difficult for this review to explain the gene-diet interactions and their effects on the development of CRC.

CONCLUSION

In this systematic review of observational studies, some interactions between *MTHFR* polymorphisms, methyl donor nutrients (such as folate) and alcohol on CRC risk are suggested. Moreover, some studies show that vitamin B_6, niacin and alcohol may affect CRC risk through not only genetic but also epigenetic regulation. In any case, this review was not able to clarify which mechanisms underlie the influence of methyl donor nutrients on DNA methylation, as well as the efficacy of methyl uptake, transportation, and the final involvement in methyl-related gene expression. Further prospective studies with large samples and long follow-up periods, as well as clinical trials that take into account the long latency period of CRC, are needed to clarify the influence of methyl group donors as epigenetic regulators, with particular emphasis on differences in CRC subsite-specific risk. Such studies may provide valuable insight into the biological mechanisms with the goal of identifying at-risk subpopulations and promoting primary prevention of CRC.

ARTICLE HIGHLIGHTS

Research background

Colorectal cancer (CRC) is the third most frequent cancer and is responsible for the second-highest mortality rate in cancer patients worldwide. The main factors which may help prevent CRC are those associated with diet, lifestyle, and prevention of metabolic diseases. With regards to the dietary component, one-carbon metabolism-related nutrients have been considered anticarcinogenic and chemotherapeutic agents in the one-carbon metabolic network. However, it is still unclear whether the influence of methyl donor intake is modified by polymorphisms in these epigenetic regulators.

Research motivation

Although screening for early detection of CRC is effective to help decrease the trends in mortality rates, understanding daily life factors is also important to prevent this type of cancer. A better understanding of the molecular basis of CRC could contribute to a better design of future research and better preventive nutritional management in this type of cancer.

Research objectives

In the present work, we reviewed previous studies that have investigated this matter to improve the current understanding of the molecular basis of CRC.

Research methods

A literature search in the Medline database, *Reference Citation Analysis* (https://www.referencecitation-analysis.com/, an artificial intelligence technology-based open multidisciplinary citation analysis database), and manual reference screening were performed to identify observational studies published from inception to May 2022. A search for relevant keywords and medical subject heading terms related to dietary methyl donors, dietary components that potentially modulate the bioavailability of methyl groups, genetic variants of methyl-metabolizing enzymes, markers of CpG island methylator phenotype and/or microsatellite instability, in combination with keywords related to CRC events was conducted. The present search was developed according to the "PRISMA Statement" guidelines. To evaluate the validity of the individual studies, two reviewers worked independently to determine the quality of the included studies based on the use of the Newcastle-Ottawa scale for case-control or cohort studies.

Research results

A total of fourteen case-control studies and five cohort studies were identified. In total, the case-control studies included 7055 cases and 9032 controls. The cohort studies included 256914 participants, with 1109 cases recorded during follow-up periods that ranged from 7.3 to 22 years. The dietary components that showed a higher association with CRC risk were folate and alcohol. Thus, high folate intake was considered a protective factor, while high alcohol consumption proved to be a risk factor. Several studies have investigated the association between methyl donor nutrients and/or methyl antagonists (*e.g.*, alcohol) and methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and have reported significant interactions. In one of those case-control studies, those with the *MTHFR* 677 TT genotype, who consume low folate diets, had a greater chance of developing CRC than people with the CC or CT genotype. Two other case-control studies reported that *MTHFR* 677 TT carriers with high (above mean) or adequate folate intake had a low risk of CRC.

Research conclusions

In this systematic review of observational studies, some interactions between *MTHFR* polymorphisms, methyl donor nutrients (such as folate), and alcohol on CRC risk are suggested. Moreover, some studies show that vitamin B_6, niacin, and alcohol may affect CRC risk through not only genetic but also epigenetic regulation.

Research perspectives

This review was not able to clarify which mechanisms underlie the influence of methyl donor nutrients on DNA methylation, as well as the efficacy of methyl uptake, transportation, and the final involvement in methyl-related gene expression. Further prospective studies with large samples and long follow-up periods, as well as clinical trials that consider the long latency period of CRC, are needed to clarify the influence of methyl group donors as epigenetic regulators, with particular emphasis on differences in CRC subsite-specific risk.

FOOTNOTES

Author contributions: The study was conceived and designed by Chávez-Hidalgo LP, Martín-Fernández-de-Labastida S, M de Pancorbo M, and Arroyo-Izaga M; The data were acquired, collated, and analysed by Chávez-Hidalgo LP and Arroyo-Izaga M; The study was drafted and revised critically for important intellectual content by all authors; The work reported in the paper has been performed by the authors, unless clearly specified in the text; All authors gave final approval of the version to be published and have contributed to the study; No ethical approval was needed.

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CASE REPORT

Percutaneous transhepatic intraportal biopsy using gastroscope biopsy forceps for diagnosis of a pancreatic neuroendocrine neoplasm: A case report

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Abstract

BACKGROUND

Pancreatic neuroendocrine neoplasms (PNENs) are a rare group of neoplasms originating from the islets of the Langerhans. Portal vein tumor thrombosis has been reported in 33% of patients with PNENs. While the histopathological diagnosis of PNENs is usually based on percutaneous biopsy or endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA), these approaches may be impeded by gastric varices, poor access windows, or anatomically contiguous critical structures. Obtaining a pathological diagnosis using a gastroscope biopsy forceps *via* percutaneous transhepatic intravascular pathway is an innovative method that has rarely been reported.

CASE SUMMARY

A 72-year-old man was referred to our hospital for abdominal pain and melena. Abdominal contrast-enhanced magnetic resonance imaging revealed a well-enhanced tumor (size: 2.4 cm × 1.2 cm × 1.2 cm) in the pancreatic tail with portal vein invasion. Traditional pathological diagnosis *via* EUS-FNA was not possible because of diffuse gastric varices. We performed a percutaneous transportal biopsy of the portal vein tumor thrombus using a gastroscope biopsy forceps. Histopathologic examination revealed a pancreatic neuroendocrine neoplasm (G2) with somatostatin receptors 2 (+), allowing systemic treatment.

CONCLUSION

Intravascular biopsy using gastroscope biopsy forceps appears to be a safe and effective method for obtaining a histopathological diagnosis. Although well-designed clinic trials are required to obtain more definitive evidence, this procedure may help improve the diagnosis of portal vein thrombosis and related

diseases.

Key Words: Percutaneous transhepatic intravascular biopsy; Portal vein tumor thrombosis; Gastroscope biopsy forceps; Pancreatic neuroendocrine neoplasms; Case report

Core Tip: Endoscopic ultrasound-guided fine-needle aspiration or percutaneous biopsy of pancreatic neuroendocrine neoplasms patients can be impeded by gastric varices and poor access windows. In this patient, we faced the challenge of obtaining biopsy of the pancreatic mass which had invaded the portal vein. We performed a percutaneous transportal biopsy of the portal vein tumor thrombosis using a gastroscope biopsy forceps. Our experience suggests that gastroscope biopsy forceps is a viable alternative to obtain biopsy in the portal vein system. Our work expands the use of diagnostic transhepatic portal catheterization. This method may also help prevent repeated liver puncture, reducing the risk of complications.

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INTRODUCTION

Pancreatic neuroendocrine neoplasms (PNENs) are rare tumors accounting for approximately 2% of all pancreatic tumors[1]. Portal vein tumor thrombosis (PVTT) was reported in approximately 33% of patients with non-functioning PNENs (NF-PNENs)[2]. Tumor thrombosis in such cases may cause splenoportomesenteric hypertension, potentially resulting in life-threatening upper gastrointestinal bleeding. Computed tomography (CT) or magnetic resonance imaging (MRI) is useful for diagnosis. Tissue samples are usually obtained *via* imaging-guided percutaneous fine-needle aspiration biopsy or core needle biopsy, endoscopic retrograde cholangiopancreatography, or endoscopic ultrasound-guided fine-needle aspiration/biopsy (EUS-FNA/B)[3]. This report describes the first diagnostic case of percutaneous transhepatic intravascular biopsy of PVTT caused by PNEN invasion using a gastroscope biopsy forceps in a patient with severe gastric varices in whom diagnosis could not be made through percutaneous or endoscopic approaches.

CASE PRESENTATION

Chief complaints

A 72-year-old man was referred to a local hospital for upper abdominal pain with melena for 10 d.

History of present illness

He was diagnosed with pancreatitis and gastric varices. The abdominal pain was relieved after somatostatin treatment for 2 d. The patient was referred to our hospital for further treatment.

History of past illness

Six months ago, he was diagnosed with gastric varices after undergoing gastroscopy as part of routine medical checkup; however, he did not undergo further evaluation.

Personal and family history

He had no family history of malignant tumors, psychological, or genetic disorders.

Physical examination

Physical examination showed pallor.

Laboratory examinations

Laboratory investigations showed three lineage pancytopenia (hemoglobin 66 g/L, WBC count: 2.4 × 10^9

/L, platelet count: 83.0 × 10^9/L). Serum transaminases, bilirubin, and amylase levels were normal. Urine routine, stool routine and occult blood test were negative. Serum tumor markers (AFP, CEA, CA199, and NSE) were all within the normal reference range.

Imaging examinations

Abdominal contrast-enhanced MRI revealed a well-enhanced tumor (size: 2.4 cm × 1.2 cm × 1.2 cm) in the pancreatic tail (Figure 1A), with portal vein invasion (Figure 1B and C), and the presence of many collateral vessels indicative of portal stenosis (Figure 1C). The mass inside the portal vein was enhanced too, suggesting that it was a tumor rather than a thrombus. Splenomegaly was also observed, which was considered as a secondary change. No metastasis was found in other organs, including the lymph nodes, liver parenchyma, and lungs.

FINAL DIAGNOSIS

Histopathologic examination revealed a NEN (G2) with somatostatin receptors 2 (SSTR2) (+) (Figure 2A-D).

TREATMENT

A multidisciplinary team meeting was held to discuss the case. Diagnosis of PNEN with portal vein invasion was highly suspected. Surgical resection was considered risky because of the involvement of adjacent organs and sinistral portal hypertension. Interventional therapy to prevent upper gastrointestinal hemorrhage and chemotherapy were suggested. However, pathological diagnosis *via* EUS-FNA seemed infeasible because of diffuse gastric varices (Figure 3A). The possibility of percutaneous CT/ultrasound-guided fine-needle aspiration biopsy was also excluded since no adequate access was identified. Finally, we performed percutaneous transportal biopsy of the PVTT using a gastroscope biopsy forceps. After percutaneous transhepatic puncture of the right-portal vein under sonographic guidance, 7F "crossover sheath" catheter was introduced into the right-portal vein and the biopsy forceps was inserted into the catheter. Under X-ray guidance, the biopsy forceps was pushed close to the thrombus and 3 samples were obtained (Figure 2E and F; Figure 4; Supplementary material) and fixed immediately. Subsequently, partial splenic embolization (PSE) was performed for treating the gastric varices. After obtaining the histopathologic results, the patient received octreotide long-acting release (LAR) injection. There were no postoperative complications, and the patient was discharged on postoperative day 8. Owing to the high risk of gastric varices hemorrhage, the patient received repeat PSE treatment.

OUTCOME AND FOLLOW-UP

Follow-up gastroscopy performed after one month showed significant alleviation of gastric varices (Figure 3B). Subsequently, the patient regularly received octreotide LAR injection at a local hospital. He experienced weight loss and fatigue for the following 2-4 mo and recovered gradually. He received no chemotherapy or CT/MRI re-examination for some personal reasons. As of 8-mo follow-up, the patient is alive and showed no discomfort.

DISCUSSION

Histological diagnosis is essential for the treatment of patients with PNEN and can be carried out using resection samples, while core biopsies can be performed in patients with advanced disease[4]. ^{68}Ga-DOTATOC-PET-CT, with high sensitivity (92%) and specificity (83%), has been recommended as part of tumor staging, preoperative imaging, and restaging[4]. The EUS features (size and irregular lesion margins) associated with malignancy/aggressiveness of iso- and hypervascular solid pancreatic lesions have also been reported[5]. However, a definitive diagnosis cannot be established based on imaging findings alone. Biopsy can be performed by EUS-FNA/B or percutaneous ultrasound/CT-guided biopsy. Currently, EUS-FNA/B represents the gold standard for diagnosis of pancreatic lesions[6]. This technique provides pathological diagnosis as well as mitotic rate and Ki-67 index which defines grading, with an overall accuracy of approximately 92%[5], leading to its fundamental role in PNENs. However, these approaches may be hampered by gastric varices, poor access windows, target mobility, adjacent critical structures, and skin-to-target remoteness.

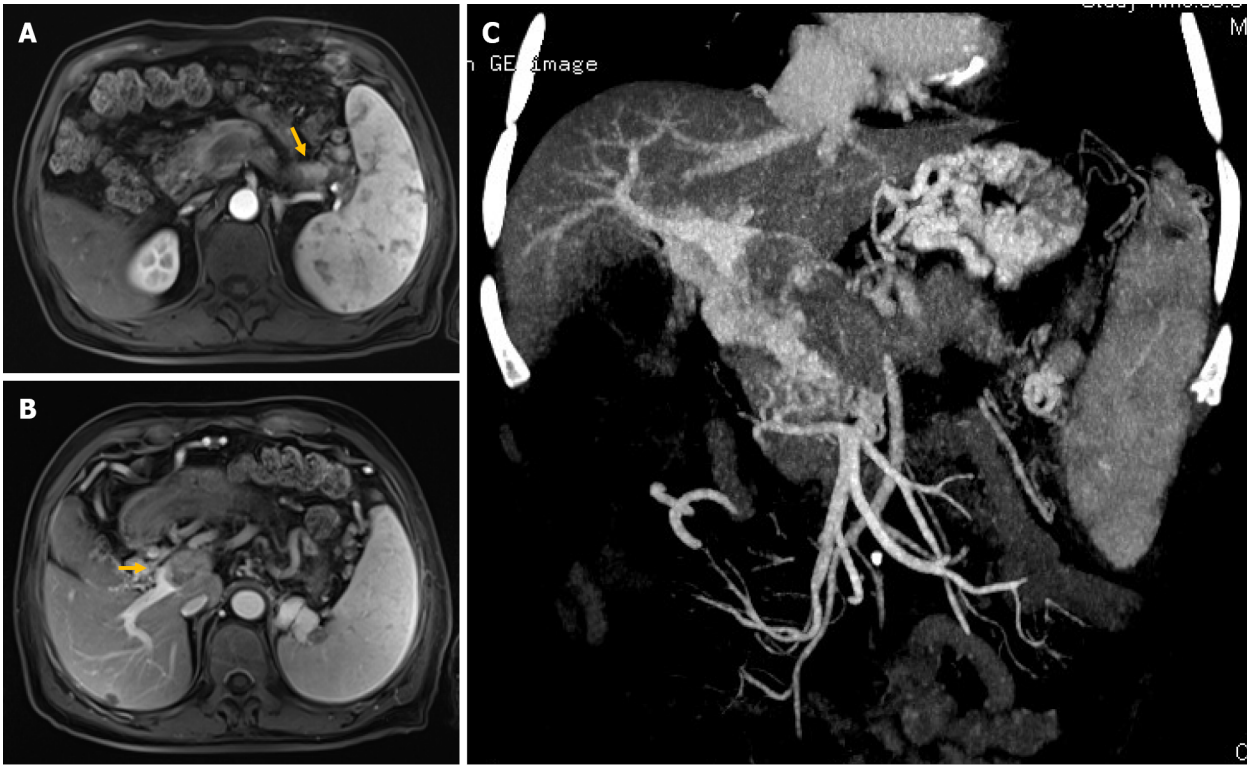

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Figure 1 Representative magnetic resonance imaging and computed tomography image on admission. A: Contrast-enhanced magnetic resonance imaging (MRI) axial view showing a well-enhanced tumor in the pancreatic tail; B: Axial view of contrast-enhanced MRI shows an intravascular filling defect (arrows) occupying the entire portal vein lumen; C: Computed tomography portal venography image showing extensive collateral venous circulation due to portal vein occlusion.

PNENs originate in the endocrine cells of the pancreas, and some of them can secrete specific hormones. Thus, PNENs can be classified as functional PNENs or non-functioning PNENs (NF-PNENs). Due to the lack of symptoms, NF-PENs tend to remain occult until a late stage of disease. In the study by Balachandran *et al*[2], venous tumor thrombus was detected by CT in 29 out of 88 (33%) patients with NF-PNEN. Six of these 29 patients showed invasion of portal vein. Portal venous tumor thrombus (PVTT) is a typical complication of hepatocellular carcinoma and is associated with a poor prognosis[7]. Other causes of PVTT include gastrointestinal and abdominal-pelvic malignant tumors[8]. Pancreatic neoplasms, especially neuroendocrine tumors, represent a possible but uncommon etiology of portal venous invasion[9,10].

Due to the PVTT in the present case, a gastroscope biopsy forceps-assisted intravascular biopsy was a safe and effective procedure for obtaining samples for histopathology, allowing the initiation of targeted drugs and chemotherapy. Portal vein catheterization has a certain learning curve, and the biopsy device must be introduced into the lesion by transhepatic approach using a large enough catheter (7 Fr). This would lead to a high risk of bleeding due to hepatic tract puncture. To prevent this complication, the puncture tract was embolized with coils, and the patient recovered well without any complications. Clinical use of percutaneous transhepatic portal catheterization was first described in 1970[11,12]. Since then, this has been proven to be a safe technique with few complications[13,14]. It is widely used to control gastroesophageal variceal bleeding[15-17] and to localize gastrointestinal hormone-producing tumors[18-21]. In hormone-producing tumors, percutaneous transhepatic portal catheterization has usually been performed for blood sampling from the portal vein. Here we expanded the use and value of transhepatic portal catheterization for diagnostic purposes.

The use of endoscopic biopsy forceps in intravenous biopsy has been reported. Guirola *et al*[22] reported an intravascular biopsy using a bronchoscopy forceps for facilitating the diagnosis of pulmonary artery intimal sarcoma. Chick JFBreported a transbiliary intravascular ultrasound-guided diagnostic biopsy of an inaccessible pancreatic head mass using core biopsy needle[3]. Sherk *et al*[23] reported a single-center retrospective analysis of 36 patients who underwent transvenous biopsy using cutting biopsy forceps; one of the patients in their study underwent a PVTT biopsy *via* transhepatic portal vein access, which is similar as our work. Apart from this, the use of biopsy forceps in portal vein biopsy has rarely been reported, let alone gastroscope biopsy forceps. Dodd GD III and Vilana R both performed Doppler-guided percutaneous portal vein thrombi biopsy using aspiration needle[24,25]. The potential side effects of percutaneous portal vein sampling include severe biliary or vascular injury. The potential complications include hepatic arterial or portal venous bleeding, bile duct laceration, and

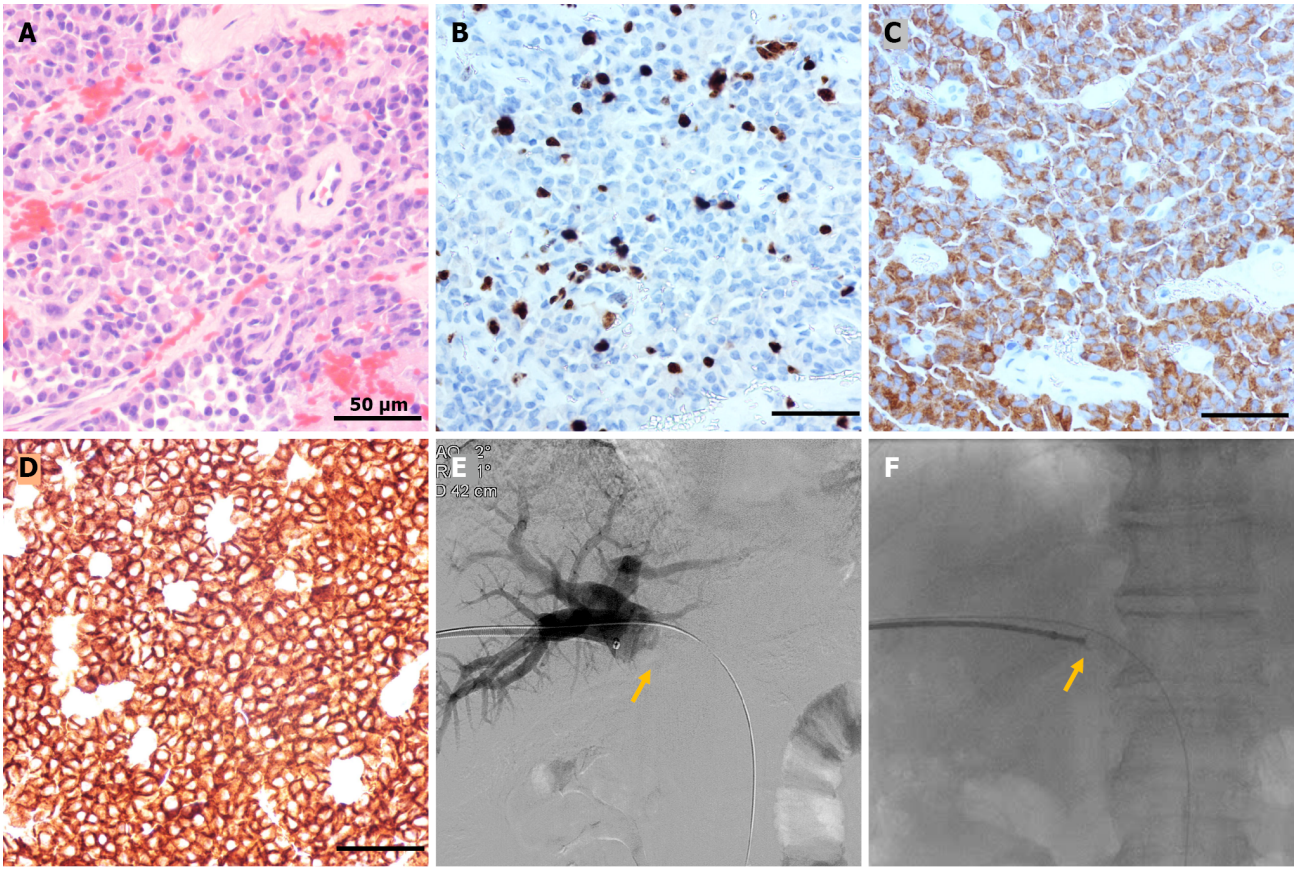

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Figure 2 Morphology and immunohistochemistry image of the tumor and radiograph from portography. A: Hematoxylin and eosin stained section of the tumor; B: Immunohistochemistry staining for Ki-67 showed Ki-67 index of 5%-8%; C and D: Immunohistochemistry staining for synaptophysin (C) and somatostatin receptors 2 (D) were positive (magnification × 400); E: Portography showing a filling defect within the intravascular space (arrows) in the main portal vein; F: Intravascular biopsy using gastroscope biopsy forceps assisted by a 7-F guide catheter located in the right portal vein (arrow).

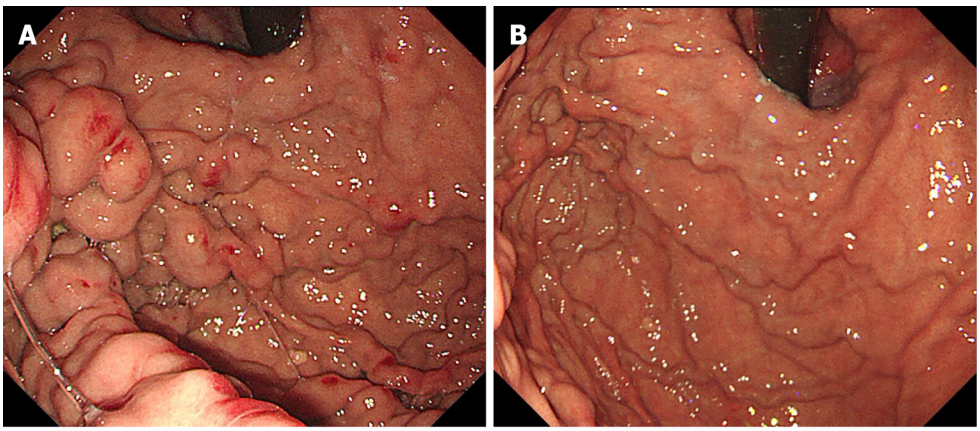

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Figure 3 Endoscopic image on admission and 1 mo later. A: Endoscopic image before partial splenic embolization (PSE) showing diffused gastric varices; B: Endoscopic image after PSE showing alleviation of gastric varices.

formation of biliary-vascular fistula, arterio-venous fistula, or pseudoaneurysm. EUS-FNA of portal venous thrombi represents an alternative approach which might overcome these limitations. Compared with fine needle aspiration, our method may help avert the need for repeated liver puncture, and reduce the risk of injury.

Currently, dual-tracer (^{68}Ga-DOTATOC and ^{18}F-FDG-)-PET/CT scan is a powerful imaging modality for the diagnosis and evaluation of PNET[26]. We did not perform the PET/CT scan owing to the patient's financial constraints. Moreover, the final therapeutic choice for the inoperable tumor would

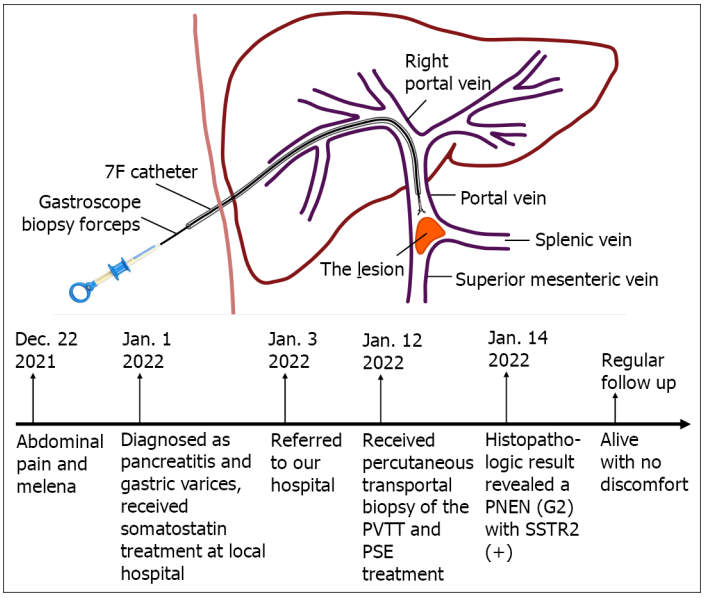

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Figure 4 Schematic illustration of the procedure and timeline. A: Schematic illustration of the procedure for percutaneous transhepatic intraportal biopsy using a gastroscope biopsy forceps; B: Timeline. PNEN: Pancreatic neuroendocrine neoplasm; SSTR2: Somatostatin receptors 2; PVTT: Portal vein tumor thrombosis; PSE: Partial splenic embolization.

not have changed regardless of the tumor stage. The validity of our method was uncertain prior to this case. Lack of malignant cells in the specimen obtained from a malignant thrombus may lead to a false-negative diagnosis. We tried to prevent false-negative result by obtaining samples for 3 times. Our positive result suggests that tumor cells can be obtained from the PVTT. Our work suggests that the gastroscope biopsy forceps of an appropriate size and function is a suitable substitute to obtain biopsy in the portal vein system. There is still a need for well-designed clinic trials, but this procedure may improve the diagnosis of portal vein thrombosis and related diseases.

CONCLUSION

Percutaneous transhepatic intraportal biopsy is valuable for the diagnosis of portal thrombosis and related diseases. Using gastroscope biopsy forceps through the sheath for tissue sample appears to be safe and effective, and can be performed in the context of other interventional procedures. More clinical trials are required to verify the safety and efficacy of this technique.

FOOTNOTES

Author contributions: Wang GC drafted the manuscript and collected data; Huang GJ performed the operation; Zhang CQ guided the operation; Ding Q guided the operation and revised the manuscript; all authors have approved the final draft submitted.

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