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LETTER TO THE EDITOR

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ABOUT COVER

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EDITORIAL

Current status of drug therapy for chronic hepatitis B

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Abstract

In this editorial, we comment on the article by Meng *et al*. Chronic hepatitis B (CHB) is a significant global health problem, particularly in developing countries. Hepatitis B virus (HBV) infection is one of the most important risk factors for cirrhosis and hepatocellular carcinoma. Prevention and treatment of HBV are key measures to reduce complications. At present, drug therapy can effectively control virus replication and slow disease progression, but completely eliminating the virus remains a challenge. Anti-HBV treatment is a long-term process, and there are many kinds of antiviral drugs with different mechanisms of action, it is essential to evaluate the safety and efficacy of these drugs to reduce side effects and improve patients' compliance. We will summarize the current status of CHB drug treatment, hoping to provide a reference for the selection of clinical antiviral drugs.

Key Words: Chronic hepatitis B; Hepatitis B virus; Anti virus; Drug treatment; Therapy

Core Tip: Chronic hepatitis B poses a serious threat to human health, and drug treatment is the most important method. The purpose of treatment is to sustainably inhibit virus replication and even achieve clinical cure, prevent or slow down disease progression, and reduce the incidence of cirrhosis and hepatocellular carcinoma. The widely used drugs at present are interferon and nucleoside/nucleotide analogue. On the premise of ensuring the efficacy, increasing drug safety and reducing side effects is an important research direction of anti-hepatitis B virus treatment.

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INTRODUCTION

Hepatitis B virus (HBV) belongs to the hepatotropic DNA virus family, with strong resistance and infectivity. When HBV is infected, virion first enters the liver cells, makes the virus relaxed circular DNA (rcDNA) invade the nucleus, under the action of various enzymes such as DNA polymerase, the gaps in rcDNA are repaired and converted into covalently closed circular DNA (cccDNA). Then, using cccDNA as a template, various mRNAs encoding different components are transcribed, and produces virion over and over. cccDNA is a key point in the HBV replication process and accompanies the entire process of HBV infection.

However, cccDNA has a long half-life and is stored in the cccDNA storage pool, which is difficult to clear completely. cccDNA is considered to be an important reason for persistent HBV infection, poor drug response and relapse after drug withdrawal, so eliminating cccDNA becomes the key to cure chronic hepatitis B (CHB)[1].

CHB is a chronic liver disease caused by persistent HBV infection, which can progress into cirrhosis and liver cancer, endangering the life safety of patients. In CHB patients, the cumulative incidence of cirrhosis over 5 years is about 10%-20%, and 2%-5% of cirrhosis progresses to liver cancer each year[2]. According to the World Health Organization, approximately 296 million people worldwide are living with CHB, with 4.5 million new infections each year, resulting in approximately 820000 deaths annually, mainly from complications such as cirrhosis and hepatocellular carcinoma (HCC). HBV infection is widely distributed around the world, with 80% of the infected patients coming from 21 countries in China, India, and Asia and Africa[3].

The genotype of HBV varies in different regions, with B and C being the main types in China. Different genotypes of HBV have different characteristics and prognosis. For example, patients with type B and type C CHB have a higher risk of mother-to-child transmission, type B is associated with HCC in young non cirrhotic patients, type C is associated with post hepatitis cirrhosis, and type C is more likely to progress to liver cancer than type B[4].

The main routes of HBV transmission include mother-to-child transmission, blood transmission, and sexual transmission. Prevention is the key to controlling the spread of HBV. Since 1982, HBV vaccine has been widely used, significantly reducing the infection rate of newborns and children, and reducing the incidence of HBV related cirrhosis and liver cancer at the source.

A study have shown that the incidence of liver cancer in HBV patients is 20-100 times higher than that in uninfected patients[5]. Therefore, the management of existing CHB patients is particularly important. In the absence of a complete cure for CHB, the management goal is mainly to reduce the risk of liver adverse events and improve the long-term prognosis of patients through active antiviral therapy[6].

DRUG THERAPY FOR CHB

From the perspective of the lifecycle and pathogenesis of HBV, theoretically, as long as any stage and pathogenic process of HBV replication can be blocked, disease progression can be slowed down or prevented. At present, anti HBV drugs in clinical practice include interferon (IFN) and nucleoside/nucleotide analogues (NAs) (Table 1).

Nucleoside/NAs

NAs are derivatives modified or substituted by chemical groups based on nucleoside/nucleotide. It's a kind of competitive inhibitor of HBV polymerase/reverse transcriptase (RT), directly inhibiting the generation of HBV DNA and is the first choice for anti-HBV therapy. In the late 1990s, the first NAs, lamivudine (LAM), was used to treat CHB patients [7]. With the continuous development of medicine, adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT), tenofovir disoproxil fumarate (TDF), tenofovir alafenamide fumarate (TAF) and tenofovir amibufenamide (TMF) have been approved for the treatment of CHB[8]. Recommended first-line NAs such as ETV, TDF and TAF have various advantages such as convenient oral administration, low cost, and high safety, and are widely used in clinical practice. NAs has good virological response, but due to the inability to clear cccDNA and poor hepatitis B surface antigen (HBSAg) seroconversion rate, it is difficult to reach the indications for drug discontinuation. Therefore, long-term medication may be required, and virological recurrence may occur once the drug is stopped[9-11].

Table 1 Characteristics of anti hepatitis B virus drugs

Drug	Approve time (year)	Classification	Mechanism	Administration method	Metabolize	Major side effect	Drug resistance
LAM	1998	NRTI	Competitive binding to HBV DNA polymerase binding site[64]	Oral administration	Kidney	Less side effects, may lead to myopathy or even rhabdomyolysis [65]	Susceptible to drug resistance, especially *YMDD* mutation[68]
LDT	2008	NRTI	Competitive binding to HBV DNA polymerase binding site[78]	Oral administration	Kidney	Myopathy and peripheral neuropathy [74]	The rate of drug resistance in long-term treatment was high, and the common drug resistance mutations were rtM204I and rtL180M [79, 85]
ADV	2002	NtRTI	Competitive binding of HBV DNA polymerase binding site and embedding into viral DNA strand[71]	Oral administration	Kidney	Renal function damage[75]	Compared to LAM, the risk of resistance is low[71]
ETV	2005	NRTI	Competitive binding to HBV DNA polymerase binding site[12,13]	Oral administration	Kidney	Less side effects, possible renal function damage[14]	Low resistance rate, but resistance to LAM-resistant HBV increased[16,20]
TDF	2008	NtRTI	Competitive binding of HBV DNA polymerase binding site and embedding into viral DNA strand[26]	Oral administration	Kidney	Bone and kidney injury[34]	Low risk of drug resistance
TAF	2016	NtRTI	Competitive binding to HBV DNA polymerase binding site[43]	Oral administration	Liver	Fewer bone and kidney side effects, but can lead to dyslipidemia[52]	Low risk of drug resistance
TMF	2021	NtRTI	Competitive binding to HBV DNA polymerase binding site[56]	Oral administration	Liver	Fewer bone and kidney side effects[58]	Low risk of drug resistance, and effective against multiple drug-resistant HBV
INF-α	1986	Standard interferon	Regulate the immune response and induce the synthesis of antiviral proteins in host cells[87]	Subcutaneous injection	Kidney	More side effects: Systemic adverse reactions	No drug resistance
Peg-IFN-α	2001	Long-acting Interferon	Regulate the immune response and induce the synthesis of antiviral proteins in host cells[87]	subcutaneous injection	Liver and kidney	Similar to INF-α	No drug resistance

NRTI: Nucleoside reverse transcriptase inhibitors, NtRTI: Nucleotide reverse transcriptase inhibitors; LAM: Lamivudine; ADV: Adefovir dipivoxil; ETV: Entecavir; LdT: Telbivudine; TDF: Tenofovir disoproxil fumarate; TAF: Tenofovir disoproxil fumarate; TMF: Tenofovir amibufenamide; IFN: Interferon; Peg-IFN-α: Polyethylene glycol interferon α; YMDD: Polymerase-tyrosine-methionine-aspartate; HBV: Hepatitis B virus.

First-line NAs

ETV: ETV is a guanine nucleoside analogue, which can be converted into ETV triphosphate by phosphokinase, and then competitively binds to the HBV DNA polymerase site, effectively inhibiting virus replication, and improving liver inflammation and fibrosis[12,13]. ETV is excreted through the kidney and may cause renal dysfunction with prolonged administration[14]. A study showed that the cumulative rates of HBV DNA seroconversion in patients receiving ETV monotherapy of 1-year and 5-year were 82.9% and 97.1%, respectively, reflecting the high virological response of ETV, but the serological conversion ability to hepatitis B e antigen (HBeAg) and HBsAg was poorer than that of IFNs[12]. A study from Korea showed a cumulative HBeAg serological clearance of 40.2% over 5 years in HBeAg-positive patients using ETV[15]. Another study from Japan showed that the cumulative HBeAg and HBsAg serological clearance rates for 5 years were 37% and 2.5%, respectively[16].

Although the long-term prognosis of ETV treatment in most CHB patients can be improved, there are some patients with HBV DNA remains at detectable levels and shows poor response. A study reported a 48-week cumulative virological response rate of 89.4% was found in patients treated with ETV at initial treatment[17]. Continuous poor response not only fails to prevent disease progression, but is a potential risk factor for cirrhosis and liver cancer. Patients with persistent hypoviremia have a 1.98 times higher risk of developing HCC than patients with persistent virological response[18,19]. Poor response often interacts with drug resistance. Long-term low-level replication of HBV DNA is a high risk factor for drug resistance, and drug resistance reduces the drug sensitivity of HBV to NAs. Long term treatment

may also cause genetic mutations leading to drug resistance, often occurring in the RT region of the HBV polymerase gene. A study has shown that the 10-year cumulative resistance rate of patients using ETV is 1.1%, while the 5-year cumulative resistance rate of patients with previous LAM resistance to ETV can reach 51%[16,20]. A study has shown that the higher the HBV DNA, the longer it takes to achieve complete virological response (CVR) during treatment, and the more likely lead to poor response[21]. When there is a poor virological response, relevant drug resistance tests can be performed to change the treatment strategy timely to avoid virological rebound. European Association for the Study of the Liver (EASL) proposed that for patients with poor response, if HBV DNA showed a downward trend after 48 weeks of treatment, the original strategy could be continued, otherwise, the drug should be changed[22].

American Association for the Study of Liver Disease (AASLD) proposed to continue the original strategy for hypoviremia[23]. Yim *et al*[24] divided CHB patients who could still detect HBV DNA after more than 48 weeks of ETV treatment into the original treatment group and the conversion to TDF group, and found that the 48-week CVR rate in the TDF group was significantly higher than that in the ETV group, and the HBV DNA decline was more obvious in the TDF group. A study found that after 24 weeks of TAF treatment, 75% of CHB patients with poor response (including 14 ETV treated patients) achieved HBV DNA negative conversion[25].

TDF: TDF is a monophosphate adenosine analogue. As the lipid precursor of tenofovir (TFV), it is rapidly hydrolyzed into TFV by non-specific carboxylesterase after entering plasma, then TFV is phosphorylated into the active product TFV diphosphate (TFV-DP) by cell kinase in hepatocytes. TFV-DP competes with the HBV DNA polymerase site to bind to the viral DNA strand, making the HBV DNA strand unable to be extended[26], thereby reducing the serum HBV-DNA load in the serum and promoting the negative transformation of HBV-DNA. A 7-year TDF treatment data showed that 99.3% of patients had HBV DNA negative conversion, and 54.5% of patients achieved HBeAg negative conversion[27]. It has been reported that 51% of patients treated with TDF for over 5 years experienced liver histological improvement and fibrosis regression[28]. Long-term follow-up has shown that TDF reduces the cumulative probability of liver decompensation, HCC, death, and liver transplantation[29]. The meta-analysis by Zuo *et al*[30] suggested that the virological response of the TDF group was superior to that of the ETV group in patients with initial CHB treatment. Chen *et al*[31] found that TDF had a stronger ability to inhibit HBV in the early stages of treatment, but no statistically significant difference was found between the two drugs at 144 weeks. In a national historical cohort study in Korea, a total of 24156 CHB patients who used ETV and TDF respectively were retrieved. The final results showed that the annual incidence rate of HCC in the TDF group (0.64 per 100 person years) was significantly lower than that in the ETV group (1.06 per 100 person years)[32].

TDF is one of the replacement drugs for ETV resistance. Mak *et al*[33] found that when TDF and ETV were used to treat CHB patients with hyperviremia, the incidence of acute kidney injury was higher in the TDF group.

TDF has a short half-life in plasma, and most of the TDF in plasma needs to be excreted through the kidney, increasing the burden on the kidney. Therefore, although TDF antiviral therapy is effective, the risk of kidney and bone damage increases with the patient's age after long-term use of TDF[34]. Multiple studies have shown that TDF can reduce blood lipid[35,36]. Studies have found that TDF up-regulates the expression of CD36 through PPAR-α activation, thereby regulating lipid metabolism[37]. CD36 is a member of the B-class scavenger receptor family, which can effectively bind to low-density lipoprotein and transport it, playing a lipid-lowering role. In a 48-week double-blind phase 3 study, the efficacy of TDF and ADV was compared. The results showed that the HBV DNA seroconversion rates of HBeAg positive and HBeAg negative patients in the TDF group were 75% and 93%, respectively, which were significantly higher than those in the ADV group (13% and 63%)[38]. Extended follow-up showed that after 4 years of treatment with TDF, 96% of HBeAg positive patients and 99% of HBeAg negative patients had HBV DNA negative conversion at the endpoint. Among HBeAg positive patients, 29% experienced HBeAg seroconversion[39,40].

TAF: As a novel nucleotide RT inhibitor, TAF is a precursor drug of TFV. Through protide technology, TAF adds a phosphoramidite structure on the basis of the structure of TFV, which better shields the active ingredient TFV, reduces the molecular polarity, improves the lipophilicity, and enhances membrane permeability. TAF can not only enter hepatocytes through passive diffusion, but also be efficiently and actively taken up by hepatocytes through liver uptake transfer proteins (OATP1B1 and OATP1B3)[41]. Studies have shown that TAF can reduce liver fibrosis both *in vivo* and *in vitro* by upregulating NS5ATP9, downregulating TGF β 1/Smad3, and NF-κB/NLRP3 inflammasome signaling pathways [42]. The mechanism of action of TAF is similar to TDF[43], but it has better stability, longer half-life, and can remain mostly intact when penetrating virus-infected cells. Therefore, the same efficacy can be achieved with less than 1/10 dose of TDF. Most of TAF is hydrolyzed into TFV in hepatocytes, which is mainly metabolized by liver and can target hepatocytes, thus reducing bone and kidney toxicity[22,44]. Studies have shown that renal function can be improved after TDF conversion to TAF treatment[45,46]. AASLD[23] and EASL[22] recommend the use of TAF in the elderly, patients with kidney damage and bone disease. Two multi-center, randomized, double-blind controlled studies demonstrated the efficacy and safety of TAF[47,48]. The two studies randomly divided target CHB patients into TAF group (25 mg/day) and TDF group (300 mg/day) in a 2:1 ratio. The results showed that the efficacy of TAF treatment for 48 weeks was not inferior to TDF, and TAF had a better ALT normalization rate than TDF. Patients treated with TAF had significantly lower bone density damage than those treated with TDF, and improved renal safety. At week 96, it was found that neither TAF nor TDF patients showed drug resistance[49]. According to the latest updated data, TAF has antiviral effects that are not inferior to TDF and better bone and kidney safety. However, multiple studies have shown that converting TDF to TAF is associated with increased body weight[35,50,51]. A large cohort study conducted by Surial *et al*[52] in Switzerland showed an average weight gain of 1.7 kg after switching to TAF for 18 months. At the same time, researchers have found that TAF can cause dyslipidemia, but the mechanism of TAF affecting blood lipids is still unclear[51,53,54]. A study have shown that statin use in patients with CHB can reduce the cumulative incidence of cirrhosis and

decompensated cirrhosis. Therefore, for patients with CHB who gain weight and increase blood lipids during the application of TAF, statin therapy can be combined[55].

TMF: TMF is a novel nucleotide RT inhibitor, with a mechanism of action similar to TDF. By optimizing the structure, the addition of a methyl group to the amidation group of TAF increases the lipophilicity of TMF, accelerates the rate of transmembrane transmission, and improves the plasma stability of the drug. It is metabolized by the liver and has lower bone and kidney adverse reactions[56,57]. A multicenter randomized clinical trial from China compared the efficacy of TMF and TDF after 48 weeks of treatment, and found that TMF exhibited similar antiviral effects as TDF and had lower bone toxicity[58]. The data updated by the researchers at week 96 confirmed this conclusion again[59]. Li *et al*[60] compared the virological response rates of TMF and TAF treatment for 24 weeks, and the results showed that TMF had a higher viral response rate (92% *vs* 74%) in the treatment naive group, while TMF and TAF had similar effects in the treatment experienced (TE) group. Zhang *et al*[61] compared the efficacy and safety of TMF and TAF in the treatment of CHB in a retrospective study involving 90 patients. After 48 weeks of treatment, they found no significant differences in virological response rates and HBeAg/HBsAg serological clearance rates (both *P* value = 1.000). Peng *et al*[62] recently published a study in the *World Journal of Gastroenterology* comparing the efficacy of TMF and TAF at 24 and 48 weeks, and found that the viral response rates were similar (24 weeks: 53.57% *vs* 48.31%, *P* value > 0.05; 48 weeks: 78.65% *vs* 78.57%, *P* value > 0.05). ALT normalization and renal safety in the TMF group were similar to those in the TAF group. However, since the study included non-alcoholic fatty liver disease (NAFLD) patients, we agree with Meng *et al*'s viewpoint[63] that in order to make the results more rigorous, subgroup analysis should be conducted on patients with and without NAFLD.

Non-first-line NAs

LAM: LAM was approved by the Food and Drug Administration (FDA) in 1998 for the treatment of CHB and was the first NAs approved. Belonging to the cytosine nucleoside class of drugs, it is converted into active lamivudine triphosphate in cells and acts by competing with the natural substrate of polymerase, which can quickly and effectively inhibit HBV replication[64]. LAM has fewer side effects, but may lead to myopathy or even rhabdomyolysis[65]. Studies have shown that oral administration of LAM 100 mg once a day can significantly inhibit HBV DNA levels. The seroconversion rate of HBeAg increases with prolonged treatment time[66]. For CHB patients with significant liver fibrosis and compensatory cirrhosis, LAM treatment for 3 years can delay disease progression, reduce liver decompensation and the incidence of HCC[67]. The main reason limiting its use is the occurrence of drug resistance. HBV DNA polymerase tyrosine methionine aspartate (*YMDD*) mutation. However, long-term treatment can cause HBV DNA polymerase tyrosine-methionine-aspartate-aspartate (*YMDD*) mutation, with a higher incidence of drug resistance. It has been reported that after 5 years of treatment, the drug resistance rate of LAM is as high as 51%, and the drug resistance rate increases with the extension of antiviral treatment time[20,68]. The weakening of antiviral effect and virus rebound can further aggravate liver disease, and liver failure or even death may occur in severe cases[69]. LAM alone has not been used as a first-line treatment for decompensated cirrhosis. For patients with LAM resistance, it is not recommended to switch to ETV because of the high possibility of subsequent drug resistance[70].

ADV: ADV was approved by the FDA for the treatment of HBV in 2002. ADV is an acyclic purine nucleoside analogue that is phosphorylated into the active diphosphate ADV through cellular kinase action *in vivo*. It competes with deoxyadenosine triphosphate substrates to inhibit the activity of HBV DNA polymerase or RT, terminate viral DNA strand extension, and thus inhibit viral replication. The mechanism of action of ADV and LAM is different. ADV is effective against both wild type and LAM resistant HBV. Therefore, ADV is also a remedy drug for LAM resistance, and the incidence of drug resistance is lower than LAM[71]. The combination of ADV and LAM can effectively inhibit HBV DNA in LAM resistant CHB, and the incidence of ADV resistance is lower in combination patients[72]. Studies have shown that when ADV is resistant, TFV can be used as a salvage treatment for ADV resistant patients[73,74]. The main metabolic pathway of ADV is completed through renal excretion, which causes significant damage to the kidneys and often leads to reduced renal function[75]. After 5 years of treatment with ADV, 3% of patients showed an increase in serum creatinine exceeding 0.5 mg/dL, but it was reversible[76,77].

LdT: LdT is a L-nucleoside anti-HBV drug, which is an artificially synthesized deoxythymidine substance[78]. It can reduce the activity of HBV DNA polymerase. Once the 5'- adenosine produced by LdT metabolism participates in HBV DNA, it prevents the elongation of HBV DNA strands. The GLOBE study found that LdT has good virus clearance ability and excellent e antigen seroconversion rate[79], and LdT is approved by FDA for anti HBV treatment in pregnancy[80]. The use of LdT in pregnant women with HBV infection can inhibit the replication of HBV and significantly reduce the risk of mother-to-child transmission of HBV, with good safety. Multiple clinical studies have shown that LdT can improve renal function impairment during anti-HBV treatment[81,82]. Lee *et al*[83] believed that ADV combined with LDT was superior to ADV alone and ADV combined with other drugs in the protection of renal function. LdT can be used even in patients with high risk factors for renal impairment[84]. LdT is classified as a pregnancy category B drug by the FDA in the United States. In women with HBV infection during pregnancy, if there is active hepatitis, LdT is selected for treatment, and it has been found to be effective and safe for both the fetus and the mother[80]. The main disadvantage of LdT is its drug resistance. The second year drug resistance rates for HBeAg positive and HBeAg negative patients were 29% and 11%, respectively. The common drug-resistant mutations are rtM204I and rtL180M[79,85]. Effective treatments for LdT resistance include addition of ADV or change to TFV monotherapy[76]. During the treatment with LdT, special attention should be paid to muscle related adverse reactions, such as elevated levels of creatine kinase and myopathy. In a small number of patients, these muscle adverse reactions may lead to severe muscle weakness and pain, and even the

need to stop treatment.

IFN (IFN-α/polyethylene glycol-IFN-α)

IFN-α is the earliest anti HBV drug. IFN is a type of cytokine that plays an important role in human immune defense, which is divided into common IFN-α and polyethylene glycol-IFN-α (Peg-IFN-α) according to the length of half-life. They have better HBeAg clearance rate than NAs[86]. IFN-α is a soluble glycoprotein secreted by infected and transformed cells, which has antiviral, anti-tumor, cell proliferation inhibiting, and immune regulating effects[87]. Peg-IFN-α is the product of pegylated IFN. Adding a large number of branched PEG molecules increases the molecular weight of IFN, reduces drug excretion, shields the antigenic determinants on the surface of IFN molecules, reduces the immunogenicity and clearance rate of IFN in the body, and the half-life can be extended to 40 hours[88]. IFN has dual effects of antiviral and immune regulation. On the one hand, it regulates T lymphocytes, NK cells, antigen-presenting cells, and promotes the secretion of cytokines such as IL-1 β, IL-6, tumor necrosis factor α (TNF-α), and IFN-γ, stimulating the host to produce anti HBV immune response[89,90]. On the other hand, it inhibits HBV replication by producing anti HBV proteins. NAs therapy does not directly target cccDNA[91]. The cccDNA persistently existing in the liver nucleus is the reason why HBV is difficult to cure[92]. IFN-α and TNF-α, as key factors produced by immune cells, can further promote inhibition of HBV replication and lead to instability of cccDNA[93]. IFN can also induce the degradation of cccDNA by promoting the expression of factors such as APOBEC3A and ISG20[94]. Peg-IFN-α can affect the epigenetic modifications of cccDNA, thereby inhibiting its transcription and reducing the replenishment of the cccDNA pool, indirectly leading to the depletion of the cccDNA pool[93]. After receiving PEG-IFN-α treatment, the cccDNA level of HBV in CHB patients was significantly reduced[95]. At present, multiple studies have shown that IFN-α has a good response to HBsAg clearance rate and HBeAg seroconversion rate[96]. The incidence of HCC in CHB patients treated with IFN-α is significantly lower than that in patients treated with NAs[97]. Long before Peg-IFN-α was widely used to treat HBV infection, Sun *et al*[98] found that the median survival of patients treated with IFN-α after HCC resection was significantly longer than that of the control group (63.8 months *vs* 38.8 months). Peg-IFN-α treatment can also achieve a higher HBsAg seroconversion rate and a lower incidence of HCC compared to NAs[99]. A Ultra-Long-term study observed that HBeAg positive CHB patients who had received IFN-α or Peg-IFN-α treatment had higher rates of HBeAg seroconversion and negative HBsAg conversion[100]. The 2012 EASL guidelines indicate that the best treatment for achieving seroconversion in HBeAg positive patients is Peg-IFN-α treatment. Several studies have shown that Peg-IFN-α can decrease serum HBsAg levels and continue to decrease during follow-up[101,102]. Despite the obvious advantages of IFN in the treatment of CHB, the use of IFN is limited by several disadvantages, such as high cost, the method of administration was subcutaneous injection, and the frequent and high incidence of side effects.

Combination therapy

Multiple studies have shown that the 5-year cumulative incidence of liver cancer is 3.6%-11.4% in patients with HBV-DNA negative conversion[103,104], 2.58% in patients with HBeAg seroconversion[105], and only about 1% in patients with HBsAg clearance[106]. At present, serum clearance of HBsAg is regarded as the optimal endpoint for anti-HBV therapy, which can significantly reduce the risk of cirrhosis and liver cancer. Studies have shown that although patients receiving NAs therapy meet the criteria recommended by the AASLD guidelines for withdrawing from NAs therapy, the cumulative virological recurrence rates during the 1-year, 2-year, 3-year, and 4-year follow-up periods are 72.5%, 77.5%, 80.0%, and 82.5%, respectively[107]. Therefore, patients who meet the criteria for discontinuation of medication still need long-term follow-up after discontinuation. Due to the different mechanisms of IFN-α and NAs in treating HBV, multiple studies have shown that IFN-based therapy can achieve higher clinical cure rates and HBeAg serologic conversion rates compared to take NAs alone, including IFN monotherapy, combination therapy, and sequential therapy. IFN monotherapy is often used for HBeAg negative patients with low viral load. A randomized controlled trial in Singapore found that CHB patients with oral NAs were treated with addition or switch to Peg-IFN-α for 48 weeks. The clinical cure rates of the control group, sequential group, and combination group were 0%, 7.8%, and 10.1%, respectively. Patients treated with Peg IFN - α had significantly higher clinical cure rates than those treated with NAs alone[108]. For patients who use ETV and other NAs to achieve virological suppression and have baseline HBsAg ≤ 1500 IU/mL, the probability of achieving HBsAg clearance after 1 year of continued NAs treatment is about 0%-3%. However, if they switch to limited course Peg-IFN-α treatment, 20% of patients can achieve HBsAg clearance after 1 year[109]. It has been reported that long-term Peg IFN - α treatment can achieve a negative HBeAg conversion rate of over 30% for HBeAg positive CHB, and for HBeAg negative CHB, NAs combined with Peg-IFN-α treatment for 48 weeks is more effective than NAs alone[6]. A randomized controlled trial included HBeAg-positive patients with consistent HBV-DNA loads. After 9-36 months of ETV treatment, the group receiving sequential combination therapy with PEG-IFN-α at 48 weeks had a higher proportion of patients with HBsAg levels < 10 IU/mL and a higher HBsAg seroconversion rate (15.9% *vs* 0%) compared to the group continuing ETV treatment[110]. Ahn *et al*[111] found that HBsAg clearance after 48 weeks of TDF combined with PEG-IFN-α was significantly higher than that of TDF alone (10.4% *vs* 3.5%) in patients receiving initial antiviral therapy. Overall, the combination therapy of NAs and Peg-IFN-α can achieve better therapeutic effects with shorter treatment time [95]. Li *et al*[112] found that patients who achieved clinical cure with IFN treatment continued to consolidate treatment for less than 12 weeks, 12-24 weeks, and more than 24 weeks. The sustained clinical cure rates of one year after discontinuation were 86.7%, 98.3%, and 91.2%, respectively. The anti HBs levels in the latter two groups were significantly higher than those in the former group, indicating that patients who have been clinically cured should continue to receive IFN consolidation therapy for 12-24 weeks to achieve a lasting clinical cure.

CONCLUSION

Vaccination and public health measures are crucial for preventing the spread of HBV. For patients already infected with HBV, NAs and IFN are the main choices for antiviral treatment of HBV. NAs first-line therapy has good viral suppression effect, many types of oral drugs can be selected. The side effects vary depending on the metabolic pathway, but overall, the side effects are relatively few. Good compliance, but limited efficacy and low possibility of cure. In contrast, IFN-based treatment can enhance the host's immune response, IFN-α has a good response to HBsAg clearance rate and HBeAg seroconversion rate, and has the potential to cure hepatitis B. However, it is expensive and has more side effects, such as fever and leukopenia. The combination of two kind of drugs can reduce resistance and improve clinical cure rates. In clinical practice, doctors should choose the most appropriate treatment plan based on the patient's specific condition, including virological characteristics, liver function status, and individual tolerance.

FOOTNOTES

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REVIEW

Bowel preparation before colonoscopy: Consequences, mechanisms, and treatment of intestinal dysbiosis

Ze-Long Zheng, Qing-Fan Zheng, Li-Qiang Wang, Yi Liu

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Abstract

The term "gut microbiota" primarily refers to the ecological community of various microorganisms in the gut, which constitutes the largest microbial community in the human body. Although adequate bowel preparation can improve the results of colonoscopy, it may interfere with the gut microbiota. Bowel preparation for colonoscopy can lead to transient changes in the gut microbiota, potentially affecting an individual's health, especially in vulnerable populations, such as patients with inflammatory bowel disease. However, measures such as oral probiotics may ameliorate these adverse effects. We focused on the bowel preparation-induced changes in the gut microbiota and host health status, hypothesized the factors influencing these changes, and attempted to identify measures that may reduce dysbiosis, thereby providing more information for individualized bowel preparation for colonoscopy in the future.

Key Words: Bowel preparation; Colonoscopy; Microbiota; Inflammatory bowel disease; Probiotics

Core Tip: Bowel preparation for colonoscopy can disrupt the gut microbiota and impact health, particularly in patients with inflammatory bowel disease. This review aims to understand these changes and minimize dysbiosis. Strategies like administration of probiotics may mitigate these effects. Moreover, the existing literature suggests that children's gut microbiota may be more resilient to the disruptions caused by bowel preparation, and that using certain agents, such as sodium picosulfate for bowel cleansing, may reduce the extent of disruption of the gut microbiota.

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INTRODUCTION

With the growing popularity of bacterial detection technologies such as 16S rRNA sequencing, substantial progress has been made in studies of the gut microbiota, which has been shown to be closely related to human health and disease[1]. Colonoscopy is a common method for diagnosing and treating early colorectal lesions, and its diagnostic and therapeutic potential largely depends on the quality of bowel preparation[2]. Bowel preparation mainly involves diet adjustment and the administration of bowel-cleansing agents, with polyethylene glycol (PEG) being the most frequently recommended agent at present[3,4]. However, bowel preparation can considerably alter the living environment of the gut microbiota, and its impact on the gut microbiota has become a topic of increasing concern and controversy. As probiotics and other microecological preparations can significantly improve dysbiosis of the gut microbiota caused by the use of antibiotics and other reasons[5,6], researchers have begun to consider whether probiotics can facilitate the recovery of bacterial dysbiosis after bowel preparation.

Inflammatory bowel disease (IBD) is a chronic and recurrent inflammatory disease mainly involving the digestive tract [7]. IBD is primarily categorized into Crohn's disease and ulcerative colitis[8]. Although the etiology of IBD is not clear, dysbiosis of the gut microbiota is one of its characteristics[9]. Colonoscopies are performed considerably more frequently in patients with IBD than in healthy people, so studying the effects of bowel preparation on the gut microbiota in patients with IBD is of particular significance.

This article will briefly introduce the common bowel-cleansing agents. Subsequently, it will focus on the changes in the gut microbiota after bowel preparation and the relationship between the related bacteria and health and disease. Additionally, the article will address the impact of bowel preparation in patients with IBD and explore the mechanism underlying these changes. Finally, it will discuss measures to improve dysbiosis.

THE CORNERSTONE OF BOWEL PREPARATION—BOWEL-CLEANSING AGENTS

The primary laxatives used for bowel preparation before colonoscopy include PEG, sodium picosulfate, sodium phosphate, mannitol, and Chinese herbal medicines such as senna. PEG is one of the most commonly used bowel-cleansing agents, and it is primarily used in single-dose and split-dose regimens, with split dosing being the preferred regimen[2]. As a bulk laxative, PEG can form hydrogen bonds with water molecules in the digestive tract, increase fecal water content and volume, stimulate intestinal peristalsis, and eventually cause diarrhea to achieve intestinal cleaning. PEG usually does not cause water and electrolyte disturbances in the short term[10] and has good efficacy[11], but the large solution volumes and poor taste of commonly used oral preparations are known to reduce compliance among participants[12,13]. The European Society of Gastrointestinal Endoscopy concluded that the bowel-preparation effect of magnesium citrate plus picosulfate (MCSP) was no worse than that of high-volume PEG[14]. Sodium picosulfate, on the other hand, is a stimulant laxative. Bacterial metabolites of sodium picosulfate can act on the colonic mucosa to stimulate colon peristalsis and increase fluid secretion, while magnesium salts can induce fluid retention in the colon by improving the osmotic pressure in the enteric cavity[15]. Because MCSP is a hypertonic mixture containing magnesium and can induce gastrointestinal mucosal inflammation, solutions containing MCSP should not be used in patients with hypermagnesemia, peptic ulcers, congestive heart disease, or severe renal impairment[14].

CHANGES IN THE DIVERSITY AND COMPOSITION OF THE GUT MICROBIOTA

Since Mai *et al*[16] proposed that bowel preparation and colonoscopy may have considerable effects on the gut microbiota, researchers have conducted a series of studies on this topic (Table 1)[17-31].

Effects of bowel preparation on the diversity of the gut microbiota

The diversity of microbiota primarily reflects the number and distribution of bacterial species in a certain environment. It is mainly represented using the terms α-diversity and β-diversity. The α-diversity refers to the richness of species within a community and the number and evenness of distribution of each species. Common descriptive metrics for α-diversity include operational taxonomic units (OTUs), Chao1 index, and the abundance-based coverage estimator (ACE) index for species richness, and the Shannon index for richness and evenness. And the β-diversity is an indicator of diversity between different communities. Most of the previous studies showed a significant reduction in the α-diversity of the gut microbiota in the colonic mucosa or stool samples taken immediately after bowel preparation. This decrease was mainly characterized by a decrease of the number of OTUs, the Chao1 index, and the ACE index[17,19,26] as well as the Shannon index[17,21]. Bowel preparation is also known to affect the β-diversity of the microbiota[17,19,26]. These changes in the α- and β-diversities have been shown to persist 1 week after bowel preparation[19,24,28]. However, they mostly

Table 1 Research associated with the effects of bowel preparation on the gut microbiota

Year of research	Ref.	Subjects	Sample	Bowel-preparation regimen	Research methods	Conclusion
2006	Mai *et al*[16]	2 Polyps + 1 intestinal inflammation + 2 negative colonoscopy	Feces	Unknown	DGGE	BP and colonoscopy may have a considerable effect on gut microbiota
2012	Harrell *et al* [17]	12 healthy adults	Mucosa	4 L PEG	16S rRNA sequencing	BP (not diet, *etc.*) affects the gut mucosal microbiota
2013	O'Brien *et al* [18]	20 adults	Feces	10 mg bisacodyl + 2 L PEG	DGGE + 16S rRNA sequencing	BP had no lasting considerable effect on gut microbiota
2013	Gorkiewicz *et al*[19]	4 healthy adults	Feces + mucosa	PEG (150 g/day, 3 days)	16S rRNA sequencing	PEG caused changes in fecal and colonic mucosal microbiota similar to the gut microbiota in IBD
2015	Jalanka *et al* [20]	23 healthy adults	Feces	PEG 1 + 1 L/2 L[1]	16S rRNA sequencing	The effect of BP on gut microbiota was transient, and the effect of 1 L + 1 L PEG regimen was smaller
2016	Drago *et al* [21]	10 adults with a positive FOBT and negative colonoscopy	Feces	PEG 4 L	16S rRNA sequencing	BP had long-lasting effects on the gut microbiota
2016	Shobar *et al* [22]	8 IBD + 10 healthy adults	Feces + mucosa	Unknown	16S rRNA sequencing	Short-term changes in fecal and luminal microbiota occurred after BP
2017	Shaw *et al* [23]	16 children	Feces + mucosa + swab	Sodium picosulfate with magnesium citrate and senna	16S rRNA sequencing	BP in the pediatric population did not result in sustained changes in the gut microbiota
2018	Chen *et al*[24]	20 overweight male adults with negative colonoscopy	Feces	Sodium phosphate	16S rRNA sequencing	In the 28 days after BP, the dominant bacteria genus changed little, and the influence on the prevotella group was relatively greater
2019	Nagata *et al* [25]	31 adults (23 control subjects were obtained from a published paper)	Feces	Sdium picosulfate with magnesium citrate and senna	16S rRNA sequencing + CE-TOF-MS	BP had a notable effect on the gut microbiota and its metabolism, but it basically recovered within 14 days
2020	Deng *et al* [26]	32 adults	Feces	PEG 2 L	16S rRNA sequencing	BP considerably altered the gut microbiota, and probiotics promoted the recovery of dysbiosis
2020	Hegelmaier *et al*[27]	16 Adults with Parkinson	Feces	Dietary intervention alone or additional enema	16S rRNA sequencing	Dietary intervention and bowel cleansing can help alleviate PD
2021	Wang *et al* [28]	128 healthy adults	Feces	Split-dose PEG	16S rRNA sequencing	Colonoscopy can cause temporary changes in gut microbiota, slow recovery in the elderly, and probiotics can speed up recovery
2022	Yang *et al*[30]	81 elderly patients with gastric cancer	Feces	PEG (2-4 L)	16S rRNA sequencing	BP altered the gut microbiota composition of patients with gastric cancer
2022	Powles *et al* [29]	2 UC + 9 healthy adults	Feces + urine	MoviPrep (with PEG, electrolyte, *etc.*)	16S rRNA sequencing	BP temporarily reduced α diversity, but had no notable effect on fecal and urine metabolic profiles
2023	Zou *et al*[31]	19 children	Feces	Split-dose PEG	Metagenomic sequencing	PEG can affect the gut microbiota in children, most of which recovered within two weeks

[1]Jalanka *et al*[20] administered a divided dose of polyethylene glycol (1 L per dose, twice) to one group of subjects, and a single dose (2 L per dose) to another group.

BP: Bowel preparation; PEG: Polyethylene glycol; UC: Ulcerative colitis; IBD: Inflammatory bowel disease.

disappeared approximately 2 weeks after bowel preparation[28,31]. Notably, while most studies used PEG regimens for bowel cleansing, two studies that reported no considerable change in microbial diversity after bowel preparation[23,25] used sodium picosulfate as the bowel-cleansing agent.

In a study on children's gut microbiota[31], although the Shannon index decreased briefly after bowel preparation, no notable change was observed in the microbiota richness. Similarly, another study in children[23] found no considerable difference in diversity. These findings suggest that children's gut microbiota may be more "resistant" to interference from bowel preparation, although one of the studies[23] did not directly characterize age or picosulfate usage as the

determining factor for this ability to resist interference.

Influence of bowel preparation on the composition of the gut microbiota

At the phylum level, the gut microbiota is mainly composed of Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. An increase in the relative abundance of Proteobacteria after bowel preparation has been reported by many studies[20,21,26]. Shaw *et al*[23] further suggested that the relative abundance of Proteobacteria could be basically restored to the level before bowel preparation after 2 weeks, but Jalanka *et al*[20] still observed a significant increase in the relative abundance of Proteobacteria after 4 weeks. However, the participants of these two studies differed in terms of age and bowel-preparation methods: The study by Jalanka *et al*[20] included adults aged 22-27 years, while Shaw *et al*[23] studied the effects of bowel preparation on children. These results provide additional evidence for the hypothesis that children's gut microbiota had stronger "resistance", as speculated above. Similarly, Zou *et al*[31] found no considerable phylum-level changes in the major components of the microbiota after bowel preparation in children. Second, Jalanka *et al*[20] and Shaw *et al*[23] used PEG and sodium picosulfate, respectively, as laxatives. Considering the weak influence of sodium picosulfate on the diversity of the gut microbiota, as described above, its use as a bowel-cleansing agent may have relatively limited effects on the gut microbiota. However, in actual clinical application, the potential risk of IBD-like mucosal inflammatory responses that may be induced or aggravated by sodium picosulfate requires consideration[11]. Although most studies suggested that the relative abundance of Firmicutes, Bacteroidetes, and Actinobacteria will change after bowel preparation, the trends in these changes were not consistent among different studies, and the abundance usually returned to the level before bowel preparation within 2 weeks[20,23].

Drago *et al*[21] collected fecal samples from 10 participants at two time points. The samples collected immediately after the use of PEG bowel preparations showed a significant increase in the abundance of gamma-Proteobacteria, a significant decrease in the abundance of Lactobacilliaceae, and an increase in the abundance of Enterobacteriaceae. One month later, when the researchers re-examined stool samples from the participants, they found that the relative abundances of gamma-Proteobacteria and Lactobacillaceae had not fully recovered, and Streptococcaceae, which had not changed considerably at the first sampling, had abnormally increased. Fecal occult blood tests in these participants showed positive results, suggesting that some intestinal diseases may cause more lasting changes in the gut microbiota. Yang *et al*[30] compared the fecal microbiota composition of patients with gastric cancer who underwent PEG-based bowel preparation and those who did not. They also found considerably fewer Lactobacillaceae and *Lactobacilli* in the group that underwent bowel preparation. However, it is important to note that the participants in this study were patients undergoing surgery, and the effects of some clinical treatments may differ from those of colonoscopy alone.

At the genus level, the abundance of *Faecalibacterium* increased in the fecal samples of adults and children after bowel preparation with PEG and sodium picosulfate[19,23]. However, in adults, simultaneous sampling of the large intestine mucosa showed that the relative abundance of *Faecalibacterium* was less than that before bowel preparation[19]. Since the structure of the fecal and large intestine mucosal microbiota in adults was inconsistent, we could not confirm which group of microbiota could better reflect the impact of bowel preparation in the participants undergoing the procedure. For *Veillonella*, two studies[30,31] conducted in older adults and children agreed that bowel preparation using PEG significantly increased its relative abundance. More genus-level research is needed to explore whether the advantage of minimal interference with the gut microbiota when using sodium picosulfate is lost in children. For other bacterial genera, similar to the phylum-level changes, most of the changes after bowel preparation did not appear consistently, but all changes essentially disappeared within 2 weeks[20,23,25].

Due to the limited identification depth of 16S rRNA gene sequencing and other methods, many sequences are not annotated at the species level[32]. Unlike traditional microbiome research methods, metagenomics directly extracts the total DNA of all microorganisms from samples for high-throughput sequencing, allowing species-level, or even strain-level, identification of microorganisms. Zou *et al*[31] recently used metagenomics to comprehensively and accurately explore the effects of PEG bowel preparation on the composition of the gut microbiota. Their study showed an increase in the abundance of *Escherichia coli* (*E. coli*), *Bacteroides fragilis,* and *Veillonella parvula* (*V. parvula*) and a decrease in the abundance of *Intestinibacter bartlettii* after bowel preparation, with the abundances returning to the pre-preparation levels within 2 weeks[31]. The increase in *Escherichia* abundance after bowel preparation in this study and the increase in Enterobacteriaceae reported by Drago *et al*[21] imply that bowel preparation can increase the abundance of *E. coli* at the family, genus, and species levels. Notably, the study by Zou *et al*[31] was conducted in minors with an average age of approximately 10 years, so the applicability of their findings to other populations needs to be evaluated using metagenomics and other deep sequencing technologies.

THE POTENTIAL EFFECTS OF BACTERIAL CHANGES - DAMAGE TO HEALTH AND AGGRAVATION OF DISEASE

As mentioned above, bowel preparation was followed by an increase in the abundance of Proteobacteria, Streptococcaceae, *Veillonella*, and *E. coli* as well as a decrease in the abundance of *Faecalibacterium* and Lactobacillaceae. Here, we discuss these bacteria in terms of their categorization as human pathobionts and beneficial microbes. Pathobionts are commensal microbes widely found in the body of healthy people, but they can be closely related to human diseases in some scenarios. As the name suggests, beneficial microbes refer to microorganisms that are beneficial to human health in most scenarios. Notably, the classification of pathobionts and beneficial microbes is not absolute, and very few bacteria are only beneficial or harmful to human health. Moreover, some of the results described in this section are based on observational data and cannot directly indicate whether dysbiosis of the microbiota is a cause or consequence of the

relevant disease.

Pathobionts

Proteobacteria exhibit varying morphologies and metabolic types, so their name is derived from Proteus, the Greek god with the ability to change appearance. Unlike the obligate anaerobes that dominate the human gut microbiota, most members of the intestinal Proteobacteria are facultative anaerobes[33]. Under normal physiological conditions, the intestinal lumen maintains an anaerobic state, and the trace amounts of oxygen entering the intestinal lumen are also consumed by facultative anaerobes such as Proteobacteria. High oxygen levels in the intestine inhibit the growth of obligate anaerobes and promote the growth of Proteobacteria, which can use oxygen for metabolism[34]. Previous studies have shown that both antibiotics and intestinal inflammation can lead to an increase in intestinal oxygen content and thus promote the proliferation of Proteobacteria[35]. Bowel preparation also disrupts the anaerobic environment in the intestinal lumen, which may partly explain the increase in Proteobacteria after bowel preparation. This change in the abundance of Proteobacteria is a marker of dysbiosis of the gut microbiota[33]. It is also associated with IBD[36-38], irritable bowel syndrome[39], colorectal cancer[40,41], obesity[42], malnutrition[43], and other diseases[44]. Several studies have suggested that the abundance of Proteobacteria is higher in the gut of patients with diabetes[45,46]. However, recent studies of placental and neonatal meconium from patients with gestational diabetes mellitus (GDM) have shown that the relative abundance of Proteobacteria in these patients was abnormally lower than that in controls[47, 48], while no considerable change was observed in the abundance of Proteobacteria in the intestinal tract of patients with GDM[49].

The abundance of Streptococcaceae is positively correlated with the production of fecal proteases[21]. Jalanka *et al*[20] previously reported an increase in fecal protease levels after bowel preparation; however, their study did not identify considerble changes in the abundance of *Streptococcus*. The authors speculated that the increase in fecal protease levels may be related to a decrease in the abundance of protease-degrading bacteria. Proteases are a diverse group of evolutionarily conserved enzymes that cut peptide bonds to hydrolyze proteins. Feces contain a variety of proteases produced by the host and the microbiota, and these enzymes play an important role in maintaining intestinal homeostasis under physiological conditions[50]. However, excessive production of proteases can damage the integrity of the intestinal epithelium[51] and promote inflammatory responses[52]. Increased levels of host- or bacteria-derived proteases have been reported in IBD[53-56], irritable bowel syndrome[57,58], and other gastrointestinal diseases. Notably, Drago *et al*[21] observed an increase in the abundance of *Streptococcus* one month after bowel preparation, while Jalanka *et al*[20] reported that the increase in protease levels was transient and that protease inhibitors in the intestine could control the damage caused by proteases[50]. Therefore, the significance of the excess fecal protease levels after bowel preparation for human health and disease remains to be further studied.

Veillonella is a gram-negative diplococcus that widely exists in the human oral cavity, intestinal tract, and urogenital tract[59]. It was first isolated from the infected human appendix in 1898 by Veillon and Zuber, and was later re-described by Prevot and categorized under the genus *Veillonella*[60]. *V. parvula* is the earliest discovered and most important species of *Veillonella*. The oral cavity serves as an endogenous reservoir of intestinal microorganisms, and oral microorganisms often spread to and colonize the gut[61]. As a major group of oral microbiota, *Veillonella* migrates into the intestine during physiological processes such as saliva swallowing[61,62], which may be one of the reasons for the abnormal increase in *Veillonella* after bowel preparation. In addition, if bowel preparation can indeed increase the abundance of *Streptococcus* as mentioned above, the resultant increase in the production of *Streptococcus* metabolites (such as lactic acid) can also provide a carbon source and energy for the growth and reproduction of *Veillonella*[63]. *Veillonella* has dual effects on human health and disease. *Veillonella*, as an important part of the biofilm in multiple parts of the human body, holds great significance in maintaining the stability of the normal human microbiota. The ability of *Veillonella* to utilize lactic acid as a carbon and energy source has attracted particular attention since lactic acid consumption by *Veillonella* may help prevent effect dental caries[64] (the overall effect of *Veillonella* on dental caries is still controversial), and the conversion of lactic acid to propionic acid in the intestine by *Veillonella* can significantly improve exercise endurance[65]. On the other hand, an increase in the abundance of *Veillonella* is also closely related to diseases such as IBD[66-68]. The lipopolysaccharides of *Veillonella* may play an important role in the occurrence and development of diseases[60,69,70]. In patients with IBD, increased levels of nitrate due to inflammation can promote the growth and colonization of *Veillonella* in the intestine through the narGHJI operon[62]. Therefore, post-colonoscopy changes in the abundance of *Veillonella* should receive attention in patients diagnosed with IBD.

E. coli is an important member of the genus *Escherichia* in Enterobacteriaceae. *E. coli* in humans can be roughly divided into four categories: Non-pathogenic, diarrheagenic, extra-intestinal, and mixed pathogenic[71]. Non-pathogenic *E. coli* are an important part of the normal gut microbiota, while pathogenic *E. coli* can cause a variety of intra- and extra-intestinal diseases[72-74]. Different types of *E. coli* have different pathogenic mechanisms, mainly relying on enterotoxins, adhesins and pili, and can also play a pathogenic role by producing proteases[50].

Beneficial microbes

Many bacteria belonging to Lactobacillaceae are beneficial to human health and widely used as probiotics, especially *Lactobacillus*, the largest genus in Lactobacilliaceae. The probiotic effect of *Lactobacillus* is achieved through the interaction of its surface-active molecules, which mainly include bacterial polysaccharides, teichoic acid, and various bacterial proteins, with the human body[75]. In addition, *Lactobacillus* metabolites and secretions play important roles in maintaining body health[76]. The relative abundance of gut *Lactobacillus* is negatively correlated with the occurrence of various digestive tract diseases, including IBD[77,78]. A recent study also showed that a reduction in the abundance of *Lactobacillus* in the gut may be related to dysbiosis of systemic glucose metabolism[79].

As one of the microorganisms with the greatest potential to become a next-generation probiotic[80], *Faecalibacterium* can help maintain host health and improve resistance to diseases. Most members of *Faecalibacterium* are strictly anaerobic bacteria[81], and their growth is inhibited in oxygen-rich environments, which may partly explain the decrease in *Faecalibacterium* after bowel preparation. *Faecalibacterium* can metabolize cellulose in the intestinal tract into butyrate[82], which can serve as a source of energy, regulate metabolism, enhance intestinal barrier function, and show anti-inflammatory and anti-tumor activities[83-85]. A number of studies have described reduced abundance of *Faecalibacterium* in IBD[86-88]. One possible mechanism is that *Faecalibacterium* can regulate the differentiation and proliferation of regulatory T cells through various pathways such as butyrate production, thereby reducing inflammatory response[89-91].

BOWEL PREPARATION MAY WORSEN IBD

Shobar *et al*[22] studied the changes in the gut microbiota of patients with IBD and a healthy control group after bowel preparation. They found that the proportion of common OTUs between sigmoid mucosal and fecal samples in patients with IBD increased after bowel preparation, while the Unifrac distance decreased. A higher number of shared OTUs and a decrease in the Unifrac distance often indicate greater similarity in microbial composition between two samples. The researchers hypothesized that bowel preparation may shift some of the bacteria in the stool to the intestinal mucosa, increasing the inflammatory response of the mucosa. Simultaneously, the study also showed that the Shannon index of the mucosal microbiota, which characterizes α-diversity, decreased only in patients with IBD and did not show such changes in healthy controls. The reduction in diversity is an important factor causing instability of the microbiota[92,93], and is also one of the characteristic changes of the gut microbiota in IBD[94]. However, in other studies on the fecal microbiota in healthy people, the Shannon index also decreased after bowel preparation. This may have occurred because mucosa-associated microbiota can better characterize the gut microbiota of IBD patients than fecal microbiota[95]. This variation may also be attributed to differences in the characteristics of the participants enrolled in various studies, specimen-collection methods, dietary interventions, and other factors.

In a study of non-IBD patients, Gorkiewicz *et al*[19] and Jalanka *et al*[20] also found similarities between the changes in the gut microbiota in non-IBD patients after bowel preparation and patients with IBD. "The oxygen hypothesis" proposes that the shift in bacterial communities from obligate to facultative anaerobes as a result of destruction of the intestinal anaerobic environment is an important factor leading to the occurrence of IBD[96]. As a chronic inflammatory response in the intestine, IBD can lead to high levels of oxidative stress, change the levels of oxygen and reactive oxygen species (ROS) in the intestinal lumen, and ultimately lead to dysbiosis of the microbiota[97,98]. Bowel preparation also increases intestinal oxygen content[99], which may be one of the reasons why bowel preparation and IBD can have similar effects on the microbiota. The close relationship between the changes in pathobionts and beneficial microbes after bowel preparation and IBD has also been discussed above. In summary, we speculate that bowel preparation may aggravate IBD in some cases, although a causal relationship between changes in the gut microbiota and IBD has not been established[100].

MECHANISMS UNDERLYING THE CHANGES IN THE GUT MICROBIOTA

Bowel preparation may change the gut microbiota by clearing intestinal mucus, flushing the bacteria themselves and the nutrients required for their metabolism, and destroying the anaerobic environment in the intestine[99]. Future studies should attempt to further verify whether changes in the intestinal oxygen level are a key pathway underlying this process.

The gut microbiota is a highly complex microbial community, and its changes are influenced by many factors[101]. Currently, there is insufficient evidence to identify the predominant factors influencing the recovery of the gut microbiota after disruption. We speculate that the following factors may play a role: (1) Microbiota homeostasis: The gut microbiota is a complex ecosystem with stability, resistance, and resilience, and it can be maintained at or restored to a stable state through various mechanisms such as competition and cooperation among microorganisms as well as interactions with the host[102-104]; (2) The oral-gut microbiome axis of the host. The oral cavity is the second-largest reservoir of bacteria in the human body[105], and its microbiota can migrate with saliva and food and colonize the colon, especially when the colonic epithelium is in an inflammatory or damaged state[106-108]. In addition to direct translocation of oral bacteria, the T cells activated by oral inflammation have been also reported to enter the gut[109]. This "flow" of bacterial and immune cells is not random, and is regulated by the oral-gut barrier. The oral-gut barrier is mainly composed of gastric acid in the stomach[110,111] and bile acid secreted into the small intestine[112], and damage to this barrier can accelerate the flow of microorganisms from the oral cavity to the gut. Proton pump inhibitors have been shown to induce or aggravate intestinal inflammation by damaging the barrier[113,114]. However, the influence of large amounts of fluids taken during bowel preparation on the oral-gut barrier and how this potential damage can be restored needs to be studied further; (3) The host immune system. The gut microbiota shows complex and close interactions with the immune system[115]. Intestinal epithelial cells and immune cells work together to precisely control the gut microbiota[116], and the immune system generally tends to mold gut bacteria into a highly diverse community dominated by obligate anaerobes[117]. In particular, intestinal mucus is involved in the composition of the intestinal barrier by selectively segregating bacteria from the immune system[118], and the abnormal immune response triggered by the destruction of the intestinal barrier may be an important link in the occurrence of IBD[119]. The use of PEG in intestinal preparation has

been reported to lead to the loss of intestinal superficial mucus[120]; (4) Host living habits. Different diets can shape different gut microbiota[121]. For example, consumption of foods rich in dietary fiber will significantly increase the diversity of the gut microbiota and orient the composition and metabolism of the gut microbiota toward a healthier direction[122,123]. Vaccaro *et al*[124] speculated that lack of sleep also disrupts the balance of the gut microbiota through the accumulation of ROS. Recent studies have even observed that large temperature differences can change the composition of the gut microbiota[125]; and (5) Mitochondria of host cells. The mitochondria of intestinal epithelial cells and the gut microbiota interact through complex mechanisms. Bacterial metabolites, bioactive substances produced by bacteria, and bacterial components can affect the function, microstructure, and dynamics of mitochondria; conversely, changes in mitochondria can also reshape the gut microbiota[126,127]. In addition to controlling ROS production as mentioned in (4), mitochondria can also influence gut microbiota by altering the oxygen consumption of metabolic processes. Butyrate, a bacterial metabolite, can activate peroxisome proliferator-activated receptor γ in colonic epithelial cells to mediate the transition of cell metabolism to β-oxidation and oxidative phosphorylation, maintaining the oxygen-consuming metabolism of cells and thus ensuring the anaerobic environment of the intestinal tract[117,128]. This is of great significance for maintaining the dominance of obligate anaerobic bacteria in the gut microbiota[129]. In addition, interactions between the gut microbiota and mitochondria are not limited to intestinal epithelial cells; researchers have identified communication between the gut microbiota and mitochondria in cells of the liver[130] and nervous system[131, 132]. Notably, the factors discussed above do not work in isolation; for example, the diet, microbiome, and immune system function as a complex network[133].

The effects of bowel preparation on the gut microbiota are short-lived in most healthy people. As mentioned above, most of the indicators return to the state before bowel preparation within 2 weeks. Sommer *et al*[134] referred to this ability of the gut microbiota to return to equilibrium after external perturbations as "the resilience phenomenon". Disturbances above a certain threshold can also cause the gut microbiota to change to another state for a long time, but no definition for this threshold has received consensus to date[135].

EXPLORATION OF GUT MICROBIOTA RECOVERY AFTER COLONOSCOPY

In the study by Wang *et al*[28], elderly Chinese adults who underwent colonoscopy using PEG for bowel preparation subsequently received *Saccharomyces boulardii* or *Bacillus subtilis* combined with *Enterococcus faecium*. This probiotic treatment significantly increased the richness and diversity of the gut microbiota. However, their study did not elaborate the effects of probiotics on the composition of the microbiota. Deng *et al*[26] found that administration of tablets containing a combination of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, and *Bacillus cereus* significantly reduced the relative abundance of Proteobacteria and increased the relative phylum-level abundance of Bacteroidetes. At the genus level, the probiotics significantly reduced the abundance of pathogenic *Acinetobacter* and *Streptococcus*, and increased the relative abundance of beneficial bacteria.

A randomized controlled trial by D'Souza *et al*[136] explored the effect of *Lactobacillus acidophilus* (*L. acidophilus*) and *Bifidobacterium lactis* (*B. lactis*) on abdominal symptoms after colonoscopy with air insufflation. Their findings showed a significant improvement in abdominal pain symptoms after the administration of probiotics. Wang *et al*[28] also reported that probiotics can significantly improve the incidence of abdominal pain, abdominal distension, and diarrhea after colonoscopy. Although their study did not clearly identify the type of gas injected during colonoscopy, most colonoscopy examinations in China use air insufflation. However, both bowel preparation before colonoscopy and air insufflation during colonoscopy can alter the gut microbiota. In another study that also used *L. acidophilus* and *B. lactis*[137], the researchers used carbon dioxide insufflation for colonoscopy and found that probiotics had a limited effect on abdominal symptoms after colonoscopy. In summary, while the use of probiotics can improve abdominal discomfort after colonoscopy, these symptoms may be attributable to the bacterial dysbiosis caused by air insufflation rather than bowel preparation.

The current strategy for bacterial recovery after colonoscopy is essentially limited to the use of probiotics, but many directions of research on promoting gut microbiota recovery after antibiotic treatment are under investigation. The use of antibiotics can also lead to a decrease in the gut microbiota diversity and an increase in facultative anaerobic bacteria [101]. Fecal microbiota transplantation[138,139] and dietary regulation[140] have been found to significantly improve gut microbiota after antibiotic use, so further studies are needed to verify whether these methods also work after colonoscopy. Measures to regulate gut microbiota from the perspective of host cell mitochondria, the immune system, and the oral-gut microbiome axis are still in the exploration stage. Recent findings showing that nicotinamide mononucleotide supplementation can reverse mitochondrial DNA mutations in intestinal cells[141] may be enlightening in this context.

CONCLUSION

Bowel preparation before colonoscopy usually causes the gut microbiota to temporarily shift to an unhealthy state, which is mainly characterized by reductions in microbiota diversity and the populations of beneficial bacteria and an expansion in the populations of harmful bacteria. These changes mostly resolve within 2 weeks due to the combined action of various potential factors. Children's gut microbiota appears to be more stable, at least in terms of the diversity and composition of the gut microbiota at the phylum level. Bowel preparation may worsen the condition of patients with IBD, highlighting the need to minimize unnecessary colonoscopies in this population. In addition, the use of sodium picosulfate as a bowel-cleansing agent and the application of probiotics may reduce the interfering effects of colonoscopy

on the gut microbiota. Some of the studies cited in this review had the following issues that require consideration: (1) The influence of confounding factors, such as differences in the participants' pre-existing gut microbiota types - enterotypes, cannot be ruled out. Different enterotypes show varying levels of resistance to external perturbations, which has not received sufficient attention in most studies. Although Chen *et al*[24] initially studied enterotype classification, they focused only on overweight men; (2) The limitations of sequencing methods were not adequately addressed. 16S rRNA gene sequencing and other methods lack sufficient depth to accurately present species-level changes in the gut microbiota. The use of metagenomics for gut microbiota analysis is also associated with problems such as susceptibility to host DNA interference; and (3) The conflicting results among different studies remain unexplained. While many studies observed a reduction in the α diversity of fecal microbiota sampled immediately after bowel preparation, Nagata *et al*[25] found no considerable change in the Shannon index, and Feng *et al*[48] even detected an increase in α diversity. Moreover, the composition of certain gut microbiota also varied across studies. Considering the current status of research on this topic, future studies may need to work on the following aspects: (1) Strict screening or control of the characteristics of the enrolled population, diet during the experiment, fecal collection method, and gas injection during colonoscopy as well as assessments on the basis of different groups of factors such as enterotypes; (2) Precise analysis of the composition and function of gut microbiota through metagenomics combined with 16S rRNA gene sequencing and other techniques; (3) Establishment of controlled trials to further validate the benefits of young age and picosulfate in preventing bowel-preparation interference; (4) Determination of the threshold for changes in the gut microbiota caused by external disturbances, such as the frequency of colonoscopies, for bowel preparation in patients with IBD; and (5) Identification of the most appropriate probiotic species and other effective strategies to promote the recovery of intestinal flora in patients with IBD.

FOOTNOTES

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REVIEW

Association between intra-pancreatic fat deposition and diseases of the exocrine pancreas: A narrative review

Jing Ye, Jian-Guo Wang, Rong-Qiang Liu, Qiao Shi, Wei-Xing Wang

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Abstract

Intrapancreatic fat deposition (IPFD) has garnered increasing attention in recent years. The prevalence of IPFD is relatively high and associated with factors such as obesity, age, and sex. However, the pathophysiological mechanisms underlying IPFD remain unclear, with several potential contributing factors, including oxidative stress, alterations in the gut microbiota, and hormonal imbalances. IPFD was found to be highly correlated with the occurrence and prognosis of exocrine pancreatic diseases. Although imaging techniques remain the primary diagnostic approach for IPFD, an expanding array of biomarkers and clinical scoring systems have been identified for screening purposes. Currently, effective treatments for IPFD are not available; however, existing medications, such as glucagon-like peptide-1 receptor agonists, and new therapeutic approaches explored in animal models have shown considerable potential for managing this disease. This paper reviews the pathogenesis of IPFD, its association with exocrine pancreatic diseases, and recent advancements in its diagnosis and treatment, emphasizing the significant clinical relevance of IPFD.

Key Words: Intrapancreatic fat deposition; Pancreatic steatosis; Nonalcoholic fatty pancreas disease; Pancreatitis; Pancreatic cancer

Core Tip: Intrapancreatic fat deposition (IPFD) is a prevalent and clinically significant condition linked to exocrine pancreatic diseases, obesity, age, and sex. Although the mechanisms driving IPFD remain poorly understood, factors like oxidative stress, gut microbiota alterations, and hormonal imbalances are thought to contribute. Imaging is currently the main diagnostic method, but emerging biomarkers and scoring systems offer new diagnostic avenues. While there are no established treatments, glucagon-like peptide-1 receptor agonists and novel experimental therapies show promise. This review highlights recent insights into IPFD's pathogenesis, diagnosis, and therapeutic potential, underscoring its clinical importance in pancreatic health management.

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INTRODUCTION

In recent years, obesity has emerged as a global issue, with the number of overweight and obese individuals increasing steadily across all age groups and socioeconomic strata, particularly in developed and some developing countries[1]. Research on obesity has revealed that the fat distribution pattern within the body plays a critical role in the development of obesity-related abnormalities in glucose and lipid metabolism. This uneven distribution can have multiple adverse effects on health. When triglyceride and free fatty acid (FFA) levels in the bloodstream exceed the metabolic capacity of adipose tissue, the excess lipids cannot be effectively processed or stored, leading to their accumulation in nonadipose tissues. This phenomenon, known as "ectopic fat deposition", can occur in several vital organs - including the liver, heart, kidneys, and pancreas - potentially compromising their normal functions[2]. Among the various forms of ectopic fat deposition, fat accumulation in the pancreas has attracted increasing attention in recent years. This condition has been described in the literature using various terms, such as "pancreatic steatosis"[3,4], "fatty pancreas"[5,6], "pancreatic lipomatosis"[7,8], "fatty infiltration of the pancreas"[9,10], "nonalcoholic fatty pancreas disease"[11-13], and "intrapancreatic fat deposition"[14,15]. The use of these different terms reflects the diversity and complexity of the current understanding of this condition (Table 1).

In this paper, we use the term "intrapancreatic fat deposition (IPFD)" to describe the abnormal accumulation of fat in the pancreas, which is defined as the diffuse presence of fat within the pancreas, excluding peripancreatic (extralobular) fat[16]. IPFD is considered a manifestation of metabolic syndrome (MetS) within the pancreas and encompasses a spectrum of pathological processes, ranging from pancreatic fat deposition to inflammation and subsequent fibrosis. Recent studies have shown that IPFD is associated not only with localized pathological changes in the pancreas but also with broader metabolic dysfunction and systemic inflammation[17,18]. Some researchers have even proposed that IPFD could represent a form of pancreatic aging, with no significant clinical implications in the absence of other risk factors[19]. Therefore, further research into IPFD is crucial to better understand its pathophysiological mechanisms in obesity and MetS, which could provide new directions for prevention and treatment strategies. In this review, existing studies on the mechanism of IFPD and the influence of current treatments on IFPD are discussed in depth, and the correlation between IFPD and exocrine diseases, such as pancreatitis and pancreatic cancer, is emphasized to report new progress in the pathogenesis and treatment of IFPD.

Prevalence

Currently, large-scale epidemiological data to accurately assess the prevalence of IPFD are lacking. However, a cross-sectional study conducted in Indonesia reported that the prevalence of IPFD was approximately 35%[20], whereas a prospective single-center study in the United States reported a prevalence rate of 27.8%[21]. In children, the estimated prevalence of IPFD is approximately 10%[22]. Another retrospective study involving 102 pediatric patients aged 5-18 years indicated that IPFD was relatively common in this group, with a prevalence rate of approximately 26.5%, and it was associated with comorbidities such as diabetes and dyslipidemia[23]. In a study from Japan, the detection rate of IPFD using abdominal ultrasound in a health screening population was reported to be 46.8%[24]. This high detection rate might have been influenced by the older age of the study participants (59.5 ± 13.2 years) and the inclusion of individuals who consumed alcohol. Another study conducted in Chile involving 203 patients without other pancreatic diseases reported that IPFD was detected in approximately 30% of the population[25]. In contrast, a population survey from Yangzhou, China, revealed a lower prevalence of IPFD, 2.7% among the 1228 individuals screened, which was lower than the rates reported in other studies conducted in China and other parts of Asia[26-30]. A meta-analysis of IPFD studies suggested that the overall prevalence was approximately 33% and strongly associated with MetS[31]. The variability in the prevalence of IPFD across different populations could be attributed to regional differences and the methods used for detection, but overall, approximately one-third of the population appears to have IPFD.

Smoking has been identified as a risk factor for IPFD. Research has demonstrated that nicotine exposure during breastfeeding can induce pancreatic steatosis in adult rats[32]. Furthermore, the prevalence of sedentary lifestyles and high-calorie diets has led to an increasing number of obese people. Multiple studies have indicated that body mass index (BMI) and obesity are associated with IPFD[33-35]. Some studies have suggested that the prevalence of IPFD increases with age

Table 1 Nomenclature of the fat in the pancreas

Name	Definition
Pancreatic steatosis	The description of fat accumulation in the pancreatic gland
Fatty pancreas	Fatty accumulation in the pancreas
Pancreatic lipomatosis	The most frequent benign pathologic condition of the adult pancreas
Fatty infiltration of the pancreas	Deposition of a large amount of fat in the pancreas
Nonalcoholic fatty pancreas disease	The accumulation of fat in pancreatic tissue (located within adipocytes)
Intra-pancreatic fat deposition	Diffuse presence of fat (measured on a continuous scale) within the pancreas; excludes peri-pancreatic (extra-lobular) fat

[28,36] and that IPFD is associated with nonalcoholic fatty liver disease. Moreover, triglyceride, lipoprotein, and adiponectin levels are independent risk factors for non-alcoholic fatty pancreatic disease exacerbation[28]. Other studies have shown that racial and ethnic differences also play a role as risk factors for IPFD, with Hispanic individuals exhibiting a higher risk of developing IPFD than African Americans[37,38]. A retrospective cohort study reported that IPFD was age-related and that the association between pancreatic fat and MetS depends on sex[39]. Age and lifestyle-related diseases may also increase the risk of IPFD[24,40].

Pathogenesis

The pathophysiological mechanisms of IPFD are not yet fully understood. Current research suggests that this disease is primarily associated with excessive fatty deposition and fatty replacement in pancreatic tissue, which involves both intralobular fat and interlobular fat[16,17]. Several processes and components may contribute to the development of IPFD, including the accumulation of excess fat within pancreatic β-cells and acinar cells, the replacement of apoptotic acinar cells with intralobular fat, the transdifferentiation of acinar cells into adipocytes, and the presence of interlobular fat cells rich in triglycerides and pancreatic stellate cells.

Oxidative stress (OS) refers to a state of imbalance between oxidation and antioxidants in the body, which can disrupt normal oxidative protein folding. Endoplasmic reticulum (ER) stress is a cellular stress response caused by abnormal protein folding and modification in the ER. Under stress conditions, OS is also the cause of ER stress, which leads to the development of pathogenic states[41]. OS has been associated with MetS and diabetes. Both IPFD and nonalcoholic fatty liver disease are considered manifestations of MetS in different organs. In the "two-hit" hypothesis of nonalcoholic steatohepatitis, OS is viewed as the "second hit" (the "two-hit" theory suggests that peripheral insulin resistance (IR) leads to the accumulation of FFAs in the liver, causing liver steatosis, which constitutes the first hit; lipid accumulation sensitizes the liver to OS, triggering a series of liver toxicity events that cause inflammation, forming the second hit); therefore, OS may also play a crucial role in the development of IPFD[42,43]. OS can lead to the accumulation of misfolded proteins, inducing ER stress. The interaction between OS and ER stress plays a significant role in cellular homeostasis dysfunction and may be associated with accelerated lipid droplet (LD) formation in the pancreas[17,41]. ER stress is closely linked to fat metabolism and influences lipid synthesis, storage, and utilization by altering lipid metabolism and LD dynamics, which can lead to lipotoxicity and metabolic disturbances[44]. In mice with glucose metabolism disorders, many lipid compounds have been observed inside and outside pancreatic cells in the form of LDs, along with abnormal ER structural abnormalities and mitochondrial structural transformation. These findings suggest a potential correlation between IPFD and OS[45]. A 2021 study using a high-fat diet model in mice also suggested that ER stress might be involved in IPFD[46].

Gut microbiota have a significant impact on human health and disease. In recent years, many metabolic diseases, including obesity, type 2 diabetes, nonalcoholic liver disease, metabolic heart disease, and malnutrition, have been linked to gut microbiota. Anatomically, the pancreas is connected to the gastrointestinal tract *via* the pancreatic duct, where a vast array of microbiota resides, which may influence pancreatic homeostasis and the development of various pancreatic diseases[47]. A 2024 study of the gut-pancreas axis revealed that the IPFD was highly correlated with the metabolic products of gut microbiota, such as trimethylamine N-oxide and butyrate levels, suggesting a possible link between IPFD and gut microbiota[48].

IPFD also seems to be associated with hormonal imbalances. Adipose tissue is an important endocrine organ that secretes various adipokines to regulate metabolic homeostasis. In response to external stimuli, adipose tissue can undergo functional impairments, structural changes, and phenotypic alterations[49,50]. Studies have shown that the gut hormone guanylin peptide can prevent β-cell steatosis under lipotoxic conditions and reduce fat accumulation in the pancreas[51]. Guanylin peptide also has a complete expression system in the pancreas and is involved in regulating electrolyte homeostasis. In palmitate-treated RIN-m5F β-cells, guanylin peptide downregulated the expression of lipogenic factors, such as Srebf1, Mogat2, and Dgat1, reducing both basal and palmitate-induced steatosis. Additionally, ghrelin appears to be related to IPFD[52]. Ghrelin, which is produced primarily by ε-cells in the fetal pancreas, may contribute to ectopic fat deposition by increasing the release of FFAs from adipocytes[53,54]. In the four models used in this study, ghrelin in the fasted state was significantly associated with IPFD, whereas postprandial pancreatic fat accumulation was not related to any of the gut hormones involved in the study. These findings suggest that gut-brain axis signaling might play a role in hormone regulation during this process.

Furthermore, previous studies have shown that the regulatory factor serine/threonine protein kinase 25, which is associated with ectopic fat storage, metabolic inflammation, and fibrosis, can exacerbate the severity of IPFD in mouse models, although the underlying mechanisms remain unclear[55]. Low levels of uric acid may also inhibit pancreatic steatosis *via* the glycerophospholipid metabolic pathway in mice[56].

DISEASE

Pancreatitis

Excessive IPFD may disrupt normal pancreatic function and affect the secretion function of the pancreas. Now IPFD, as an increasingly common pancreatic disease, plays an important role in pancreatitis, the most common exocrine disease of the pancreas. In patients with pancreatitis, pancreatic fat deposition levels are elevated[52,57]. A 2021 study evaluated the association between IPFD and acute pancreatitis (AP), suggesting that IPFD might be a risk factor for AP and hypothesizing that IPFD may mediate the onset of AP by inducing endogenous tissue stress or low-grade inflammation[58]. Recently, a prospective cohort study involving 42599 patients demonstrated a significant association between increases in IPFD and AP[59]. Another 2023 study explored the relationship between IPFD and the severity of AP, indicating that IPFD might be an important indicator of the severity of AP[60]. In this study, patients with IPFD had higher systemic inflammatory response syndrome scores than those without IPFD[60]. IPFD is associated with substantial damage in AP. The increased volume of pancreatic fat cells in obese individuals might have caused more extensive pancreatic necrosis during AP. Moreover, the increased systemic inflammatory response in AP might be related to adipokines released from peripancreatic adipose tissue[61,62]. Additionally, IPFD was associated not only with the occurrence of AP but also with pancreatic size and exocrine function following an AP episode[63] (Figure 1). Currently, research on the association between IPFD and chronic pancreatitis is limited. A six-year prospective cohort study revealed that IPFD may be a risk factor for subclinical chronic pancreatitis[5]. Another retrospective study involving 118 patients indicated that chronic pancreatitis was associated with higher pancreatic fat scores[64]. A 2022 quantitative magnetic resonance imaging (MRI) study assessing chronic pancreatitis reached a similar conclusion[65].

Pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic malignancy, accounting for approximately 90% of malignant pancreatic tumors. By 2030, PDAC is projected to become the second leading cause of cancer-related death[66]. IPFD is currently considered significantly associated with PDAC, and IPFD is believed to promote the spread of PDAC and worsen the prognosis of tumor patients[59,67-71]. A recent prospective cohort and Mendelian randomization study suggested that IPFD was a high-risk factor for PDAC and may not be related to overall body fat [14]. This association may involve inflammatory and cancer-promoting signals from local adipocytes. This study also revealed that the characteristics of pancreatic steatosis potentially differ from those of fat deposition in other organs[14]. Another study involving overweight patients revealed that pancreatic steatosis indicated by computed tomography (CT) before the diagnosis of PDAC was an independent risk factor for PDAC[72].

Fatty infiltration in the pancreas falls into one of two categories: Intralobular fatty infiltration and extralobular fatty infiltration. The lipid compositions of these types differ and play distinct roles in oncogenesis development and progression, especially in obese patients. In patients with malignant tumors, pancreatic intraepithelial tumors affect specific lipids found in extralobular fatty infiltration, whereas obesity affects the composition of both intralobular and extralobular fatty infiltration[9]. This study also suggested that intralobular fatty infiltration plays a major role in acinar modifications and the development of precancerous lesions in obese patients. In contrast, extralobular fatty infiltration was more likely to contribute to the progression of PDAC.

LDs are organelles that regulate intracellular lipid homeostasis. In healthy tissues, intrapancreatic adipocytes and LDs store lipids for metabolic needs. In cases of obesity or visceral fat accumulation, LDs increase the intracellular levels of cytotoxic FFAs, inducing mitochondrial damage and the unfolded protein response[73]. Additionally, IPFD may drive the development of early pancreatic cancer, with inflammatory processes in the fat-rich pancreas being a crucial trigger for PDAC development[74,75]. High levels of proinflammatory adipokines and cytotoxic FFAs released by pancreatic fat cells contribute to the recruitment of tumor-promoting immune cells and create a microenvironment conducive to cancer formation. Furthermore, the size of LD pools in cancer cells is an important determinant of tumor metastatic potential. In addition, tumor lipid reprogrammed metabolism is potentially regulated by intrapancreatic adipocytes and abundant LDs[75,76] (Figure 2).

Exocrine dysfunction

IPFD is also associated with impaired pancreatic exocrine function. Fecal elastase-1, a highly stable enzyme, is commonly used to assess pancreatic exocrine function. Individuals with impaired pancreatic exocrine function harbor significantly more pancreatic fat, which negatively correlated with fecal elastase levels in the tested population[77,78]. Lipids may affect acinar cells or replace lost acinar cells and necrotic apoptotic endocrine tissue, leading to pancreatic exocrine dysfunction[77].

Others

Pancreatic fistula is a common and serious complication following pancreatic surgery. The development of postoperative pancreatic fistula is associated with the softness of the pancreatic parenchyma, and IPFD can increase pancreatic gland

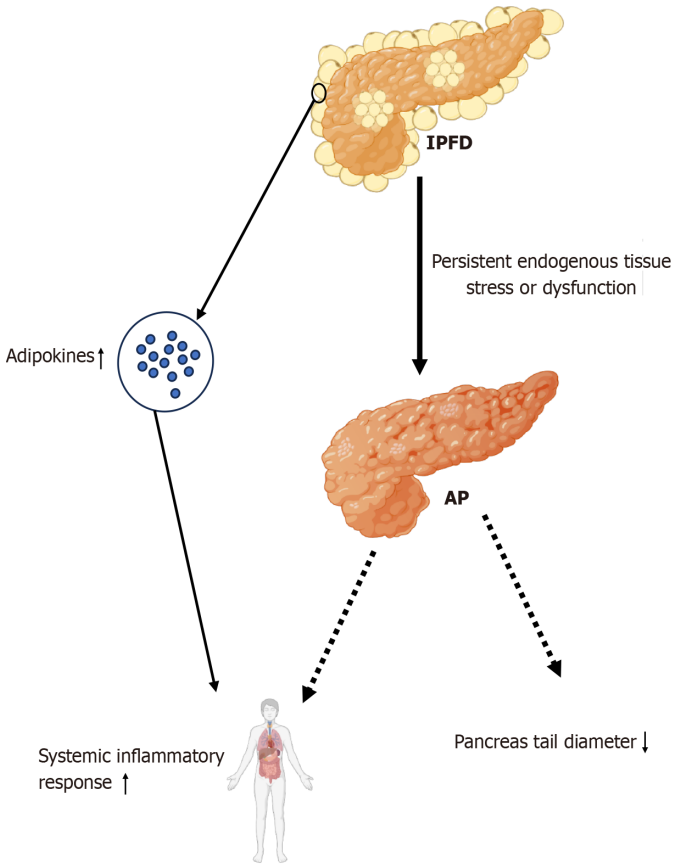

Figure 1 Mechanism of intrapancreatic fat deposition-induced pancreatitis. In intrapancreatic fat deposition, chronic endogenous tissue stress and dysfunction initiate a persistent inflammatory response. Over time, this inflammation escalates, eventually manifesting as clinically significant acute pancreatitis. Additionally, adipokines secreted from adipose tissue surrounding the pancreas further exacerbate systemic inflammation. IPFD: Intrapancreatic fat deposition; AP: Acute pancreatitis.

softness[79-81]. Additionally, MetS and type 2 diabetes have been linked to IPFD, as obesity - a condition closely associated with MetS - leads to fat accumulation in various organs, including the pancreas. IPFD is increasingly regarded as a manifestation of MetS within the pancreas[82]. Several studies have reported elevated pancreatic fat content in patients with type 2 diabetes, and this increase may also occur in individuals with pre-diabetes[18,83,84]. Furthermore, a 2014 study indicated that IPFD is associated with carotid atherosclerosis and may serve as a risk marker for cardiovascular disease[85] (Table 2).

Diagnosis

At present, no universally accepted standard exists for diagnosing pancreatic fat content, and imaging remains the primary method for assessing and diagnosing IPFD. Histological examination of pancreatic samples has revealed fat cell infiltration and LDs localized within acinar cells[42,86,87]. However, due to the retroperitoneal location of the pancreas [31], pancreatic biopsy is challenging. Due to the uneven distribution of fat in the pancreas among IPFD patients, histological examinations might not have accurately reflected fat deposition throughout the entire pancreas. Furthermore, pancreatic tissue samples can be obtained only during pancreatic surgery, which is limited to specific indications. Most individuals at risk for IPFD have never undergone pancreatic surgery, making the specific evaluation of pancreatic fat content *via* histological methods difficult.

Imaging techniques, including abdominal ultrasound, endoscopic ultrasound, CT, proton magnetic resonance spectroscopy, and MRI, are commonly used to detect pancreatic fat content. An abdominal ultrasound diagnosis of IFPD is usually compared with the echoes of the liver, kidney cortex, and spleen; the main manifestation of IFPD is diffuse enhancement of the pancreatic parenchymal echo[27,30,88,89]. Ultrasound evaluation of the echogenicity of the pancreas may have predictive value for prediabetes/diabetes[89,90]. Ultrasound shear wave dispersion measurements are considered useful for objectively assessing changes in fat content in the pancreas because of their good reproducibility[91]. Additionally, endoscopic ultrasound may provide more detailed pancreatic images and simultaneously image other organs, making it more reliable for assessing the degree of pancreatic fat infiltration[21,92,93]. Compared with the other two impact tests, ultrasound is widely available and inexpensive, and endoscopic ultrasound can simultaneously image neighboring organs, such as the liver and spleen, in real time. These advantages make ultrasound widely used in the determination of pancreatic fat content. However, transendoscopic ultrasonography is invasive and difficult to evaluate as a whole. Therefore, both abdominal ultrasound and endoscopic ultrasound are associated with difficulties in attempts to accurately assess the overall pancreatic fat content, which limits their application in the diagnosis of IFPD.

Table 2 Disease

Disease	Mechanism	Ref.
Pancreatitis	Inflammation induced by fat deposition and adipokine secreted by adipose tissue promote the occurrence and development of pancreatitis	Sbeit and Khoury[58], 2021; Tirkes *et al*[64], 2019
Pancreatic cancer	The inflammatory process of the pancreas in the context of fatty pancreas is an important inducible factor, and the different types of fatty infiltration of the pancreas also play a role in the different processes of tumor development	Frendi *et al*[9], 2024; Lilly *et al*[75],2023
Exocrine dysfunction	Lipids have affected acinar cells or replaced lost acinar cells and necrotic apoptotic endocrine tissue	Tahtacı *et al*[77], 2018
POPF	IPFD increases the softness of the pancreatic glands and raises the risk of POPF	Gaujoux *et al*[79], 2010; Dei *et al*[81], 2022
Type 2 diabetes mellitus	IPFD leads to dysfunction of islet beta cells, affecting insulin secretion and exacerbating insulin resistance	Lu *et al*[83], 2019; Chin *et al*[84], 2021
Metabolic syndrome	IPFD is associated with components of metabolic syndrome such as obesity, hyperglycemia, dyslipidemia, *etc*	Smits and van Geenen [82], 2011
Cardiovascular disease	IPFD is associated with abnormal fat distribution and metabolic disorders throughout the body, which may affect the development of atherosclerosis	Kim *et al*[85], 2014

IPFD: Intrapancreatic fat deposition; POPF: Postoperative pancreatic fistula.

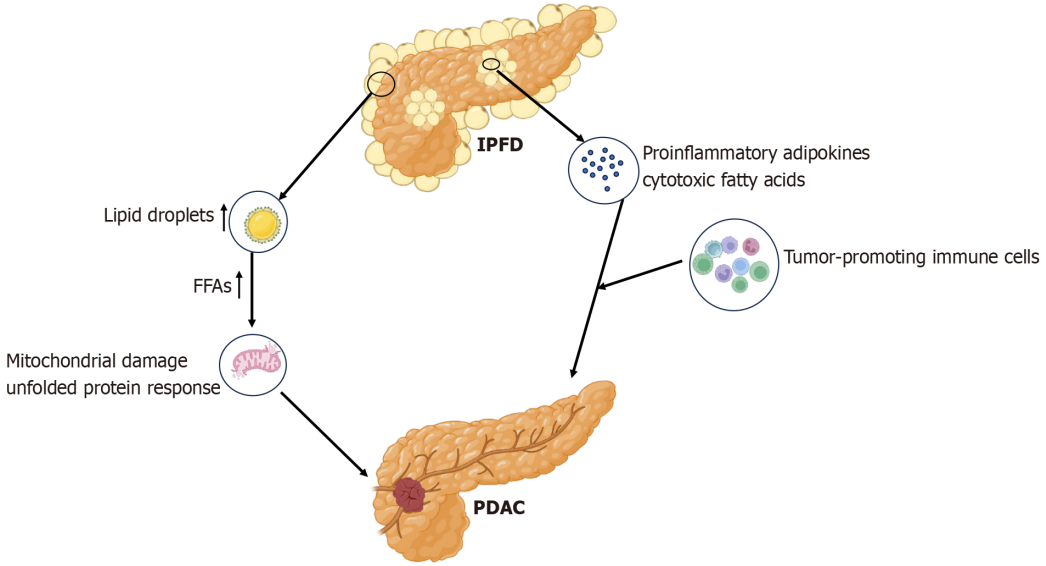

Figure 2 Mechanism of intrapancreatic fat deposition-induced pancreatic cancer. During intrapancreatic fat deposition, the accumulation of intracellular lipid droplets leads to an elevation in toxic free fatty acids, which induces mitochondrial dysfunction and triggers the unfolded protein response. These events contribute to the development of pancreatic cancer. Moreover, pro-inflammatory cytokines and cytotoxic fatty acids released by pancreatic adipocytes in the intrapancreatic fat deposition environment foster an inflammatory microenvironment. This microenvironment facilitates the proliferation of transformed cells with carcinogenic potential by promoting the recruitment of tumor-supportive immune cells. IPFD: Intrapancreatic fat deposition; PDAC: Pancreatic ductal adenocarcinoma; FFA: Free fatty acid.

CT can provide a (semi)quantitative analysis of pancreatic fat *via* attenuation of the pancreas, depending on the contrast with the spleen[94,95], with unenhanced CT often providing more accurate results than enhanced CT[96]. The combination of quantitative CT parameters, specifically pancreas attenuation and pancreas surface lobularity, enhances the accuracy of detecting pancreatic fat infiltration. In the validation cohort, this method yielded negative and positive predictive values of 91% and 70%, respectively[94]. A visceral fat tissue area threshold of ≥ 107.2 cm² may predict pancreatic fat degeneration[97]. CT attenuation indices and visual assessments of CT images have aided in studying the clinical relevance of IPFD with impaired glucose metabolism and type 2 diabetes[98,99]. Additionally, the CT fat volume fraction can automatically measure the severity of IPFD[100]. Moreover, quantitative CT assessments of pancreatic fat infiltration location and content are accurate[101], which could help in preoperative planning and postoperative complications[102-105]. Compared with other imaging methods, CT is more accurate and convenient for measuring pancreatic volume and abdominal fat content. However, CT involves potential radiation exposure and is relatively expensive, and it does not show a significant advantage over ultrasound in the quantitative assessment of pancreatic fat[18,97].

MRI is based on signal differences between water and fat. It offers better soft tissue contrast and higher sensitivity, playing an important role in the assessment and clinical application of IPFD. Chemical shift-encoded MRI is based on the difference in resonant frequencies caused by the different distributions of the surrounding electron clouds of hydrogen protons in water and adipose tissue, and its measured fat fraction is highly correlated with the actual fat fraction[16,36,106]. It maps the region of interest in the caput, corpus, and cauda of the pancreas to minimize the influence of other visceral fat. Compared with nuclear magnetic resonance spectroscopy, it needs less time and is more convenient. The proton density fat fraction is an objective measure of the intrinsic properties of tissues. The use of MRI to quantify the proton density fat fraction can standardize and objectively measure fat content, with good repeatability, and can reflect the overall fat content[107-109]. Moreover, multiparametric MRI of the pancreas provides more reliable and accurate fat quantification than conventional MRI[110]. 1H-magnetic resonance spectroscopy is considered the gold standard to noninvasively measure pancreatic fat content, with accuracy comparable to that of histological and biochemical markers [111]. Another new technique, 3D-IDEAL (iterative decomposition with echo asymmetry and least squares estimation), provides faster imaging, less signal contamination from surrounding fat, and reduced susceptibility to respiratory motion effects, potentially offering superior performance in evaluating fat content in small organs such as the pancreas[112,113]. However, MRI-related imaging technology is also associated with several problems, such as high costs and long scanning intervals, which affect its application.

With the increasing influence of artificial intelligence (AI) on diagnostic technologies, recent studies have proposed that AI-assisted MRI and CT models could be utilized as objective, automated, and reproducible tools for assessing pancreatic fat infiltration[114]. Additionally, several emerging biomarkers have been identified as potential screening tools for patients with IPFD. The level of serum fibroblast growth factor-21 (FGF-21), a cytokine intimately involved in glucose and lipid metabolism, has been shown to be closely associated with metabolic diseases. A previous study indicated that serum FGF-21 was strongly linked to IPFD and could serve as a reliable screening marker for the disease[115]. However, the transabdominal ultrasound used in the diagnosis of IPFD in this study can only be used to identify is the presence fat infiltration in the pancreas, not its quantity. Therefore, the correlation between the serum FGF-21 level and the degree of fat infiltration in the pancreas was difficult to explore in this study, and whether the serum FGF-21 level is a prognostic indicator for patients with an IPFD also needs to be further explored. In the lipidomic analysis of serum, triglycerides were identified as significant markers for the development of IPFD following AP, which can be used to assess and identify individuals at high risk of IPFD[116]. IPFD in healthy nonobese individuals in this study was not significantly associated with any markers of lipid metabolism, and new prospective longitudinal studies are needed to validate this finding and to focus on changes in IPFD over time and its association with markers of lipid metabolism. Furthermore, an Egyptian study revealed statistically significant differences in fatty acid-binding protein-1 (FABP-1) levels based on the presence of fatty deposits among fatty pancreas patients[117]. Thus, FABP-1 may be a direct and noninvasive predictor of IPFD[117]. Although the diagnosis of IPFD was graded in this study, verifying whether the level of FABP-1 was correlated with the severity of IPFD was difficult because the diagnosis was based on transabdominal ultrasound, and the correlation between the FABP level and the IPFD grade was not discussed. The hypertriglyceridemic waist phenotype, a clinical indicator of visceral fat accumulation that reflects visceral obesity and metabolic disorders, was found to be significantly associated with IPFD and could be a useful screening reference, particularly for Asians who are underweight but prone to developing visceral obesity[118]. A new diagnostic score that includes obesity, hyperlipidemia and fatty liver has also been developed to initially identify IPFD[119]. Common anthropometric parameters and biochemical markers also demonstrated good predictive value, including weight-related parameters such as BMI and triglyceride-related measures, with a combination of multiple parameters offering potentially greater predictive accuracy[120].

TREATMENT

Nonpharmacological treatment

Currently, the clinical significance and related mechanisms of IPFD are not fully understood, and routine, effective management strategies are lacking. Lifestyle modification appears to be the most effective treatment approach available. Exercise is considered beneficial in reducing ectopic fat accumulation in the pancreas[121]. However, this finding was based on a small study focusing only on individuals with prediabetes and type 2 diabetes. The effects of exercise on pancreatic fat accumulation in other normal populations, as well as the optimal exercise duration and intensity, remain unstudied. Additionally, low-calorie diets have been found to help reduce pancreatic fat accumulation in type 2 diabetes patients[122]. Altering the intake of other nutrients also seemed to influence the incidence of IPFD. Lactoferrin, an iron-binding protein with numerous biological functions and significant immunomodulatory effects, was found to improve pancreatic fat levels and function and to mitigate weight gain when administered as a supplement[123]. Probiotics have shown great potential in inhibiting the development of IPFD and alleviating related metabolic and pathological abnormalities[124]. Moreover, reducing branched-chain amino acid intake might improve IPFD by activating the liver kinase B1/AMP-activated protein kinase pathway or inhibiting the mechanistic target of rapamycin complex 1 pathway [125]. However, these dietary changes have only been validated in animal models and lack supporting clinical data.

Lifestyle modifications appear to be a promising approach for the prevention and treatment of IPFD. However, existing studies examining the effects of lifestyle changes on pancreatic fat content have primarily focused on diabetic patients and animal models, leaving a gap in data across broader population samples. Additionally, risk factors for IPFD, such as smoking, high BMI, and obesity, are strongly linked to lifestyle habits, suggesting that future studies targeting lifestyle interventions could potentially enable early intervention and achieve preventive effects for IPFD.

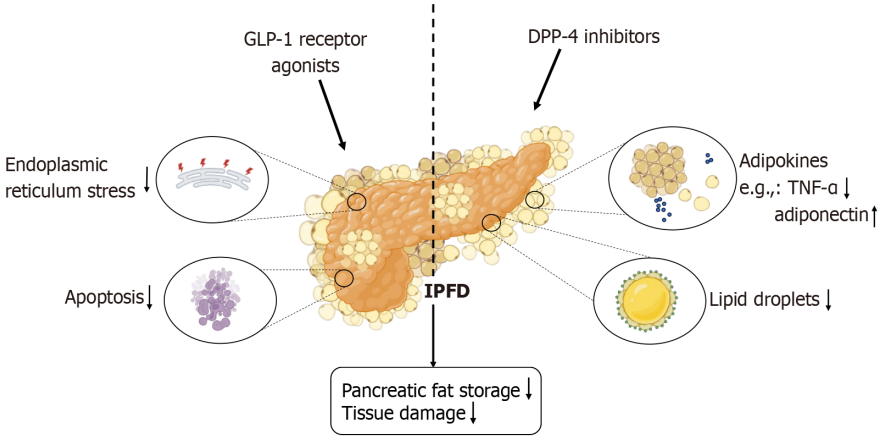

Figure 3 Therapeutic mechanism of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in intrapancreatic fat deposition. Glucagon-like peptide-1 receptor agonists potentially mitigate intrapancreatic fat deposition and its complications by modulating the endoplasmic reticulum stress pathway and downstream apoptosis signaling. In contrast, dipeptidyl peptidase-4 inhibitors exert therapeutic effects by regulating adipokines - such as reducing tumor necrosis factor-α levels and enhancing adiponectin production - and by modulating intracellular lipid droplet content. IPFD: Intrapancreatic fat deposition; GLP-1: Glucagon-like peptide-1; DPP-4: Dipeptidyl peptidase-4; TNF: Tumor necrosis factor.

Pharmacotherapy

Evidence-based medicine for the pharmacological treatment of IPFD is lacking. Glucose-lowering drugs, lipid-regulating medications, angiotensin II receptor blockers, somatostatin receptor agonists, and antioxidants are promising candidates for improving IPFD[126]. In preclinical studies, glucose-lowering drugs such as glucagon-like peptide-1 (GLP-1) receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors, lipid-regulating statins, pancreatic lipase inhibitors, somatostatin receptor agonists such as octreotide, and antioxidants such as ascorbic acid have shown favorable results[127]. Metformin reduced IPFD severity in animal models, but imaging studies before and after metformin administration did not reveal significant changes in the clinical setting[128]. Newer therapies [such as GLP-1 receptor agonists, DPP-4 inhibitors, and sodium-glucose co-transporter type 2 (SGLT-2) inhibitors] and classical therapies (such as statins, metformin, and angiotensin II receptor blockers) at least partially reduce pancreatic ER stress[129]. The administration of the GLP-1 receptor agonist liraglutide in high-fat diet-fed mouse models suggested that it might reduce IPFD by modulating ER stress pathways and downstream apoptosis signals[46,130,131]. A retrospective study in Japan suggested that SGLT-2 inhibitors might reduce pancreatic fat accumulation in type 2 diabetes patients, but this study had a small sample size and could not exclude the effects of other antidiabetic drugs on fat accumulation, indicating that the results were only suggestive[132,133]. A 2021 study of the SGLT-2 inhibitor empagliflozin revealed that it effectively reduced liver fat in mice and humans, but the changes in pancreatic fat were not statistically significant[134].

Additionally, some new drug therapies have appeared to offer new possibilities based on animal model studies. Oral glucosamine may provide some protection against fat accumulation and pancreatic tissue damage in rats fed a high-fat diet[135]. Sodium butyrate improved metabolic disorders as well as pancreatic lipid accumulation and uric acid metabolism abnormalities in rats[136]. Simvastatin was found to improve pancreatic fat accumulation[137,138]. Specifically, the simvastatin-A indica extract combination resulted in a lower pancreatic fat percentage than the single treatment, although the difference was not statistically significant. The active compound 6-gingerol from ginger appeared to reduce pancreatic lipid accumulation by lowering body weight, which in turn reduced OS and inflammation in the pancreas to improve the incidence of IPFD[139]. Additionally, benzyl propylene glycoside, a cGAS-STING pathway modulator, was found to inhibit pancreatic inflammation and fibrosis in high-fat high-sucrose diet-fed rat models, improving IPFD and reducing metabolic disorders in nonalcoholic fatty pancreatic inflammation animal models[140].

Currently, there is no evidence-based medical guideline recommending specific, effective drugs for the treatment of IPFD. Among the potential therapeutic options, GLP-1 receptor agonists and DPP-4 inhibitors show the most promise, but further large-scale clinical studies are necessary to evaluate their effectiveness in treating IPFD. Such studies could facilitate the development of reliable therapies aimed at reducing pancreatic fat accumulation and mitigating the pathological damage caused by excessive fat deposition (Figure 3). Although new treatment approaches for IPFD have been tested in animal models, these studies have primarily relied on high-fat diet models and focused on the impact of these factors on blood lipids. The mechanisms of lipid deposition in the pancreas have been explored only briefly, and there is a lack of genetic models tailored to IPFD, limiting the depth of mechanistic investigation. Consequently, further research is needed to assess the efficacy of these potential therapies and to build a foundation for targeted treatment options (Table 3).

CONCLUSION

The understanding of IPFD has remained insufficient. Although it is a very common condition, it has not been adequately addressed clinically. More multicenter, large-scale population surveys are needed to clarify the prevalence and related

Table 3 Summary of intrapancreatic fat deposition treatment methods

Treatment method	Category	Mechanism of action	Research evidence	Ref.
Low-calorie diets	Non-pharmacological	Reduces insulin secretion and decreases adipose tissue accumulation in the pancreas and body	Evidence indicates that low-carbohydrate diets effectively diminish pancreatic and visceral fat deposition in individuals with obesity and type 2 diabetes	Taylor *et al*[122], 2018
Exercise	Non-pharmacological	Enhances overall insulin sensitivity and reduces pancreatic fat content	Numerous small clinical trials have demonstrated that physical activity can lower pancreatic fat deposition, regardless of the baseline glucose tolerance of participants	Heiskanen *et al*[121], 2018
Lactoferrin	Non-pharmacological	Improves lipid profiles, pancreatic function, and histological integrity	Supplementation with lactoferrin has been shown to mitigate weight gain, improve lipid levels, and enhance pancreatic function in models fed a high-fat diet	Hassan *et al*[123], 2022
Metformin	Pharmacological	Improves insulin resistance and reduces pancreatic and overall adipose tissue	In studies involving high-fat diet-fed mouse models, metformin significantly decreased pancreatic fat deposition and ameliorated insulin resistance	Souza-Mello *et al*[128], 2010
GLP-1 receptor agonists	Pharmacological	Stimulates insulin secretion, inhibits glucagon release, promotes weight loss, and decreases pancreatic fat	A growing body of clinical and preclinical evidence supports the efficacy of GLP-1 receptor agonists in reducing fat accumulation in both the pancreas and liver while enhancing pancreatic function	Kuriyama *et al*[131], 2024; Vanderheiden *et al*[130], 2016; Fang *et al*[46], 2021
SGLT-2 inhibitors	Pharmacological	Increases glucose excretion through urine, reduces lipogenesis, and improves pancreatic fat deposition	Research has demonstrated that SGLT-2 inhibitors, such as dapagliflozin, effectively improve body fat distribution and reduce pancreatic fat accumulation in patients with type 2 diabetes	Ghosh *et al*[133], 2022; Shi *et al*[132], 2023
DPP-4 inhibitors	Pharmacological	Enhances insulin secretion from pancreatic beta cells, suppresses glucagon secretion from alpha cells, and improves fat accumulation	Clinical trials and animal studies indicate that the DPP-4 inhibitor sitagliptin can effectively manage pancreatic steatosis and prevent the progression of pancreatic diseases	Souza-Mello *et al*[128], 2010; Nag *et al*[127], 2024
Statins	Pharmacological	Lowers blood lipid levels, inhibits pancreatic cell proliferation, and alleviates endoplasmic reticulum stress	Animal studies have demonstrated that statins significantly reduce pancreatic fat accumulation in models subjected to high-fat diets	Chen *et al*[138], 2014; Krisnamurti *et al*[137], 2022
Angiotensin II receptor blockers	Pharmacological	Mitigates intracellular calcium overload and lipid accumulation, improving insulin sensitivity and preventing fat degeneration	Various animal studies suggest that angiotensin II receptor blockers can alleviate pancreatic steatosis by enhancing metabolic conditions in diabetic patients	Souza-Mello *et al*[128], 2010; Lee *et al*[129], 2023

GLP-1: Glucagon-like peptide-1; DDP-4: Dipeptidyl peptidase-4; SGLT-2: Sodium-glucose co-transporter type 2.

risk factors for IPFD in different regions and populations. Given the rise in obesity, the systemic metabolic disturbances caused by excessive fat accumulation warrant significant attention, especially because the pancreas is an important organ related to metabolism. The pathogenesis of IPFD is not fully understood, and further clinical and basic research is needed to explore its detailed mechanisms and validate its clinical significance. IPFD is highly related to exocrine pancreatic diseases, with excessive lipid accumulation in the pancreas creating an inflammatory microenvironment that promotes pancreatitis and pancreatic cancer, which further impairs pancreatic function and leads to more severe clinical outcomes. Furthermore, existing animal models of IPFD have primarily used high-fat diet models, and genetic models that specifically investigate the regulation of pancreatic fat cell accumulation are lacking. Future research might involve the development of relevant genetic animal models to further explore the mechanisms of IPFD.

Given the numerous and serious clinical consequences of IPFD, there is a pressing need for more accessible and efficient screening methods. These methods could complement traditional examinations to assess pancreatic fat content, facilitate timely identification of IPFD, and help prevent its worsening effects on the endocrine environment, thereby reducing the risk of progression to malignant disease. Furthermore, robust studies are needed to clarify specific pancreatic fat thresholds for IPFD diagnosis and classification using standard imaging techniques, ultimately working toward a diagnostic gold standard for IPFD. Although specific drugs for treating pancreatic fat changes have not yet been approved, repurposing antidiabetic drugs, statins, and other medications has shown promise in reducing the burden of IPFD. However, research on the effects of these drugs on IPFD has been limited to small-scale preclinical and prospective clinical studies. More reliable clinical research is needed to explore and validate the effectiveness of these drugs in preventing and treating IPFD. Interdisciplinary collaboration is essential to fully understand IPFD and to provide reliable diagnostic and therapeutic strategies for clinical practice.

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FOOTNOTES

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Trends of alkaline phosphatase to prealbumin ratio in patients with hepatitis B linked to hepatocellular carcinoma development

Wen-Chong Zhen, Jing Sun, Xue-Ting Bai, Qian Zhang, Zi-Han Li, Yi-Xin Zhang, Rong-Xuan Xu, Wei Wu, Zhi-Han Yao, Chun-Wen Pu, Xiao-Feng Li

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Abstract

BACKGROUND

Chronic hepatitis B often progresses silently toward hepatocellular carcinoma (HCC), a leading cause of mortality worldwide. Early detection of HCC is crucial, yet challenging.

AIM

To investigate the role of dynamic changes in alkaline phosphatase to prealbumin ratio (APR) in hepatitis B progression to HCC.

METHODS

Data from 4843 patients with hepatitis B (January 2015 to January 2024) were analyzed. HCC incidence rates in males and females were compared using the log-rank test. Data were evaluated using Kaplan–Meier analysis. The Linear Mixed-Effects Model was applied to track the fluctuation of APR levels over time. Furthermore, Joint Modeling of Longitudinal and Survival data was employed to

investigate the temporal relationship between APR and HCC risk.

RESULTS

The incidence of HCC was higher in males. To ensure the model's normality assumption, this study applied a logarithmic transformation to APR, yielding ratio. Ratio levels were higher in females (t = 5.26, P < 0.01). A 1-unit increase in ratio correlated with a 2.005-fold higher risk of HCC in males (95%CI: 1.653-2.431) and a 2.273-fold higher risk in females (95%CI: 1.620-3.190).

CONCLUSION

Males are more prone to HCC, while females have higher APR levels. Despite no baseline APR link, rising APR indicates a higher HCC risk.

Key Words: Alkaline phosphatase to prealbumin ratio; Chronic hepatitis B patients; Hepatocellular carcinoma; Retrospective cohort study; Linear Mixed-Effect Mode; Joint Modelling of Longitudinal and Survival data

Core Tip: Joint Modeling of Longitudinal and Survival data analysis revealed no association between baseline alkaline phosphatase to prealbumin ratio (APR) levels and Hepatocellular carcinoma (HCC) risk, yet a significant correlation exists between the increasing trend of APR and heightened HCC risk.

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INTRODUCTION

Hepatitis B virus (HBV) infection remains an important global public health problem with significant morbidity and mortality[1-3]. About 257 million people worldwide live with chronic HBV infection. HBV causes over 850000 deaths annually and is the most common cause of hepatocellular carcinoma (HCC; 44%-55%)[3-7]. In China, hepatitis B-related liver cancer constitutes 90% of liver cancer cases. Despite treatment, the 5-year survival rate of patients with HCC remains low compared to other cancers due to late diagnosis[8]. Growing evidence suggests that the host immune response is crucial in the natural history of HBV infection and HCC development. Among these responses, systemic inflammatory response is a significant component, with its imbalance promoting HCC occurrence[9-12].

Alkaline phosphatase (ALP) is a hydrolase primarily concentrated in the liver. Cytological studies have shown that high ALP levels may be associated with cancer cell proliferation[13]. Prealbumin (PA), a reliable indicator of inflammatory stress and nutritional status, has a short half-life, rendering it a sensitive biomarker for gauging morbidity, mortality, and tumor progression. Empirical research has demonstrated a significant correlation between PA levels and HCC, suggesting its clinical utility[14,15]. The ALP to PA ratio (APR), introduced by Li *et al*[16], is a straightforward, accessible, and economical marker of inflammation. High APR can indicate disease outcomes.

In patients with chronic hepatitis B (CHB), dynamic changes in APR may reflect alterations in the inflammatory and nutritional conditions during disease progression, potentially associated with the risk of HCC development. Previous studies using cross-sectional data have not thoroughly examined APR evolution or its impact on the risk of HCC development. Additionally, traditional survival analysis methods may not accurately identify the complex relationship between APR and HCC risk due to limitations in handling dynamic data.

This study dynamically monitors the APR in patients with CHB using Joint Modeling of Longitudinal and Survival data (JLMS) to analyze the association between APR trends and HCC risk. By collecting multiple follow-up data points, the study constructs a trajectory of APR's longitudinal changes and assesses their impact on the risk of HCC development using survival analysis models. This research further clarifies the APR mechanism in CHB development and complications and provides new insights and evidence for individualized risk assessment and early intervention.

MATERIALS AND METHODS

Study design and setting

This study is a single-center retrospective cohort study. Participants were patients diagnosed with CHB who sought medical treatment at a hospital in Dalian City, China, spanning from January 2015 through January 2024. This study was

approved by the ethics committee of Dalian Public Health Clinical Center (No. 2024-026KY-001). All data were anonymized.

Participants

Inclusion criteria involved the following: (1) CHB diagnosis indicated by positive HBsAg for ≥ 6 months or serum HBV DNA > 2000 IU/mL; (2) Age between 20 and 85 years with complete clinical data; (3) HCC diagnosed *via* pathological examination or confirmed by liver DSA iodol staining showing tumor staining; (4) Absence of serious complications or organ failure; and (5) Liver cirrhosis diagnosed by imaging or portal hypertension signs.

Exclusion criteria were as follows: (1) CHB in a patient with hepatitis A, hepatitis C, or hepatitis E Virus infections; (2) CHB in a patient with acquired immunodeficiency syndrome or autoimmune diseases; (3) Pregnancy or lactation; and (4) CHB in a patient with gallbladder stones and obstructive jaundice.

Patients diagnosed with HCC during follow-up or by January 2024 were considered for follow-up termination, with their post-diagnosis data excluded from the study.

From the original cohort of 5143 patients with CHB, 300 were excluded due to missing ALP and PA data or meeting the exclusion criteria (during follow-up). Thus, 4843 patients were included (effective cohort; Figure 1).

Data collected

Data included age, gender, and results of routine tests (blood-RT, urine-RT, liver function tests, kidney function tests, coagulation function tests), the findings of imaging evaluation (abdominal ultrasound, upper abdominal computed tomography, magnetic resonance imaging, *etc.*), and pathological diagnosis.

Statistical analysis

Normally distributed data are reported as mean ± SD. Non-normally distributed continuous variables are presented as median with interquartile range and compared using the Wilcoxon rank-sum test. Categorical variables, expressed as frequencies and percentages, are compared utilizing χ^2. The analysis was conducted using SPSS 27.0 and R 4.3.3, with $P < 0.05$ indicating statistical significance. HCC incidence rates in males and females were compared using the log-rank test. Data were evaluated using Kaplan–Meier analysis. Linear Mixed-Effects Models (LMEs) were employed to assess the temporal dynamics of APR in patients with CHB. JMLS was utilized to evaluate the longitudinal association between APR and HCC. Variable generation (for the models) and model principles[17,18] are outlined in Supplementary material 1.

Baseline reference was established using initial data from participants fulfilling the inclusion criteria, with each contributing at least two data points. The primary outcome was HCC diagnosis during follow-up, denoted by setting their status in the respective year to 1. To meet the model's assumption of normal distribution, a logarithmic transformation was applied to APR values, particularly ln(10APR), referenced as the ratio.

The fundamental descriptions of the variables are shown in Supplementary Table 1 (Supplementary material 1); the variable setup for the models is detailed in Supplementary material 2. Full model equations for LME and JMLS are included in Supplementary Table 2.

All abbreviations in this study are explained in Supplementary Table 3 (Supplementary material 2).

RESULTS

Baseline characteristics and follow-up outcomes

Study cohort inclusion and follow-up: The study sample comprised 5143 individuals, forming the original cohort. After applying the exclusion criteria, 4843 patients (effective cohort) were analyzed. Supplementary Table 4 (Supplementary material 2) demonstrates that the effective cohort is representative of the original cohort.

The effective cohort consisted of 3087 males (63.74%) and 1756 females (34.51%), with ages at baseline spanning from 20 to 85 years. The overall median baseline age was 49 years, with males averaging 49 years and females 50 years. The median follow-up duration for the entire study population was 4.22 years (IQR: 2.24-6.0), with males at 4.20 years (IQR: 2.23-6.02) and females at 4.23 years (IQR: 2.25-6.11).

HCC occurrence during follow-up: During follow-up, 355 patients with CHB developed HCC, with a cumulative incidence of 7.33% and an incidence density of 0.81/100 PY. Among them, 261 males had a higher cumulative incidence (8.45%) and density (0.94/100 PY), while 94 females had lower rates (5.35% and 0.59/100 PY). The Log-rank test showed a statistically significant gender difference in HCC incidence ($P < 0.01$; Figure 2).

APR levels in HCC and non-HCC groups during follow-up

Supplementary Table 5 indicates that the average APR levels in the HCC group consistently exceeded those in the non-HCC group across all follow-up years for both genders. APR levels declined over time and were higher in females than males, with significant differences observed between follow-up years ($P < 0.01$).

LME Results for dynamic changes in the APR

LME for the overall population (LME_1) shows that the ratio in females was higher than in males by 0.085. For non-HCC patients, the average annual growth rate of the ratio was -0.093. The annual growth rate increased by 0.003 in those who

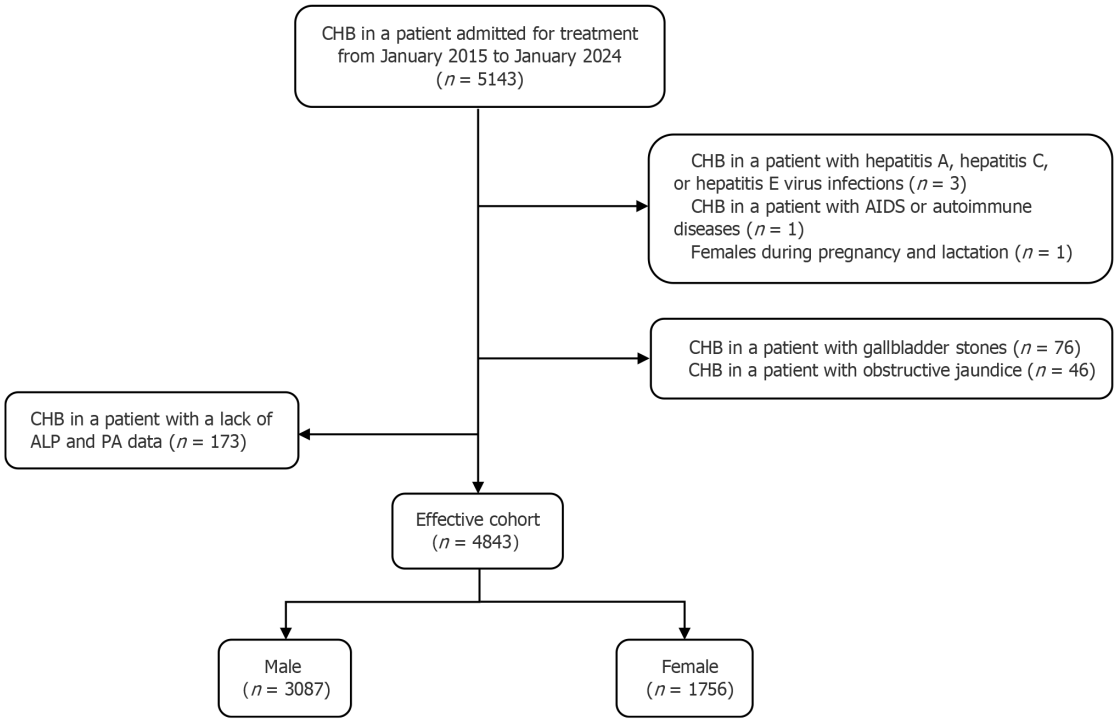

Figure 1 Effective cohort flowchart. CHB: Chronic hepatitis B; AIDS: Acquired immunodeficiency syndrome; ALP: Alkaline phosphatase; PA: Prealbumin.

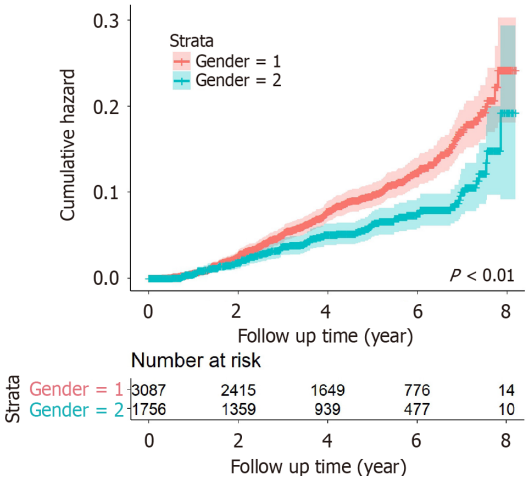

Figure 2 Cumulative incidence of hepatocellular carcinoma. 1: Males; 2: Females.

were 1 year older at baseline (Table 1).

The LME for males and LME for females, derived from the LME_1 model by incorporating gender as a stratification factor, reveal the following outcomes. At baseline, males with cirrhosis had a ratio 0.446 units higher than those without cirrhosis, while females with cirrhosis exhibited a ratio 0.458 units higher. Additionally, for every year increase in baseline age, the average ratio for males decreased by 0.006, whereas for females, it increased by 0.005. Male patients with HCC showed an average ratio 0.325 higher than their non-HCC counterparts, who averaged 1.234. Conversely, female patients with HCC had an average ratio 0.317 higher than female non-HCC patients, who averaged 1.239. Among male non-HCC patients, the average annual ratio decrease was -0.093; a 1-year increase in baseline age yielded an annual ratio increase of 0.003. Among female non-HCC patients, the average annual growth rate of the ratio was -0.089; an increase of 1 year in baseline age correlated with an annual ratio decrease of 0.002. Statistical analysis is detailed in the Supplementary Tables 6, 7 and 8.

JMLS results for APR and HCC risk

Table 2 illustrates the results from the survival sub-models of JMLS in terms of the HR (95%CI) and *P* value. All the JMLS are time-dependent relative risk models with a baseline risk function. Thus, HRs can be interpreted similarly to those from a proportional hazard model, such as a Cox PH model. JMLS for the overall population (JMLS1) exhibits an HR of

Table 1 Summary of Linear Mixed-Effects Model findings

Variable	LME_1 (total)		LME_2 (males)		LME_3 (females)	
	β	*P* value	β	*P* value	β	*P* value
Intercept	1.147	< 0.01	1.234	< 0.01	1.292	< 0.01
Gender	0.085	< 0.01	-	-	-	-
Liver cirrhosis	0.456	< 0.01	0.446	< 0.01	0.458	< 0.01
Time	-0.093	< 0.01	-0.093	< 0.01	-0.089	< 0.01
Cage	-0.002	< 0.01	-0.006	< 0.01	0.005	< 0.01
State	0.314	< 0.01	0.325	< 0.01	0.317	< 0.01
Time:Cage	0.003	< 0.01	0.004	< 0.01	0.002	< 0.01
Time:State	0.006	> 0.05	0.006	> 0.05	-0.003	> 0.05

LME_1: Linear Mixed-Effects Model for the overall population; LME_2: Linear Mixed-Effects Model for males; LME_3: Linear Mixed-Effects Model for females; Intercept: The average intercept of all subjects; Liver cirrhosis: Whether cirrhosis had occurred at baseline; Time: The interval between each detection time and the first detection time (years); Cage: Baseline age after centralization; State: Outcome state; Time:Cage: The interaction of time and cage; Time:State: The interaction of time and state; β: Fixed-effect regression coefficient.

Table 2 Event summary of Joint Modeling of Longitudinal and Survival data and hazard ratios

Variable	$JMLS_1$ (total)		$JMLS_2$ (males)		$JMLS_3$ (females)	
	HR	*P* value	HR	*P* value	HR	*P* value
Ratio	1.069 (0.923–1.216)	> 0.05	1.034 (0.882–1.213)	> 0.05	1.149 (0.865–1.526)	> 0.05
Cage	1.069 (1.057–1.081)	< 0.01	1.068 (1.055, 1.082)	< 0.01	1.071 (1.045–1.098)	< 0.01
Gender	0.480 (0.377–0.610)	< 0.01	-	-	-	-
Liver cirrhosis	4.570 (3.376–6.185)	< 0.01	4.896 (3.428, 6.994)	< 0.01	3.690 (2.075–6.561)	< 0.01
α	2.065 (1.749-2.439)	< 0.01	2.005 (1.653–2.431)	< 0.01	2.273 (1.620–3.190)	< 0.01

$JMLS_1$: Joint Modeling of Longitudinal and Survival data for the overall population; $JMLS_2$: Joint Modeling of Longitudinal and Survival data for males; $JMLS_3$: Joint Modeling of Longitudinal and Survival data for females; Ratio: Ln(10 alkaline phosphatase to prealbumin ratio); Cage: Baseline age after centralization; Liver cirrhosis: Whether cirrhosis had occurred at baseline; α: The joint coefficient of the joint model.

2.065 (1.749-2.439) suggesting a 2.065-fold increase in the hazard of HCC per unit increase of ratio.

The JMLS for males ($JMLS_2$) and JMLS for females ($JMLS_3$), derived from $JMLS_1$ by incorporating gender as a stratification factor, demonstrate the following outcomes. $JMLS_2$ exhibits an HR of 2.005 (1.653-2.431), suggesting a 2.005-fold increase in the hazard of HCC per unit increase in the ratio. $JMLS_3$ exhibits an HR of 2.273 (1.620-3.190), suggesting a 2.237-fold increase in the hazard of HCC per unit increase in the ratio. Detailed statistical analysis is included in Supplementary Tables 9, 10 and 11.

Notably, all three models suggest that baseline ratio levels are not associated with HCC risk, whereas the longitudinal increase in the ratio elevates HCC risk.

Analysis of JMLS

Upon examining the diagnostic outcomes, it became evident that the model fit was satisfactory for both male and female cohorts. This conclusion is supported by the residual Q-Q plots that align with normality, the marginal survival and cumulative hazard function estimation curves that closely match their theoretical values, and the Martingale and Cox-Snell residuals that are appropriately centered around zero. Notably, in E-th sub-figure, the solid black line, which depicts the Loess smoothing curve fitted to the standardized edge residuals, hovers near zero, indicating an excellent fit for both genders. Likewise, in F-th sub-figure, the gray Loess smoothing curve, based on the martingale residuals, stays near the horizontal axis at zero, signifying that the model assumptions regarding the relationship between the time-dependent longitudinal component and the risk function hold true for both genders (Figure 3 and Figure 4).

Patient-specific prognosis

Figure 5 and Supplementary Tables 12 and 13 show predictions of JMLS for survival rates in male and female patients with CHB at median age. The model estimates a 10-year survival probability of 72% (95%CI: 0.54-0.85) for males and 95% (95%CI: 0.87-0.98) for females.

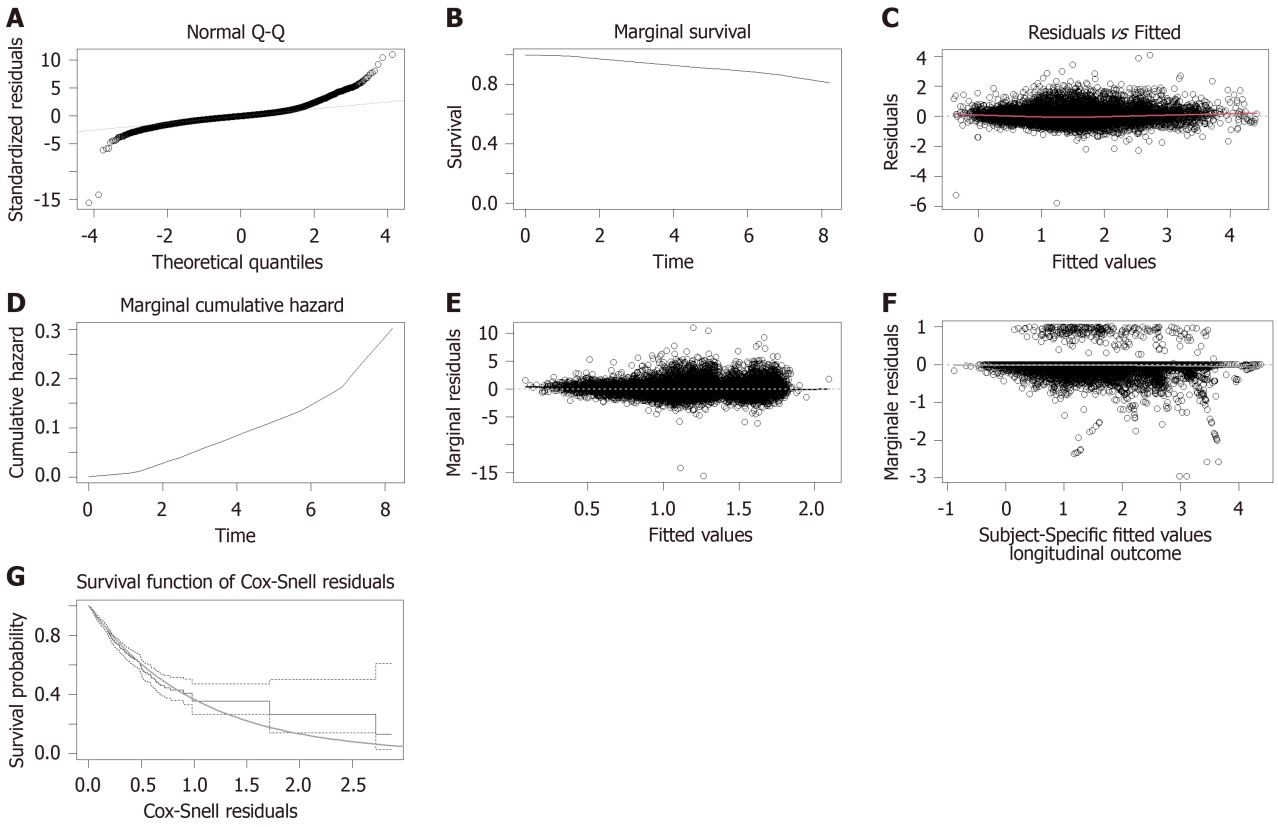

Figure 3 Analysis of the Joint Modeling of Longitudinal and Survival data for male. A: Normal Q-Q plot of the residuals in the longitudinal process; B: Marginal survival function estimation curve during survival; C: Residuals and fitted data values in the longitudinal process; D: Marginal cumulative hazard function estimation curve throughout survival; E: Marginal residuals of the longitudinal portion of the joint model. The solid black line is the Loess smoothing curve fitted and based on standardized edge residuals, indicating a good fit as it approximates 0; F: Martingale residuals of the surviving portion of the joint model. The gray Loess smoothing curve, obtained from martingale residuals, is close to the 0 horizontal line, indicating reasonable assumptions between the time-dependent longitudinal part and the risk function; G: Cox–Snell residuals of the survival portion of the joint model. The gray curve is the cumulative risk value corresponding to the survival function. The Kaplan–Meier estimation is close to the exponential distribution of the cumulative risk function of the survival function. All diagnostic graphs showed that Joint Modeling of Longitudinal and Survival data fit well.

DISCUSSION

HBV infection accounts for the majority of liver cancer cases in most Asian countries, with prevalence rates reaching 60%-80% in endemic areas[19]. Risk factors for HCC in carriers of HBV include male sex, older age, family history, viral type, and cirrhosis[5]. In sub-Saharan Africa, HBV-related HCC is diagnosed at a median age of 45 years[20], compared to 52, 69, 65, and 62 years in China, Japan, Europe, and North America, respectively[21,22].

This retrospective cohort study shows that the cumulative incidence of HCC in patients with CHB is 7.33%. The incidence was higher in males (8.45%) than females (5.35%). Most studies have found that HCC incidence and mortality vary by sex, with males being 1.2-3.6 times more likely than females to develop HCC[23,24]. A cohort study by Liao *et al* [25] similarly showed that men are at higher risk of being diagnosed with HCC than women, with an HR of 3.9 (95%CI: 3.6-4.2) for HCC. Similarly, this study found that men have a higher incidence of HCC than women. Gender differences in HCC incidence can be attributed to several factors, such as higher prevalence of intravenous drug use, viral infections, and excessive alcohol consumption in men, as well as differences in sex hormones[26,27]. A clinical trial has shown that testosterone can promote HCC development by reducing the activity of inhibitory enzymes[28]. Additionally, another clinical study has shown that estrogen protects against HCC development through estrogen-mediated inhibition of IL-6 production by Kupffer cells[29].

ALP is an enzyme widely distributed in the liver, bone, intestine, kidney, placenta, and various tumors. ALP is crucial in regulating inflammatory responses and immune functions and can be used to predict liver cancer rupture, assist in tumor imaging, and differentiate bone tumors[30-32]. PA, primarily produced by the liver, is a sensitive biomarker for plasma proteins and reliably indicates a patient's nutritional status and liver disease severity[33,34]. ALP serves as an independent predictor of significant liver inflammation; preoperative levels can help in monitoring recurrence in patients at high risk for HCC[35]. Additionally, a low PA level independently indicates shorter overall survival[36]. During HCC progression, the APR may undergo dynamic changes. These changes may be associated with tumor growth, treatment efficacy, and the patient's physiological state. Monitoring these changes can help predict the occurrence and development of HCC more intuitively.

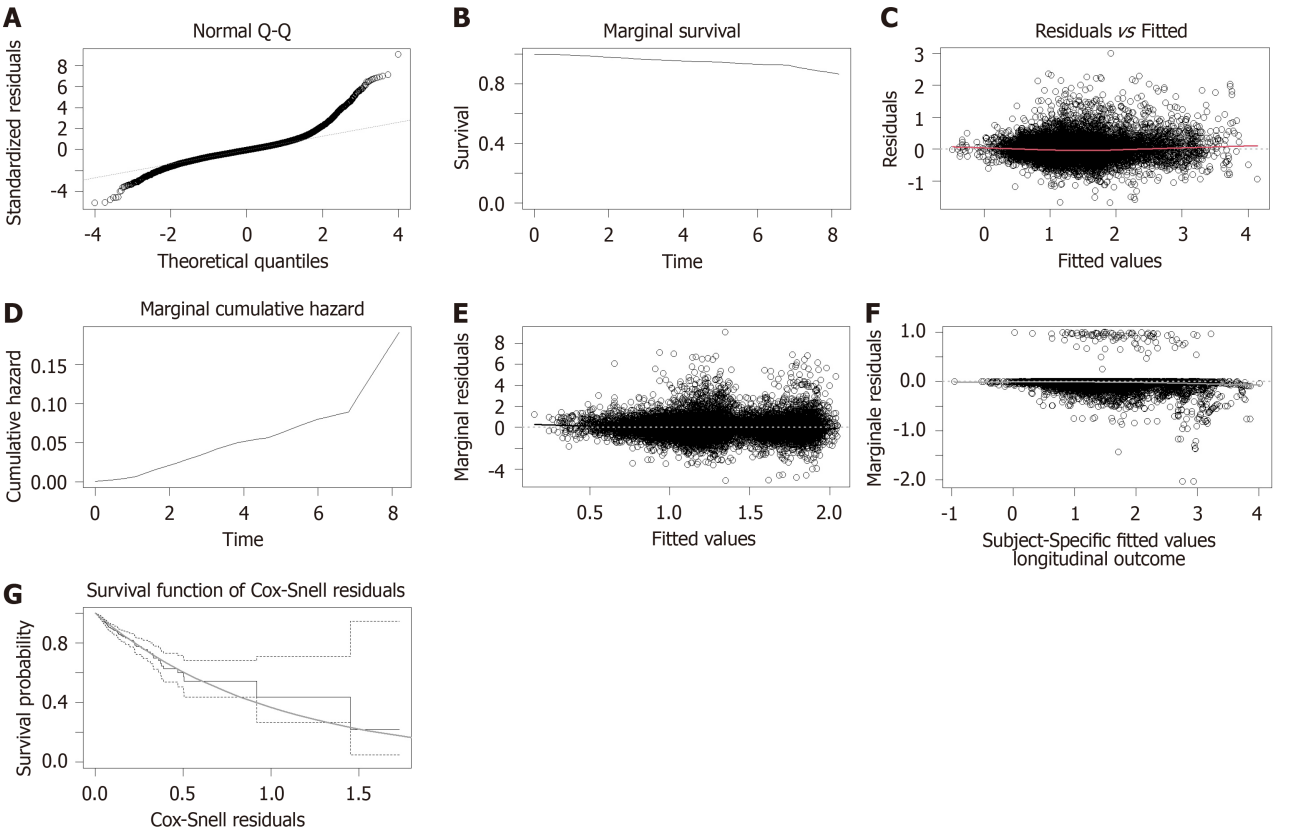

Figure 4 Analysis of the Joint Modeling of Longitudinal and Survival data for female. A: Normal Q-Q plot of the residuals in the longitudinal process; B: Marginal survival function estimation curve during survival; C: Residuals and fitted data values in the longitudinal process; D: Marginal cumulative hazard function estimation curve throughout survival; E: Marginal residuals of the longitudinal portion of the joint model. The solid black line is the Loess smoothing curve fitted and based on standardized edge residuals, indicating a good fit as it approximates 0; F: Martingale residuals of the surviving portion of the joint model. The gray Loess smoothing curve, obtained from martingale residuals, is close to the 0 horizontal line, indicating reasonable assumptions between the time-dependent longitudinal part and the risk function; G: Cox–Snell residuals of the survival portion of the joint model. The gray curve is the cumulative risk value corresponding to the survival function. The Kaplan–Meier estimation is close to the exponential distribution of the cumulative risk function of the survival function. All diagnostic graphs showed that Joint Modeling of Longitudinal and Survival data fit well.

Our LME_1 results showed a higher baseline APR level in females than males. The results of the models showed that the average APR level in the baseline HCC group was higher than in the non-HCC group in both sex groups. Mean APR levels were lower in older males at baseline but tended to increase with age. In older females, mean APR levels were higher at baseline but also tended to increase with age.

JMLS is a model that allows for the simultaneous analysis of longitudinal repeated covariates and event time data[37]. JMLS is used to assess the association of a biomarker with the hazard of an event. It serves as a sensitivity analysis to reduce bias due to censoring or mortality. It also enables personalized prognostication by accounting for measurement error, different follow-up times, and efficiency through combining data (*i.e.*, smaller standard error)[38]. In this study, all APR measurements from the past 9 years for each participant were included in the model analysis. The dynamic change of APR was analyzed using the LME; the association between the longitudinal change of APR and HCC was analyzed by combining the COX model. The results show that a longitudinal increase in APR increases HCC risk. Specifically, the longitudinal increase in the ratio was a risk factor for HCC in both males (HR = 2.005, 95%CI: 1.653-2.431) and females (HR = 2.273, 95%CI: 1.620-3.190). JLMS revealed that an increase in APR would increase HCC risk; the HCC risk was not related to baseline APR levels in individual subjects. However, the longitudinal dynamic change in individual APR significantly impacted HCC development. Additionally, this study found that age and baseline cirrhosis were risk factors for HCC in both male and female groups. Males' HCC risk increased by 1.068-fold annually (95%CI: 1.055-1.082). Patients with baseline cirrhosis had a 4.896 times higher HCC risk (95%CI: 3.428-6.994). Females also had a 1.071-fold annual increase in HCC risk (95%CI: 1.045-1.097); those with baseline cirrhosis have a 3.690 times higher risk (95%CI: 2.075-6.561). An increase in the APR indicates a rise in ALP levels and a decrease in PA levels. Hann *et al*[18] found that ALP elevation was associated with HCC development. Huo *et al*[39] and Zhou *et al*[40] found a close relationship between reduced PA levels and postoperative adverse outcomes in patients with cancers (*e.g.*, in the liver or stomach). These studies align with our results. The physiological effects of ALP and PA are complex and diverse. Although the exact pathophysiological relationship cannot be determined from our data, the current results may help further research clarify the association between APR levels and HCC risk in those with HBV.

Clinicians often rely on biomarker trends for decision-making, making it crucial to understand how these parameters link to clinical practice. This study highlights gender differences in HCC risk factors, facilitating personalized medical strategies. These strategies involve customized prevention and treatment plans based on gender and APR levels.

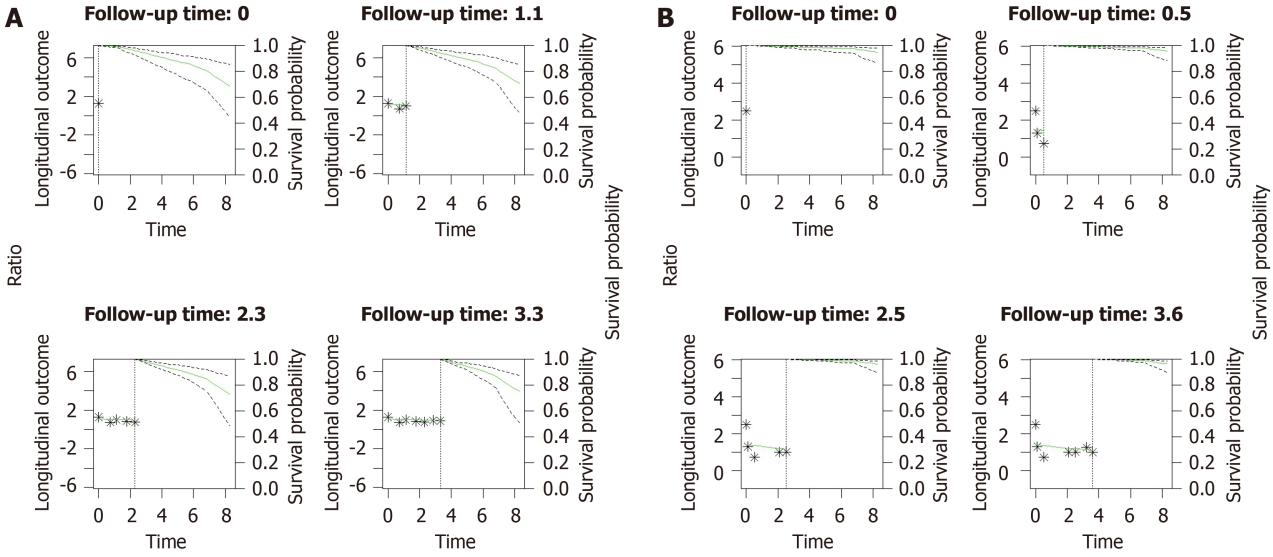

Figure 5 Prediction chart of the survival. A: Males; B: Females. The dotted left line represents the longitudinal measurement trajectory of the patient; the solid and dashed lines on the right represent the survival predictive value and its 95%CI, respectively; ratio: Ln(10 alkaline phosphatase to prealbumin ratio).

Furthermore, we advocate for additional research, especially into the causes of non-cirrhotic HCC in women, to develop more effective methods of prevention and treatment. Timely identification and treatment of HCC can significantly enhance patient outcomes and survival rates, thereby reducing HCC-related mortality. Supplementary Table 14 concisely summarizes existing studies on the role of inflammatory markers in disease progression in patients with CHB. Thus, our study further consolidates the relationship between inflammatory markers and fibrosis, providing new insights that enrich the current scientific discourse.

This study has several limitations. First, the study's population composition ratio varies significantly between genders; relying solely on data from one hospital population may introduce selection biases. Selection bias occurs when the study sample differs from the larger population in characteristics or behaviors, potentially yielding inaccurate effect estimates. Despite the steps taken to ensure a representative sample, our sample may not fully reflect the broader population. Future research should consider larger and more diverse samples to mitigate the risk of selection bias and enhance the generalizability of the findings. Second, the follow-up period is insufficient to observe the dynamic changes of APR and its relationship with HCC in patients with CHB. Third, joint modeling assumes that censoring is independent of random effects. Violation of this assumption would bias parameter estimates. However, simulation studies suggest that joint models' estimates are robust to misspecification of the distribution of random effects[37].

CONCLUSION

HCC incidence rates were higher in males, with females exhibiting higher APR levels. Baseline APR levels did not predict HCC risk. However, an increasing APR over time correlated with an elevated HCC risk in both genders.

FOOTNOTES

Author contributions: Zhen WC and Sun J contribute equally to this study as co-first authors; Pu CW and Li XF contribute equally to this study as co-corresponding authors; Zhen WC and Li XF designed the study and drafted the initial manuscript; Sun J participated in the statistical analysis and interpretation of the data; Bai XT, Zhang Q, Li ZH, Zhang XY, Xu RX, Wu W, Yao ZH participated in the acquisition, analysis; Pu CW Provided clinical data, Li XF performed the review and editing.

Institutional review board statement: The study was reviewed and approved by the Dalian Public Health Clinical Center Institutional Review Board (Approval No. 2024-026KY-001).

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

Conflict-of-interest statement: The authors of this research hereby confirm that there are no potential conflicts of interest to disclose.

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Clinical effects of phospholipase D2 in attenuating acute pancreatitis

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Abstract

BACKGROUND

The objective of the current study was to elucidate the clinical mechanism through which phospholipase D2 (PLD2) exerted a regulatory effect on neutrophil migration, thereby alleviating the progression of acute pancreatitis.

AIM

To elucidate the clinical mechanism through which PLD2 exerted a regulatory effect on neutrophil migration, thereby alleviating the progression of acute pancreatitis.

METHODS

The study involved 90 patients diagnosed with acute pancreatitis, admitted to our hospital between March 2020 and November 2022. A retrospective analysis was conducted, categorizing patients based on Ranson score severity into mild (n = 25), moderate (n = 30), and severe (n = 35) groups. Relevant data was collected for each group. Western blot analysis assessed PLD2 protein expression in patient serum. Real-time reverse transcription polymerase chain reaction was used to evaluate the mRNA expression of chemokine receptors associated with neutrophil migration. Serum levels of inflammatory factors in patients were detected using enzyme-linked immunosorbent assay. Transwell migration tests were conducted to compare migration of neutrophils across groups and analyze the influence of PLD2 on neutrophil migration.

RESULTS

Overall data analysis did not find significant differences between patient groups (P > 0.05). The expression of PLD2 protein in the severe group was lower than that in the moderate and mild groups (P < 0.05). The expression level of PLD2 in the moderate group was also lower than that in the mild group (P < 0.05). The severity of acute pancreatitis is negatively correlated with PLD2 expression (r = -0.75, P = 0.002). The mRNA levels of C-X-C chemokine receptor type 1, C-X-C chemokine receptor type 2, C-C chemokine receptor type 2, and C-C chemokine receptor type 5 in the severe group are significantly higher than those in the

moderate and mild groups ($P < 0.05$), and the expression levels in the moderate group are also higher than those in the mild group ($P < 0.05$). The levels of C-reactive protein, tumor necrosis factor-α, interleukin-1β, and interleukin-6 in the severe group were higher than those in the moderate and mild groups ($P < 0.05$), and the levels in the moderate group were also higher than those in the mild group ($P < 0.05$). The number of migrating neutrophils in the severe group was higher than that in the moderate and mild groups ($P < 0.05$), and the moderate group was also higher than the mild group ($P < 0.05$). In addition, the number of migrating neutrophils in the mild group combined with PLD2 inhibitor was higher than that in the mild group ($P < 0.05$), and the number of migrating neutrophils in the moderate group combined with PLD2 inhibitor was higher than that in the moderate group ($P < 0.05$). The number of migrating neutrophils in the severe group + PLD2 inhibitor group was significantly higher than that in the severe group ($P < 0.05$), indicating that PLD2 inhibitors significantly stimulated neutrophil migration.

CONCLUSION

PLD2 exerted a crucial regulatory role in the pathological progression of acute pancreatitis. Its protein expression varied among patients based on the severity of the disease, and a negative correlation existed between PLD2 expression and disease severity. Additionally, PLD2 appeared to impede acute pancreatitis progression by limiting neutrophil migration.

Key Words: Phospholipase D2; Neutrophil migration; Acute pancreatitis; Retrospective analysis; Inflammatory response

Core Tip: This research substantiated the indispensable role of phospholipase D2 (PLD2) in the pathological progression of acute pancreatitis. Moreover, the varying protein expression levels of PLD2 among patients with distinct degrees of acute pancreatitis exhibited an inverse relationship with disease severity. Moreover, this study revealed PLD2's potential to mitigate acute pancreatitis by restricting neutrophil movement.

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INTRODUCTION

Acute pancreatitis, a prevalent condition characterized by an acute pancreatic response with intricate and incompletely elucidated pathophysiology[1], poses a substantial global health burden. The United States grapples with severe cases of this condition, leading to significant clinical and economic repercussions[2,3]. Despite relentless research, the underlying causes of acute pancreatitis remains inadequately comprehended. Thus, delving into its pathogenesis and discovering effective treatment strategies is imperative from both theoretical and clinical perspectives. Phospholipase D2 (PLD2), a member of the phospholipase family, facilitates the breakdown of phosphatidylcholine into phosphatidylcholine and choline. It exerts influence over diverse cellular functions encompassing apoptosis, survival, migration, and adhesion[4-6]. PLD2 is associated with the development of various diseases, such as vascular, immune, and neurological disorders. Notably, PLD2 inhibition exhibits potential in ameliorating intestinal mucosal inflammation in inflammatory bowel disease[7]. Furthermore, PLD2 is implicated in cellular response to tissue damage, including acute inflammatory conditions. According to previous research, PLD2 induces the migration of macrophages from the vascular system in cases of acute pancreatitis[8]. While PLD2 appears to play a role in modulating inflammatory conditions like acute pancreatitis, the precise function of PDL2 remains uncertain.

Neutrophil migration plays a crucial role in the progression of acute pancreatitis[9,10], a sudden inflammation of the pancreas that involves a multitude of numerous cells and molecules, including neutrophil migration. Neutrophil migration constitutes a vital component of both inflammation and immune responses[11], a complex process regulated by various factors, including chemokines and their receptors. Chemokines, substances with the capability to attract and guide neutrophil movement, bind to receptors located on the exterior of neutrophils. In acute pancreatitis, the up-regulation of chemokine receptors can trigger neutrophil migration, driven by the release of inflammatory mediators[12]. This study is a retrospective analysis aimed at exploring the clinical mechanism of PLD2 in the development of acute pancreatitis, with a particular focus on its regulatory role in neutrophil migration.

MATERIALS AND METHODS

General information of patients

A retrospective analysis was done on 90 hospitalized patients with acute pancreatitis at our hospital from March 2020 to November 2022. The age of the patients ranged from 25 to 64 years, with an average age of 46.24 ± 5.33 years. Among them, 53 were male and 37 were female. Based on the Ranson score, the severity of the patients' conditions was categorized into three groups: Mild (n = 25), moderate (n = 30), and severe (n = 35) groups. The age range of the patients is 25 to 64 years, with an average age of 46.24 ± 5.33 years. There were 53 males and 37 females. According to the Ranson score, the severity of the patients was divided into three groups: Mild (n = 25), moderate (n = 30), and severe (n = 35). Notably, the difference was not statistically significant for this particular test (P > 0.05). This study has been approved by the ethics committee, and all participants have signed informed consent forms. The Ranson score is a scoring system used to assess the severity of acute pancreatitis, primarily based on the patient's clinical presentation and laboratory test results (Table 1). This study was approved by the Ethics Committee of China-Japan Friendship Hospital and informed consent was waived because the research was retrospective and conducted on anonymized data.

Inclusion criteria

Patients at least 18 years old with characteristic abdominal pain and increased levels of amylase and/or lipase (> three times the normal upper limit), diagnosed with acute pancreatitis through clinical and imaging assessments.

Exclusion criteria

Patients with pancreatitis caused by trauma, chronic pancreatitis, or pancreatic tumor; patients with congestive heart failure, chronic obstructive pulmonary disease, liver cirrhosis, renal insufficiency, autoimmune diseases, active acute infections, and malignant tumors were excluded due to potential confounding effects of systemic inflammatory markers.

Steps for collecting clinical data

The steps of collecting clinical data in this study include medical history collection, physical examination, and laboratory and auxiliary examinations. The prerequisite for obtaining effective data is proficient questioning and physical examination skills: (1) Medical history collection: The main method of collecting medical history is through consultation, which also includes reviewing various medical records of patients. The main body of medical history is symptoms, and the characteristics, occurrence, development, and evolution of symptoms play an important role in forming a diagnosis. But symptoms are not diseases, and physicians should combine medical knowledge and clinical experience in medical history collection to understand and explore the essence of the patient's disease; (2) Physical examination: Based on the collection of medical history, a comprehensive, standardized, and correct physical examination is conducted on the patient. The positive signs and negative manifestations discovered can become important basis for diagnosing diseases. During the physical examination process, one should investigate and ask questions, think while examining, consider the relationship between symptoms, signs, and diagnosis, verify, supplement, and improve evidence; and (3) Laboratory and auxiliary examinations: Based on obtaining medical history and physical examination data, considering the available laboratory and auxiliary examinations, selecting necessary examinations reasonably will undoubtedly make clinical diagnosis more accurate and reliable.

Sample collection

Blood samples were obtained from each patient after fasting. The blood was collected in red cap tubes, left to clot for at least 20 minutes, and then centrifuged at a 3000 rpm for 10 minutes at room temperature. The resulting serum was stored in 1.8 mL freezing tubes for subsequent analysis.

Western blot analysis of PLD2 protein expression

Proteins were extracted from serum using RIPA lysis buffer and a protease inhibitor. The protein concentration was determined using the BCA protein method. Loading buffer measuring 5 L was added to each sample, followed by denaturation at 95 °C for 5 minutes and additional denaturation on ice for 5 minutes. Protein samples were loaded onto sodium-dodecyl sulfate gel electrophoresis gels for electrophoresis. Subsequently, proteins were transferred to a polyvinylidene fluoride membrane and blocked using 5% skim milk powder for 60 minutes. The primary antibody for PLD2 (diluted 1:2000) was applied to the membrane and incubated overnight at 4 °C. After a Tris-buffered saline with Tween wash, the membrane was incubated with alkaline phosphatase-coupled goat anti-rabbit secondary antibody (diluted 1:2000) for 1 hour. Following another Tris-buffered saline with Tween wash, the membrane was treated with 5 mL BCIP/NBT chromogenic agent, visualization was achieved using the Quality image analysis system.

RNA extraction and quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from serum samples using Trizol. The RNA underwent reverse transcription at 42 °C for 60 minutes, followed by an additional step at 70 °C for 5 minutes. The quantitative real-time polymerase chain reaction (qRT-PCR) reaction mixture consisted of 20 μL in total, comprising 10 μL SYBR Green PCR Master Mix (2 ×), 2 μL cDNA template, 0.5 μL forward primer, 0.5 μL reverse primer, and 7 μL DDH_2O. The cDNA template was used for PCR and agarose gel electrophoresis to establish the PCR product's standard curve. The qRT-PCR reaction program included an initial denaturation step at 95 °C for 5 minutes, followed by denaturation at 95 °C for 10 seconds and annealing at 60 °C for 30 seconds, repeated for 39 cycles. Amplification efficiency was approximately 90%-100%. Following qRT-PCR, the

Table 1 General statistics, *n* (%)

Parameter	Mild group (*n* = 25)	Moderate group (*n* = 30)	Severe group (*n* = 35)	Variance ratio	*P* value
Gender (male:female)	Eleven past two p.m.	Thirteen past five p.m.	Thirteen past ten p.m.	3.109	0.413
Age (years)	45.17 ± 4.28	46.83 ± 4.66	45.92 ± 5.18	5.226	0.385
BMI (kg/m^2)	22.46 ± 1.75	23.56 ± 1.49	22.85 ± 1.88	4.035	0.307
Smoker	8 (32.00)	10 (33.33)	12 (34.28)	2.461	0.216
Alcohol consumption	6 (24.00)	8 (26.67)	11 (31.42)	1.975	0.445

amplicon underwent electrophoresis on a 2% agarose gel, and analysis was performed using the $2^{-\Delta\Delta Ct}$ method.

Enzyme-linked immunosorbent assay

Plasma samples were obtained by centrifuging a 5 mL venous blood sample at 3000 rpm for 10 minutes and separating the plasma. Enzyme-linked immunosorbent assay (ELISA) kits (Thermo Fisher Scientific Shier Technology, United States) were utilized to measure the levels of C-reactive protein (CRP), tumor necrosis factor (TNF)-α, IL-6, and IL-1β in plasma, following the manufacturer's instructions. Three samples were collected for each parameter.

Transwell migration test

After collecting peripheral blood samples from each patient group, the samples were centrifuged at 800 × *g* for 10 minutes to separate the serum. This serum was then divided into cryopreservation tubes and stored at -80 °C. Neutrophils were isolated using a commercial neutrophil isolation kit according to instructions. Isolated neutrophils were added into the culture medium, combined with 30% patient serum, and placed in a Transwell orifice plate. In the Transwell setup, neutrophils were placed in the upper chamber, and a culture medium-filled orifice plate was placed in the lower chamber. The Transwell orifice plate was placed in an incubator at a constant temperature, allowing neutrophils to migrate from the upper to the lower chamber in the culture medium over a 6-hour period. After incubation, the upper section of the Transwell was removed, and the cells in the lower section were immobilized and stained. The migrating cells in the lower chamber were observed and counted in several randomly chosen visual fields under a microscope. For the *in vitro* neutrophil migration test, a PLD2 inhibitor (Cmpd101) at a concentration of 5 μM was prepared by dissolving it in DMSO and adding it to the patient's serum.

Statistical analysis

Statistical analysis was conducted using IBM's SPSS software. The data were presented as the mean ± SD from a minimum of three trials. One-way ANOVA was initially conducted, followed by further analyses using Tukey's and Student's *t*-tests to explore the differences among the groups. A significance level of less than 0.05 was considered statistically significant.

RESULTS

Protein expression analysis of PLD2

The analysis indicated that the severe group exhibited a decreased protein expression compared to both the moderate and mild groups ($P < 0.05$), while the moderate group also displayed a lower protein expression in comparison to the mild group ($P < 0.05$). Pearson correlation coefficient analysis revealed a negative correlation between the severity of acute pancreatitis and PLD2 expression ($r = -0.75$, $P = 0.002$) (Figure 1 and Table 2).

Expression analysis of chemokine receptors associated with neutrophil migration

The mRNA expression of chemokine receptors linked to neutrophil migration was detected using RT-PCR. The results revealed significantly elevated mRNA expression levels of C-X-C chemokine receptor type 1 (*CXCR1*), CXCR2, C-C chemokine receptor type 2 (CCR2), and CCR5 in the severe group compared to both the moderate and mild groups ($P < 0.05$). Additionally, the moderate group displayed elevated mRNA expressions of *CXCR1*, *CXCR2*, *CCR2*, and *CCR5* compared to the mild group ($P < 0.05$) (Figure 2 and Table 3).

ELISA detection of inflammatory factors

Serum inflammatory factors were detected using ELISA. The severe group exhibited elevated levels of CRP, TNF-α, IL-1β, and IL-6 compared to both the moderate and mild groups ($P < 0.05$). Additionally, the moderate group had higher levels of CRP, TNF-α, IL-1β, and IL-6 than the mild group ($P < 0.05$) (Table 4).

Transwell migration test

The investigation demonstrated that the severe group exhibited an increased count of migrating neutrophils compared to both the moderate and mild groups ($P < 0.05$). Additionally, the moderate group had a higher count of migrating

Table 2 Protein expression analysis of phospholipase D2

Group	PLD2
Mild group (n = 25)	1.49 ± 0.10
Moderate group (n = 30)	1.03 ± 0.05
Severe group (n = 35)	0.54 ± 0.02
Variance ratio	13.226
P value	0.015

PLD2: Phospholipase D2.

Table 3 The mRNA expression analysis of neutrophil migration-related chemokine receptor

Group	CXCR1	CXCR2	CCR2	CCR5
Mild group (n = 25)	1.02 ± 0.01	0.97 ± 0.01	1.06 ± 0.02	1.01 ± 0.01
Moderate group (n = 30)	2.28 ± 0.07	2.05 ± 0.03	1.98 ± 0.06	1.49 ± 0.05
Severe group (n = 35)	4.16 ± 0.16	4.94 ± 0.15	3.02 ± 0.13	1.98 ± 0.17
Variance ratio	9.336	12.208	13.115	10.624
P value	0.002	0.014	0.005	0.006

CXCR1: C-X-C chemokine receptor type 1; CXCR2: C-X-C chemokine receptor type 2; CCR2: C-C chemokine receptor type 2; CCR5: C-C chemokine receptor type 5.

Table 4 Enzyme-linked immunosorbent assay detection of inflammatory factors

Group	CRP (mg/L)	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
Mild group (n = 25)	75.68 ± 10.43	36.45 ± 8.24	14.39 ± 3.18	135.88 ± 15.05
Moderate group (n = 30)	136.29 ± 10.67	68.24 ± 10.67	36.27 ± 6.33	536.72 ± 25.46
Severe group (n = 35)	194.18 ± 17.34	105.67 ± 12.49	58.41 ± 8.62	768.55 ± 36.19
Variance ratio	15.389	11.663	9.419	12.056
P value	0.015	0.026	0.003	0.006

CRP: C-reactive protein; TNF: Tumor necrosis factor; IL: Interleukin.

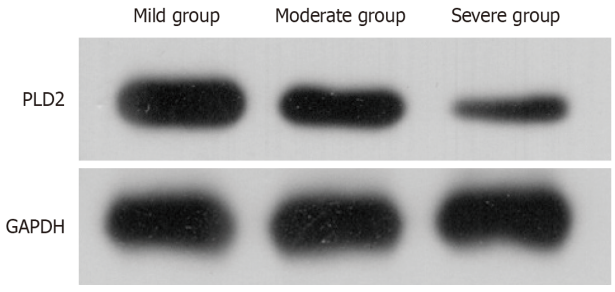

Figure 1 Expression levels of phospholipase D2 protein in different severity groups of acute pancreatitis by western blot analysis. PLD2: Phospholipase D2.

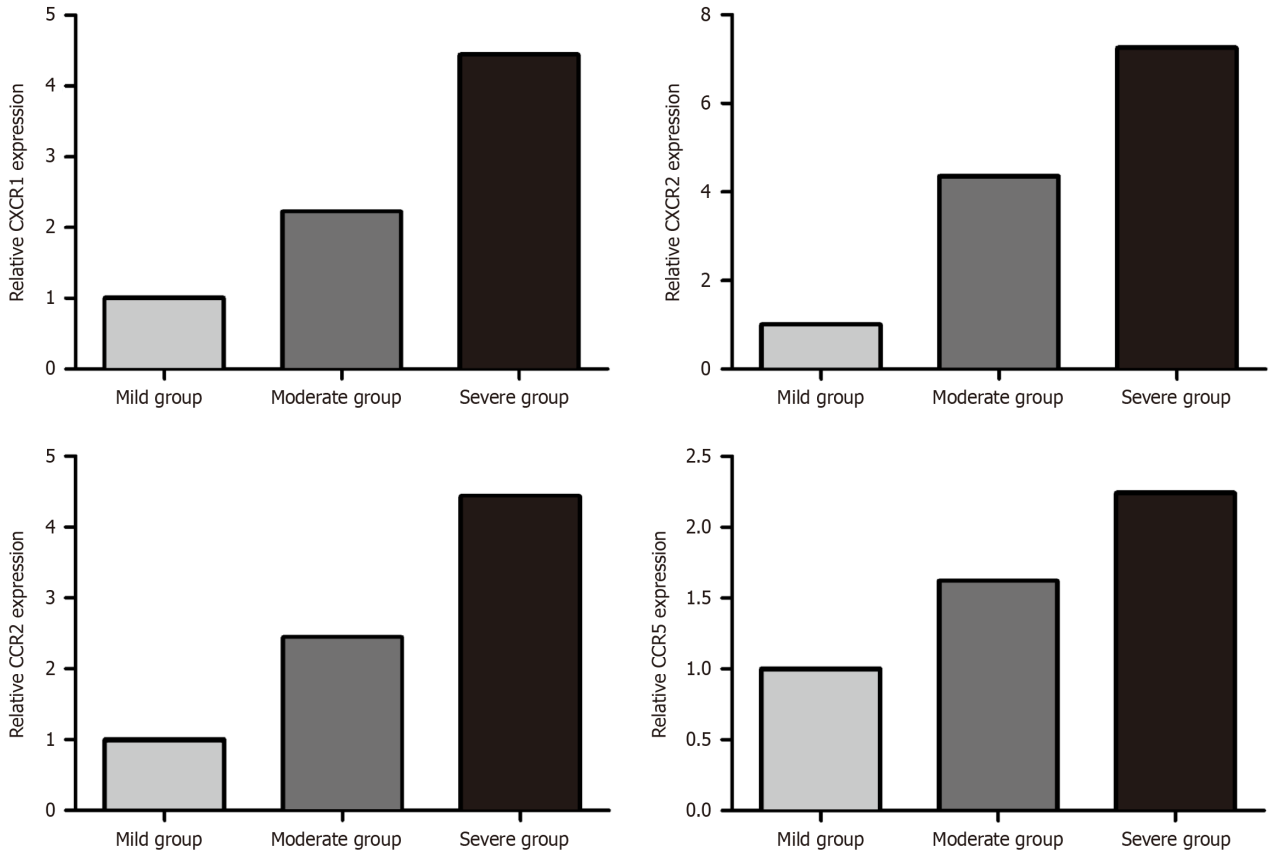

Figure 2 The mRNA expression of chemokine receptor detected by reverse transcription-polymerase chain reaction. CXCR1: C-X-C chemokine receptor type 1; CXCR2: C-X-C chemokine receptor type 2; CCR2: C-C chemokine receptor type 2; CCR5: C-C chemokine receptor type 5.

neutrophils than the mild group ($P < 0.05$) (Table 5).

Neutrophil migration and PLD2

The Transwell migration test was applied to assess the impact of PLD2 on neutrophil migration. Results indicated that the neutrophil migration count was higher in the mild group + PLD2 inhibitor group when compared to the mild group ($P < 0.05$). Similarly, the moderate group + PLD2 inhibitor group showed higher neutrophil migration compared to the moderate group ($P < 0.05$). The severe group + PLD2 inhibitor group displayed increased neutrophil migration compared to the severe group ($P < 0.05$). The addition of the PLD2 inhibitor significantly promoted neutrophil migration *in vitro* (Table 6).

DISCUSSION

An array of triggers, including the activation of pancreatic digestive enzymes, gallstone-related conditions, unhealthy dietary habits, alcohol misuse, and other factors damage to the pancreatic tissue, give rise to a series of symptoms and complications[13,14]. The severe manifestations of pancreatitis can result in significant complications including pancreatic necrosis, abdominal infections, multiple-organ failure, and potentially fatal outcomes[15-17]. Amidst this backdrop, PLD2 emerges as a significant player. Belonging to the phospholipase group, PLD2 facilitates the cleavage of phosphatidylcholine into phosphatidylcholine acid on cellular membranes. This process generates hemolytic phosphatidylcholine and diacylglycerophosphate. Of note, PLD2 wields substantial influence across diverse biological processes encompassing cell growth, signal transmission, cellular motility, and inflammatory responses. There is growing indication that PLD2 could play a regulatory part in the pathogenesis and progression of acute pancreatitis[18]. Multiple studies indicated that PLD2 might influence inflammatory response regulation, activation of immune cells, and cell migration. Consequently, it holds the potential to impact the progression of acute pancreatitis[19,20]. Over the past few years, PLD2 has garnered significant attention as a regulator of inflammation[21].

To assess the severity of the disease, we categorized the patients into mild, moderate, and severe groups based on their Ranson score. Significant differences in PLD2 protein expression were observed among patients with varying degrees of acute pancreatitis, particularly in the severe group. This observation indicated that PLD2 might exert a detrimental regulatory function in the inflammatory process. Alternatively, its expression could be suppressed due to inflammation. Furthermore, Pearson correlation coefficient analysis revealed a negative correlation between acute pancreatitis severity and PLD2 expression. The identification highlighted a possible control mechanism of PLD2 in the pathological

Table 5 *In vitro* migration test of neutrophils

Group	Number of neutrophil migration (*n*)
Mild group (*n* = 25)	235.62 ± 23.15
Moderate group (*n* = 30)	568.39 ± 49.37
Severe group (*n* = 35)	856.27 ± 54.28
Variance ratio	13.226
P value	0.015

Table 6 *In vitro* migration test of neutrophils

Group	Number of neutrophil migration (*n*)
Mild group (*n* = 25)	212.24 ± 24.66
Mild group + PLD2 inhibitor group (*n* = 25)	473.69 ± 35.17
Moderate group (*n* = 30)	536.19 ± 44.58
Moderate group + PLD2 inhibitor group (*n* = 30)	755.03 ± 50.19
Severe group (*n* = 35)	828.61 ± 58.21
Severe group + PLD2 inhibitor group (*n* = 35)	1126.75 ± 64.38
Variance ratio	16.204
P value	0.001

PLD2: Phospholipase D2.

progression of acute pancreatitis. The inverse relationship indicated that PLD2 might play a specific role in suppressing the inflammatory response, and its reduced expression might contribute to aggravated inflammation.

Substantial increases in mRNA expression of neutrophil chemokine receptors (such as *CXCR1*, *CXCR2*, *CCR2*, and *CCR5*) were observed in severe acute pancreatitis patients. This phenomenon might signify active immune cell infiltration regulation triggered by inflammation during the course of acute pancreatitis. Receptors CXCR1 and CXCR2 linked with chemokine IL-8 significantly influence inflammation and the immune responses[22]. Compared with similar studies, this study found that receptors CCR2 and CCR5 are tied to chemokines CCL2 and CCL5 playing a regulatory function in inflammation and immunity[23]. This increased receptor expression could represent the body's response to inflammatory signals, resulting in neutrophil accumulation at inflammation sites and amplification of the inflammatory response[24]. Although increased receptor expression in severe patients could indicate a natural immune response, it might also lead to excessive neutrophil migration, aggravating inflammatory and tissue damage. This aligned with the pathophysiological mechanism of acute pancreatitis, characterized by cell infiltration and inflammation-induced tissue damage. Hence, increased chemokine receptor expression in neutrophils could significantly impact the pathological progression of acute pancreatitis. The escalation of inflammatory markers additionally reinforced the pathological features of acute pancreatitis. CRP, TNF-α, IL-1β, and IL-6 are common indicators of acute inflammation, and their rise is strongly associated with the inflammatory response. This surge in inflammatory markers may reflect the immune system's reaction to inflammation, but it could also contribute to pancreatic tissue harm. Phosphatidylcholine specific PLD2 is a key signaling molecule involved in regulating various cellular processes, including cell survival, proliferation, and death. In pancreatitis, changes in the expression and activity of PLD2 may affect the fate of pancreatic cells[25]. The results indicated that PLD2 potentially regulated the pathological progression of pancreatitis. The decrease in PLD2 expression could potentially be associated with increased neutrophil chemokine receptor expression and elevated levels of inflammatory factors, consequently impacting neutrophil migration and the inflammatory process.

The pathways of antioxidant response transcription factors and key regulatory factors of inflammatory response play important roles in cellular response to oxidative stress and inflammation. During pancreatitis, PLD2 may affect the antioxidant capacity and inflammatory response of cells by regulating oxidative stress and inflammatory cyto-kine pathways, thereby affecting the activation of apoptosis pathways and intervening in the progression of inflammatory diseases[26]. The outcomes of the Transwell migration test strongly supported the significance of neutrophils in the progression of acute pancreatitis. Neutrophil migration plays a crucial role in the development and advancement of inflammation during acute pancreatitis. The observed disparities in the Transwell migration test results demonstrated the quantifiable changes in neutrophil migration across different severity groups. This indirectly reaffirmed the crucial involvement of neutrophil migration in the progression of acute pancreatitis. Simultaneously, the introduction of a PLD2 inhibitor led to an elevation in neutrophil movement across varying severity levels, potentially introducing a novel therapeutic intervention. The effect of PLD2 could be manifested through its capability to constrain neutrophil

movement. Given that extensive neutrophil movement can exacerbate the inflammatory response during acute pancreatitis, the involvement of PLD2 might partially inhibit this process, exerting a beneficial influence on disease progression. This investigation uncovered the clinical mechanism of PLD2 in acute pancreatitis by conducting a retrospective analysis, specifically emphasizing on its role in restricting neutrophil movement. We uncovered the crucial governing function of PLD2 in the development of acute pancreatitis, as well as its impact on both neutrophil movement and the generation of inflammatory agents. Consequently, this deeper understanding of acute pancreatitis can offer valuable insights for the formulation of future therapeutic strategies involving PLD2.

CONCLUSION

In summation, this research substantiated the indispensable role of PLD2 in the pathological progression of acute pancreatitis. Moreover, the varying protein expression levels of PLD2 among patients with distinct degrees of acute pancreatitis exhibited an inverse relationship with disease severity. Moreover, this study revealed PLD2's potential to mitigate acute pancreatitis by restricting neutrophil movement. PLA2 is an early biomarker for a series of complications in severe pancreatitis, and has important clinical significance for evaluating pancreatitis and various organ injuries. If the research results of PLD2 are applied to patient treatment in this study, it will help to develop more targeted nursing strategies for patients with pancreatitis. This study has some limitations, such as a small sample size and lack of regional representativeness. In subsequent research, multi center large sample studies will be organized to improve the credibility of the research results.

FOOTNOTES

Author contributions: Niu JW and Li CF contributed to the conception and design of the study; Niu JW, Zhang GC, Ning W, Liu HB, Yang H, and Li CF participated in the clinical practice, including diagnosis, treatment, consultation and follow up of patients; Zhang GC and Ning W contributed to the acquisition of data; Liu HB and Yang H contributed to the analysis of data; Niu JW wrote the manuscript; Li CF revised the manuscript. All authors approved the final version of the manuscript.

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ORIGINAL ARTICLE

Retrospective Study

Imaging characteristics of hypervascular focal nodular hyperplasia-like lesions in patients with chronic alcoholic liver disease

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Abstract

BACKGROUND

Focal nodular hyperplasia (FNH)-like lesions are hyperplastic formations in patients with micronodular cirrhosis and a history of alcohol abuse. Although pathologically similar to hepatocellular carcinoma (HCC) lesions, they are benign. As such, it is important to develop methods to distinguish between FNH-like lesions and HCC.

AIM

To evaluate diagnostically differential radiological findings between FNH-like lesions and HCC.

METHODS

We studied pathologically confirmed FNH-like lesions in 13 patients with alcoholic cirrhosis [10 men and 3 women; mean age: 54.5 ± 12.5 (33-72) years] who were negative for hepatitis-B surface antigen and hepatitis-C virus antibody and underwent dynamic computed tomography (CT) and magnetic resonance imaging (MRI), including superparamagnetic iron oxide (SPIO) and/or gadoxetic acid-enhanced MRI. Seven patients also underwent angiography-assisted CT.

RESULTS

The evaluated lesion features included arterial enhancement pattern, washout appearance (low density compared with that of surrounding liver parenchyma), signal intensity on T1-weighted image (T1WI) and T2-weighted image (T2WI), central scar presence, chemical shift on in- and out-of-phase images, and uptake

pattern on gadoxetic acid-enhanced MRI hepatobiliary phase and SPIO-enhanced MRI. Eleven patients had multiple small lesions (< 1.5 cm). Radiological features of FNH-like lesions included hypervascularity despite small lesions, lack of "corona-like" enhancement in the late phase on CT during hepatic angiography (CTHA), high-intensity on T1WI, slightly high- or iso-intensity on T2WI, no signal decrease in out-of-phase images, and complete SPIO uptake or incomplete/partial uptake of gadoxetic acid. Pathologically, similar to HCC, FNH-like lesions showed many unpaired arteries and sinusoidal capillarization.

CONCLUSION

Overall, the present study showed that FNH-like lesions have unique radiological findings useful for differential diagnosis. Specifically, SPIO- and/or gadoxetic acid-enhanced MRI and CTHA features might facilitate differential diagnosis of FNH-like lesions and HCC.

Key Words: Focal nodular hyperplasia; Alcoholic liver disease; Hepatocellular carcinoma; Magnetic resonance imaging; Liver

Core Tip: Two enhancement patterns were observed for the hepatobiliary phase on gadoxetic acid-enhanced magnetic resonance imaging (MRI): Heterogeneous hyperintense (43%) and ring-like enhancement (57%), and all lesions exhibited a marked homogeneous uptake pattern on superparamagnetic iron oxide-enhanced MRI in patients with focal nodular hyperplasia-like lesions. This finding is of clinical relevance because it is useful for the differential diagnosis of hypervascular liver nodules in patients with chronic liver disease.

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INTRODUCTION

Focal nodular hyperplasia (FNH)-like lesions were initially reported by Gluud *et al*[1] as hyperplastic formations in patients with micronodular cirrhosis and a history of alcohol abuse. These lesions, now widely identified in patients with alcoholic liver cirrhosis, are histopathologically similar to FNH in non-cirrhotic livers[2-7]. However, FNH-like lesions further exhibit unique vascular characteristics, including hypervascularity and sinusoidal capillarization[7], which are also common in hepatocellular carcinoma (HCC). Conversely, unlike HCC, FNH-like lesions are generally considered benign and non-progressive, particularly following alcohol abstinence. Despite these findings, FNH-like lesions are not yet classified in major diagnostic guidelines, such as those of the International Working Party[8] or the World Health Organization (WHO)[9], presenting challenges in diagnosis and clinical management. Clinically, the differentiation between FNH-like lesions and HCC is essential as the treatment approach differs significantly. While HCC commonly requires aggressive treatment options, including surgery, ablation, or systemic therapies, FNH-like lesions may not require any interventions, making an accurate diagnosis crucial for appropriate patient care. The current literature on FNH-like lesions primarily addresses their pathology, and there are relatively few studies on their imaging characteristics, making non-invasive diagnosis challenging.

This study aimed to address these gaps by examining the radiological features of FNH-like lesions in alcoholic liver cirrhosis using dynamic computed tomography (CT), superparamagnetic iron oxide (SPIO), and gadoxetic acid-enhanced magnetic resonance imaging (MRI). By identifying specific imaging criteria, we aimed to support non-invasive differentiation of FNH-like lesions from early-stage HCC, potentially reducing the need for invasive biopsy and providing clearer diagnostic pathways for clinicians.

MATERIALS AND METHODS

Patients

This retrospective study was approved by the institutional ethics committee of Kindai University, Faculty of Medicine (ethical identification No. 27-067). All patients provided informed consent for the use of their CT and MRI, as well as their biopsy specimens. The study protocol conformed to the ethics guidelines of the 2002 Declaration of Helsinki.

This study enrolled 13 patients with pathologically confirmed FNH-like lesions who underwent dynamic CT, unenhanced MRI, and SPIO- and/or gadoxetic acid-enhanced MRI between 2000 and 2020. In all the patients, liver cirrhosis was determined through clinical examination and blood chemistry tests (for aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, albumin, and globulin). None of the patients included in this study had

comorbidities such as diabetes, hypertension, or metabolic syndrome, as verified by assessment of their medical histories and clinical examinations. Further, each patient had at least one FNH-like nodule pathologically diagnosed through biopsy, with the remaining lesions being diagnosed based on clinical findings indicating no decrease in size after abstinence from alcohol during the 1-year follow-up.

Imaging technique

Plain and three-phase contrast-enhanced CT (CE-CT) scans were obtained in a craniocaudal direction using Sensation 64 CT (Siemens, Erlangen, Germany) or Aquilion multi 64 (Canon Medical System, Otawara, Japan) scanners. Routine scanning was conducted at a 5-mm section thickness and 5-mm scan increment. Subsequently, the scans were reconstructed with a 2-mm thickness using a routine abdominal algorithm. An injector system was used to intravenously administer 100-150 mL non-ionic contrast media (Iopamiron®; 300/370 mgI/mL; Bayer Schering Pharma, Berlin, Germany) at a rate of 3–3.5 mL/s.

All MRIs were performed on a 1.5-T system using the Magnetom Avanto (Siemens Healthcare, Erlangen, Germany) or Signa HDxt (GE Healthcare, Waukesha, WI, United States) scanner. Baseline MRI were acquired using a respiratory-triggered T2-weighted turbo spin-echo (TSE) sequence, breath-hold T2*-weighted gradient-echo (GRE) imaging with steady-state precession (FISP) sequence, and breath-hold T1-weighted GRE sequence. All images were obtained in the transaxial plane, using a phased-array multi-coil. For all sequences, a 7-mm slice thickness was used, with a 10% intersection gap and a field of view of 35-40 cm, depending on the liver size. SPIO-enhanced MRI comprised the respiratory-triggered T2-TSE sequence, breath-hold T2*-weighted FISP sequence, and breath-hold T1-weighted GRE sequence, with the same parameters as those used in baseline MRI. Fercarbotran (Resovist®; Bayer Yakuhin, Osaka, Japan) at a dose of 8.0 μmol iron per kg (body weight) was manually injected as a rapid bolus through a filter with 5-μm pore size, which was immediately followed by a 10 mL saline solution flush. Imaging was then performed after 7 minutes. Gadoxetic acid-enhanced MRI comprised dynamic images constructed using fat-suppressed T1-weighted GRE images obtained before (pre-contrast) as well as 14-30 seconds (arterial phase), 70 seconds, and 3 minutes after intravenous administration of 0.025 mmol of gadoxetic acid (Primovist®, Bayer Schering Pharma, Berlin, Germany) per kg (body weight) at a rate of 2.0 mL/s, followed by a 20 mL saline flush. Hepatobiliary phase (HBP) images were obtained 20 minuntes after gadoxetic acid injection.

All CT during arterial portography and double-phase CT during hepatic angiography (CTHA) examinations were performed using a unified angiography and CT system (Multistar Plus/Somatom Plus 4 Volume Zoom; Siemens, Erlangen, Germany). These images were subsequently obtained in a craniocaudal direction with 5-mm section thickness and 5-mm reconstruction intervals. For double-phase CTHA, first-phase data acquisition was started 5 seconds after second-phase data acquisition, which was performed 21-26 seconds after initiation of a transcatheter hepatic arterial injection of a fixed dose of 20 mL iohexol (Omnipaque 300, 300 mgI/mL; Daiichi-Sankyo Parma, Tokyo, Japan) diluted with 33% sterile water (iodine, 100 mg iodine/mL) at a rate of 2 mL/s.

Pathological analysis

All patients underwent ultrasound-guided core needle biopsy, following which the liver samples were fixed in 10% neutral-buffered formalin, routinely processed for paraffin embedding, sectioned (4 μm), and stained with hematoxylin and eosin or Masson trichrome (Muto Pure Chemicals, Tokyo, Japan) according to standard procedures. Immunohistochemical analysis for OATP8 and CD68 antigens were performed on formalin-fixed, paraffin-embedded specimens extracted from seven patients. The stained sections were evaluated under a microscope. The expressions of OATP8 and CD68 antigen were typically assessed by determining the intensity and localization of staining within hepatocytes. The intensity was graded semi-quantitatively (as either none, weak, moderate, or strong), and the localization was confirmed as predominantly cytoplasmic and/or membrane-associated, depending on the known expression pattern of OATP8. The percentage of positively stained cells was also recorded.

Image analysis

Two radiologists with > 10 years of experience evaluated the following lesion characteristics: Number and size (largest axial diameter) of each lesion; dynamic enhancement pattern on dynamic CT and CTHA (1 and 2) for qualitative imaging analysis; and other MRI features (3-8) as follows: (1) Arterial enhancement pattern of the dynamic CT and/or first phase of the CTHA; (2) Washout appearance (defined as low density compared with the density of the surrounding liver parenchyma) on the portal venous phase (PVP) of the dynamic CT and/or second phase of the CTHA; (3) Signal intensity on T1-weighted image (T1WI); (4) Signal intensity on T2-weighted image (T2WI); (5) Central scar (defined as a central T2 hyperintensity and/or T1 hypointensity representing fibrotic tissue); (6) Chemical shift (defined as a drop in signal intensity on out-of-phase images compared with that on in-phase images); (7) Uptake pattern on the HBP of the gadoxetic acid-enhanced MRI; and (8) Uptake pattern on the SPIO-enhanced MRI. Arterial enhancement was classified as homogeneous (peripherally or entirely) or heterogeneous. Signal intensity on T1WIs was classified as hypointensity, iso- or hyperintensity, or heterogeneous signal intensity. The signal intensity on T2WIs was classified as iso-intensity or hyperintensity. Uptake on the HBP of gadoxetic acid- and SPIO-enhanced MRI was classified as homogeneous (peripherally or entirely) or heterogeneous. In case of interobserver discordance, the radiologists re-evaluated the images and reached a consensus. Both radiologists were informed about the location of each lesion; however, they were blinded to the clinical information and final diagnosis.

Table 1 Summary of clinical findings

Patient No.	Age (years)	Sex	Background of liver failure	Child Pugh grade	AFP	PIVKA-II	Diagnosis	Lesion growth at imaging follow up
1	41	M	Alcoholic	A	6	23	FNB	N/A
2	33	F	Alcoholic	A	7	23	FNB	No growth
3	34	F	Alcoholic	B	4	10	FNB	Increased number
4	60	M	Alcoholic	B	9	20	FNB	No growth
5	58	M	Alcoholic	A	7	40	FNB	No growth
6	57	M	Alcoholic	B	6	19	FNB	N/A
7	53	M	Alcoholic	B	23[1]	33	FNB	No growth
8	65	M	Alcoholic	A	10	18	FNB	Increased number
9	63	M	Alcoholic	A	6	12	FNB	No growth
10	48	F	Alcoholic	B	11	18	FNB	No growth
11	55	M	Alcoholic	B	5	16	FNB	N/A
12	70	M	Alcoholic	A	5	22	FNB	No growth
13	72	F	Alcoholic	A	4	15	FNB	No growth

[1]Case 7 was only the patient with hepatocellular carcinomas.

PIVKA-II: Prothrombin induced by vitamin K deficiency or antagonist-II; FNB: Fine needle biopsy; AFP: Alpha-fetoprotein; N/A: Not available.

RESULTS

This study included 13 patients [10 men and 3 women; mean age: 54.5 ± 12.5 (33-72) years] with chronic liver failure due to alcoholic liver disease. Table 1 presents a summary of the clinical findings. All patients with FNH-like lesions were pathologically diagnosed using fine-needle biopsy. The levels of alpha-fetoprotein (AFP; ng/mL) and prothrombin induced by vitamin K deficiency or antagonist-II (mAU/mL) were normal in all patients with FNH-like lesions, except for one patient with HCC who showed elevated AFP (ng/mL) levels. Among the 10 patients with FNH-like lesions, 2 (20%) showed new lesions following abstinence from alcohol. The size of the FNH-like lesions remained stable or decreased in eight (80%) patients.

Table 2 presents the CT findings of the FNH-like lesions. Among the 13 patients with 26 FNH-like lesions, seven had a single lesion, while six had multiple lesions (two, one, and three patients had two, three, and over four lesions, respectively). The lesion size ranged from 10 mm to 30 mm (mean ± SD, 14.0 ± 5.5 mm). All 26 FNH-like lesions exhibited marked hypervascularity during the arterial phase on CE-CT and CTHA (seven patients) as well as washout appearance during the portal venous and equilibrium phases on CE-CT. Pathological examination revealed numerous unpaired arteries and sinusoidal capillarization in all patients, which were similar to those in HCC.

Table 3 presents the MRI findings of the FNH-like lesions. Among the 13 patients with FNH-like lesions, 11 (85%) showed high or iso-high intensity on T1WI, seven (54%) showed iso-high intensity on T2WI, six (46%) showed low intensity, three (23%) showed high-intensity central scars, and 10 (77%) showed no central scars on T2WI. Chemical shift artefacts were observed in all 13 patients with FNH-like lesions (Figures 1 and 2). In the six patients who underwent gadoxetic acid-enhanced MRI (Figures 3 and 4), all lesions exhibited marked homogeneous enhancement during the arterial phase. Two enhancement patterns were observed on the HBP: Heterogeneous hyperintense (n = 3, 43%) and ring-like enhancement (n = 4, 57%). In the eight patients who underwent SPIO-enhanced MRI, all lesions exhibited a marked homogeneous uptake pattern on post-SPIO-enhanced MRI (Figures 1 and 3).

DISCUSSION

We studied pathologically confirmed FNH-like lesions in 13 patients with alcoholic cirrhosis. The radiological features of FNH-like lesions included hypervascularity, despite small lesion sizes, lack of a "corona-like" enhancement in the late phase on CTHA, high-intensity on T1WI, slightly high- or iso-intensity on T2WI, no signal decrease in out-of-phase images, and complete SPIO uptake or incomplete/partial uptake of gadoxetic acid. There have been several recent reports of FNH-like lesions in cirrhotic livers[1-6]. Although there is currently no established definition for FNH-like lesions/nodules, FNH is known to develop in normal livers. Accordingly, FNH-like lesions refer to similar nodules observed in patients with chronic liver disease[7]. These lesions have been extensively reported in alcoholic liver disease[8-10]. Most of these hyperplastic lesions are highly vascular and relatively small; additionally, they are difficult to differentiate from HCC lesions, especially early-stage HCC lesions[7-12]. We included 13 patients with alcoholic liver disease and

Table 2 Summary of tumor characteristics, dynamic patterns assessed using computed tomography and computed tomography hepatic arteriography, and pathological findings

Patient No.	No. of nodules	Size	Arterial enhancement pattern	Washout appearance	Modality	Central scar	Unpaired arteries and capillarization	Liver cell atypia	Scar-like fibrosis
1	2	15	Hyper	No	CECT/CTHA	-	+	-	-
2	1	15	Hyper	No	CECT/CTHA	-	+	-	-
3	> 4	10-15	Hyper	No	CECT/CTHA	-	+	-	-
4	> 4	10-30	Hyper	No	CECT/CTHA	+	+	-	+
5	1	10	Hyper	No	CECT	-	+	-	-
6	3	10-30	Hyper	No	CECT/CTHA	-	+	-/+	-
7	1	15	Hyper	No	CECT/CTHA	+	+	-	+
8	1	10	Hyper	No	CECT	-	+	-	-
9	1	10	Hyper	No	CECT	-	+	-	-
10	> 4	10-15	Hyper	No	CECT/CTHA	-	+	-	-
11	2	15	Hyper	No	CECT	-	+	-	-
12	1	20	Hyper	No	CECT	-	+	-	-
13	1	14	Hyper	No	CECT	+	+	-	-

CT: Computed tomography; CECT: Contrast-enhanced computed tomography; CTHA: Computed tomography hepatic arteriography.

Table 3 Summary of magnetic resonance imaging characteristics and findings

Patient No.	T1-weighted images	T2-weighted images	Central scar on magnetic resonance imaging	Chemical shift	Uptake pattern on the HBP	Uptake pattern on the SPIO
1	High	Iso-high	No	No	N/A	Homogenous
2	High	Iso-high	No	No	N/A	N/A
3	High	Low	No	No	N/A	Homogenous
4	Iso-high	Low	Yes	No	N/A	Homogenous
5	High	Iso-high	No	No	Ring	Homogenous
6	Low-high	Low	No	No	N/A	Homogenous
7	High	Iso	Yes	No	N/A	Homogenous
8	High	Low	No	No	Ring/hetero	Homogenous
9	High	Iso-high	No	No	N/A	Homogenous
10	High	Iso-high	No	No	Hetero	N/A
11	High	Low	No	No	Ring	N/A
12	Iso	Low	No	No	Hetero	N/A
13	Iso-high	Iso-high	Yes	No	Ring	N/A

SPIO: Superparamagnetic iron oxide; N/A: Not available.

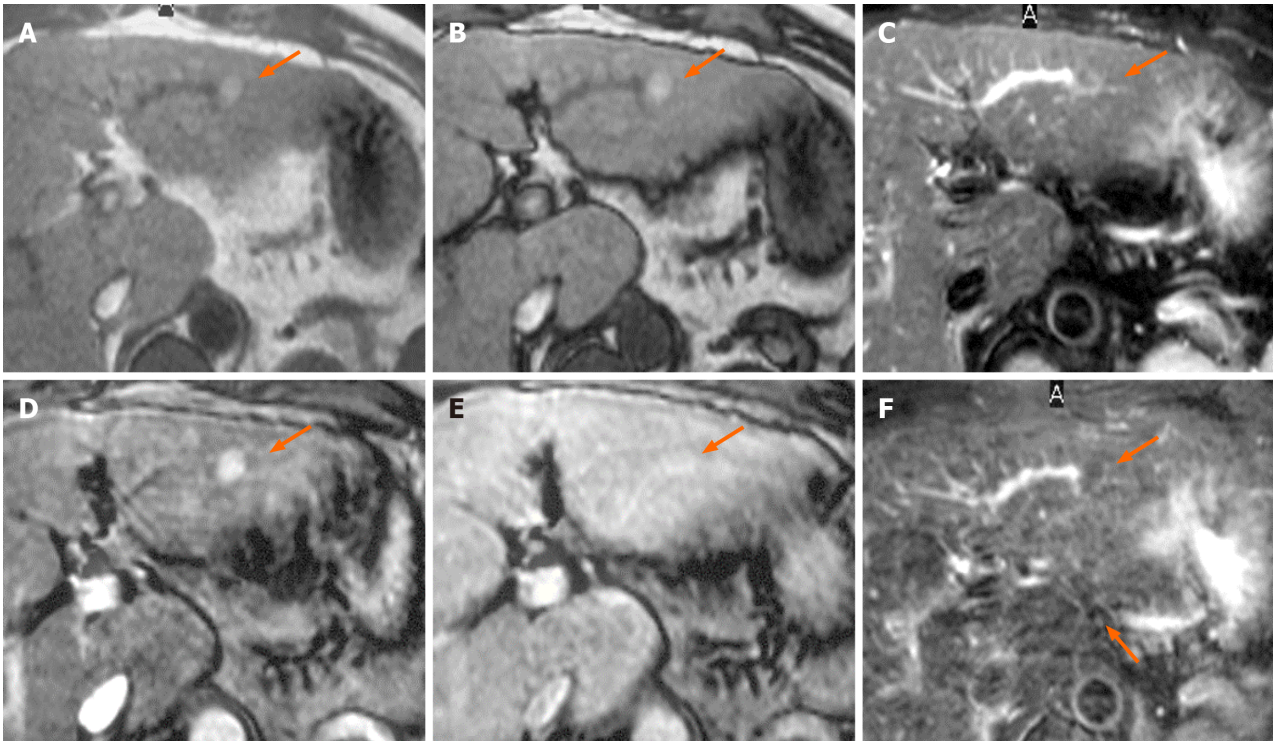

Figure 1 A 58-year-old man with alcoholic liver cirrhosis (patient No. 5). A and B: T1-weighted gradient-echo (GRE) magnetic resonance (MR) (A: In-phase; B: Opposed-phase) image shows a small and high-signal-intensity nodule (arrow) in the lateral segment; C: T2-weighted fast spin-echo MR imaging (MRI) shows iso-intensity nodule (arrow); D and E: Gadolinium-enhanced T1-weighted GRE MRI obtained during the arterial- and portal venous phase reveals a nodule with arterial phase hyperenhancement (arrow) and without washout (arrow); F: The superparamagnetic iron oxide (SPIO)-enhanced T2*-weighted GRE MRI shows the lesion as a low-signal intensity nodule (arrow) with SPIO uptake compared with the surrounding liver parenchyma.

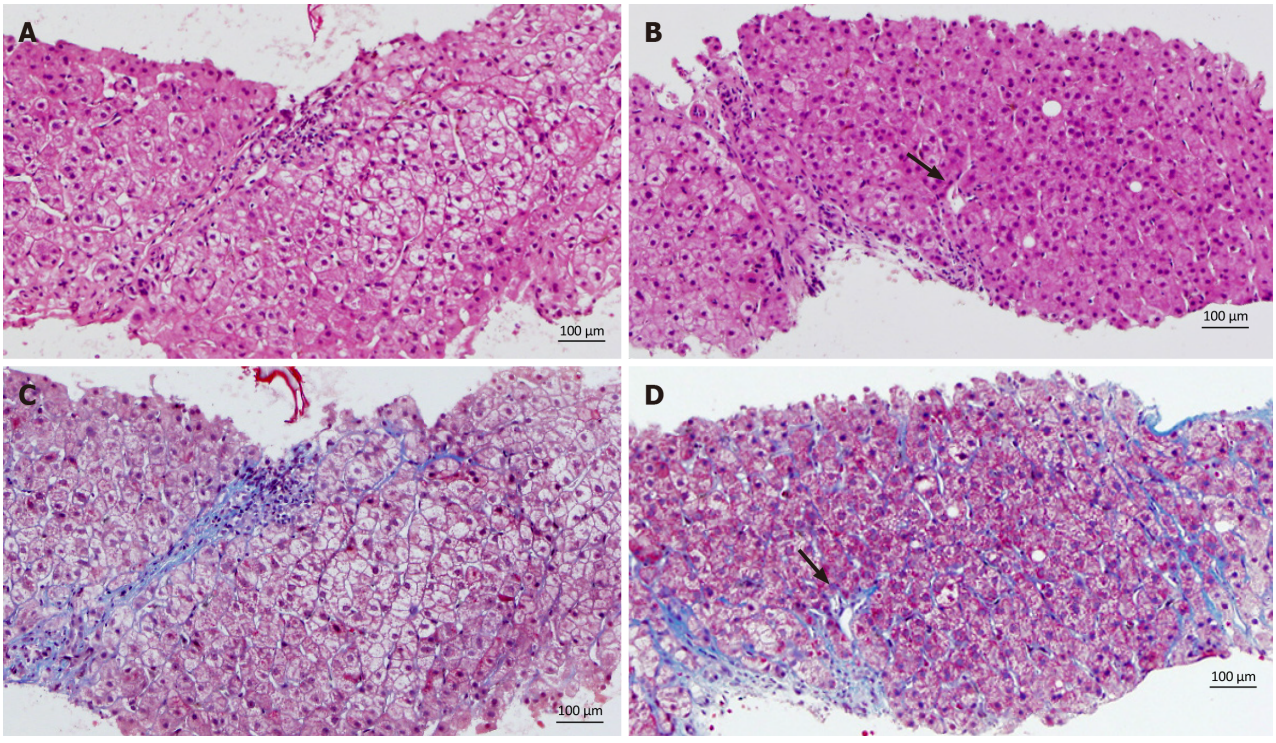

Figure 2 Histopathological features of the non-nodular and nodular portions. A: Non-nodular portion showing chronic hepatitis with pericellular fibrosis and diffuse capillarization of sinusoids, likely due to alcoholic injury. Mild inflammatory activity and moderate fibrosis are observed (hematoxylin and eosin staining, × 100); B: Nodular portion displaying a hyperplastic nodule with a mild increase in cell density and pericellular fibrosis. Unpaired arteries (arrow) are present in the nodule. (Hematoxylin and eosin staining, × 100); C: Non-nodular portion highlighting pericellular fibrosis and sinusoidal capillarization (Masson trichrome staining, × 100); D: Nodular portion illustrating pericellular fibrosis and unpaired arteries (arrow) within the hyperplastic nodule (Masson trichrome staining, × 100).

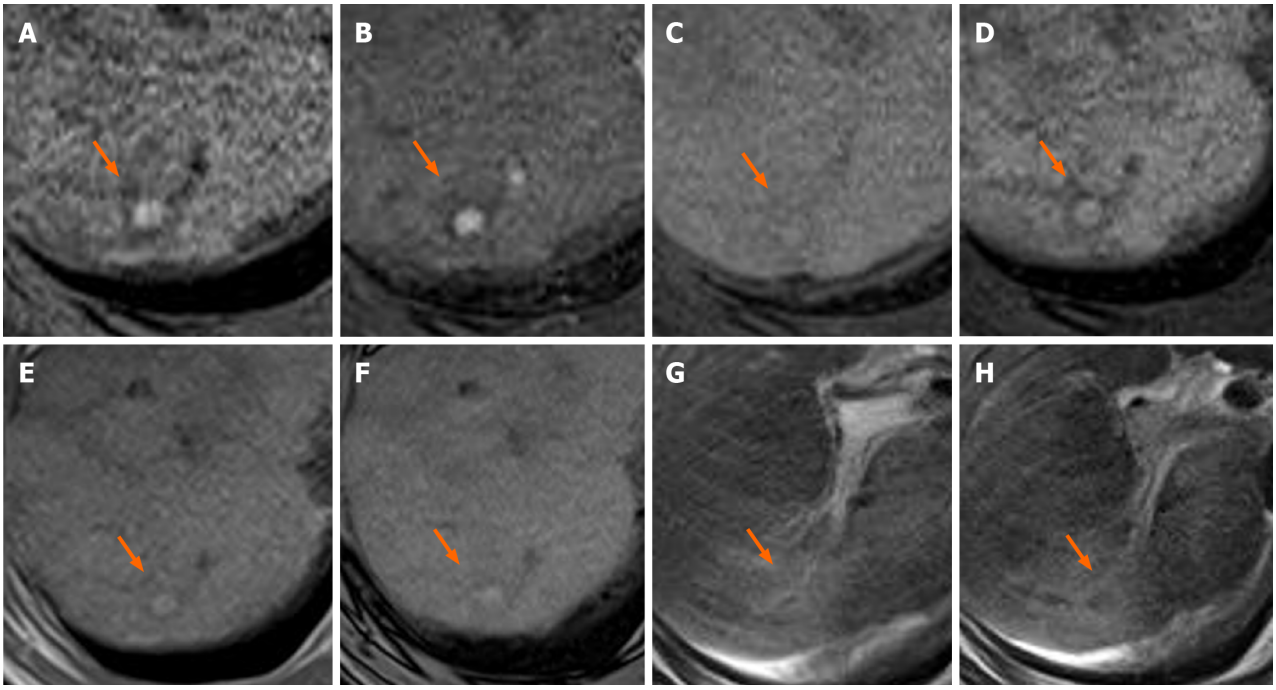

Figure 3 A 65-year-old man with alcoholic liver cirrhosis (patient No. 8). A and B: T1-weighted gradient-echo (GRE) magnetic resonance (MR) (A: In-phase; B: Opposed-phase) image shows a small and high-signal-intensity nodule (arrow) in segment 7 of the liver; C: Pre-contrast T1-weighted GRE MR imaging (MRI) shows iso-slightly high-intensity nodule (arrow); D and E: Gadoxetic acid-enhanced T1-weighted GRE MRI obtained during the arterial-and portal venous phase reveal a nodule with arterial phase hyperenhancement (arrow) and without washout (arrow); F: The hepatobiliary phase of the gadoxetic acid-enhanced T1-weighted GRE MRI shows homogeneous high-intense uptake (arrow); G: T2*-weighted GRE MRI shows iso-intensity nodule (arrow); H: The superparamagnetic iron oxide (SPIO)-enhanced T2*-weighted GRE MRI shows the lesion as a low-signal intensity nodule (arrow) with SPIO uptake compared with the surrounding liver parenchyma.

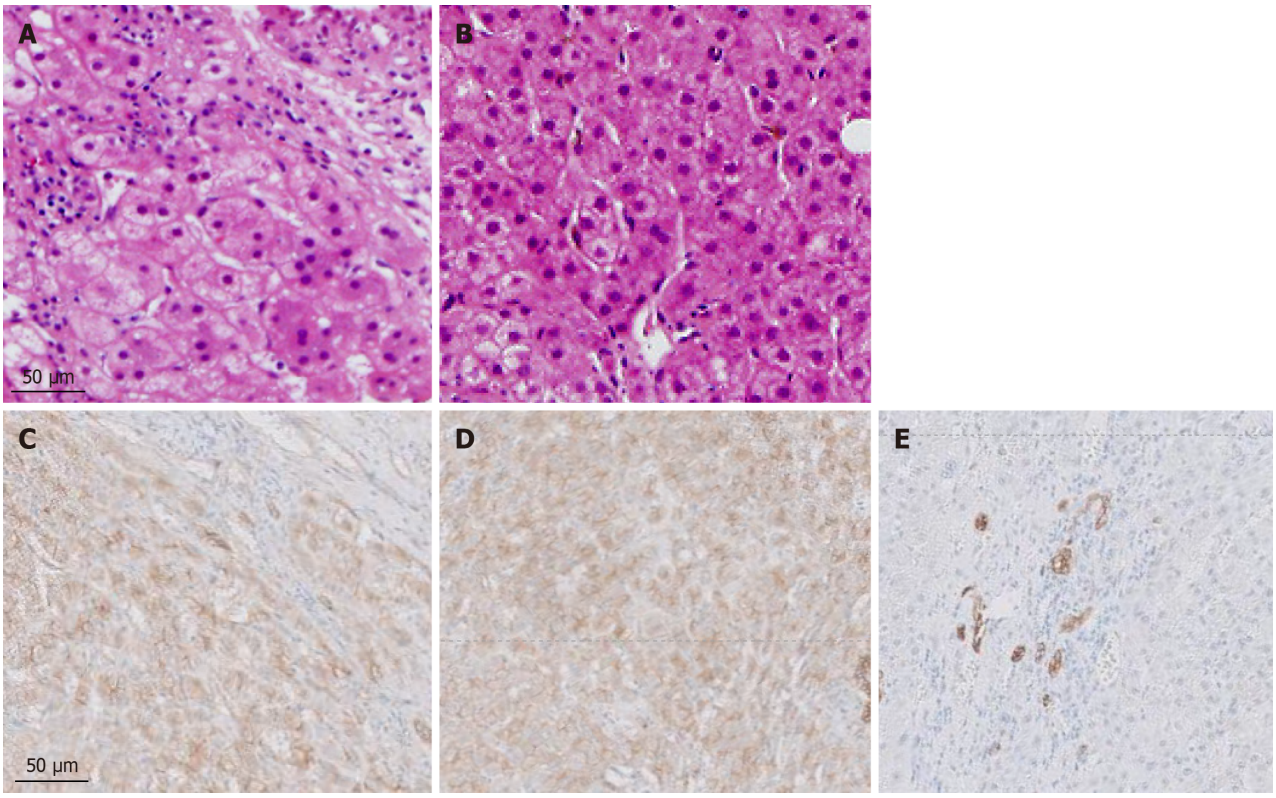

Figure 4 Histopathological and Immunohistochemical features of the non-nodular and nodular portions (A, C: Non-nodular, B, D, E: Nodular). A and B: Cell density, iron deposits, and sinusoidal capillarization in the surrounding liver tissue and the focal nodular hyperplasia (FNH)-like nodule. The

FNH-like nodule shows increased cell density (A: hematoxylin and eosin staining × 400); C and D: OATP8 (red-orange color) is expressed on the cellular membrane of hepatocytes at the sinusoidal side. The expression of OATP8 is nearly absent in the nodule (B: × 400); however, it is diffusely found in the surrounding tissue (C: × 400). OATP8 is immunohistochemically detected using anti-OATP8; E: Immunohistochemical staining of CD68 antigen showing Kupffer cells. The staining of nodule CD68 antigen shows diffuse Kupffer cell infiltration.

hyperplastic liver lesions who were negative for hepatitis virus markers. Here, we discuss the imaging findings with respect to reported imaging characteristics of such lesions[13-20].

In our cases, except for a normal hepatic background and absence of a central scar, the pathological findings were consistent with those of FNH; accordingly, the patients were diagnosed with FNH-like lesions, based on the original text in the WHO classification (version 5)[9]. There has been no previous report on the radiographic findings of FNH-like lesions based on our exact definition since there remains no established definition. There were no differences in imaging diagnoses according to age or gender, as in previous reports. However, considering the similarities of the histopathology and imaging findings with those of FNH, it is highly likely that FNH-like lesions have been previously described as atypical FNH based on imaging findings. Approximately 80% of FNH cases are reported to be atypical on imaging[21]. Typical FNH on plain CT is characterized by hypo- to iso-attenuation, with homogeneous attenuation in early-phase contrast enhancement, and isoattenuation with the surrounding liver from the PVP to the equilibrium phase. On MRI, these lesions present as iso- to moderately hypointense on T1WIs, iso- to somewhat hyperintense on T2WIs, and iso- to somewhat hyperintense or ring-like hyperintense in the hepatocyte phase after gadoxetic acid-enhanced-MRI[13-22]. The central scar is generally hypointense on plain CT and early-phase contrast enhancement CT, with delayed enhancement into the equilibrium phase. Additionally, the central scar is hypointense on T1WIs and hyperintense on T2WIs; further, the region, including the surrounding area, has low uptake of gadoxetic acid during the hepatocyte phase. However, a central scar is only observed in approximately 50% of cases[23].

Single-level dynamic CTHA has indicated that blood flows from a dilated feeding artery inside the central scar; moreover, blood within the lesion directly flows into the hepatic vein *via* the fibrous septum and dilated veins at the margin of the lesion[24]. Grazioli *et al*[22] did not report the imaging characteristics of six nodules with hypointense enhancement; as such, whether there was a complete lack of enhancement currently remains unclear. Notably, the enhancement was relatively lower than that of the surrounding liver; moreover, the heterogeneity was similar to that in our cases; however, their nodules exhibited overall hypointense enhancement. In addition, similar to our cases, the nodule lacked a central scar. Furthermore, although there is heterogeneity in the signal intensity in the hepatocyte phase of typical FNH, which ranges from iso- to hyperintense enhancement, the mechanisms underlying these findings remain unclear. Moreover, the cause of the relatively low enhancement in the uptake area within the nodule remains unclear.

This study has several limitations. First, this retrospective study had inevitable selection bias. Additionally, all patients with FNH-like lesions were pathologically confirmed using fine-needle biopsy specimens rather than surgical specimens. Finally, there was no standard follow-up imaging of the FNH-like lesions; further, we did not describe the frequency of this phenomenon. Further large-scale multicenter studies using surgical specimens are warranted.

CONCLUSION

In conclusion, the present study showed that FNH-like lesions in chronic alcoholic liver disease demonstrate distinct imaging features, including small lesion size, hypervascularity, high signal on T1WIs, and characteristic uptake patterns on SPIO or gadoxetic acid-enhanced MRI. These features support a non-invasive differentiation from HCC, thereby reducing the need for invasive diagnostics and aiding appropriate clinical management. Overall, our findings provide valuable imaging criteria that can improve diagnostic accuracy and patient outcomes.

FOOTNOTES

Author contributions: Urase A, Tsurusaki M, Sofue K, Kozuki R, Kono A, Ishii K wrote the paper; Urase A and Tsurusaki M designed research; Tsurusaki M performed research; Urase A, Tsurusaki M, Sofue K, Kozuki R, Kono A contributed new reagents or analytic tools; Tsurusaki M analyzed data.

Institutional review board statement: This retrospective study was performed with the approval of the institutional ethics committee of Kindai University, Faculty of Medicine.

Informed consent statement: Informed consent for using their computed tomography and magnetic resonance images and biopsy specimens was obtained from all patients.

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Data sharing statement: Raw data used in this study are available from the corresponding author upon reasonable request.

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ORIGINAL ARTICLE

Retrospective Study

Predicting colorectal adenomatous polyps in patients with chronic liver disease: A novel nomogram

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Abstract

BACKGROUND

Colorectal polyps are commonly observed in patients with chronic liver disease (CLD) and pose a significant clinical concern because of their potential for malignancy.

AIM

To explore the clinical characteristics of colorectal polyps in patients with CLD, a nomogram was established to predict the presence of adenomatous polyps (AP).

METHODS

Patients with CLD who underwent colonoscopy at Tianjin Second People's Hospital from January 2020 to May 2023 were evaluated. Clinical data including laboratory results, colonoscopy findings, and pathology reports were collected. Key variables for the nomogram were identified through least absolute shrinkage and selection operator regression, followed by multivariate logistic regression. The performance of the model was evaluated using the area under the receiver

area under curve, as well as calibration curves and decision curve analysis.

RESULTS
The study enrolled 870 participants who underwent colonoscopy, and the detection rate of AP in patients with CLD was 28.6%. Compared to individuals without polyps, six risk factors were identified as predictors for AP occurrence: Age, male sex, body mass index, alcohol consumption, overlapping metabolic dysfunction-associated steatotic liver disease, and serum ferritin levels. The novel nomogram (AP model) demonstrated an area under curve of 0.801 (95% confidence interval: 0.756-0.845) and 0.785 (95% confidence interval: 0.712-0.858) in the training and validation groups. Calibration curves indicated good agreement among predicted and actual probabilities (training: χ^2 = 11.860, P = 0.157; validation: χ^2 = 7.055, P = 0.530). The decision curve analysis underscored the clinical utility of the nomogram for predicting the risk of AP.

CONCLUSION
The AP model showed reasonable accuracy and provided a clinical foundation for predicting the occurrence of AP in patients with CLD, which has a certain predictive value.

Key Words: Metabolic dysfunction associated steatotic liver disease; Fatty liver; Chronic liver disease; Colorectal adenomas; Hepatitis; Risk factors

Core Tip: This study is the first effort to develop and validate a predictive model for assessing the risk of adenomatous polyps in patients with chronic liver disease. Based on six risk factors, the developed adenomatous polyps model will function as an effective means of identifying and stratifying patients undergoing management for chronic liver disease, thereby helping to reduce the risk of colorectal cancer in these individuals.

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INTRODUCTION

Chronic liver disease (CLD) affects an estimated 1.5 billion individuals globally, necessitating diverse healthcare interventions owing to its multi-organ and systemic impact. Although the prevalence of viral hepatitis has been decreasing in high-income nations, CLD remains a significant healthcare burden[1,2]. Non-alcoholic fatty liver disease (NAFLD) has a worldwide prevalence of 30%, and has emerged as a major contributor to liver-related morbidity and mortality[3]. Colorectal cancer (CRC) is a highly malignant cancer of the gastrointestinal tract. As reported in the World Health Organization's 2020 Global Cancer Statistics, CRC ranks as the third most common cancer globally and is the second leading cause of cancer-related deaths[4]. The development of CRC occurs through a well-established "adenoma-cancer" sequence in which precursor lesions, primarily adenomatous colon polyps, undergo genetic and epigenetic alterations over a period of 10-15 years before progressing to CRC[5]. Potential risk factors for colorectal polyps include age, sex, smoking, alcohol consumption, high-fat diet, metabolic syndrome (MS), and gallbladder disease[6-8]. CLD has been reported to promote lesions in colorectal adenoma-carcinoma sequence[9]. Primary sclerosing cholangitis, NAFLD, and viral liver disease are linked to an increased likelihood of colorectal lesions, particularly adenomas and hyperplastic polyps[10-13]. However, the relationship between the contributing factors for colorectal polyps in patients with CLD and the potential impact of different CLD etiologies on colorectal polyp detection rates remains poorly understood. Effective predictive models for adenomatous polyps (AP) in patients with CLD are unavailable. Therefore, this study aimed to identify the risk factors for colorectal polyps in this population more efficiently to reduce the need for invasive colonoscopies.

MATERIALS AND METHODS

Study population

The study included participants recruited from individuals undergoing colonoscopy at Tianjin Second People's Hospital from January 2020 to May 2023. Initially, 1693 patients with CLD were enrolled. The exclusion criteria were as follows: 12 patients were excluded due to age (either aged < 18 years or > 80 years), 468 patients due to poor bowel preparation and incomplete pathological analysis, 115 patients with familial adenomatous polyposis or inflammatory bowel disease, and

86 patients due to a history of diseases such as acquired immunodeficiency syndrome, tuberculosis, and syphilis. In addition, 98 patients had incomplete medical records and laboratory tests; 20 were excluded because of a family history of CRC in first-degree relatives, and 24 were excluded because of unexplained liver disease or an unclear diagnosis. Finally, 870 patients were enrolled in the study: 347 without polyps, 274 with non-AP, and 249 with AP. Figure 1 illustrates the flowchart of the study design.

Data collection

Data regarding age, sex, smoking status, alcohol consumption, body mass index (BMI), hypertension, diabetes, and MS were obtained from the patients' medical records. Smoking was categorized as the intake of at least one cigarette daily for a minimum of one year, and alcohol consumption was defined as having at least one alcoholic drink per week over the course of one year. Blood samples were obtained from the participants after an eight-hour fasting period *via* venipuncture of the antecubital vein. The samples were analyzed using an automatic biochemical analyzer (Model 7180; HITACHI, Japan) to assess a range of biochemical parameters. All analyses were conducted in a single laboratory to ensure the methodological consistency and reliability of the results. The biochemical parameters evaluated comprised total high-density lipoprotein (HDL), low-density lipoprotein, cholesterol, triacylglycerol, fasting plasma glucose (FPG), serum total protein, serum albumin (ALB), platelet count, determine serum iron, serum ferritin (SF), transferrin, serum thyroid stimulating hormone (TSH), free T4, free T3, gamma-glutamyl transferase (GGT), aspartate aminotransferase, and alanine aminotransferase.

Liver disease etiology diagnosis

Population demographics and clinical data were extracted from the medical records of hospitals using standard protocols. All patients with CLD underwent routine examinations to identify common etiologies, such as hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, alcoholic liver disease (ALD), autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and drug-induced liver injury (DILI). Patients with HBV or HCV were identified through hepatitis B surface antigen testing and positive serum anti-HCV antibodies (confirmed by HCV RNA)[14,15]. ALD was defined as having a long-term history of alcohol consumption, typically exceeding 5 years, with an equivalent ethanol intake of ≥ 40 g/day for men and ≥ 20 g/day for women, or a history of heavy drinking within 2 weeks with an equivalent ethanol intake of > 80 g/day[16]. AIH was diagnosed based on simplified criteria that utilized autoimmune markers and liver biopsy[17]. PBC was diagnosed based on cholestatic liver biochemistry, autoimmune antibody reactivity, and/or liver biopsy[18]. DILI was diagnosed using the Roussel Uclaf Causality Assessment Method scale and/or liver biopsy[19]. Experienced radiologists performed abdominal ultrasonography using ultrasound scanners. The diagnosis of fatty liver disease was based on the presence of an enhanced hepatorenal echo, characterized by differences in echo patterns between the liver and kidney parenchyma, attenuation of the deep ultrasound beam, blurring of vascular structures, and a straightened hepatic vein lumen[20]. Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by the presence of hepatic steatosis alongside a cardiometabolic risk factor, without any identifiable cause: (1) BMI ≥ 23 kg/m^2; (2) Fasting serum glucose ≥ 5.6 mmol/L or 2-hour post-load glucose level ≥ 7.8 mmol/L or glycated hemoglobin ≥ 5.7% or on specific drug treatment; (3) Blood pressure ≥ 130/85 mmHg or specific drug treatment; (4) Plasma triglycerides ≥ 1.70 mmol/L or specific drug treatment; and (5) Plasma HDL < 1.0 mmol/L for men and < 1.3 mmol/L for women or specific drug treatment[21].

Diagnosis and classification of colorectal polyp

Experienced endoscopists performed the colonoscopies, and bowel preparation was performed on all study participants by adept gastroenterologists before the colonoscopies, utilizing a standardized protocol with an OLYMPUS equipment (Tokyo, Japan), the procedure aligned with the protocols for diagnostic colonoscopies[22]. The number of polyps, including both single and multiple polyps, were recorded. After detection during colonoscopy, all abnormalities were subjected to biopsy, with pathological confirmation conducted in accordance with contemporary clinical guidelines, specifically the World Health Organization Classification of Digestive Tumors: Fifth Edition[23]. Pathological diagnosis: Pathological assessments were performed by two seasoned pathologists, supported by at least one chief pathologist, to ensure rigorous examination and interpretation. Immunohistochemistry was used to stain the pathological specimens. Pathological findings from these examinations led to the categorization of polyps into adenomatous and non-adenomatous types based on their histological characteristics.

Statistical analysis

Statistical analyses were conducted using SPSS version 25.0 software. For the groups without polyps, AP, and non-AP, variables that adhered to or closely approximated a normal distribution were compared using one-way analysis of variance. Variables that did not satisfy the normality assumption were assessed using the Kruskal-Wallis test. Post-hoc comparisons were conducted using the least significant difference method and Tamhane's T2 tests. For the training and validation groups, normally distributed continuous data were analyzed with the *t*-test, whereas non-normally distributed data were evaluated using the Mann-Whitney *U* test. Descriptive statistics are presented as mean ± SD for variables with normal distribution and as median with interquartile range (25th-75th percentile) for those with non-normal distribution. All count data are presented as the number of subjects and percentages, with comparisons made using the χ^2 test. Bonferroni correction was implemented as multiple pairwise comparisons where necessary. This predictive model was developed using R Software v.4.0.2. Initially, the least absolute shrinkage and selection operator regression method was used to filter variables and select predictors. Subsequently, a multivariate logistic regression was used for further variable selection. The nomogram was constructed using the "rms" package in R Software. Statistical significance was set at P <

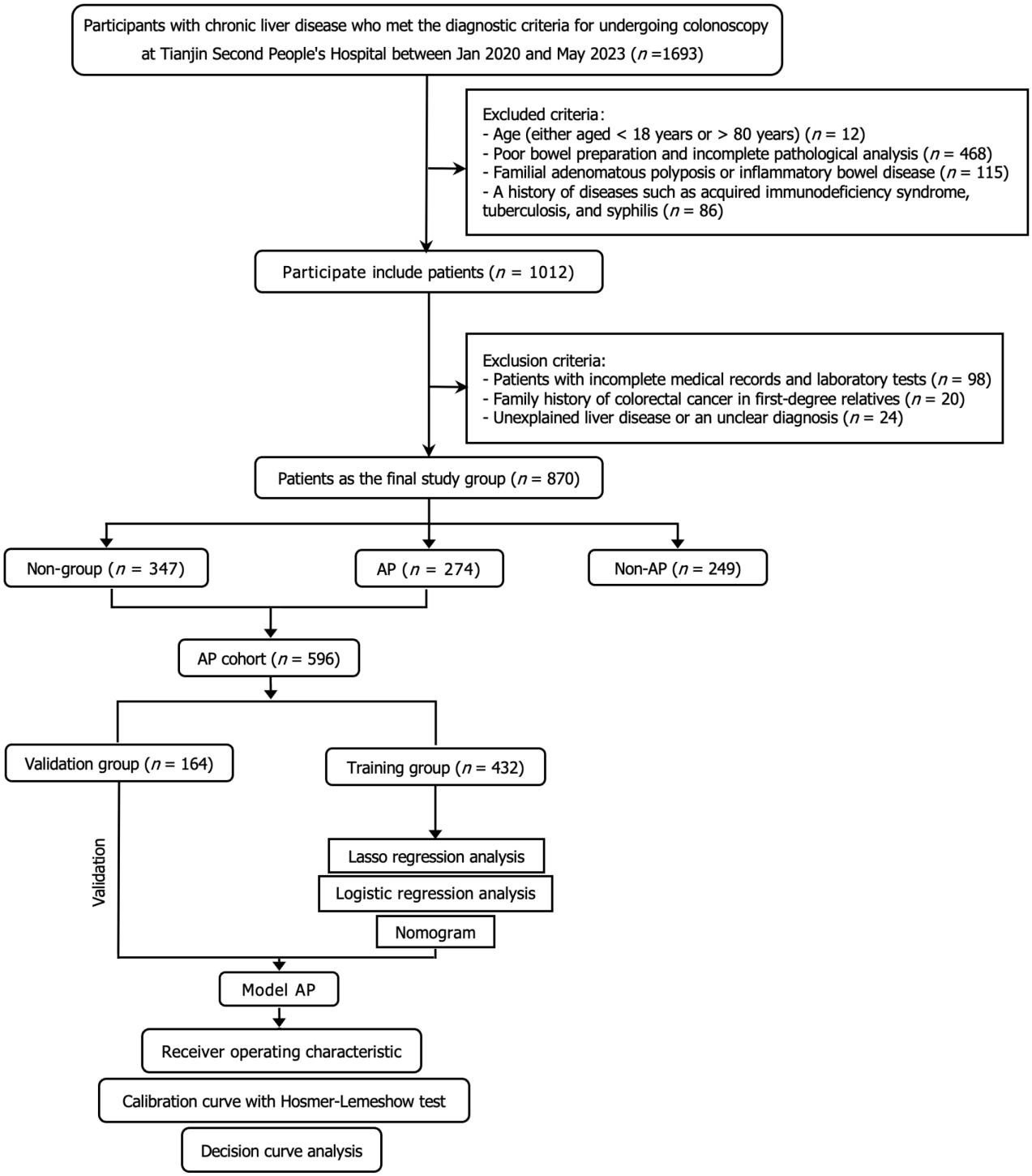

Figure 1 Flowchart of this study. AP: Adenomatous polyps.

0.05.

RESULTS

Baseline demographics and laboratory findings

A total of 870 participants were included in this study, of whom 347 (39.9%) were diagnosed with non-polyps, 249 (28.6%) with AP, and 274 (31.5%) with non-adenomatous (including hyperplastic and inflammatory) polyps. The cohort had an average age of 53.21 ± 10.67 years. The detection rate of AP in male patients was 32.9% (160/486), whereas that in female patients was 22.1% (85/384). Compared to the non-polyp group, the AP group showed a higher proportion of men, older age, elevated serum iron and SF levels, higher BMI, and greater proportions of alcohol consumption and smoking. Significant differences were found in hypertension, MS, and FPG, HDL, ALB, TSH, and GGT levels ($P < 0.05$). However,

Table 1 Clinical and laboratory data of patients with chronic liver disease

Variables	Non-polyp (*n* = 347)	Colorectal polyp (*n* = 523)		*F*/*H*/χ^2	*P* value
		Adenomatous (*n* = 249)	Non-adenomatous (*n* = 274)		
Male, *n* (%)	160 (46.1)[a]	164 (65.9)	162 (59.1)	24.67	< 0.001
Age (year), mean ± SD	50.8 ± 11.3[a]	56.08 ± 10.0	53.6 ± 9.6[a]	18.662	< 0.001
BMI (kg/m²), mean ± SD	25.05 ± 3.92[a]	26.50 ± 3.80	25.39 ± 3.72[a]	10.846	< 0.001
Alcohol, *n* (%)	71 (20.5)[a]	108 (43.4)	67 (24.5)[a]	40.41	< 0.001
Smoking, *n* (%)	88 (25.4)[a]	105 (42.2)	81 (29.6)[a]	19.676	< 0.001
Diabetes, *n* (%)	79 (23.0)	74 (29.7)	72 (26.4)	3.4	0.183
Hypertension, *n* (%)	80 (23.1)[a]	74 (29.7)	73 (26.6)[a]	8.632	0.013
MS, *n* (%)	45 (13.0)[a]	51 (20.5)	61 (22.3)	10.343	0.006
Cirrhosis, *n* (%)	172 (49.6)	126 (46.0)	113 (45.4)	1.272	0.529
FPG (mmol/L), mean ± SD	5.9 ± 1.6[a]	6.4 ± 2.5	6.1 ± 1.7	4.017	0.018
HDL (mmol/L), mean ± SD	1.3 ± 0.4[a]	1.2 ± 0.3	1.3 ± 0.5[a]	4.103	0.017
CHO (mmol/L), median (IQR)	4.56 (3.88, 5.39)	4.51 (3.78, 5.35)	4.51 (3.79, 5.63)	1.056	0.59
TG (mmol/L), mean ± SD	1.3 ± 1.2	1.4 ± 0.6	1.4 ± 1.2	0.579	0.561
LDL (mmol/L), mean ± SD	2.8 ± 0.8	2.9 ± 0.9	2.9 ± 1.0	1.745	0.175
PLT (10^9/L), median (IQR)	161.00 (103.00, 203.00)	153.00 (93.00, 213.00)	168.50 (112.00, 231.25)	5.841	0.054
TP (g/L), mean ± SD	71.7 ± 7.7	71.3 ± 8.1	70.4 ± 8.4	1.964	1.141
ALB (g/L), mean ± SD	41.4 ± 5.8	39.9 ± 6.5	40.2 ± 6.0	4.148	0.016
SI (μmol/L), mean ± SD	20.8 ± 9.4[a]	23.6 ± 11.7	24.5 ± 13.6[a]	11.607	< 0.001
SF (μg/L), median (IQR)	125.00 (60.00, 247.00)[a]	215.00 (101.00, 356.50)	174.00 (79.75, 363.50)[a]	29.928	< 0.001
TRF (g/L), median (IQR)	2.55 (2.22, 3.17)	2.57 (2.20, 3.17)	2.56 (2.31, 3.22)	0.3	0.985
TSH (mIU/L), mean ± SD	2.6 ± 3.5[a]	2.1 ± 1.3	2.2 ± 1.7[a]	4.5	0.011
FT4 (pmol/L), mean ± SD	16.7 ± 13.0	17.6 ± 7.0	19.7 ± 9.8	2.829	0.06
FT3 (pmol/L), mean ± SD	4.9 ± 1.3	5.0 ± 0.7	5.1 ± 1.7	0.955	0.382
ALT (IU/L), median (IQR)	30.00 (18.3, 65.3)	37.00 (21.4, 70.10)	34.85 (20.55, 72.60)	1.087	0.336
AST (IU/L), median (IQR)	31.80 (21.70, 62.20)	36.70 (23.30, 68.77)	34.85 (22.00, 70.62)	3.411	0.182
GGT (IU/L), median (IQR)	52.90 (23.00, 96.50)[a]	57.60 (28.60, 142.00)	59.00 (25.97, 189.07)	7.632	0.022

[a]*P* < 0.05 *vs* adenomatous polyp.

BMI: Body mass index; MS: Metabolic syndrome; FPG: Fasting plasma glucose; HDL: High-density lipoprotein; CHO: Cholesterol; TG: Triacylglycerol; LDL: Low-density lipoprotein; PLT: Platelet count; TP: Total protein; ALB: Albumin; SI: Serum iron; SF: Serum ferritin; TRF: Transferrin; TSH: Thyroid-stimulating hormone; FT3: Free T3; FT4: Free T4; ALT: Alanine aminotransferase; AST: Aspartate transaminase; GGT: Gamma-glutamyl transferase; IQR: Interquartile range.

no significant differences were observed between non-adenomatous and AP regarding MS, FPG, ALB, and GGT. Additionally, no significant correlations were found among the three groups with respect to diabetes, liver cirrhosis, and cholesterol, triacylglycerol, low-density lipoprotein, platelet count, serum total protein, transferrin, free T4, free T3, alanine aminotransferase, and aspartate aminotransferase levels ($P > 0.05$) (Table 1).

Baseline features of the training and validation groups

In total, 596 eligible patients, including those in the non-polyp and AP groups, were enrolled in the AP cohort, which had an average age of 53.02 years. 249 patients were identified as having AP: 40.5% and 45.1% in the training and validation groups. No significant differences were found between the training and validation groups according to the statistical analysis ($P > 0.05$) (Table 2).

Table 2 Baseline characteristics of adenomatous polyp cohort in training and validation group

Variables	Total (*n* = 596)	Training group (*n* = 432)	Validation group (*n* = 164)	$\chi^2/t/Z$	*P* value
Male, *n* (%)	324 (54.4)	90 (54.9)	234 (54.2)	0.024	0.876
Age (year), mean ± SD	53.02 ± 11.14	53.04 ± 11.24	52.96 ± 10.91	0.089	0.929
BMI (kg/m²), mean ± SD	25.66 ± 3.94	25.78 ± 3.94	25.31 ± 3.93	1.323	0.186
Alcohol, *n* (%)	179 (30.0)	127 (29.4)	52 (31.7)	0.302	0.583
Smoking, *n* (%)	193 (32.4)	145 (33.6)	48 (29.3)	1.002	0.317
Diabetes, *n* (%)	154 (25.8)	120 (27.8)	34 (20.7)	3.080	0.079
Hypertension, *n* (%)	165 (27.7)	120 (27.8)	45 (27.4)	0.007	0.934
MS, *n* (%)	96 (16.1)	69 (16.0)	27 (16.5)	0.021	0.884
Cirrhosis, *n* (%)	285 (47.8)	215 (49.8)	70 (42.7)	2.392	0.122
FPG (mmo/L), mean ± SD	6.15 ± 2.07	6.23 ± 2.16	5.93 ± 1.85	-1.573	0.116
HDL (mmo/L), mean ± SD	1.33 ± 0.40	1.33 ± 0.41	1.33 ± 0.38	0.105	0.916
CHO (mmo/L), median (IQR)	4.54 (3.82, 5.38)	4.57 (3.81, 5.40)	4.41 (3.85, 5.41)	-0.714	0.458
TG (mmo/L), mean ± SD	1.42 ± 1.12	1.44 ± 1.22	1.35 ± 0.82	-0.826	0.409
LDL (mmo/L), mean ± SD	2.81 ± 0.90	2.75 ± 0.82	2.83 ± 0.93	-0.979	0.382
TP (g/L), mean ± SD	71.54 ± 7.98	71.29 ± 7.76	72.19 ± 8.51	1.239	0.216
ALB (g/L), mean ± SD	40.75 ± 6.14	40.83 ± 6.07	40.52 ± 6.34	-0.551	0.582
SI (μmol/L), mean ± SD	22.36 ± 10.67	22.37 ± 10.15	22.33 ± 11.97	-0.040	0.968
SF (ug/L), median (IQR)	165.00 (70.25, 303.25)	163.00 (76.00, 304.00)	174.00 (54.50, 295.00)	-0.721	0.471
TRF (g/L), mean ± SD	2.75 ± 0.89	2.75 ± 0.91	2.76 ± 0.83	0.203	0.840
TSH (mIU/L), median (IQR)	1.85 (1.33, 2.83)	1.94 (1.30, 2.89)	1.72 (1.34, 2.66)	-1.435	0.151
FT4 (pmol/L), mean ± SD	17.10 ± 14.18	17.31 ± 9.77	16.57 ± 9.62	-0.698	0.485
FT3 (pmol/L), mean ± SD	4.98 ± 1.00	5.01 ± 1.02	4.90 ± 0.94	-1.245	0.214
ALT (IU/L), median (IQR)	33.20 (19.40, 78.57)	32.20 (19.10, 76.57)	34.75 (19.65, 79.25)	-0.610	0.542
AST (IU/L), median (IQR)	33.75 (22.35, 72.47)	33.75 (22.02, 65.60)	34.10 (22.62, 78.75)	-0.491	0.624
GGT (IU/L), median (IQR)	53.85 (24.35, 116.77)	54.55 (24.97, 100.57)	50.05 (22.07, 163.45)	-0.274	0.708

BMI: Body mass index; MS: Metabolic syndrome; FPG: Fasting plasma glucose; HDL: High-density lipoprotein; CHO: Cholesterol; TG: Triacylglycerol; LDL: Low-density lipoprotein; PLT: Platelet count; TP: Total protein; ALB: Albumin; SI: Serum iron; SF: Serum ferritin; TRF: Transferrin; TSH: Thyroid-stimulating hormone; FT3: Free T3; FT4: Free T4; ALT: Alanine aminotransferase; AST: Aspartate transaminase; GGT: Gamma-glutamyl transferase; IQR: Interquartile range.

Development of prediction AP model

Predictive factors for the identification of AP were explored. Variables were collected from the patients. Initially, the collected clinical data, including demographics, laboratory data, and liver etiology variables were incorporated. Least absolute shrinkage and selection operator regression analysis identified nine potential predictive factors with non-zero coefficients: Age, male sex, BMI, alcohol consumption, and overlapping MASLD, SF, ALB, and TSH levels (Figure 2). Subsequently, these nine predictive factors were examined using a multivariate logistic regression. Compared to individuals without polyps, age [odds ratio (OR) = 1.069, 95% confidence interval (CI): 1.044-1.094, $P < 0.001$], male sex (OR = 3.217, 95%CI: 1.902-5.444, $P < 0.001$), BMI (OR = 1.098, 95%CI: 1.025-1.175, $P = 0.008$), history of alcohol consumption (OR = 1.956, 95%CI: 1.065-3.593, $P = 0.031$), overlapping MASLD (OR = 2.301, 95%CI: 1.384-3.826, $P = 0.008$), and SF (OR = 1.001, 95%CI: 1.000-1.010, $P = 0.047$) were identified as independent risk factors for AP (Table 3). The regression equation of the model was derived as logit, AP model = -6.188 + 0.066 age + 1.16 male sex + 0.093 BMI + 0.067 alcohol consumption + 0.833 other liver diseases (OLDs)-MASLD + 0.001 SF.

Validation of prediction AP model

The above-mentioned equation was used to construct a column chart, providing a user-friendly and simple nomogram for predicting AP probability (Figure 3A). Each variable was represented on its respective axis to assess the risk of developing AP in patients with CLD. Vertical lines were extended from each value on the upper scale to identify the

Table 3 The multivariate logistic regression analyses of the risk for model

Variables	B	Standard error	Wals	*P* value	Expect (B)	95%CI
Age	0.066	0.012	30.283	< 0.001	1.069	1.044-1.094
Male	1.169	0.268	8.971	< 0.001	3.217	1.902-5.444
BMI	0.093	0.035	7.113	0.008	1.098	1.025-1.175
Alcohol consumption	0.671	0.310	4.677	0.031	1.956	1.065-3.593
OLDs-MASLD	0.833	0.259	10.312	0.001	2.301	1.384-3.826
SF	0.001	0.001	2.627	0.047	1.001	1.000-1.010
Smoking	0.311	0.293	1.129	0.288	1.365	0.769-2.423
ALB	0.033	0.021	2.499	0.114	0.968	0.929-1.008
TSH	0.160	0.083	3.738	0.053	0.852	0.724-1.002

CI: Confidence interval; BMI: Body mass index; OLDs-MASLD: Other liver diseases overlapping metabolic dysfunction associated steatotic liver disease; SF: Serum ferritin; ALB: Albumin; TSH: Thyroid stimulating hormone.

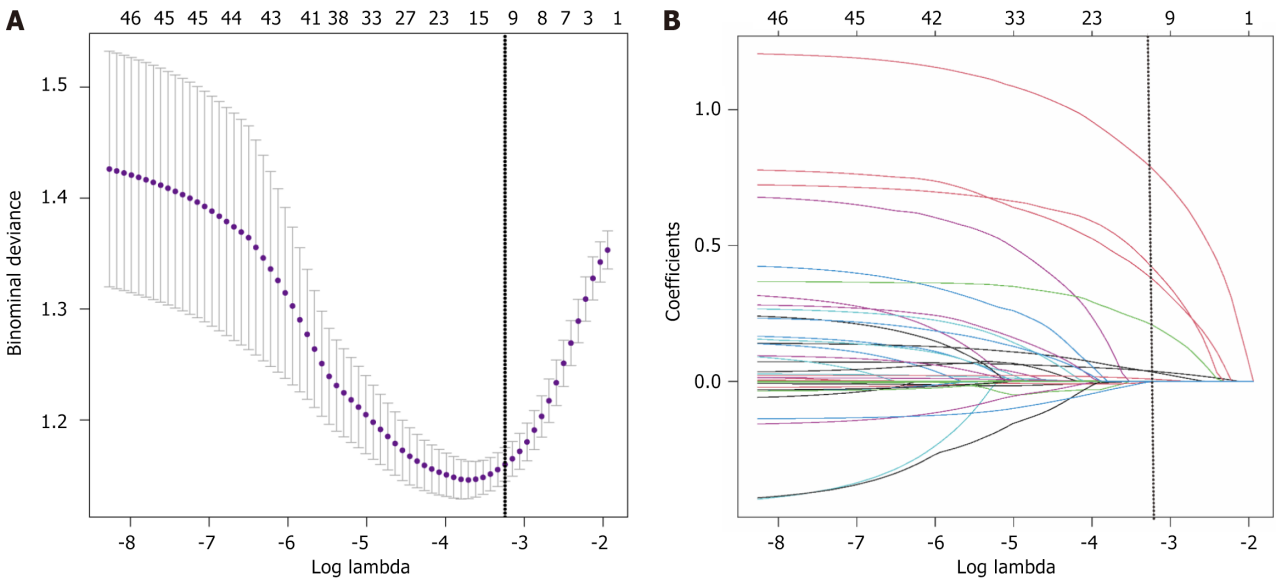

Figure 2 Least absolute shrinkage and selection operator regression analysis identified potential predictive factors. A: The least absolute shrinkage and selection operator model was optimized by selecting parameters based on the criteria method, which added one standard error to the minimum error. This process utilized five-fold cross-validation, resulting in λ = 0.03902279 and log(λ) = -3.241, satisfying the one standard error criteria for a simpler and more regularized model; B: The regression coefficients varied as λ increased, smaller λ values were associated with larger coefficients for most features, while larger λ values resulted in coefficients being reduced towards zero or reaching zero, a total of nine variables with non-zero coefficients were identified.

corresponding scores. These scores were then aggregated and mapped onto a total-score scale. Ultimately, the total score was projected vertically onto the lower axis to ascertain the personalized risk for AP. To determine the discriminatory power of the predictive AP model, in the training group, the area under curve (AUC) was found to be 0.801 (95%CI: 0.756-0.845) (Figure 3B). The best specificity was 0.704, corresponding to a sensitivity of 0.791 and accuracy of 0.739. In the validation group, the AUC was 0.785 (95%CI: 0.712-0.858) (Figure 3C). Internal validation for different etiologies of liver disease showed the receiver operating characteristic curve results (Figure 3D). The AUC for patients with PBC reached its peak at 0.970 (95%CI: 0.964-0.990), while the AUC for HBV registered the lowest value at 0.769 (95%CI: 0.710-0.827).

Evaluation of prediction AP model

Additionally, nomogram accuracy was evaluated using a calibration plot. The calibration curve indicated no significant difference in the comparison of the estimated and observed probabilities of AP occurrence in both the training and validation groups (χ^2 = 11.860, *P* = 0.157) and validation groups (χ^2 = 7.055, *P* = 0.530) (Figure 4A and B). To determine the clinical applicability of the AP model, a decision curve analysis was conducted, and net benefits were calculated based on the relative risks of false positives and false negatives, along with the difference between the proportions of individuals with true-positive results and those with false-positive results. The resultant curve demonstrated a significant net benefit for patients with AP, encompassing risk threshold probability ranges of 1.8%-80.2% in the training group and 1.3%-70.1%

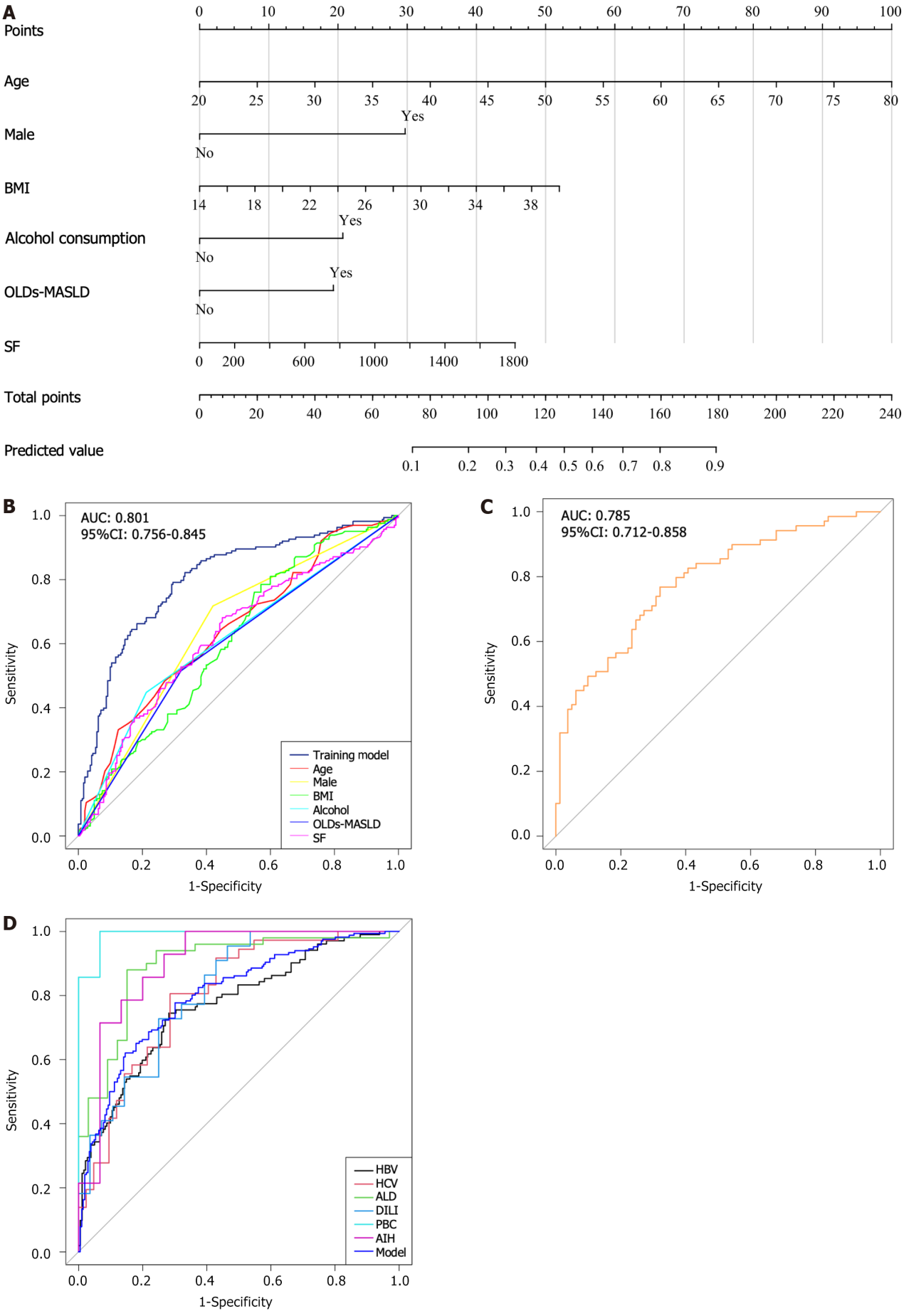

Figure 3 Nomogram and performance metrics for predicting the risk of adenomatous polyps. A: Nomogram; B: In the training group, the area

under curve (AUC) was 0.801 (95% confidence interval: 0.756-0.854). The impact on AUC from age, gender, body mass index, alcohol consumption, serum ferritin, and overlapping metabolic dysfunction-associated steatotic liver disease was 0.64, 0.65, 0.61, 0.62, 0.62, and 0.60, respectively; C: In the validation group, the AUC was 0.785 (95% confidence interval: 0.712-0.858); D: Internal validation among chronic liver disease patients yielded the following AUCs: Hepatitis B virus: 0.769, hepatitis C virus: 0.800, alcoholic liver disease: 0.892, drug-induced liver injury: 0.812, primary biliary cholangitis: 0.970, autoimmune hepatitis: 0.900. BMI: Body mass index; OLDs-MASLD: Other liver diseases overlapping metabolic dysfunction associated steatotic liver disease; SF: Serum ferritin; AUC: Area under curve; CI: Confidence interval; HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALD: Alcoholic liver disease; DILI: Drug-induced liver injury; PBC: Primary biliary cholangitis; AIH: Autoimmune hepatitis.

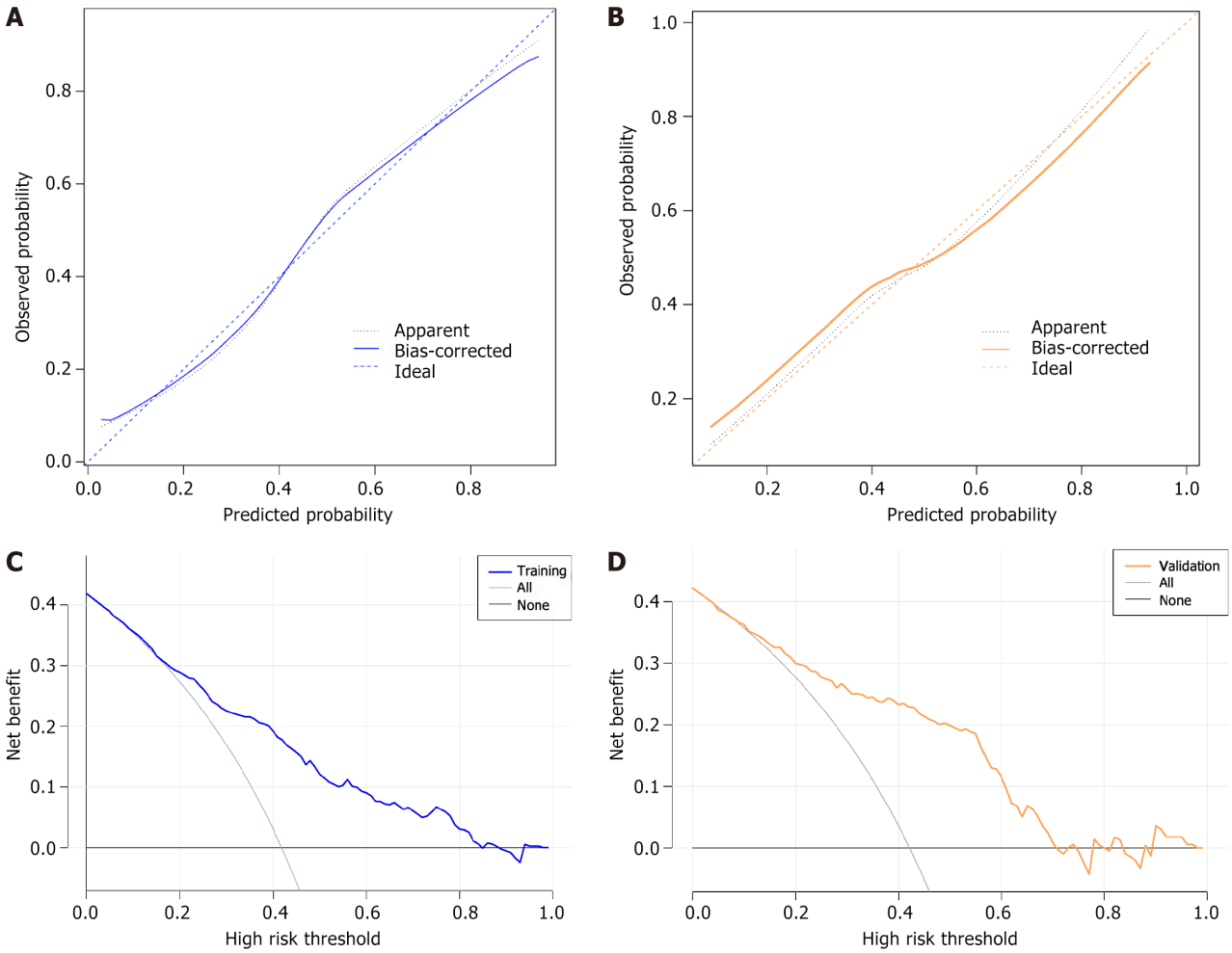

Figure 4 Calibration and decision curve analysis for predicting adenomatous polyps. A: The training group (χ^2 = 11.860, P = 0.157); B: The validation group (χ^2 = 7.055, P = 0.530); C and D: Decision curve analysis for the adenomatous polyps (AP) nomogram, blue line and orange line corresponds to the risk nomogram results for the training group and validation group, respectively. Black line: The assumption of no patient having AP; gray line: Assumes that all patients experienced AP.

in the validation group (Figure 4C and D).

Variety in liver disease etiologies

Subsequent subgroup analysis, as illustrated in Figure 5, showed a significant enhancement in the detection rate of AP to 28.8% when MASLD coexisted with HBV infection, compared with 21.6% without MASLD (P < 0.001). Additionally, compared to OLDs and pure MASLD, the detection rate of AP in MASLD overlapping with OLDs was significantly higher (P < 0.001). In patients with ALD, the prevalence of AP was significantly higher than that of non-AP (61.1% *vs* 24.3%, P < 0.001). No significant statistical differences were observed in patients with HCV compared to those with DILI; However, the percentage of AP was greater in patients with concurrent MASLD than in those without MASLD. A statistical difference was observed in patients with AIH, potentially because only two patients with AIH without concurrent MASLD were included (Table 4).

Table 4 Differences of etiology in colorectal polyps in chronic liver disease, *n* (%)

Etiology	Non-polyps	Non-adenomatous polyps	Adenomatous polyps	*P* value
OLDs (n = 815)				
OLDs-MASLD	96 (27.2)[a]	133 (37.7)[a]	124 (35.1)	< 0.001
No MASLD	225 (48.7)[a]	129 (27.9)[a]	108 (23.4)	
Pure MASLD (n = 55)				
Pure MASLD	26 (47.3)[a]	12 (21.8)	17 (30.9)	< 0.001
OLDs-MASLD	96 (27.2)[a]	133 (37.7)	124 (35.1)	
HBV (n = 417)				
MASLD	47 (28.8)[a]	69 (42.3)[a]	47 (28.8)	< 0.001
No MASLD	134 (52.8)[a]	65 (25.6)[a]	55 (21.6)	
AIH (n = 44)				
MASLD	7 (25.9)	13 (48.1)	7 (25.9)	0.046
No MASLD	8 (47.0)	2 (11.8)	7 (41.2)	
ALD (n = 124)				
MASLD	8 (14.8)[a]	13 (24.1)[a]	33 (61.1)	< 0.001
No MASLD	25 (35.7)[a]	28 (40.0)[a]	17 (24.3)	
HCV (n = 108)				
MASLD	17 (30.4)	20 (35.7)	19 (33.9)	0.090
No MASLD	25 (48.1)	10 (19.2)	17 (32.7)	
PBC (n = 37)				
MASLD	5 (33.3)	6 (40.0)	4 (26.7)	0.570
No MASLD	10 (45.5)	9 (40.9)	3 (13.6)	
DILI (n = 75)				
MASLD	12 (31.6)	12 (31.6)	14 (36.8)	0.327
No MASLD	16 (43.2)	13 (35.1)	8 (21.6)	

[a]$P < 0.05$ *vs* adenomatous polyps.

This study included 10 cases of other liver diseases, such as Budd-Chiari syndrome and genetic metabolic liver diseases, which were excluded from detailed analysis due to their small sample size. OLDs: Other liver diseases; MASLD: Metabolic dysfunction-associated steatotic liver disease; HBV: Hepatitis B virus; HCV: Hepatitis C virus; ALD: Alcoholic liver disease; AIH: Autoimmune hepatitis; PBC: Primary biliary cholangitis; DILI: Drug-induced liver injury.

DISCUSSION

This study explored the clinical characteristics of colorectal polyps and the detection rates of adenomatous and non-AP in patients with CLD. Additionally, a personalized prediction nomogram for incident AP in Chinese adults with CLD was developed and validated. The prediction model incorporated six parameters: Age, sex, BMI, alcohol consumption, OLDs-MASLD, and SF. Model assessment and internal validation consistently demonstrated strong predictive performance of the nomogram. First, we found that 28.6% of the participants were diagnosed with AP, whereas 31.5% were diagnosed with non-AP. The occurrence of colorectal polyps varies according to geographic location and ethnicity, exhibiting the highest levels in Western nations, while Africa and Southern Asia report the lowest rates. In Asian countries, a prevalence of approximately 20% is observed in populations with average-risk colorectal polyps[24,25], with older adults and men exhibiting higher prevalence rates. Yang *et al*[26] reported that the prevalence of colorectal adenomas among average-risk individuals was 34.5% in men and 20.0% in women, with an increasing trend observed over the study period. An earlier study from Germany indicated that 26% of patients with CLD had AP, which focused on alcoholic and viral liver diseases, including 73 cases (23.6%) of ALD and 63 cases (23.1%) of viral liver diseases[9]. This study included a notable number of patients with HBV-related liver diseases comprising 417 patients (47.9%). Differences in the distribution of liver disease etiologies between studies could potentially contribute to variations in the prevalence of AP.

Unhealthy lifestyle habits, such as smoking and alcohol consumption, are more likely to be associated with the presence of polyps in patients with CLD. In this study, the training group showed a 1.95-fold increased risk of AP in

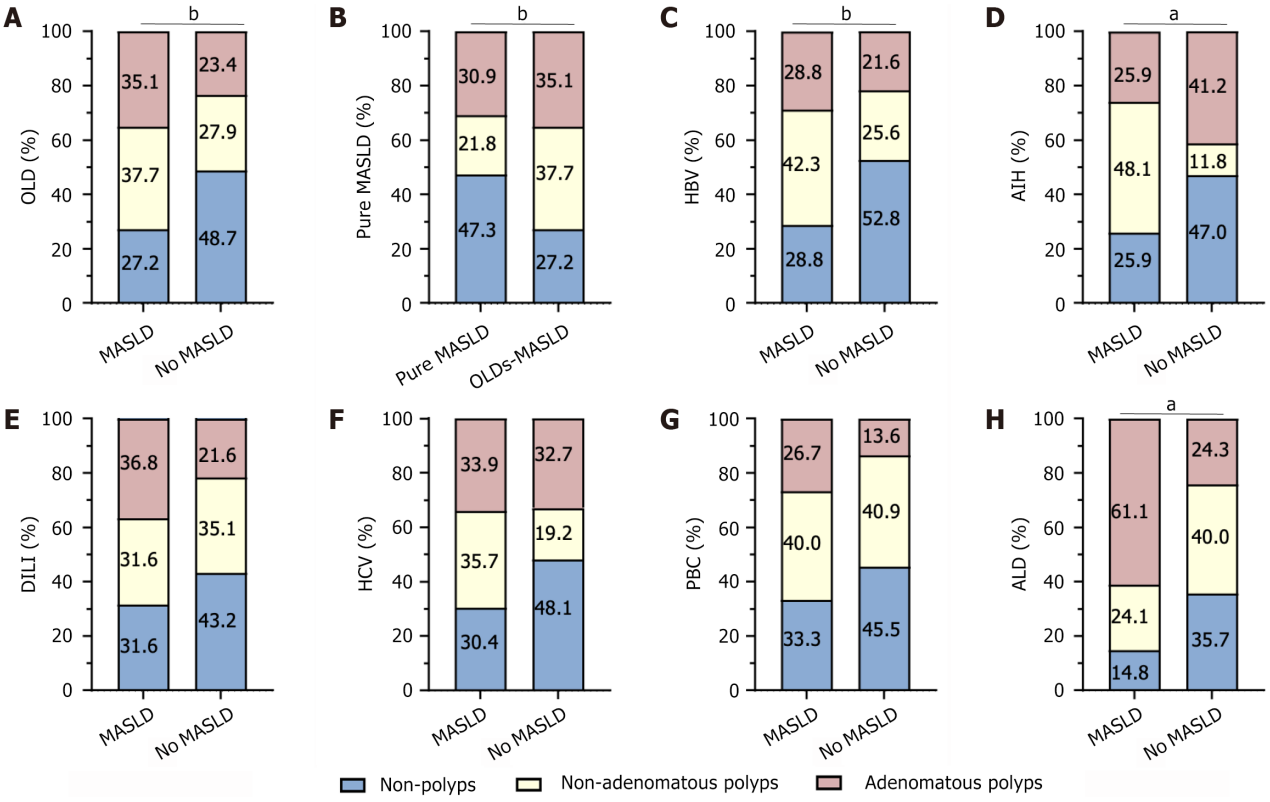

Figure 5 Percentage of chronic liver disease etiology combined with metabolic dysfunction-associated steatotic liver disease and no metabolic dysfunction-associated steatotic liver disease. A: Other liver diseases; B: Pure metabolic dysfunction-associated steatotic liver disease; C: Hepatitis B virus; D: Autoimmune hepatitis; E: Drug-induced liver injury; F: Hepatitis C virus; G: Primary biliary cholangitis; H: Alcoholic liver disease. [a]*P* < 0.05; [b]*P* < 0.01. OLD: Other liver diseases; MASLD: Metabolic dysfunction-associated steatotic liver disease; HBV: Hepatitis B virus; AIH: Autoimmune hepatitis; DILI: Drug-induced liver injury; HCV: Hepatitis C virus; PBC: Primary biliary cholangitis; ALD: Alcoholic liver disease.

patients who consumed alcohol than those without polyps. Bailie *et al*[8] reported a 2.5-fold increased risk of serrated polyps in smokers and a 1.33-fold increased risk in alcohol consumers (95%CI: 1.17-1.52). The underlying mechanisms are unclear; however, the inflammatory stimulus of alcohol may contribute to the development of polyps. Long-term feeding of mice with ethanol has been shown to enhance inflammation and elevate pro-inflammatory cytokines and chemokines, thereby promoting the occurrence of colorectal tumors[27]. In the multivariate analysis, smoking was not established as a standalone contributing factor for AP. This might be explained by the stronger influence of other variables such as age, sex, BMI, alcohol consumption, OLDs-MASLD, and SF in the multivariate analysis, which weakened the significance of smoking as an independent risk factor. In addition, several studies have shown that BMI is linked to an increased risk of developing AP in men and women[28-30]. The hypothesis suggests that being overweight can be viewed as a condition of chronic mild inflammation, and that obesity is connected to the onset of CRC through increased chronic low-grade inflammation and increased adipokine production[31,32].

When OLDs coexisted with MASLD, the combined condition became an independent risk factor. Previous reports have indicated that the association between MAFLD and cancer risk varies by site, with an 1.14 times increased risk for CRC [33]. The exact pathophysiological mechanisms connecting MASLD and colorectal AP are not completely understood. Patients with NAFLD have lower numbers of $CD4^+$ and $CD8^+$ T cells in their blood circulation than healthy participants [34]. Similarly, patients with CRC exhibited significant differences in the abundance of these T cells. Low $CD8^+$ T cell levels are associated with the advancement of original and metastatic colorectal adenomatous carcinoma[35,36]. This suggests that NAFLD, which is characterized by low levels of $CD4^+$ and $CD8^+$ T cells, may create an advantageous setting for the growth of colonic tumor cells. Related to NAFLD, tumor necrosis factor alpha has been reported to promote epithelial-mesenchymal transition in colon carcinoma cells[37]. Moreover, dysregulation of endoplasmic reticulum homeostasis and elevated endoplasmic reticulum stress are associated with epithelial-mesenchymal transition, which are conditions present in NAFLD[38]. Additionally, the gut microbiota significantly influences the regulation of immune responses. An altered composition of gut microbiota has been connected to the onset of NAFLD, including an increased Bacteroidetes to Firmicutes ratio and decreased abundance of butyrate-producing ruminococcaceae[39]. This suggests that enterotoxins produced by *Bacteroides fragilis* can trigger significant colonic inflammation in mice, thereby mimicking inflammation-driven colon carcinogenesis observed in humans[40]. The persistence of such bacterial populations in NAFLD and early CRC could help identify individuals with a background of fatty liver disease at increased risk for colorectal adenomas. A report published in 2023 suggested that serum microRNA levels could serve as biomarkers for diagnosing NAFLD in Chinese patients with colorectal polyps[24]. Further studies are required to fully investigate the association between MASLD and colorectal adenomas.

Studies indicate that irregular iron metabolism, whether due to genetic mutations or high dietary iron consumption, is a risk factor for CRC[41,42]. Abnormal iron status has been a topic of concern in CLD and is connected to the accelerated development of liver fibrosis[43,44]. The occurrence of AP may be associated with the inflammatory state of the liver. However, the study found no association between cirrhosis and AP. Interestingly, compared with patients with polyps, those without polyps had higher TSH levels (P = 0.011). As previously reported in a population of 139426 individuals, hypothyroidism was linked to a 22% reduction in CRC risk (OR = 0.78; 95%CI: 0.65-0.94; P = 0.08), and the strongest inverse relationship between rectal cancer and hypothyroidism was particularly evident in patients aged > 50 years[45]. Additional research is needed to ascertain if hypothyroidism may offer protection against the development of polyps in patients[45]. The advantages of this study include the comprehensive recording of histopathological information that confirmed the diagnosis of colorectal polyps based on patient pathology reports. Nonetheless, this study had several limitations. First, this was a retrospective analysis conducted at a single institution. The limited number of patients, particularly in the subgroup analysis, may have influenced the statistical power and reliability of the findings. Selection and information biases may also have occurred. In the future, we intend to conduct a prospective multicenter study that includes diverse regions and ethnicities to further validate the predictive performance of the nomogram model, thereby enhancing its generalizability. Second, establishing a causal relationship between MASLD and the risk of colorectal polyps is challenging when relying on cross-sectional studies. Moreover, the establishment and evaluation of this model were performed internally owing to the availability of data, enabling us to refine the model in a more controlled setting. However, this limitation affects the generalizability of the findings to other institutions. Finally, certain potential factors related to AP, such as detailed dietary habits, exercise patterns, medication history, and patient genetic information, were not included in our retrospective analysis because we were limited to the data accessible in the medical database. Future model development should consider these potentially influential factors.

CONCLUSION

To the best of our knowledge, this is the first study to create and validate a nomogram for predicting AP in patients with CLD. We established a simple model and internally validated it, thereby demonstrating its utility as a risk assessment tool. The predictive model developed is valuable for assisting in the evaluation of whether an endoscopic examination is necessary in patients with CLD.

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FOOTNOTES

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ORIGINAL ARTICLE

Observational Study

Prognostic model for esophagogastric variceal rebleeding after endoscopic treatment in liver cirrhosis: A Chinese multicenter study

Jun-Yi Zhan, Jie Chen, Jin-Zhong Yu, Fei-Peng Xu, Fei-Fei Xing, De-Xin Wang, Ming-Yan Yang, Feng Xing, Jian Wang, Yong-Ping Mu

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Abstract

BACKGROUND

Rebleeding after recovery from esophagogastric variceal bleeding (EGVB) is a severe complication that is associated with high rates of both incidence and mortality. Despite its clinical importance, recognized prognostic models that can effectively predict esophagogastric variceal rebleeding in patients with liver cirrhosis are lacking.

AIM

To construct and externally validate a reliable prognostic model for predicting the occurrence of esophagogastric variceal rebleeding.

METHODS

This study included 477 EGVB patients across 2 cohorts: The derivation cohort (n = 322) and the validation cohort (n = 155). The primary outcome was rebleeding events within 1 year. The least absolute shrinkage and selection operator was applied for predictor selection, and multivariate Cox regression analysis was used to construct the prognostic model. Internal validation was performed with bootstrap resampling. We assessed the discrimination, calibration and accuracy of the model, and performed patient risk stratification.

RESULTS

Six predictors, including albumin and aspartate aminotransferase concentrations, white blood cell count, and the presence of ascites, portal vein thrombosis, and bleeding signs, were selected for the rebleeding event prediction following endoscopic treatment (REPET) model. In predicting rebleeding within 1 year, the REPET model exhibited a concordance index of 0.775 and a Brier score of 0.143 in the derivation cohort, alongside 0.862 and 0.127 in the validation cohort. Furthermore, the REPET model revealed a significant difference in rebleeding rates (P < 0.01) between low-risk patients and intermediate- to high-risk patients in both cohorts.

CONCLUSION

We constructed and validated a new prognostic model for variceal rebleeding with excellent predictive performance, which will improve the clinical management of rebleeding in EGVB patients.

Key Words: Esophagogastric variceal bleeding; Variceal rebleeding; Liver cirrhosis; Prognostic model; Risk stratification; Secondary prophylaxis

Core Tip: Rebleeding is a serious complication in liver cirrhosis patients following esophagogastric variceal bleeding, and there is no widely recognized prognostic model to reliably predict this risk. To address this gap, we developed and externally validated the rebleeding event prediction following endoscopic treatment model, which incorporates readily accessible clinical variables from multiple domains. The rebleeding event prediction following endoscopic treatment model enables effective risk stratification, facilitating improved patient management and the tailoring of follow-up and treatment strategies.

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INTRODUCTION

Esophagogastric variceal bleeding (EGVB) is a major consequence of portal hypertension in patients with liver cirrhosis and is associated with considerable mortality[1]. Rebleeding is a serious complication following the recovery from EGVB. In patients who do not receive secondary prophylaxis, the incidence of rebleeding can reach up to 60%, accompanied by a higher risk of mortality[2,3]. The Baveno VII workshop guidelines recommend the use of a combination of endoscopic variceal ligation and nonselective beta-blockers (NSBB) as a first-line therapy to prevent variceal rebleeding[1], and this method has been widely adopted. However, a recent meta-analysis revealed that the rebleeding rate following standard secondary prophylaxis still ranged from 22% to 33%[4]. Given the varied risk of rebleeding among patients, there is a clear need to tailor therapeutic interventions and follow-up regimens on the basis of each individual patient's anticipated risk.

There is a lack of widely recognized prognostic models that effectively predict esophagogastric variceal rebleeding in patients with liver cirrhosis[1]. Several models for predicting rebleeding have been reported[5-9], but these models are limited in their selection of predictive factors, focusing primarily on laboratory indicators while overlooking a wide range of clinical information, such as cirrhosis complications, endoscopic features, and treatment regimens. As a result, the predictive performance of these models is constrained. Furthermore, the lack of external validation reduces their generalizability. Therefore, there is an urgent need for the development of a prognostic model for variceal rebleeding using long-term follow-up cohorts, a multidimensional collection of clinical data and reliable validation.

In the present study, we screened EGVB patients who underwent endoscopy combined with NSBB secondary prophylaxis, with the aim of constructing and externally validating a reliable prognostic model for variceal rebleeding. The developed model not only facilitates active monitoring and treatment of high-risk patients but also helps spare low-risk patients from unnecessary treatment[10], thereby optimizing the allocation of medical resources.

MATERIALS AND METHODS

Study population

The study was performed in patients with cirrhosis and EGVB who were derived from two retrospective cohorts. The derivation cohort included patients with cirrhosis and EGVB who underwent endoscopy combined with NSBB secondary prophylaxis at Shuguang Hospital Affiliated with Shanghai University of Traditional Chinese Medicine between December 2015 and April 2023. External validation was performed on patients who were treated with endoscopy combined with NSBB for EGVB between April 2022 and April 2023 at Zhongshan Hospital Affiliated with Fudan University. The clinical data were collected retrospectively, with each collection independently gathered and verified by two data managers. All patients were followed up by telephone, outpatient visits, and inpatient records until rebleeding, or up to 1 year. This study was performed in accordance with the transparent reporting of a multivariable prediction model for individual prognosis or diagnosis guidelines (Supplementary Table 1)[11]. The study was approved by the local ethics committees and complied with the Declaration of Helsinki. The raw data did not contain any personal identifying information that could be linked to particular individuals and were anonymized before use. Given that all the clinical data were obtained retrospectively, the need for informed consent was waived.

Inclusion criteria

The inclusion criteria for the derivation and validation cohorts were as follows: (1) Had a diagnosis of cirrhosis (on the basis of clinical characteristics and laboratory and imaging tests or liver biopsy); (2) Were admitted due to EGVB confirmed by endoscopy; (3) ≥ 18 years of age; and (4) Underwent endoscopy combined with NSBB secondary prophylaxis for the first time.

Exclusion criteria

The exclusion criteria were as follows: (1) Previous endoscopic primary or secondary prophylaxis; (2) Previous transjugular intrahepatic portosystemic shunt (TIPS), balloon-occluded retrograde transvenous obliteration, splenectomy, liver transplantation, or pericardial vascular dissections; (3) Emergency endoscopic hemostasis only; (4) Diagnosis of hepatocellular carcinoma (HCC) or other malignant tumors at the time of recruitment; (5) Combined serious disease of important organs with insufficient function; and (6) Follow-up of less than one year.

Therapeutic interventions

Patients were managed according to the Baveno consensus[1,12] and the American Association for the Study of Liver Diseases guidelines[13,14] at the time of treatment. The patients were initially treated with vasoactive drugs and prophylactic antibiotics within 12 hours, and endoscopic emergency hemostasis was performed when necessary. After control of the index bleed, secondary prophylaxis with endoscopy combined with NSBB was instituted to prevent rebleeding. Decisions to institute therapeutic modifications were made according to individual center policy and the clinical assessment of the patient by the attending physician.

Each center has two senior endoscopists with more than five years of therapeutic experience who are in charge of endoscopic examinations and treatments. Esophageal varices were treated with endoscopic variceal ligation, with ligation from the cardia to the oral side using a commercial multiband device. When combined with gastric varices, intravenous injections of lauroylmorpholine and tissue adhesive were performed using a therapeutic endoscope and a transparent Teflon syringe.

Outcomes

The primary outcome was defined as rebleeding events within 1 year. The secondary outcomes were rebleeding events within 6 weeks and 2 years. Rebleeding was defined as the reoccurrence of a clinically significant active bleeding event after bleeding control (hematemesis, dark stools, or melena; decrease in systolic blood pressure > 20 mmHg or increase in heart rate > 20 beats/minute; decrease in hemoglobin > 30 g/L in the absence of transfusion)[15]. The assessment of rebleeding events was performed by senior endoscopists at each center.

Candidate predictors and data collection

The candidate predictors were restricted to variables collected at admission on the first visit. The candidate predictors are

listed in Table 1. Information on esophageal varices and gastric varices was recorded according to the classification of the Japanese Research Society for Portal Hypertension[16]. Bleeding signs, as observed through endoscopy, were defined as gushing bleeding, spurting bleeding, oozing bleeding, red plug, or white plug[16,17]. Bacterial infections were defined as spontaneous peritonitis, pulmonary infections or urinary tract infections, depending on the collected clinical data.

Sample size calculations

The derivation cohort sample size was calculated using the methodology proposed by Riley *et al*[18]. The calculation was based on the assumption of 6 predictive parameters in the model, an adjusted R-square of 0.2, a shrinkage of 10%, and a 22% incidence of the primary outcome (rebleeding within 1 year). Given this, the minimum sample size required for model development was 239 patients with 53 outcome events. The external validation sample size was calculated with reference to a statistical article published by Riley *et al*[19]. The calculation was based on the assumption of an observed/expected statistic of 1, a target confidence interval width of 0.7 for observed/expected statistic, and a 22% incidence of rebleeding within 1 year. Thus, at least 77 participants (approximately 17 events) were required to satisfy these criteria.

Statistical analysis

Statistical analyses were conducted using R 4.3.2 (https://www.R-project.org). Descriptive results are presented as *n* (%) or mean ± SD, as appropriate. Missing data were imputed with multiple imputations. Variables for which more than 10% of the values were missing were excluded (the excluded data are shown in Supplementary Table 2). Group comparisons of continuous variables were made using Student's *t*-test for normally distributed data and the Mann-Whitney *U* test for non-normally distributed data. Group comparisons of categorical variables were made using Pearson's χ^2 test or Fisher's exact test. A 2-sided $P < 0.05$ was considered to indicate statistical significance.

Least absolute shrinkage and selection operator (LASSO) was applied to screen independent variables of the training set for selection of candidate predictors. This approach performs simultaneous feature selection by applying a regularization penalty term to the model that causes some coefficients to shrink to zero. The Cox proportional hazards model was constructed using the features that had nonzero coefficients in the LASSO regression model. Proportionate risk assumptions were tested *via* the Global Schoenfeld Test. Multicollinearity was assessed using the variance inflation factor (VIF), and a VIF < 5 indicates the absence of multicollinearity. We also developed and used a nomogram to calculate the predicted probability of rebleeding at 6 weeks, 1 year, and 2 years.

Model performance was assessed using the concordance index (C-index), area under the receiver operating curve (AUC), calibration plots, Brier scores and decision curve analysis (DCA). The predictive performance of the prognostic model was compared with that of the Child-Turcotte-Pugh (CTP) score, the model for end-stage liver disease (MELD) score, albumin-bilirubin grade and fibrosis 4 (FIB-4) score[20,21] (the calculations are shown in Supplementary Table 3). Internal validation was performed *via* the bootstrap resampling method with 1000 repetitions. For external validation, prognostic scores were calculated for each individual using the formula developed for the derivation cohort, and the performance of the prognostic scores was subsequently evaluated. We used X-tile software[22] to determine the optimal threshold for prognostic scores and plotted Kaplan-Meier survival curves.

Given that the incidence of rebleeding after emergency endoscopic hemostasis is significantly higher[23,24], we performed a sensitivity analysis to exclude patients who underwent emergency endoscopic hemostasis. To assess whether there was heterogeneity in the predictive value of the prognostic model, we assessed the prognostic scores of the subgroups individually according to the etiology of cirrhosis (hepatitis B virus, autoimmune hepatitis, alcoholic hepatitis, and other etiologies), variceal type (esophageal varices, combined with gastric varices), sex (male or female), age (< 60, ≥ 60), and use of NSBB (non-NSBB group, NSBB group).

RESULTS

Baseline of dataset

A total of 997 patients with liver cirrhosis and EGVB were enrolled during the study period. Of these, 520 were excluded for the reasons illustrated in Figure 1. The remaining 477 patients were included in subsequent model development and validation, with the derivation cohort comprising 322 patients and the external validation cohort comprising 155 patients. The baseline characteristics of the patients are presented in Table 1, with missing values noted. A total of 56 variables were included in the statistical analyses after excluding variables with more than 10% missing values. There was no significant difference in the distribution of cirrhosis etiology between the two cohorts ($P = 0.8$). Despite notable differences in liver function tests and variceal types between the derivation and validation cohorts, the rebleeding rates within 6 weeks (8.07% *vs* 4.52%, $P = 0.21$), 1 year (25.78% *vs* 23.23%, $P = 0.62$), and 2 years (34.47% *vs* 32.90%, $P = 0.81$) did not significantly differ.

All patients were followed up until rebleeding occurred or for up to 1 year. The median follow-up times for the two cohorts were 408.5 days and 447 days, respectively. During the follow-up period, 25 patients developed HCC. Two patients underwent TIPS and 1 patient underwent splenectomy before rebleeding occurred. A total of 27 patients died, with 26 deaths attributed to hemorrhagic shock or multi-organ failure related to EGVB, and 1 death resulting from HCC.

Model development

A total of 56 independent variables from the training set were screened using LASSO regression to identify candidate predictors (Supplementary Figure 1A and B). To facilitate the model's practical clinical application, we selected the most

Table 1 Baseline characteristics and outcomes of patients

Characteristic	Derivation cohort (*n* = 322)	Missing data in derivation cohort (%)	Validation cohort (*n* = 155)	Missing data in validation cohort (%)	*P* value
Demographics					
Age (year)	58.38 ± 11.86	0	57.7 ± 13.37	0	0.57
Sex, *n* (%)		0		0	0.36
Female	132 (40.99)		56 (36.13)		
Male	190 (59.01)		99 (63.87)		
Smoking history, *n* (%)	52 (16.25)	0.6	26 (16.77)	0	0.99
Drinking history, *n* (%)	74 (23.12)	0.6	30 (19.35)	0	0.42
Surroundings, *n* (%)		0		0	0.67
Rural	93 (28.88)		50 (32.26)		
Suburban	58 (18.01)		24 (15.48)		
Urban	171 (53.11)		81 (52.26)		
Symptom, *n* (%)		0		0	0.44
Melena or hematochezia	115 (35.71)		49 (31.61)		
Hematemesis	207 (64.29)		106 (68.39)		
Etiology, *n* (%)		0		0	0.8
Chronic HBV infection	156 (48.45)		69 (44.52)		
Autoimmune liver disease	55 (17.08)		31 (20.00)		
Alcohol-related	50 (15.53)		23 (14.84)		
Other	61 (18.94)		32 (20.65)		
Course of cirrhosis (year)	2 (0.5-6)	3.7	2 (0.2-6)	0	0.46
Family history of cirrhosis, *n* (%)	52 (16.20)	0.3	7 (4.52)	0	< 0.01
Endoscopic treatment and presentation					
Received emergency endoscopic hemostasis, *n* (%)	28 (8.70)	0	10 (6.45)	0	0.5
Types of varices, *n* (%)		0		0	< 0.01
EV	177 (54.97)		28 (18.06)		
EV combined with GV	145 (45.03)		127 (81.94)		
Red color sign, *n* (%)	302 (93.79)	0	150 (96.77)	0	0.25
Bleeding signs, *n* (%)	49 (15.22)	0	29 (18.71)	0	0.4
Form of varices, *n* (%)		0		0	0.57
F1	56 (17.39)		23 (14.84)		
F2-F3	266 (82.61)		132 (85.16)		
Location of varices, *n* (%)		0.3		0	< 0.01
Low to middle	252 (78.50)		146 (94.19)		
High	69 (21.50)		9 (5.81)		
Numbers of varices	4 (4-4)		4 (4-4)		0.73
Diameter of varices (cm)	0.9 (0.8-1)	0.6	1 (0.8-1)	0	0.53
Number of EVL (times)	2 (1-3)	0	2 (1.5-3)	0	0.05
Received sclerotherapy injections, *n* (%)	139 (43.17)	0	74 (47.74)	0	0.4

Received tissue glue injections, *n* (%)	125 (38.82)	0	65 (41.94)	0	0.58
Comorbidities and complications					
Ascites, *n* (%)	220 (68.32)	0	95 (61.29)	0	0.16
Bacterial infection, *n* (%)	48 (14.91)	0	12 (7.74)	0	0.04
Encephalopathy, *n* (%)	13 (4.04)	0	3 (1.94)	0	0.36
Diabetes, *n* (%)	67 (20.87)	0.3	31 (20.00)	0	0.92
Hypertension, *n* (%)	78 (24.30)	0.3	20 (12.90)	0	0.01
Hemorrhagic shock, *n* (%)	10 (3.12)	0.3	2 (1.29)	0	0.38
Heart disease, *n* (%)	16 (4.98)	0.3	7 (4.52)	0	1.00
Laboratory tests					
Total bilirubin (μmol/L)	21.3 (15-32.02)	3.1	15.8 (10.85-20.85)	0	< 0.01
Alanine aminotransferase (U/L)	25 (17-36)	4.3	21 (15-30)	0	0.01
Aspartate aminotransferase (U/L)	35 (26-47)	4.3	28 (21-38.5)	0	< 0.01
Alkaline phosphatase (U/L)	87 (68-130)	5	83 (63.25-107)	0.6	0.05
Total protein (g/L)	62.45 ± 8.92	3.8	62.9 ± 9.16	0	0.62
Albumin (g/L)	31.89 ± 6.07	3.1	35.3 ± 5.94	0	< 0.01
Globulin (g/L)	29.55 (25.9-34.42)	5.6	27 (23-32)	0	< 0.01
A/G	1.05 (0.87-1.3)	5.6	1.33 (1.08-1.59)	0	< 0.01
Blood urea nitrogen (mmol/L)	5.72 (4.2-7.72)	6.8	5.2 (4.1-6.18)	0.6	< 0.01
Creatinine (μmoI/L)	65.4 (55-79)	4.7	69 (57.25-80.5)	0.6	0.14
Uric acid (μmoI/L)	298 (238-372)	4.7	288 (239-357)	0	0.26
Total cholesterol (mmol/L)	3.17 (2.61-3.94)	7.5	3.17 (2.6-3.94)	25.8	0.94
White blood cell (× 10^9/L)	3.1 (2.09-4.75)	5.6	2.66 (1.92-4.47)	0	0.07
Neutrophil (× 10^9/L)	1.96 (1.25-3.52)	5.3	1.6 (1.1-2.77)	0.6	< 0.01
Lymphocyte (× 10^9/L)	0.7 (0.5-1.03)	5.9	0.7 (0.5-1)	1.9	0.69
Red blood cell (× 10^9/L)	3.05 ± 0.74	3.4	3.19 ± 0.77	0	0.08
Hemoglobin (g/L)	88.37 ± 24.42	1.9	89.1 ± 22.33	0	0.76
Platelet count (× 10^9/L)	68 (45.75-91.25)	3.1	68 (45.5-103)	0	0.68
Prothrombin time (second)	14.8 (13.6-16.25)	3.4	14.4 (13.7-15.6)	0	0.07
INR (unit)	1.27 (1.15-1.4)	4	1.26 (1.17-1.37)	0	0.48
D-dipolymer (μg/mL)	1.45 (0.56-3.34)	7.1	1.1 (0.45-2.37)	1.9	0.12
Thrombin time (second)	17.8 (16.9-19.1)	6.5	17.8 (16.67-18.72)	1.9	0.34
Fibrinogen (g/L)	200.26 ± 80.04	5.6	185.67 ± 69.84	1.9	0.06
CTP (points)	7 (6-8)	0	6 (6-7)	0	< 0.01
CTP class, *n* (%)		0		0	< 0.01
A (5-6)	101 (31.37)		83 (53.55)		
B (7-9)	190 (59.01)		69 (44.52)		
C (10-13)	31 (9.63)		3 (1.94)		
MELD (points)	11 (9-13)	0	9 (8-11)	0	< 0.01
Radiological features					
Portal vein thrombosis, *n* (%)	68 (21.12)	1.2	49 (31.61)	0	0.02
Splenic vein diameter (mm)	9.6 ± 3.45	6.8	9.08 ± 3.94	4.5	0.16

Inner diameter of portal vein trunk (mm)	13.17 ± 2.34	6.5	12.7 ± 2.97	4.5	0.07
Use of medication					
Use of NSBB, *n* (%)	314 (65.83)	0	204 (63.35)	0	0.12
Use of PPI, *n* (%)	450 (94.34)	0	306 (95.03)	0	0.47
Use of antibiotics, *n* (%)	335 (70.23)	0	219 (68.01)	0	0.16
Outcomes					
Rebleeding within 6 weeks, *n* (%)	26 (8.07)	0	7 (4.52)	0	0.21
Rebleeding within 6 months, *n* (%)	60 (18.63)	0	22 (14.19)	0	0.28
Rebleeding within 1 year, *n* (%)	83 (25.78)	0	36 (23.23)	0	0.62
Rebleeding within 2 years, *n* (%)	111 (34.47)	0	51 (32.90)	0	0.81
Follow-up time (day)	408.5 (322-885)	0	447 (366-1108)	0	0.06

EV: Esophageal varices; GV: Gastric varices; EVL: Endoscopic variceal ligation; INR: International normalized ratio; A/G: Albumin/globulin; CTP: Child-Turcotte-Pugh score; MELD: Model for end-stage liver disease score; NSBB: Non-selective beta-blockers; PPI: Proton pump inhibitor.

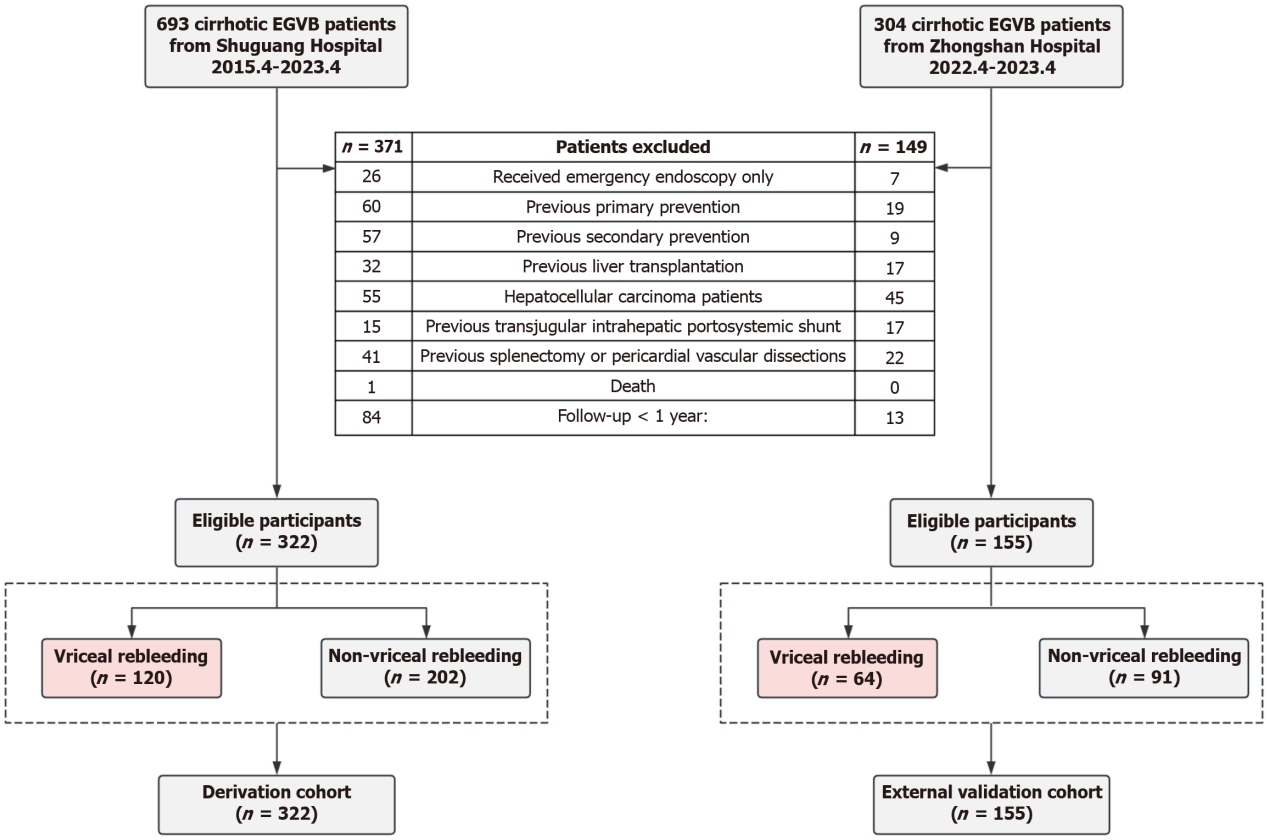

Figure 1 Study flow chart. EGVB: Esophagogastric variceal bleeding.

concise scheme (the lambda.1se scheme) for the final modeling variables[25], which included albumin (ALB) and aspartate aminotransferase (AST) concentrations, white blood cell (WBC) count, and the presence of ascites, portal vein thrombosis (PVT), bacterial infection, and recent bleeding manifestations. Owing to their closely related clinical significance, bacterial infection and WBC count were considered collinear variables. After careful discussion between two senior physicians, it was decided to exclude bacterial infection from the model to enhance the model's practical clinical application. Therefore, six predictors were ultimately selected for the rebleeding event prediction following endoscopic treatment (REPET) model, including ALB, AST, and WBC, and the presence of ascites, PVT, and bleeding signs.

We then performed variable analysis using Cox multivariable regression, constructed a forest plot of risk factors for rebleeding (Supplementary Figure 1C), and constructed a Cox proportional risk prognostic model. The global Schoenfeld test yielded a P value > 0.5, indicating that the prognostic model complies with the proportional risk assumption (Supplementary Figure 2). Multicollinearity analysis revealed no covariance (VIF < 5) (Supplementary Figure 3).

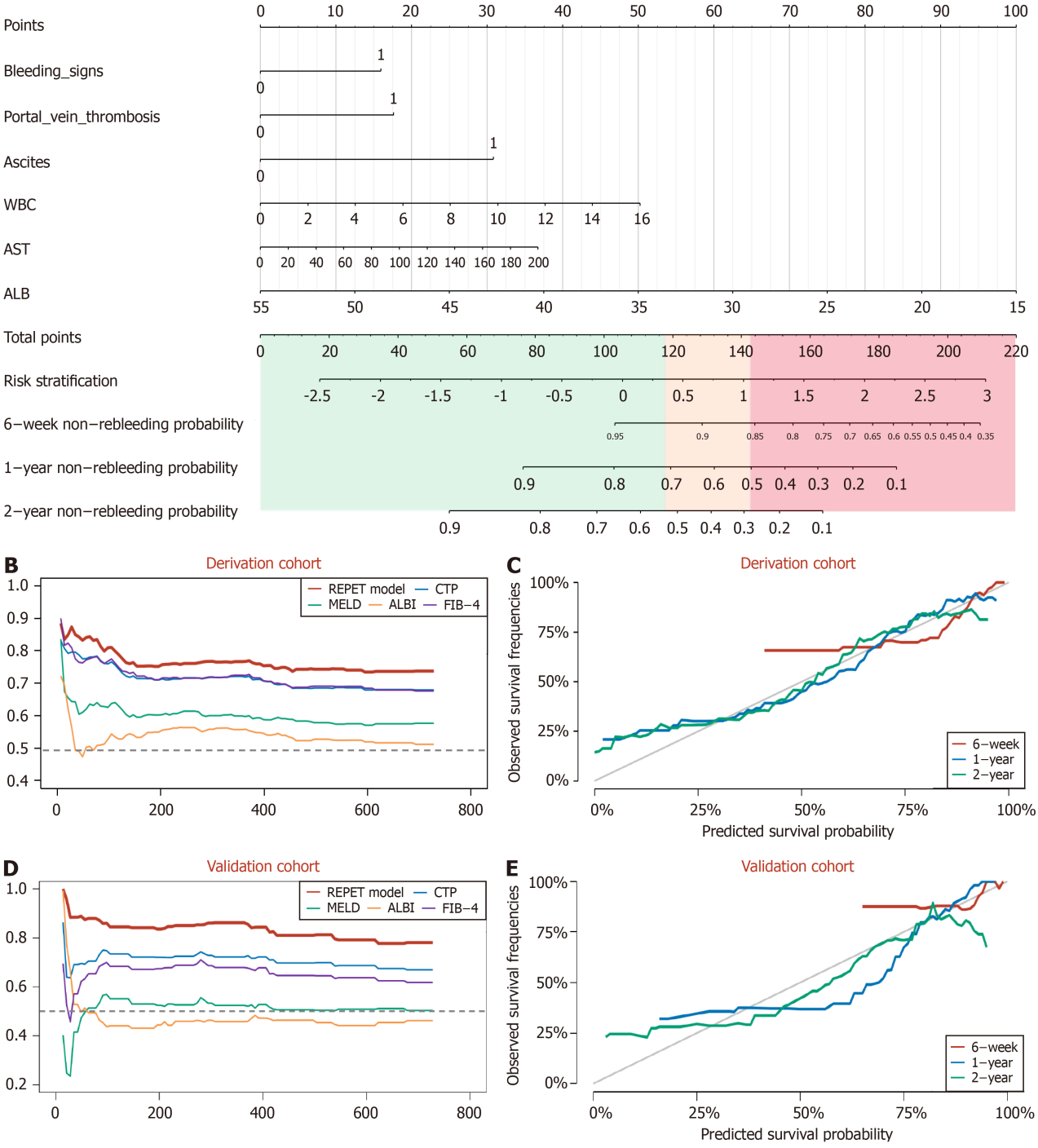

Figure 2 The nomogram, time-dependent concordance index and calibration curve. A: The nomogram for predicting variceal rebleeding; B-E: Risk stratification was based on the total points: Low-risk group (score < 117.3, green background), medium-risk (score 1173-142.7, yellow background) and high-risk (score > 142.7, red background). The time-dependent concordance index (C-index) of the rebleeding event prediction following endoscopic treatment model compared with other existing scores/criteria for the predicting variceal rebleeding in the derivation cohort (B) and in the external validation cohort (D). Calibration curves for 6 weeks, 1 year, and 2 years variceal rebleeding prediction in the derivation cohort (C) and in the external validation cohort (E). WBC: White blood cell; AST: Aspartate aminotransferase; ALB: Albumin; REPET: Rebleeding event prediction following endoscopic treatment.

A nomogram based on the REPET model was developed (Figure 2A). Using easily accessible clinical characteristics, clinicians can calculate a risk score according to the following formula: REPET score = 137.5 - 2.5 × ALB (g/L) + 30.799 × Ascites (1, 0) + 17.609 × PVT (1, 0) + 15.927 × bleeding signs (1, 0) + 0.184 × AST (U/L) + 3.138 × WBC (10^9/L). The incidences of rebleeding at 6 weeks, 1 year and 2 years can be obtained by combining the REPET score with the nomogram.

REPET performance evaluation and internal validation

The C-index values of the REPET for predicting 6-week, 1-year and 2-year rebleeding in the derivation cohort were 0.857, 0.775, and 0.741, respectively. As shown in Figure 2B, the C-index values of the REPET consistently exceeded those of the

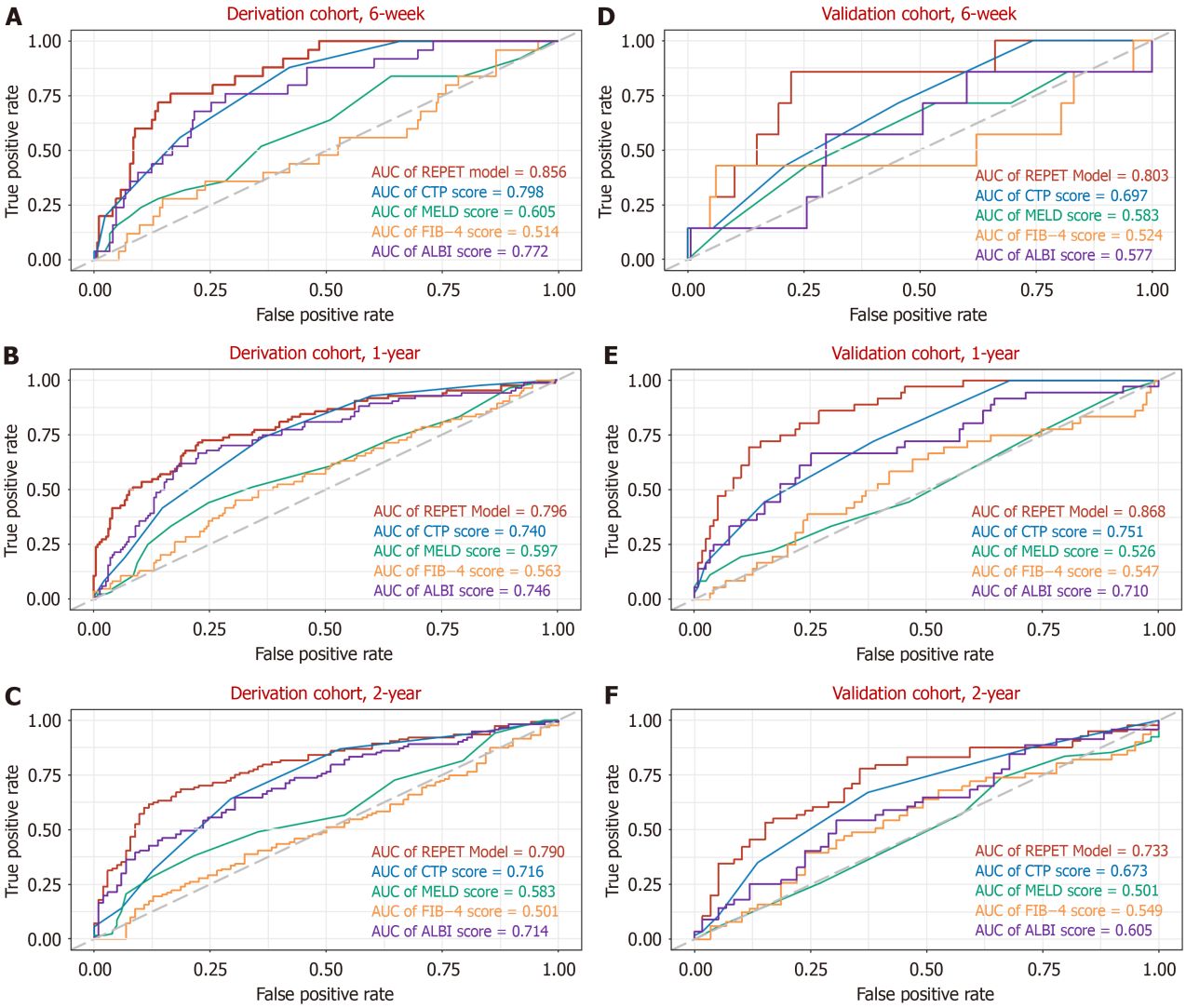

Figure 3 Area under receiver operating curve for variceal rebleeding in the derivation cohort and external cohort with 6 weeks, 1 year, and 2 years. A: Area under receiver operating curve for variceal rebleeding in the derivation cohort with 6 weeks; B: Area under receiver operating curve for variceal rebleeding in the derivation cohort with 1 year; C: Area under receiver operating curve for variceal rebleeding in the derivation cohort with 2 years; D: Area under receiver operating curve for variceal rebleeding in external cohort with 6 weeks; E: Area under receiver operating curve for variceal rebleeding in external cohort with 1 year; F: Area under receiver operating curve for variceal rebleeding in external cohort with 2 years. AUC: Area under the receiver operating curve; REPET: Rebleeding event prediction following endoscopic treatment; CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; FIB-4: Fibrosis 4; ALBI: Albumin-bilirubin.

other scores across all time points. The AUC values for the ability of the REPET to predict rebleeding were 0.856, 0.796, and 0.790 at 6 weeks, 1 year, and 2 years, respectively, which also surpassed those of the comparator scores (Figure 3A-C). Additionally, compared with the other scores, the DCA of the REPET model demonstrated a greater net benefit (Figure 4A-C). The calibration plot revealed that the REPET model had acceptable calibration at 6 weeks and good calibration at 1 year and 2 years (Figure 2C). The Brier scores for the REPET model at various time points were consistently less than 0.25, indicating excellent model accuracy (Table 2).

The REPET model was internally validated using 1000 bootstrap resampling data points. Our prognostic model showed good discriminative ability, with C-index values of 0.842, 0.759, and 0.735 at 6 weeks, 1 year, and 2 years, respectively (Supplementary Figure 4), and AUC values of 0.841, 0.783, and 0.779, respectively (Supplementary Figure 5). DCA for internal validation revealed favorable clinical net benefit (Supplementary Figure 6). The internal validation data showed good calibration at 1 year and 2 years but had limited calibration at 6 weeks (Supplementary Figure 7). Moreover, the internal validation Brier scores confirmed the excellent accuracy of the model (Table 2).

External validation

In the external validation set, the REPET model maintained impressive discriminative ability, with C-index values of 0.885, 0.862, and 0.768 at 6 weeks, 1 year, and 2 years, respectively, outperforming the compared models (Figure 2D). The AUC values for predicting rebleeding were 0.803, 0.868, and 0.733 at 6 weeks, 1 year, and 2 years, respectively, further demonstrating the solid predictive performance of the REPET model (Figure 3D-F). Similarly, DCA of the external validation cohort demonstrated the greatest clinical net benefit among all the models tested (Figure 4D-F). The REPET

Table 2 Predictive performance of the rebleeding event prediction following endoscopic treatment model

	C-index	AUC (95%CI)	Brier (95%CI)
The derivation cohort			
6 weeks	85.7	0.856 (0.794-0.918)	0.062 (0.042-0.083)
1 year	77.5	0.796 (0.737-0.855)	0.143 (0.121-0.165)
2 years	74.1	0.790 (0.727-0.853)	0.177 (0.149-0.205)
Internal validation			
6 weeks	84.2	0.841 (0.755-0.925)	0.066 (0.036-0.102)
1 year	75.9	0.783 (0.712-0.842)	0.153 (0.127-0.185)
2 years	73.5	0.779 (0.685-0.867)	0.184 (0.146-0.233)
External validation			
6 weeks	88.5	0.803 (0.647-0.959)	0.04 (0.014-0.066)
1 year	86.2	0.868 (0.808-0.928)	0.127 (0.096-0.159)
2 years	76.8	0.733 (0.634-0.832)	0.194 (0.151-0.237)

C-index: Concordance index; AUC: Area under the receiver operating curve; CI: Confidence interval.

model displayed good calibration at 1 and 2 years but limited calibration at 6 weeks (Figure 2E). The REPET model also exhibited exceptional accuracy, with Brier scores of 0.04, 0.127, and 0.194 at 6 weeks, 1 year, and 2 years, respectively.

Sensitivity and subgroup analysis

In the sensitivity analysis, we excluded patients who received emergency endoscopic hemostasis, and surprisingly, the REPET model maintained similarly excellent discriminatory ability (C-index: 6 weeks/1 year/2 years: 0.847/0.78/0.724; AUC: 6 weeks/1 year/2 years: 0.847/0.8/0.75) and accuracy (Brier scores: 6 weeks/1 year/2 years: 0.053/0.136/0.188) (Table 3). In the subgroup analyses, the REPET model showed good to excellent performance in predicting rebleeding at 6 weeks, 1 year, and 2 years across subgroups according to the etiology of cirrhosis, types of varices, sex, age and use of NSBB (Table 3).

Recurrence risk stratification

In the derivation cohort, X-tile identified 2 optimal thresholds (117.3 and 142.7) that classified patients into three distinct risk groups with significantly different probabilities of rebleeding: Low-risk (score < 117.3, n = 211), intermediate-risk (score between 117.3 and 142.7, n = 66), and high-risk (score > 142.7, n = 45) (Figure 5A). The cumulative probabilities of rebleeding for the low-risk, intermediate-risk and high-risk groups were 1.9%, 9.09%, and 33.33%, respectively, at 6 weeks, 11.85%, 37.88%, and 77.78%, respectively, at 1 year, and 30.46%, 77.78%, and 92.5%, respectively, at 2 years (Figure 5B). In the external cohort, the cumulative probabilities of rebleeding for the low-risk, intermediate-risk and high-risk groups were 1.67%, 13.04%, and 16.67%, respectively, at 6 weeks, 11.67%, 56.52%, and 75%, respectively, at 1 year, and 33.33%, 69.57%, and 83.33%, respectively, at 2 years (Figure 5C).

DISCUSSION

Variceal rebleeding is a concerning complication following EGVB in patients with liver cirrhosis, yet a recognized prognostic model that effectively predicts rebleeding is lacking[1-3]. In this longitudinal study, we investigated 56 clinical characteristics to construct and validate an easy-to-apply model comprising 6 items (ALB and AST concentrations, the WBC count, and the presence of ascites, PVT, and bleeding signs) to assist clinicians in risk stratification of rebleeding in cirrhotic EGVB patients who undergo endoscopic therapy combined with NSBB secondary prophylaxis. The model exhibited good discrimination and accuracy in predicting short-term (6 weeks) to long-term (1 and 2 years) variceal rebleeding risk, outperforming existing liver function assessment tools (CTP, MELD, albumin-bilirubin) and noninvasive fibrosis markers (FIB-4)[20,21]. There are several strengths of our study. First, the selection of predictive factors incorporated variables from multiple clinical domains. This study pioneers the integration of endoscopic features and the presence of significant complications into the model, thereby fully utilizing clinically available information to increase the model's predictive accuracy. Second, all patients were followed up until rebleeding or for up to 1 year, with 67.92% of the cohort being followed for up to 2 years or until rebleeding occurred. This comprehensive follow-up allows the REPET model to be used for predicting long-term rebleeding risk. Third, the study proposes a risk stratification scheme for rebleeding, which can assist clinicians in tailoring treatment plans according to patient risk levels. Finally, the external validation of the model with an independent cohort enhances the generalizability of our findings.

Table 3 Model performance in sensitivity analysis and subgroup analyses

	C-index	AUC (95%CI)	Brier score (95%CI)
Sensitivity analysis			
Exclude patients underwent emergency endoscopy			
6 weeks	0.847	0.847 (0.781-0.913)	0.053 (0.036-0.07)
1 year	0.78	0.8 (0.749-0.85)	0.136 (0.117-0.156)
2 years	0.724	0.75 (0.691-0.81)	0.188 (0.163-0.213)
Subgroup analysis			
Etiology of cirrhosis			
Chronic HBV infection			
6 weeks	0.9	0.915 (0.858-0.973)	0.04 (0.021-0.059)
1 year	0.779	0.793 (0.718-0.867)	0.137 (0.11-0.164)
2 years	0.717	0.731 (0.646-0.816)	0.195 (0.158-0.232)
Autoimmune liver disease			
6 weeks	0.913	0.884 (0.761-1)	0.048 (0.011-0.085)
1 year	0.84	0.892 (0.814-0.97)	0.122 (0.083-0.162)
2 years	0.798	0.821 (0.69-0.953)	0.154 (0.103-0.206)
Alcohol-related			
6 weeks	0.803	0.817 (0.705-0.929)	0.093 (0.04-0.147)
1 year	0.786	0.828 (0.723-0.934)	0.138 (0.094-0.183)
2 years	0.757	0.783 (0.66-0.907)	0.174 (0.126-0.222)
Other			
6 weeks	0.745	0.718 (0.532-0.903)	0.067 (0.025-0.109)
1 year	0.812	0.793 (0.695-0.892)	0.153 (0.11-0.196)
2 years	0.743	0.777 (0.658-0.897)	0.2 (0.141-0.259)
Types of varices			
EV			
6 weeks	0.862	0.868 (0.796-0.94)	0.061 (0.036-0.087)
1 year	0.836	0.854 (0.799-0.909)	0.129 (0.103-0.155)
2 years	0.776	0.822 (0.747-0.896)	0.164 (0.131-0.197)
EV combined with GV			
6 weeks	0.845	0.823 (0.727-0.919)	0.05 (0.029-0.071)
1 year	0.768	0.791 (0.726-0.857)	0.144 (0.119-0.17)
2 years	0.715	0.726 (0.65-0.802)	0.203 (0.169-0.237)
Sex			
Male			
6 weeks	0.872	0.894 (0.845-0.943)	0.062 (0.04-0.084)
1 year	0.796	0.809 (0.75-0.869)	0.139 (0.115-0.163)
2 years	0.719	0.724 (0.651-0.798)	0.2 (0.167-0.234)
Female			
6 weeks	0.703	0.71 (0.539-0.882)	0.044 (0.02-0.067)
1 year	0.781	0.831 (0.764-0.899)	0.136 (0.108-0.164)
2 years	0.772	0.831 (0.756-0.907)	0.164 (0.13-0.197)

Age			
< 60			
6 weeks	0.889	0.862 (0.77-0.954)	0.046 (0.025-0.067)
1 year	0.771	0.783 (0.711-0.854)	0.134 (0.107-0.161)
2 years	0.706	0.731 (0.649-0.812)	0.191 (0.155-0.227)
≥ 60			
6 weeks	0.814	0.826 (0.742-0.909)	0.064 (0.039-0.089)
1 year	0.801	0.848 (0.792-0.904)	0.142 (0.117-0.166)
2 years	0.761	0.796 (0.723-0.87)	0.181 (0.150-0.213)
Use of NSBB			
Non-NSBB group			
6 weeks	0.846	0.837 (0.753-0.921)	0.079 (0.046-0.113)
1 year	0.802	0.835 (0.762-0.908)	0.146 (0.114-0.178)
2 years	0.794	0.871 (0.805-0.937)	0.141 (0.11-0.171)
NSBB group			
6 weeks	0.85	0.846 (0.754-0.939)	0.042 (0.025-0.06)
1 year	0.79	0.806 (0.75-0.862)	0.134 (0.112-0.156)
2 years	0.708	0.705 (0.631-0.78)	0.21 (0.177-0.242)

C-index: Concordance index; AUC: Area under the receiver operating curve; CI: Confidence interval; HBV: Hepatitis B virus; EV: Esophageal varices; GV: Gastric varices; NSBB: Nonselective beta-blocker.

To date, several alternative models/scores have been proposed to predict variceal rebleeding risk, but the results have been mixed. Wang *et al*[7] attempted to repurpose commonly used cirrhosis scoring systems for predicting rebleeding, concluding that the MELD-Na and MELD were effective predictors, with AUC values of 0.85 and 0.80, respectively, whereas the CTP demonstrated lower predictive accuracy, with an AUC of 0.65. In 2023, Liu *et al*[5] developed a prognostic model for esophageal variceal rebleeding in hepatitis B-associated cirrhosis patients that was independent of those risk scores, comprising the body mass index, liver stiffness measurement, NSBB usage, platelet count, and hemoglobin concentration, and achieved good predictive results (C-index: 0.772). However, the model's predictive accuracy may be weakened by two key issues: The inaccuracy of liver stiffness measurement in patients with ascites and its limited use in EGVB patients, along with the assumption of uniform NSBB effectiveness without considering variations in patient adherence, dosage, and individual response. These factors could undermine the model's relevance and reliability in critical clinical scenarios. Furthermore, the lack of external validation for the above studies makes it challenging to assess the generalizability of these models. Recently, in a multicenter cohort study encompassing 581 individuals, Balcar *et al*[26] concluded that routine clinical indicators and cirrhosis prognostic scoring systems (CTP, MELD, and MELD-Na) were insufficient for predicting rebleeding. However, this conclusion might be limited by the fact that many clinical characteristics potentially influencing rebleeding were not considered.

The most commonly reported variables influencing rebleeding can be categorized as follows: Liver dysfunction, severity of portal hypertension, infection indicators, and variceal features[1,17,27-32]. The present study revealed that higher serum ALB concentrations protect against EGVB, whereas elevated AST levels and WBC counts are risk factors. A decrease in ALB levels typically indicates impaired liver synthetic function, whereas elevated AST reflects hepatocellular injury, and both of these changes are manifestations of liver dysfunction. An elevated WBC indicates the presence of an infection or an inflammatory response in the body. Consistent with our findings, bacterial infection has been identified as a significant risk factor for rebleeding, underscoring the critical importance of prophylactic antibiotic use in managing EGVB[1]. In addition to routine biochemical indicators, our model also assessed comorbidities and complications at admission. Ascites detected *via* ultrasonography reflected, in part, liver dysfunction and the severity of portal hypertension[33]. PVT was diagnosed *via* computed tomography, highlighting an aspect that may exacerbate portal hypertension[34]. Moreover, we comprehensively assessed the impact of endoscopic examination and treatment characteristics on esophagogastric varices and found that bleeding signs under endoscopy are a valid predictor of rebleeding, a fact that has been neglected in previous studies[17].

By integrating clinical characteristics from several domains, we developed a novel model (the REPET model) to predict the risk of experiencing rebleeding in patients with cirrhosis and EGVB. The REPET model achieved great prognostic performance, as confirmed in an independent cohort. In the external cohort, 77.42% of patients were classified as low risk, and their rebleeding rates at 6 weeks and 1 year were 1.67% and 11.67%, respectively. However, the risk of rebleeding increased sharply to 33.33% at 2 years. These findings suggest that follow-up at approximately 1 year is critical for low-risk patients, as it may help identify and prevent rebleeding events effectively. The remaining 22.58% of patients were

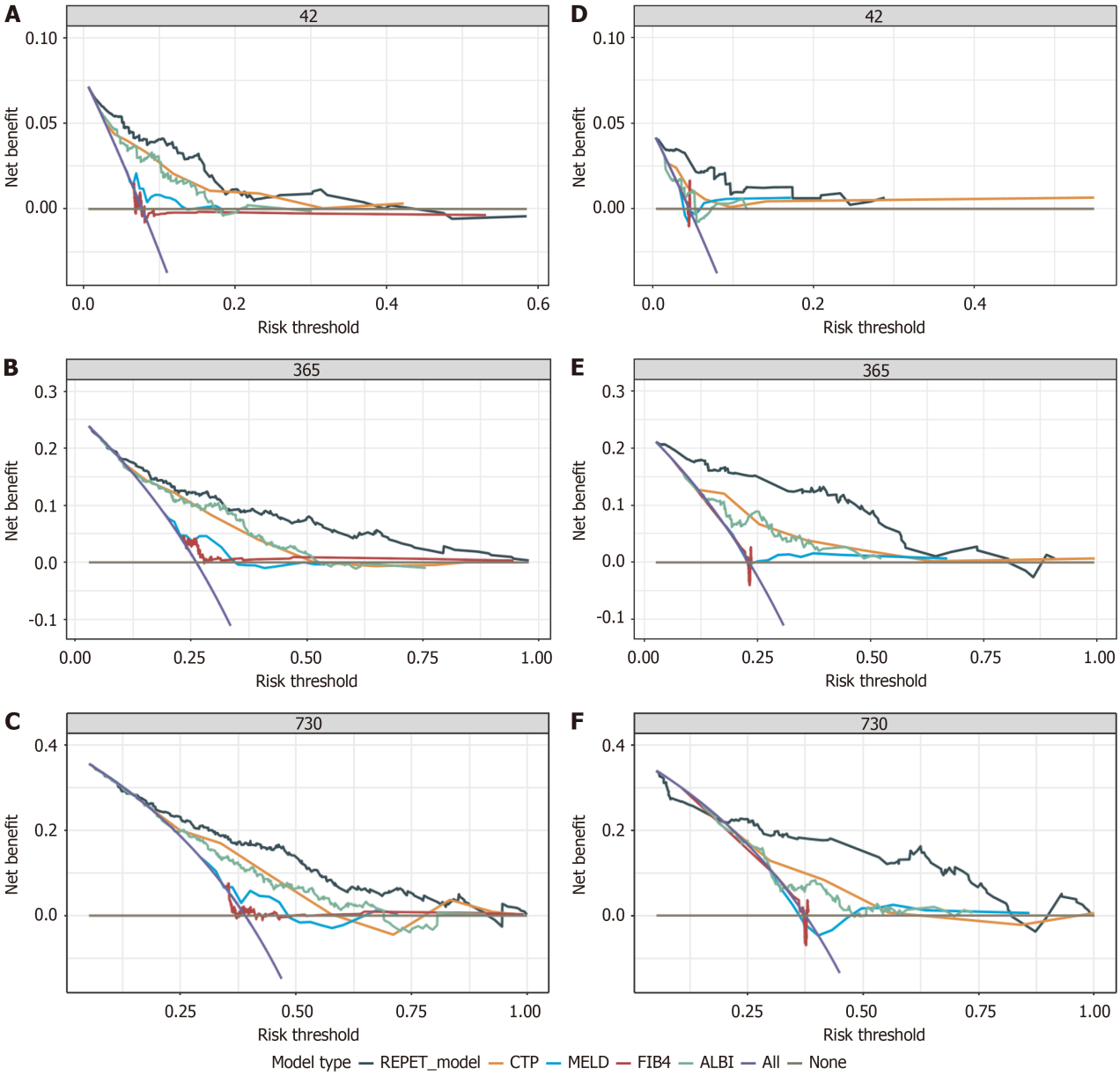

Figure 4 Decision curve analysis for variceal rebleeding in the derivation cohort and external cohort with 6 weeks, 1 year, and 2 years. A: Decision curve analysis for variceal rebleeding in the derivation cohort with 6 weeks; B: Decision curve analysis for variceal rebleeding in the derivation cohort with 1 year; C: Decision curve analysis for variceal rebleeding in the derivation cohort with 2 year; D: Decision curve analysis for variceal rebleeding in external cohort with 6 weeks; E: Decision curve analysis for variceal rebleeding in external cohort with 1 year; F: Decision curve analysis for variceal rebleeding in external cohort with 2 years. REPET: Rebleeding event prediction following endoscopic treatment; CTP: Child-Turcotte-Pugh; MELD: Model for end-stage liver disease; FIB-4: Fibrosis 4; ALBI: Albumin-bilirubin.

categorized as intermediate- to high-risk, corresponding to a greater risk of variceal rebleeding. Thus, more frequent follow-up and more aggressive prophylaxis may be needed for this population. In summary, the REPET score can distinguish between low-risk patients and intermediate- to high-risk patients well, which may be useful for guiding follow-up and treatment regimen adjustment.

We subsequently performed further sensitivity analyses and subgroup analyses based on patient characteristics. Notably, the REPET model maintained excellent predictive performance in all of the sensitivity analyses and subgroup analyses. This demonstrates the robustness and generalizability of our risk stratification system to "real-world" clinical practice, where standardization may be lacking[35]. Our research has certain limitations. First, in this study, we utilized retrospective data to construct and validate a predictive model, which has inherent information and recall bias, requiring further validation *via* prospective data. Accurate recording of patients' conditions and regular follow-up at each medical center may help to reduce these biases. Second, the model cannot predict rebleeding in patients who are undergoing other treatment regimens, such as TIPS and balloon-occluded retrograde transvenous obliteration. Prediction of rebleeding risk in these patients require the development of additional specialized models that consider factors such as preoperative imaging assessments, the hepatic venous pressure gradient, the portal pressure gradient, and other intraoperative parameters. Additionally, given the increased rebleeding risk and distinct metabolic, tumor, and treatment profiles in HCC patients, we excluded them from this study. Therefore, further validation of the REPET model is needed

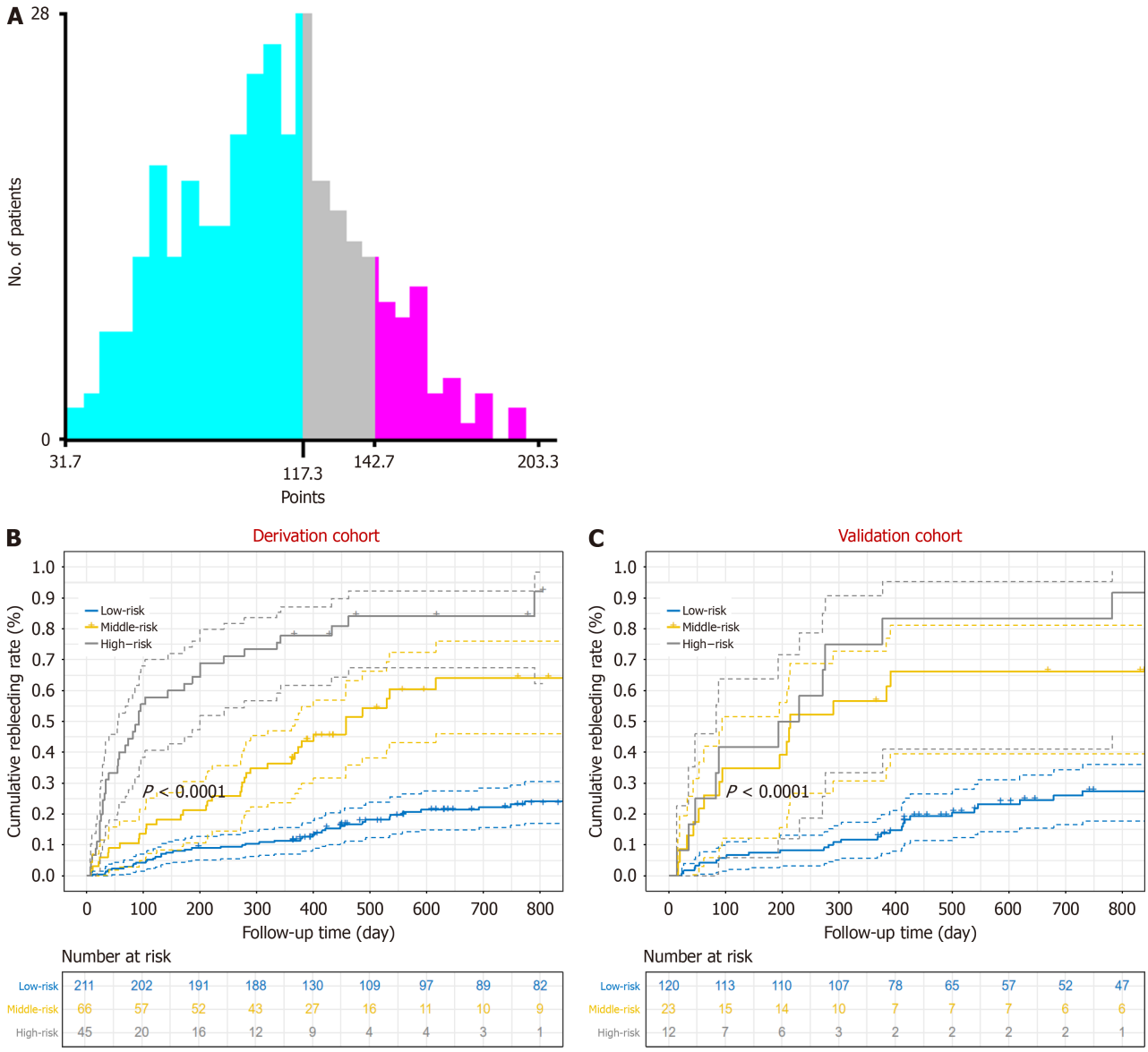

Figure 5 Risk stratification for variceal rebleeding. A: Optimal thresholds for prognostic scores (using X-tile software); B and C: The Kaplan-Meier curves for the different risk groups in the derivation (B) and validation cohorts (C).

for the HCC population. Third, although the REPET model can be used to effectively distinguish low-risk patients from intermediate- to high-risk patients, its ability to discriminating between intermediate- and high-risk patients in external validation cohort is lower than expected. One possible reason for this may be sampling bias and reduced statistical power due to the small sample size in the high-risk group. Thus, these findings need further validation in a large independent cohort. The current REPET model can be used for clinical risk stratification to some extent, as up to 69.39% of patients were classified as low risk and were spared from aggressive treatment. Fourth, there were statistically significant differences between the two cohorts in certain serological markers. These differences may be attributed to variations in sample size and time span, reflecting the complexity of real-world data. Nevertheless, the REPET model consistently maintained robust predictive performance across both the training and external validation sets, further demonstrating the model's strong generalizability. Finally, although a comprehensive investigation of the available clinical characteristics was performed in this study, certain gaps in the data persist, and potential risk factors for rebleeding may have been missed. Future prospective studies should implement standardized protocols for data evaluation and collection and should take into account potential rebleeding risk factors such as liver stiffness, spleen stiffness, and relevant blood electrolyte concentrations.

CONCLUSION

In conclusion, we developed and externally validated a new REPET model for predicting rebleeding in patients with cirrhosis and EGVB comprising a set of broadly available clinical variables obtained from multiple perspectives. By revealing the expected probability of rebleeding for individuals at different time points, the REPET model allows for

rational risk stratification of patients, which helps optimize follow-up and treatment regimens. In addition, the model presented here should be further evaluated in prospective cohorts and in independent cohorts at other centers.

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FOOTNOTES

Author contributions: Zhan JY and Chen J have contributed equally to this work and share first authorship; Mu YP and Wang J have contributed equally to this work and share co-corresponding authorship; Mu YP, Wang J, and Zhan JY carried out study concept and design; Zhan JY carried out interpretation of data and drafting of the manuscript; Zhan JY, Chen J, Yu JZ, Xu FP, Xing FF, Wang DX, Yang MY, and Xing F carried out collection of data; Zhan JY, Chen J, and Yu JZ were involved in analysis of data; Mu YP, Wang J, and Chen J were involved in critical revision for important intellectual content; and all authors have read and approve the final manuscript.

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Data sharing statement: Statistical code and the dataset supporting the findings of this study are available from the corresponding author upon reasonable request. Requests should be directed to ypmu8888@126.com. Data will be shared following approval of a reasonable request and may require a signed data use agreement to ensure the protection of sensitive information.

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ORIGINAL ARTICLE

Prospective Study

Vedolizumab serum trough concentrations with and without thiopurines in ulcerative colitis: The prospective VIEWS pharmacokinetics study

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Abstract

BACKGROUND

Ulcerative colitis (UC) is a chronic inflammatory condition requiring continuous treatment and monitoring. There is limited pharmacokinetic data on vedolizumab during maintenance therapy and the effect of thiopurines on vedolizumab trough concentrations is unknown.

AIM

To investigate the exposure-response relationship of vedolizumab and the impact

of thiopurine withdrawal in UC patients who have achieved sustained clinical and endoscopic remission during maintenance therapy.

METHODS

This is a post-hoc analysis of prospective randomized clinical trial (VIEWS) involving UC patients across 8 centers in Australia from 2018 to 2022. Patients in clinical and endoscopic remission were randomized to continue or withdraw thiopurine while receiving vedolizumab. We evaluated vedolizumab serum trough concentrations, presence of anti-vedolizumab antibodies, and clinical outcomes over 48 weeks to assess exposure-response association and impact of thiopurine withdrawal.

RESULTS

There were 62 UC participants with mean age of 43.4 years and 42% were females. All participants received vedolizumab as maintenance therapy with 67.7% withdrew thiopurine. Vedolizumab serum trough concentrations remained stable over 48 weeks regardless of thiopurine use, with no anti-vedolizumab antibodies detected. Patients with clinical remission had higher trough concentrations at week 48. In quartile analysis, a threshold of > 11.3 μg/mL was associated with sustained clinical remission, showing a sensitivity of 82.4%, specificity of 60.0%, and an area of receiver operating characteristic of 0.71 (95%CI: 0.49-0.93). Patients discontinuing thiopurine required higher vedolizumab concentrations for achieving remission.

CONCLUSION

A positive exposure-response relationship between vedolizumab trough concentrations and UC outcomes suggests that monitoring drug levels may be beneficial. While thiopurine did not influence vedolizumab levels, its withdrawal may necessitate higher vedolizumab trough concentrations to maintain remission.

Key Words: Pharmacokinetic; Vedolizumab; Thiopurine; Ulcerative colitis; Trough concentration; Antibody; Inflammatory bowel diseases

Core Tip: This prospective study investigated the pharmacokinetic relationship between vedolizumab trough concentrations and clinical outcomes in ulcerative colitis patients who had achieved clinical remission, on vedolizumab with or without thiopurine. Vedolizumab levels remained stable over 48 weeks, regardless of thiopurine withdrawal. Significant associations between vedolizumab trough concentrations and endoscopic, histologic, and histo-endoscopic remission at week 48 suggest that monitoring vedolizumab levels could be beneficial, with a threshold of > 11.3 μg/mL linked to sustained clinical remission. Although thiopurine withdrawal did not affect vedolizumab levels, it may necessitate higher vedolizumab trough concentrations to maintain remission.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory intestinal disease that results in a substantial burden on patients' quality of life and to the healthcare system[1,2]. A significant proportion of patients suffer from moderate-to-severe disease activity necessitating treatment with an advanced therapy[3]. Long-term maintenance of biological therapy helps sustain clinical remission and prevent relapse. However, Immunogenic- and non-immunogenic causes of loss of response occur at a rate of 13% per year, sometimes requiring dose escalation or reduction in dose frequency of the biological agents. Optimization of these drugs through therapeutic drug monitoring has become a pivotal strategy that might sustain therapeutic efficacy[4,5]. This approach has demonstrated significant benefits, particularly evident with anti-tumor necrosis factor (TNF) therapy, where higher drug concentrations correlate with increased rates of remission[6,7]. Studies have also shown that thiopurine co-therapy is more effective than anti-TNF monotherapy in inducing clinical remission, primarily by reducing the formation of anti-drug antibodies[8,9].

Vedolizumab, an IgG monoclonal antibody with target binding of the α4β7 integrin on the surface of T-lymphocytes selectively inhibit the migration of lymphocytes into the gastrointestinal tract[10]. The exposure-efficacy relationship of vedolizumab in inflammatory bowel diseases (IBDs) is not well-defined. Post-hoc analyses of the GEMINI trial[11,12] and the recent ERELATE study[13] revealed a positive exposure-response relationship of vedolizumab. These studies

suggested that vedolizumab concentration can serve as a predictor for both short- and long-term outcomes. In contrast, data from the ENTERPRET trial[14] and other observational studies[15,16] found no association between trough concentration and improved clinical outcomes. Furthermore, there is almost an absence of therapeutic drug monitoring data during the maintenance phase of UC treatment, and the potential impact of concomitant thiopurine therapy on vedolizumab trough concentrations in prospective studies. Our objective, therefore, was to prospectively evaluate the exposure-response relationship of vedolizumab on UC patients in clinical-endoscopic remission either with continuation of thiopurine-combination or after withdrawal of thiopurine but continuation of vedolizumab monotherapy.

MATERIALS AND METHODS

Patient population

We performed a prospective controlled trial on UC patients treated with vedolizumab that were randomized to either continue or to withdraw from thiopurine (VIEWS)[17]. Between 2018 to 2022, 8 IBD centers in Australia enrolled UC patients aged > 18 years treated with 300 mg intravenous vedolizumab every 8 weeks combined with an optimized thiopurine. All eligible participants were in corticosteroid-free clinical remission, with partial Mayo score < 2 without any subscore > 1 for at least 6 months, and in endoscopic improvement with Mayo endoscopic subscore 0-1. Participants at a ratio of 2:1 were randomized to either withdraw- or to continue their thiopurine whilst continuing vedolizumab. Every participant continued to receive eight weekly vedolizumab infusions with regular vedolizumab therapeutic drug monitoring, alongside eight weekly complete blood count, liver function test, serum C-reactive protein (CRP), electrolytes, urea, and creatinine. Fecal calprotectin and 6-thioguanine nucleotides (6-TGN) levels were measured at week 0 and 48. The study was approved by Sydney Local Health District Human Research Ethics Committee (HREC/17/CRGH/22) and Australian New Zealand Clinical trials registry (registration number ACTRN12618000812291). All participants provided written informed consent.

Vedolizumab serum trough concentration, anti-vedolizumab antibody and fecal calprotectin testing

Vedolizumab serum trough concentration and anti-vedolizumab antibody testing were performed at week 0, 24, and 48. Samples collected prior to vedolizumab infusions were centrifuged, stored at -70 °C and 5 μL of serum was batch tested by the Quest Pharmaceutical Services Holdings LLC (QPS, Newark, Delaware, United States) core laboratory. Enzyme-linked immunosorbent assay was performed in accordance with standard operating procedures and quality-controlled. Qualitative anti-vedolizumab antibodies, including neutralizing antibodies, were analyzed using electrochemiluminescence independent of drug concentrations. Fecal calprotectin levels were analyzed at Queensland Medical Laboratory Pathology (QML, Murarrie, Queensland, Australia) using the DiaSorin Liaison Calprotectin assay (DiaSorin, Saluggia, Italy).

Endpoints

The endpoints of interest of this post-hoc study were vedolizumab serum trough concentrations, antibodies to vedolizumab, their differences over time from week 0 to week 48, and between subjects on combination therapy with thiopurine and those on monotherapy. Clinical assessment was conducted every 8 weeks and measured by individual components of the partial Mayo clinical score as well as the composite score (range 0-9). Clinical remission was defined as partial Mayo score < 2 with no subscore exceeding 1, and the absence of corticosteroid-use. Biochemical remissions were determined through serum CRP level < 5 mg/L and fecal calprotectin concentration < 150 μg/g. Colonoscopy was performed at week 48 to assess endoscopic and histologic activity. Endoscopic improvement was defined as a Mayo endoscopic score of 0-1. Histologic remission was defined as a Nancy histological index of 0. Corticosteroid-free clinical remission was also evaluated at 2 years after study commencement.

Statistical analyses

Non-parametric continuous variables were presented as median and interquartile range and analyzed by the Mann-Whitney *U* test. Normally-distributed data were described by mean with standard deviation and analyzed by the *t*-test. Categorical variables were expressed as proportions and analyzed by χ^2 test. A two-tailed *P* value of < 0.05 was deemed statistically significant. A Spearman correlation was performed to assess the association between vedolizumab serum trough concentrations and clinical outcomes at each measurement time point.

For longitudinal analysis, a mixed-effects logistic regression for repeated measures was utilized to investigate the association between remission rates and vedolizumab serum trough concentrations over time, assuming an unstructured covariance and adjusting interaction terms for concomitant thiopurine use, visit, interaction between outcome group and visit. Area of receiver operating characteristic (AUROC) curves were evaluated to identify optimal vedolizumab trough concentration with the maximal diagnostic yield. The analyses were performed using STATA version 17.0 (Stata Corporation, United States).

RESULTS

Patient characteristics and disease outcomes

In total, 62 UC (42% females, mean age 43.4 years) patients in clinical and endoscopic remission were prospectively recruited. Figure 1 illustrates the study enrollment process. Patients had typically failed corticosteroids and immunomodulators, and 22.6% had exposed to anti-TNF. Among the participants, 43 were previously on azathioprine 25-100 mg (69.3%), 16 (25.8%) on 6-mercaptopurine 12.5-100 mg, and 3 (4.8%) on thioguanine 20 mg. Two-thirds of the patients (n = 42) were randomized to withdraw thiopurines, including 27 previously on azathioprine, 13 on 6-mercaptopurine, and 2 on thioguanine. Table 1 provides the baseline characteristics and comparison between the thiopurine withdrawal and continuation groups. At week 0, the median 6-TGN levels were comparable between the withdrawal group and the continuation group, at 248 (186-326) *vs* 282 (186-367.5) pmol/8 × 10^8 red blood cell (RBC), respectively (P = 0.72). At 48 weeks, the median TGN level in the continuation group was 214 (163.5-358) pmol/8 × 10^8 RBCs, while levels were undetectable in the withdrawal group. A total of 82.3% of subjects remained in clinical remission, 79.6% in CRP biochemical remission, and 91.5% in fecal calprotectin biochemical remission. In addition, 68.4% of subjects were in endoscopic improvement and 58.2% were in histologic remission. Clinical relapse occurred in 11 patients (17.7%), with a median partial Mayo score of 4 and Mayo endoscopic score of 3. Two patients (10%) were in the continuation group and 9 patients (21.4%) were in the withdrawal group (P = 0.27). Corticosteroids were required in 5 patients in the withdrawal group, while no patients in the continuation group needed corticosteroids. The total corticosteroid-free clinical remission at 2 year was 77.4%.

Vedolizumab serum trough concentrations and anti-vedolizumab antibodies

In total, 180 serum samples were tested for vedolizumab serum trough concentrations and anti-vedolizumab antibodies at 3 time points. The median (interquartile range) vedolizumab serum trough concentrations were 15.6 μg/mL (10.8-20.8), 16.9 μg/mL (12.0-22.1), and 15.4 μg/mL (11.3-21.7) at week 0, 24, and 48, respectively. We observed significant positive correlations of vedolizumab serum trough concentrations at each measurement time point, with Spearman's rho of 0.77 (P < 0.001) between week 0 and week 24, and 0.80 (P < 0.001) between week 0 and week 48. These findings suggest the stability of vedolizumab concentrations over the course of the study. Figure 2 shows distribution of vedolizumab serum trough concentrations at week 0 and 48. Irrespective of whether patients continued and withdrew thiopurine, there was no discernible difference in vedolizumab concentrations between the two groups. No patients tested positive for anti-vedolizumab antibodies throughout the study.

Association between vedolizumab serum trough concentrations and treatment endpoints

We analyzed the association between vedolizumab serum trough concentrations against UC treatment endpoints. The median vedolizumab serum trough concentrations at week 48 was statistically significantly higher in patients who achieved clinical remission *vs* those that did not (P = 0.04, Figure 3). At week 0, higher vedolizumab serum trough levels were observed in the clinical remission group compared to the non-remission group, but without statistical significance (P = 0.47). No significant associations were found between trough levels at week 0 and CRP (P = 0.96), fecal calprotectin (P = 0.32), endoscopic improvement (P = 0.69), histologic (P = 0.44), and histo-endoscopic remission (P = 0.68) at week 48 (Table 2). Similarly, trough levels at week 48 did not show significant associations with CRP (P = 0.08), fecal calprotectin (P = 0.14), endoscopic improvement (P = 0.09), histologic (P = 0.78), and histo-endoscopic remission (P = 0.27) at the same time point. Histologic remission at baseline was significantly associated with higher vedolizumab serum trough levels at week 48 (P < 0.01). Patients achieving clinical remission after a 2-year follow-up showed higher vedolizumab serum trough concentrations at week 48 compared to those who did not (P = 0.02).

The longitudinal analysis was performed through mixed model regressions, incorporating interaction terms to assess the association between vedolizumab serum trough concentrations and various clinical outcomes over specified time intervals. A trend towards significant association between vedolizumab serum trough concentrations and clinical remission at week 48 was observed (P = 0.10). Notably, the trough concentrations interaction with time had significant influence on endoscopic improvement (P = 0.02), histologic remission (P = 0.04), and histo-endoscopic remission (P = 0.02) (Figure 4). No association was observed for CRP (P = 0.37) and fecal calprotectin (P = 0.57) remission.

Vedolizumab serum trough concentrations quartile and threshold analysis

We analyzed vedolizumab serum trough concentrations by quartiles to assess whether a threshold level correlated with outcomes (Table 3). In subjects with vedolizumab serum trough concentrations corresponding to quartile 2, there was a higher likelihood of achieving clinical, endoscopic, and histologic remission at week 48 compared to quartile 1. In subjects continuing thiopurine compared to those who withdrew thiopurine, there was a trend suggesting that lower vedolizumab serum trough levels were sufficient to achieve remission (Figure 5). Week 0 vedolizumab serum trough level > 11.5 μg/mL was associated with clinical remission at week 48 with a sensitivity of 76.5%, specificity of 45.5%, and an AUROC of 0.57 (95%CI: 0.35-0.79). Week 48 vedolizumab serum trough level > 11.3 μg/mL was associated with clinical remission with sensitivity of 82.4%, specificity of 60.0%, and an AUROC of 0.71 (95%CI: 0.49-0.93).

DISCUSSION

In this prospective study, we investigated the association between vedolizumab serum trough concentrations and clinical

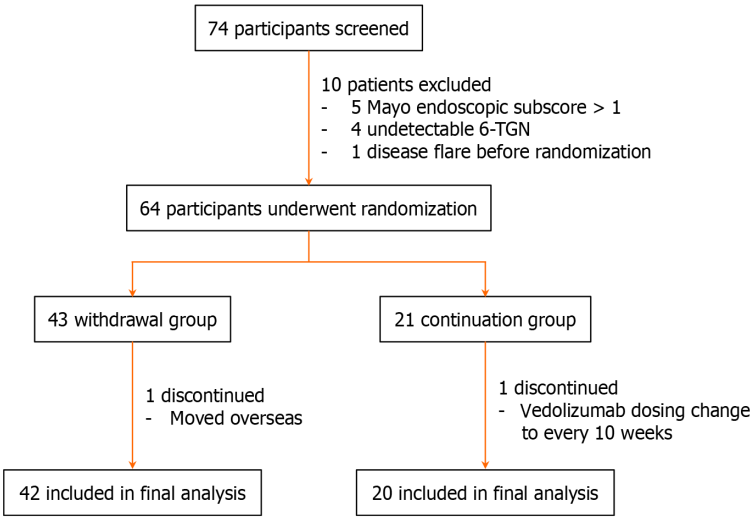

Figure 1 Study enrollment process. TGN: Thioguanine nucleotides.

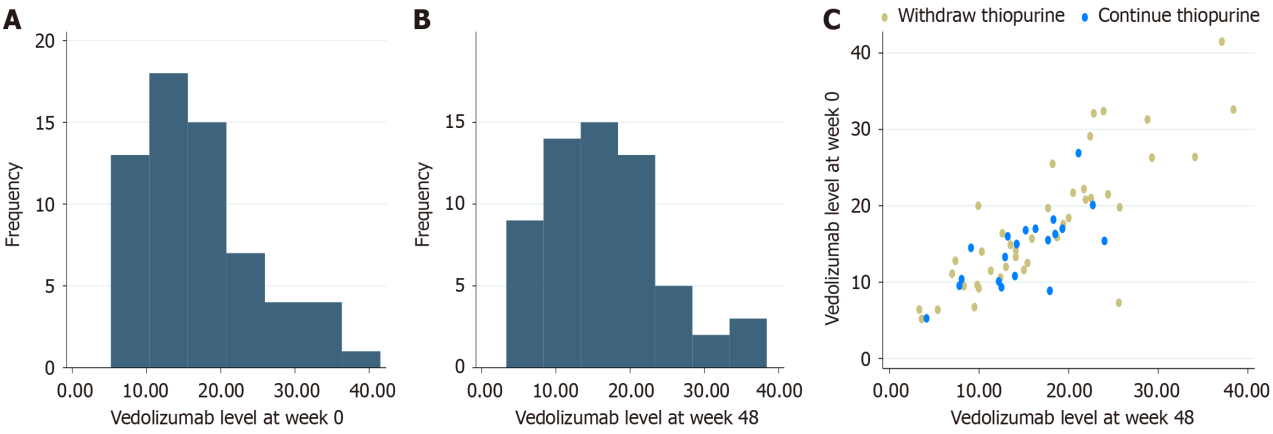

Figure 2 Histograms demonstrates the distribution of vedolizumab serum trough concentrations. A: At week 0; B: At week 48; C: Scatter plot illustrates the relationship between vedolizumab serum trough concentrations at week 0 and week 48, categorized by thiopurine use.

endpoints while continuing vedolizumab as maintenance treatment with either continued or withdrew thiopurine. We observed a significant correlation of vedolizumab trough concentrations between three time points within 48 weeks. The continuation or withdrawal of thiopurine did not affect vedolizumab concentrations and no anti-vedolizumab antibodies were detected during the study. An exposure-response relationship was identified between trough concentrations at week 48 and clinical remission at both week 48 and 2 years. In longitudinal analysis, higher vedolizumab serum trough concentrations were associated with increased likelihood of endoscopic, histologic, and histo-endoscopic remission. A vedolizumab cut-off level of > 11.3 μg/mL showed highest diagnostic value for predicting long-term clinical remission during maintenance treatment. Patients who discontinued thiopurine may require higher vedolizumab trough concentrations to attain remission endpoints.

Several studies have investigated the association between vedolizumab serum trough concentrations during induction to early maintenance phase and clinical and endoscopic remission[12-15,18-23]. Most of these studies identified an exposure-efficacy relationship, underscoring the value of therapeutic drug monitoring. However, few studies have specifically addressed this association during long-term maintenance treatment[22,24-28]. A real-world study of 258 IBD patients, including 45% with UC treated with vedolizumab for median of 38 weeks, reported significantly higher vedolizumab concentrations in those achieving clinical, biochemical, or endoscopic remission[26]. However, the association in UC subgroup was marginally significant. Another retrospective study showed that histologic healing defined by Nancy index < 1 was associated with higher vedolizumab trough levels during maintenance treatment[25]. After 2.4 years of maintenance treatment with vedolizumab, the TUMMY study, which included 159 IBD patients (including 100 with UC) with 81% having prior exposure to anti-TNF, demonstrated an association between vedolizumab trough concentrations and biochemical, but not clinical remission[24]. A very low incidence of anti-vedolizumab antibodies was consistently found across all studies[22,29]. Discrepancies of pharmacokinetic findings among these studies can be due to various factors, including nature of the study, a mixed population of UC and CD, sample size, diverse measures of primary outcomes, varied duration of vedolizumab therapy, different intervals of vedolizumab infusion, prior exposure to anti-TNF, and distinct methodologies for measuring vedolizumab trough concentrations.

Table 1 Baseline characteristics and comparison between thiopurine withdrawal and continuation groups, *n* (%)/mean ± SD/ median (interquartile range)

	Total (*n* = 62)	Withdrawal group (*n* = 42)	Continuation group (*n* = 20)
Demographics			
Female sex	26 (41.9)	21 (50)	5 (25)
Age (years)	43.4 ± 18.0	43.7 ± 19.0	42.8 ± 16.1
Current/ex-smoker	16 (25.8)	11 (26.2)	5 (25)
Disease extent			
Left sided colitis	41 (66.1)	27 (64.3)	14 (70)
Pancolitis	21 (33.9)	15 (35.7)	6 (30)
Disease duration (years)	7.5 (4-12)	8 (3-13)	6.5 (4-11.8)
Prior treatments			
Corticosteroids	57 (91.9)	39 (92.9)	18 (90)
Immunomodulators	62 (100)	42 (100)	20 (100)
Anti-TNF	14 (22.6)	10 (23.8)	4 (20)
Baseline assessment			
Partial mayo score	0 (0-0)	0 (0-0)	0 (0-0)
Mayo endoscopic score, 0-1 (*n* = 57)	57 (100)	42 (100)	20 (100)
Histologic remission (*n* = 54)	41 (75.9)	25 (69.4) (*n* = 36)	16 (88.9) (*n* = 18)
Laboratory values at baseline			
Hemoglobin (g/L)	139.6 ± 12.9	137.8 ± 13.8	143.2 ± 10.5
Fecal calprotectin (μg/g)	23.7 (7.9-65)	17.7 (6.7-51.8)	44.3 (10.5-124.6)
Albumin (g/L)	41.4 ± 4.5	41.4 ± 5.0	41.2 ± 3.2
CRP (mg/L)	1.25 (0.5-3)	1.3 (0.5-3.3)	0.9 (0.2-2.5)
Vedolizumab serum trough concentrations			
Week 0 (μg/mL)	15.6 (10.8-20.8)	16.2 (11.5-22.2)	15.2 (10.3-16.9)
Week 24 (μg/mL) (n= 57)	16.9 (12.0-22.1)	17.8 (10.5-25.7)	14.7 (12.2-19.7)
Week 48 (μg/mL) (n= 61)	15.4 (11.3-21.7)	15.9 (10.3-22.5)	14.7 (12.4-18.4)
Outcomes at week 48			
Partial mayo score	0 (0-0)	0 (0-0)	0 (0-0)
Clinical remission	51 (82.3)	33 (78.6)	18 (90)
CRP (mg/L)	1.2 (0-3.4)	2.4 (0-6.4) (*n* = 32)	1.2 (0-2.8) (*n* = 17)
CRP remission	39 (79.6)	24 (75)	15 (88.2)
Fecal calprotectin (μg/g)	15.4 (5.5-47.7)	15.3 (5.5-43.2) (*n* = 31)	17.5 (6.4-48.2) (*n* = 16)
Fecal calprotectin remission	43 (91.5)	27 (87.1)	16 (100)
Endoscopic improvement (*n* = 57)	39 (68.4)	23 (62.6) (*n* = 37)	16 (80) (*n* = 20)
Histologic remission (*n* = 55)	32 (58.2)	18 (48.7) (*n* = 37)	14 (77.8) (*n* = 18)
Histo-endoscopic remission (*n* = 55)	25 (45.5)	12 (32.4) (*n* = 37)	13 (72.2) (*n* = 18)
Clinical remission at 2 years	48 (77.4)	30 (71.4)	18 (90)

Anti-TNF: Anti-tumor necrosis factor; CRP: C-reactive protein.

Table 2 Associations between vedolizumab serum trough concentrations and variable treatment endpoints, median (interquartile range)

Median vedolizumab serum trough concentrations (μg/mL)	No clinical remission	Clinical remission	*P* value	No endoscopic improvement	Endoscopic improvement	*P* value	No histologic remission	Histologic remission	*P* value
Week 0[1]	14.0 (6.4-22.2)	15.7 (11.5-20.1)	0.47	13.9 (10.4-22.2)	15.9 (11.5-20.1)	0.69	16.0 (11.1-21.7)	15.5 (11.2-19)	0.44
Week 48	9.2 (5.4-21.7)	16.3 (12.5-22.4)	0.04	12.3 (8.1-21.9)	17.7 (13.5-21.1)	0.09	15.4 (9.8-21.9)	17 (12.6-21.0)	0.78
2 years[2]	12.4 (7.0-15.0)	17.7 (12.6-22.5)	0.02						

[1]Vedolizumab serum trough concentrations at week 0 *vs* remission rates at week 48.
[2]Vedolizumab serum trough concentrations at week 48 *vs* clinical remission at 2 years.

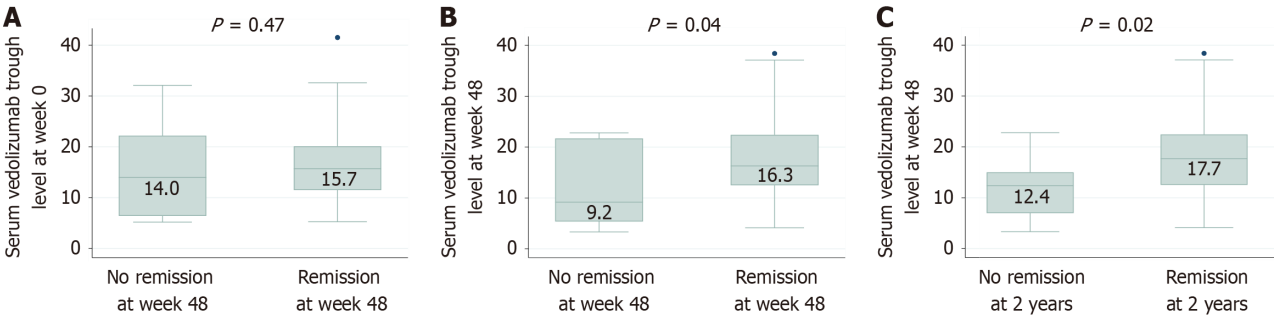

Figure 3 Association of vedolizumab serum trough concentrations (μg/mL) with clinical remission. A: Concentration at week 0 *vs* clinical remission at week 48; B: Concentration at week 48 *vs* clinical remission at week 48; C: Concentration at week 48 *vs* clinical remission at 2 years.

The median vedolizumab trough levels for favorable endpoints in UC have mainly been studied in induction phase. Reported values across studies range from 24.0 to 34.7 μg/mL[11,23,27]. A lower threshold for achieving remission was observed during the maintenance phase. A cohort study indicated vedolizumab concentrations in the range of 10.7-17 μg/mL during maintenance were associated with highest remission rates, recommending levels above 11.5 μg/mL for achieving corticosteroid-free clinical remission and biochemical remission[26]. Another recent study identified similar threshold of 11.9 μg/mL for clinical remission and a higher threshold of 15.3 μg/mL for objective remission at 1 year[22]. Consistent with these findings, our study found that the second quartile concentration (10.75-15.60 μg/mL) was associated with the highest rates of clinical and objective remission, suggesting a cut-off of > 11.3 μg/mL for predicting clinical remission during maintenance. While our findings suggest an exposure-response relationship for vedolizumab, this effect may reflect increased drug clearance during periods of disease activity. Interventional study is necessary to establish whether higher vedolizumab levels are indeed linked to improved outcomes.

Unlike anti-TNF therapy, the role of adding immunomodulator to vedolizumab in IBD patients remains controversial. Previous large retrospective analyses showed no discernible difference in clinical response or remission between combination therapy compared to vedolizumab monotherapy[30]. Similarly, a meta-analysis of 16 studies on vedolizumab found no clinical benefit associated with combination therapy during either induction or maintenance period, although these findings were limited by small participant numbers and retrospective study designs[31]. VIEWS study demonstrated that withdrawal of thiopurine in UC patients with remission may lead to increased biochemical, histological, and histo-endoscopic activity[17]. In this post-hoc analysis, we observed stable vedolizumab concentrations over 48 weeks regardless of thiopurine continuation or withdrawal. Outcomes remained consistent after adjusting thiopurine use in mixed-effects regression analysis. However, patients who discontinued thiopurine appeared to require higher vedolizumab levels to achieve remission, suggesting a potential pharmacokinetic interaction that favors the continuation of thiopurines in some patients. Additionally, patients with baseline histologic remission showed higher serum vedolizumab trough concentrations, suggesting that mucosal integrity may influence drug retention and subsequent clinical response, similarly to findings in infliximab studies[32,33].

Our study's prospective randomized controlled design allowed for comprehensive baseline characterization and complete data collection, including endoscopic and histologic assessments, which strengthened the reliability of the findings. Serial measurements of vedolizumab trough concentrations at three time points enabled a robust longitudinal analysis, reinforcing the observed associations. However, several limitations should be noted. The relatively small sample size may have limited the statistical power to detect smaller but clinically relevant differences, especially in post-hoc analyses. Additionally, the inclusion of anti-TNF exposed patients may have influenced responses to vedolizumab, and variability in treatment duration at initiation could have affected trough concentrations and clinical outcomes. While we

Table 3 Quartile analysis of vedolizumab serum trough concentrations and variable outcomes at week 48, *n* (%)

Quartile at week 0	Vedolizumab trough concentrations (μg/mL)	Clinical remission	CRP remission	Fecal calprotectin remission	Endoscopic improvement	Histologic remission	Histo-endoscopic remission (%)
1	5.19-10.75	11 (73.3)	8 (80.0)	8 (80.0)	8 (61.5)	7 (58.3)	6 (50.0)
2	10.75-15.60	14 (87.5)	12 (85.7)	13 (92.9)	10 (66.7)	10 (66.7)	7 (46.7)
3	15.60-20.85	14 (87.5)	9 (69.2)	12 (100)	12 (80.0)	9 (60.0)	7 (46.7)
4	20.85-41.50	12 (80.0)	10 (83.3)	10 (90.9)	9 (64.3)	6 (46.2)	5 (38.5)
Week 48							
1	3.33-11.30	9 (60.0)	5 (62.5)	4 (57.1)	4 (33.3)	5 (45.5)	3 (27.3)
2	11.30-15.40	16 (94.1)	12 (75.0)	15 (100.0)	14 (82.4)	10 (62.5)	7 (43.8)
3	15.40-21.70	13 (86.7)	10 (90.9)	12 (100)	12 (80.0)	9 (60.0)	8 (53.3)
4	21.70-38.40	13 (86.7)	12 (85.7)	12 (92.3)	9 (69.2)	8 (61.5)	7 (53.9)

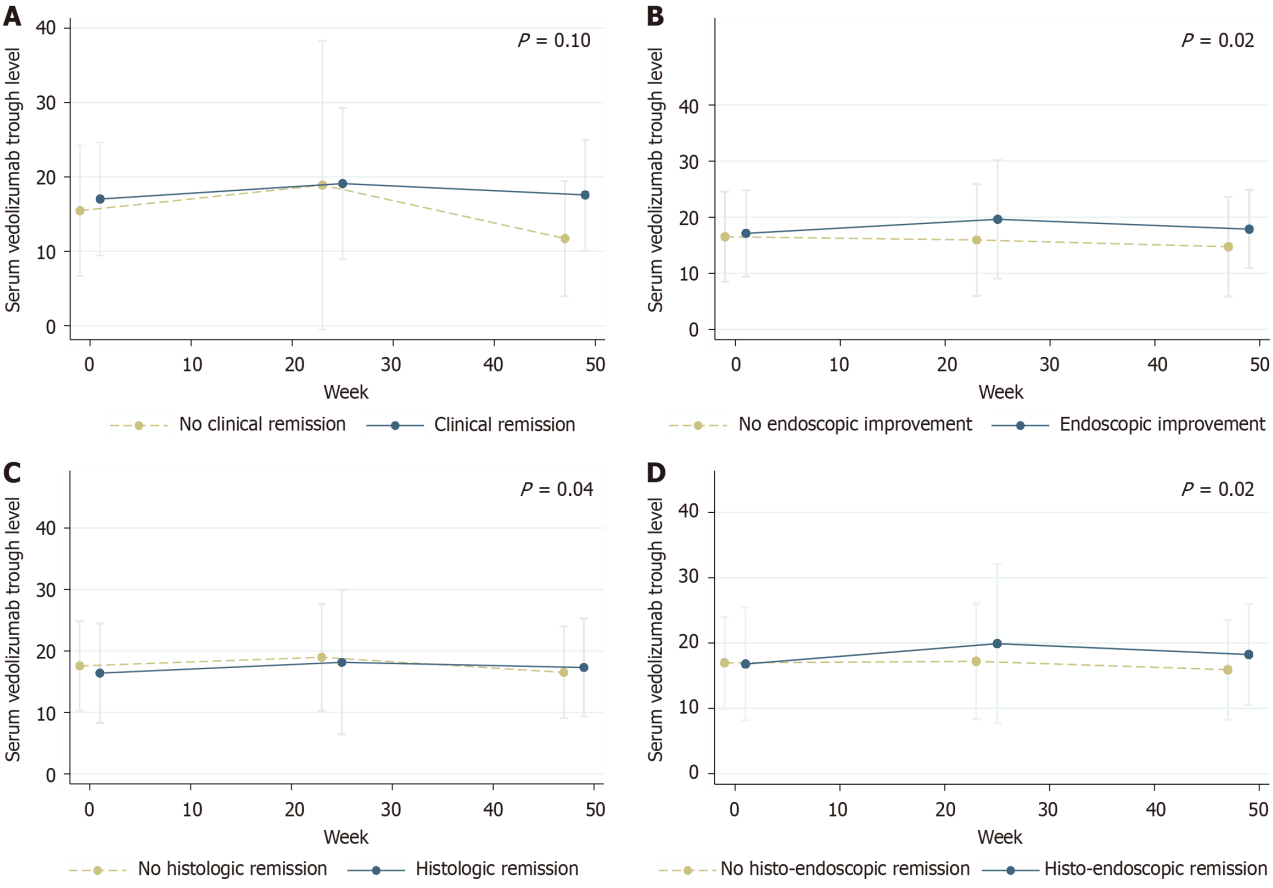

Figure 4 Longitudinal association between vedolizumab serum trough concentrations at 3 distinct time points. A: Clinical; B: Endoscopy; C: Histology; D: Histo-endoscopic outcomes over 48 weeks.

explored the exposure-response relationship, causation remains uncertain, and the pharmacodynamic mechanisms of vedolizumab are not fully understood. These highlight the need for further research to confirm our findings in larger and more homogeneous populations.

CONCLUSION

Our study highlights the utility of vedolizumab trough concentrations for predicting outcomes in UC patients on combination therapy. Lower vedolizumab trough levels were associated with poorer outcomes, suggesting therapeutic

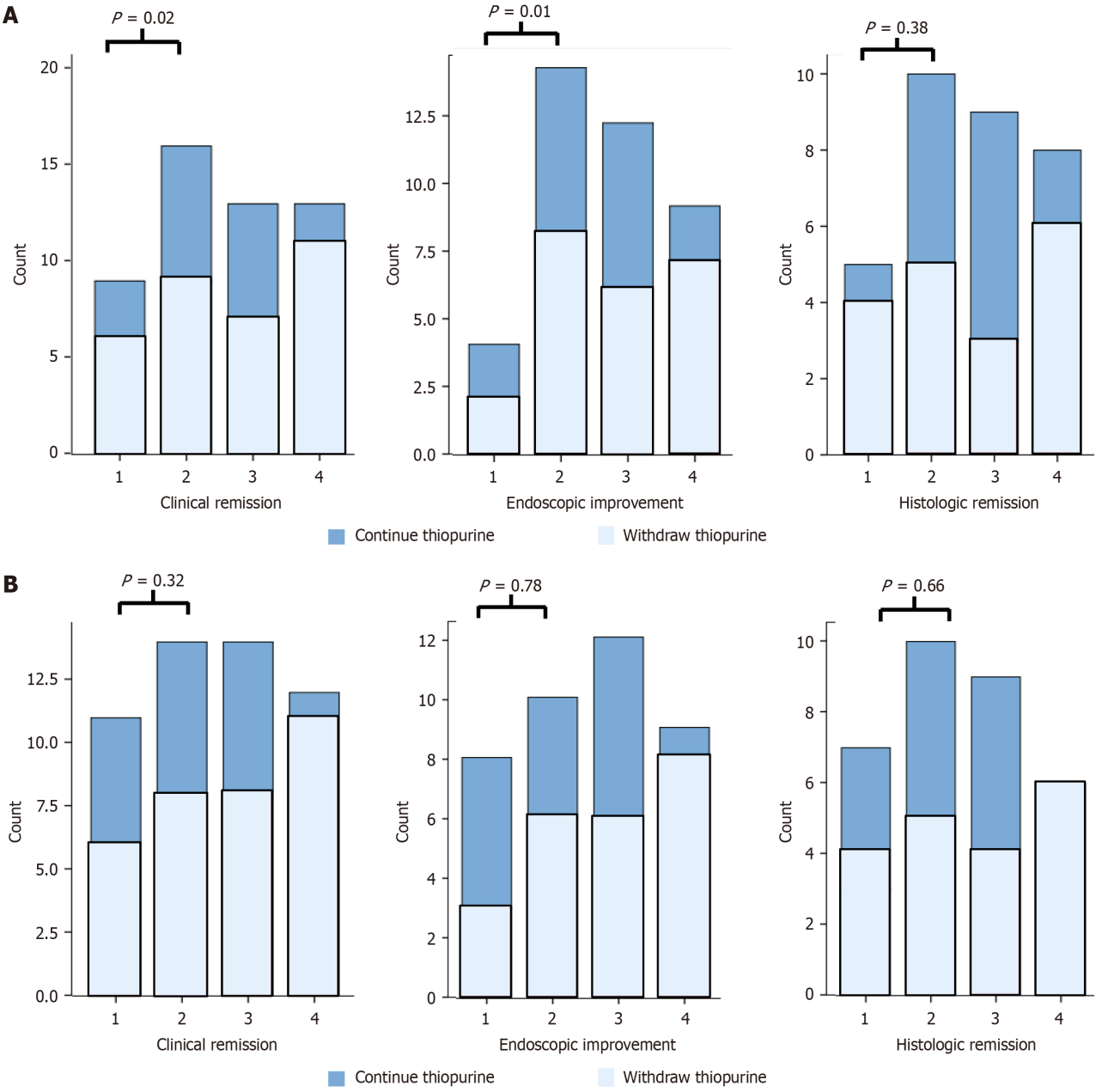

Figure 5 Remission rates expressed per quartile. A: At week 0; B: At week 48.

drug monitoring might be clinically useful, particularly when treatment efficacy declines. Although vedolizumab serum trough concentrations remained consistent over time regardless of thiopurine continuation, withdrawing thiopurine may necessitate higher vedolizumab trough levels to achieve remission.

FOOTNOTES

Author contributions: Leong RW designed and supervised the study; Chaemsupaphan T, Pudipeddi A, Paramsothy S, Leong RW were involved in methodology; Chaemsupaphan T, Pudipeddi A, Kermeen M, and Kariyawasam VC conducted the investigation; Chaemsupaphan T, Pudipeddi A, and Lin HY performed data analysis; Chaemsupaphan T and Leong RW wrote the original draft. All authors have edited and approved the final manuscript.

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ORIGINAL ARTICLE

Basic Study

RGS4 promotes the progression of gastric cancer through the focal adhesion kinase/phosphatidyl-inositol-3-kinase/protein kinase B pathway and epithelial-mesenchymal transition

Peng-Yu Chen, Pei-Yao Wang, Bang Liu, Yang-Pu Jia, Zhao-Xiong Zhang, Xin Liu, Dao-Han Wang, Yong-Jia Yan, Wei-Hua Fu, Feng Zhu

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Abstract

BACKGROUND

Regulator of G protein signaling (RGS) proteins participate in tumor formation and metastasis by acting on the α-subunit of heterotrimeric G proteins. The specific effect of RGS, particularly *RGS4*, on the progression of gastric cancer (GC) is not yet clear.

AIM

To explore the role and underlying mechanisms of action of *RGS4* in GC development.

METHODS

The prognostic significance of *RGS4* in GC was analyzed using bioinformatics based public databases and verified by immunohistochemistry and quantitative polymerase chain reaction in 90 patients with GC. Function assays were employed to assess the carcinogenic impact of *RGS4*, and the mechanism of its possible influence was detected by western blot analysis. A nude mouse xenograft model was established to study the effects of *RGS4* on GC growth *in vitro*.

RESULTS

RGS4 was highly expressed in GC tissues compared with matched adjacent normal tissues. Elevated *RGS4* expression was correlated with increased tumor-node-metastasis stage, increased tumor grade as well as poorer overall survival in patients with GC. Cell experiments demonstrated that *RGS4* knockdown suppressed GC cell proliferation, migration and invasion. Similarly, xenograft experiments confirmed that RGS4 silencing significantly inhibited tumor growth. Moreover, RGS4 knockdown resulted in reduced phosphorylation levels of focal adhesion kinase, phosphatidyl-inositol-3-kinase, and protein kinase B, decreased vimentin and N-cadherin, and elevated E-cadherin.

CONCLUSION

High *RGS4* expression in GC indicates a worse prognosis and *RGS4* is a prognostic marker. *RGS4* influences tumor progression *via* the focal adhesion kinase/phosphatidyl-inositol-3-kinase/protein kinase B pathway and epithelial-mesenchymal transition.

Key Words: Gastric cancer; Prognosis; Regulator of G protein signaling 4; Focal adhesion kinase; Epithelial-mesenchymal transition

Core Tip: This study explored the relevant role of regulator of G protein signaling 4 (RGS4) in gastric cancer (GC) and its possible mechanism. Through analysis of public databases, validation of clinical specimens, cell experiments and xenograft model, our study confirmed that RGS4 promotes GC progression through the focal adhesion kinase/phosphatidyl-inositol-3-kinase/protein kinase B pathway and epithelial-mesenchymal transition. RGS4 is a potential therapeutic target for GC and our study may further guide clinical practice.

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INTRODUCTION

Gastric cancer (GC) is a prevalent malignant tumor of the digestive tract, which is the fifth most common cancer and the fifth leading cause of cancer mortality worldwide[1]. Despite there has been significant progress in multidisciplinary treatment modalities, the overall survival (OS) rate remains low, particularly among patients in advanced stages of GC [2]. The poor prognosis of GC underscores the importance of identifying biomarkers related with cancer development. As one of the most widely known and ubiquitous membrane receptor families, G-protein-coupled receptors (GPCRs) are essential in a variety of physiological processes, such as cellular proliferation, motility, and gene expression[3]. GPCRs play an integral role in tumor's malignant transformation[3,4]. The regulator of G protein signaling (RGS) protein family, comprising over 20 members, modulates cellular responses mediated by GPCRs through a conserved RGS domain with guanosine triphosphate (GTP)ase-activating protein activity[5], which interacts with GTP-bound Gα subunits of activated G-proteins and accelerates GTP hydrolysis, causing rapid G-protein deactivation and termination of GPCR signaling[6]. In cancer progression, RGS proteins act as pivotal gating switches that regulate the growth, division, differentiation, and mobility of tumor cells[7,8]. It is reasonable to speculate that the *RGS* gene family may be important molecular markers for GC diagnosis, treatment due to the significant role of GPCRs in this GC[9,10].

In our previous research, we found that *RGS1* was associated with poor prognosis in GC[11], which has increased our interest in the *RGS* gene family. *RGS4* belongs to the B/R4 *RGS* gene family and serves as a negative regulator of GPCRs, inhibiting the transmission of related signaling factors by hastening the hydrolysis of GTP molecules bound to the Gα subunit[12]. Interacting with various receptors, effectors, scaffolding proteins, and signaling entities, *RGS4* forms intricate transduction assemblies that affect the localization, functionality, and stability of signals within the cells[5]. This protein exerts a crucial regulatory effect on tumor tissues or cells[13-16]. Given these biological functions, we speculate that *RGS4* are essential in GC progression. Based on the public database and 90 patients with GC from Tianjin Medical University General Hospital, we aimed to investigate *RGS4* expression in GC tissues and the relationship between *RGS4* expression levels, clinicopathological parameters as well as OS of GC. Additionally, we examined the role of *RGS4* in GC cell behavior and investigated its potential molecular mechanisms.

MATERIALS AND METHODS

Data sources

The normalized gene expression matrix, OS data, and relevant clinical and pathological parameters were obtained from The Cancer Genome Atlas Stomach Adenocarcinoma (TCGA-STAD) dataset (https://portal.gdc.cancer.gov/) and the Genotype-Tissue Expression dataset (https://xenabrowser.net/). Following data preprocessing steps such as probe annotation, normalization, and correction, the R software was used to analyze the gene expression differences of RGS4 between GC and normal tissues. Clinical and pathological data from 90 patients who underwent gastrectomy and were pathologically confirmed to have GC were collected at Tianjin Medical University General Hospital between 2017 and 2020. Each patient provided samples of GC and para-carcinoma normal tissues. All patients provided their consent.

Pathway analysis and survival analysis

The gene expression and OS data of *RGS4* from TCGA-STAD database and clinical samples were organized. Whole group were categorized into *RGS4* high-expression and *RGS4* low-expression groups based on the median *RGS4* expression value. The correlation between *RGS4* expression and the prognosis of GC patients was analyzed using R software (survey package). Differences in gene expression were analyzed using Gene Set Enrichment Analysis (GSEA) (GSEA v3.0). Each analysis involved 1000 gene set permutations, with pathways deemed significantly enriched if they met the following criteria: Enrichment score exceeding 0.6 and P value < 0.05.

Cell lines source and cell culture

The human gastric mucosal cell line GES-1 and the human GC cell line MGC-803 were purchased from icellbioscience Co., Ltd. The human GC cell line NCI-N807 was purchased from ATCC Co., Ltd. AGS and HGC27 cell lines (two human GC cell lines) were acquired from Wuhan Prosa Life Science Co., Ltd. MGC-803 and NCI-N87, HGC27, and GES-1 cell lines were cultivated in RPMI 1640 medium (Gibco). AGS cells were grown in F-12 medium. All cells were cultivated with 50 mL/L CO_2 at 37 °C.

Immunohistochemistry analysis

The tumor and normal tissue specimens were incubated with the corresponding anti-*RGS4* (Abcam, ab97037, 1:1000) overnight at 4 °C. Subsequently, the secondary antibody was used to incubate the specimens. The signals were detected using a DAB staining kit (Solarbio, China). The immunohistochemistry (IHC) scoring method was the same as that used by Chen *et al*[17].

Real-time quantitative polymerase chain reaction analysis

Basic RNA extraction as well as real-time quantitative polymerase chain reaction (qPCR) methods were conducted as our previous research[11]. The primers used were shown below: *RGS4* upstream-primer, 5′-ACATCGGCTAGGTTTC-3′, downstream-primer, 3′-GTTGTGGGAAGAATTGTGTTCAC-5′; GAPDH downstream-primer, 5′-GGTGAAGGTCG-GAGTCAACG-3′, downstream-primer, 5′-CAAAGTTGTCATGGATGHACC-3′.

Western blot analysis

RIPA lysis buffer supplemented with phosphatase and protease inhibitors was used to isolate total protein. The proteins were transferred to polyvinylidene fluoride membranes following separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Next, primary antibodies [*RGS4*, ab308160, 1:1500, Abcam; focal adhesion kinase (FAK), ab40794, 1:2000, Abcam; p-FAK, ab81298, 1:1500, Abcam; phosphatidyl-inositol-3-kinase (PI3K), ab302958, 1:1500, Abcam; p-PI3K, ab182651, 1:200, Abcam; protein kinase B (AKT), ab8805, 1:500, Abcam; p-AKT, ab38449, 1:1500, Abcam; E-cadherin, ab40772, 1:1500, Abcam; N-cadherin, ab76011, 1:5000, Abcam; vimentin, ab20346, 1:1500, Abcam; β-actin, ab8227, 1:2000, Abcam] were used to nurture membranes. After incubation with the primary antibody, secondary antibodies were used to nurture membranes for 2 hours.

Cell transfections

RGS4 short hairpin ribonucleic acid (shRNA) was synthesized by Tsingke Biotech Co. (Beijing, China). Supplementary Table 1 showed the top- and bottom-strand primer sequences of shRNA. ShRNA was cloned into the *EcoRI/BamHI* sites to generate recombinant plasmids. The recombinant plasmid was then transfected into cells to obtain MGC-803 cell line with RGS4 knockdown, and the vector plasmid was used as a blank control. To induce *RGS4* overexpression, we integrated lentiviral expression vectors with DNA fragments of human *RGS4* open reading frames to generate recombinant lentivirus, which was used to generate AGS cell lines overexpressing *RGS4*.

Cell counting kit-8 assay

AGS and MGC-803 cells (2.0×10^3 cells/well) after transfection were cultivated in 96-well plates for 0, 12, 24, 48, 72, 96 hours with 50 mL/L CO_2 at 37 °C. The cell counting kit-8 (CCK-8) reagent was then added at the endpoint time. Subsequently, cells were incubated for 1 hour. Finally, the microplate reader was used to measure the optical density.

Colony formation assay

Transfected AGS and MGC-803 cells were seeded in 6-well plates and hatched at 37 °C for 10 days to form single-cell colonies. Subsequently, the cells were fixed in 4% paraformaldehyde and then stained with 0.5% w/v crystal violet

solution.

Wound healing assay

Transfected cells were seeded into 6-well plates (4×10^5 cells/well) and cultured in medium until 80% confluence. The tip of the 200 μL pipette was used to leave scratch. After 0, 24, and 48 hours post-scratch, wound closure was monitored.

Transwell assay

The 24-well transwell chambers (Corning, United States) were coated overnight at 4 °C with or without 100 μL Matrigel matrix (Corning). A total of 1×10^4 cells in 150 μL of serum-free medium were placed in the upper chamber, with 500 μL of 10% foetal bovine serum culture medium added to the lower chamber. After 24 hours of incubation, cells that migrated to the lower chamber were stained with 0.5% crystal violet and counted.

Xenograft model

BALB/c nude mice (4-6 weeks old) were randomly divided into four groups (n = 5 mice/group). The same amounts (1×10^7) of MGC-803 cells which were transfected with *RGS4* (MGC803-shRGS4), blank control cells (MGC803-NC), AGS cells stably overexpressing *RGS4* (AGS-*RGS4*), and blank control cells (AGS-NC) were injected into the axillary skin of nude mice. We measured the long diam and short diam of the tumor every two days. The tumor volume = $(A \times B^2)/2$ (A, long diam; B, short diam). After two weeks, the nude mice were executed. The removed tumor tissues were harvested for IHC as well as western blot analyses, and tumor sizes were analyzed, weighed, and photographed.

Statistical analysis

Data were analyzed and graphed using GraphPad Prism 10 and SPSS 25.0 software. Categorical variables were expressed as the frequency and percentages and continuous variables as means ± SD deviation. Kaplan-Meier method was used to plot survival curves. Significant differences in categorical variables were analyzed using the χ^2 test and continuous variables were analyzed using Student's t-test or one-way analysis of variance (ANOVA). Two tails P value < 0.05 was considered statistically significant.

RESULTS

The expression level and prognostic analysis of RGS4 in GC in public databases

The results of bioinformatics showed that in the TCGA-STAD dataset, the expression level of *RGS4* was not significantly different between the two groups - GC tissue (n = 375) and normal tissue (n = 32) (Figure 1A). To validate the expression of *RGS4* at larger samples, the TCGA and Genotype-Tissue Expression datasets were integrated. The integrated analysis showed that compared to normal tissues (n = 88), the *RGS4* was significantly increased in GC tissues (n = 375) (Figure 1B). From the results of the survival analysis, we can know that GC patients with high expression levels of *RGS4* had a worse prognosis (P = 0.011, Figure 1C). Besides, the high expression of *RGS4* was related with advanced T stage, tumor-node-metastasis (TNM) stage, and tumor grade (P < 0.05, Figure 1D).

Validation of the prognostic analysis of RGS4 in patients with GC

We collected the clinical and pathological information of 90 patients with GC at our center, as well as their tissues including tumor and para-carcinoma. Subsequently, we performed qPCR and IHC assays on the tissue samples from each patient. As shown in Figure 1E, *RGS4*'s expression level in GC tissues was significantly higher than that in para-carcinoma normal tissues (0.9278 ± 0.5081 *vs* 0.5464 ± 0.4425, P < 0.05). IHC confirmed higher levels of *RGS4* expression at protein level in GC tissues than in para-carcinoma tissues (2.254 ± 0.6992 *vs* 1.772 ± 0.6745, P < 0.01; Figure 1F and G). According to the expression level of the *RGS4* gene, patients with GC (n = 90) were separated into an *RGS4* high expression group (n = 45) and an *RGS4* low expression group (n = 45). Survival curves displayed that the high expression group of *RGS4* had a worse prognosis and shorter OS (P = 0.004, Figure 1H), which validated the results obtained in TCGA-STAD dataset (Figure 1C). The analysis of clinicopathological parameters of patients with GC in our center indicated that patients with high *RGS4* expression had advanced T stage, N stage, tumor grade, and TNM stage (Table 1).

RGS4 knockdown inhibited GC cells proliferation, migration, and invasion

The expression levels of *RGS4* in GES-1, NCI-N87, AGS, HGC27, and MGC-803 were assessed using qPCR. Figure 2A showed that the expression levels of *RGS4* in NCI-N87, AGS, HGC27, and MGC803 GC cell lines were higher than those in the GES-1 cell line (P < 0.05). Due to MGC-803 cell lines showed the highest expression of *RGS4*, it was selected for subsequent experiments. MGC803 cells were transfected with shRNA-NC, *RGS4* #1, or *RGS4* #2. Transfection efficiency was shown in Figure 2B. *RGS4* expression was significantly decreased following transfection with *RGS4* #1 shRNA, leading to its selection for further experiments. CCK-8 proliferation assays revealed that *RGS4* knockdown inhibited the proliferation of MGC-803 cells which were transfected compared with blank control cells (1.761 ± 0.054 *vs* 1.268 ± 0.054, P < 0.05; Figure 2C). Similarly, colony formation assays (Figure 2D) demonstrated that the number of cell colonies decreased significantly after *RGS4* knockdown (133.3 ± 15.3 *vs* 12.0 ± 3.0, P < 0.05). Furthermore, wound healing (49% ± 3.61% *vs* 22% ± 2.65%, P < 0.05) and transwell assays (migration: 352.7 ± 11.2 *vs* 165.7 ± 10.1, P < 0.05; invasion: 201.7 ± 7.6 *vs* 94.3 ± 6.4, P < 0.05) indicated that *RGS4* suppression significantly impaired cell migration and invasion (Figure 2E and F).

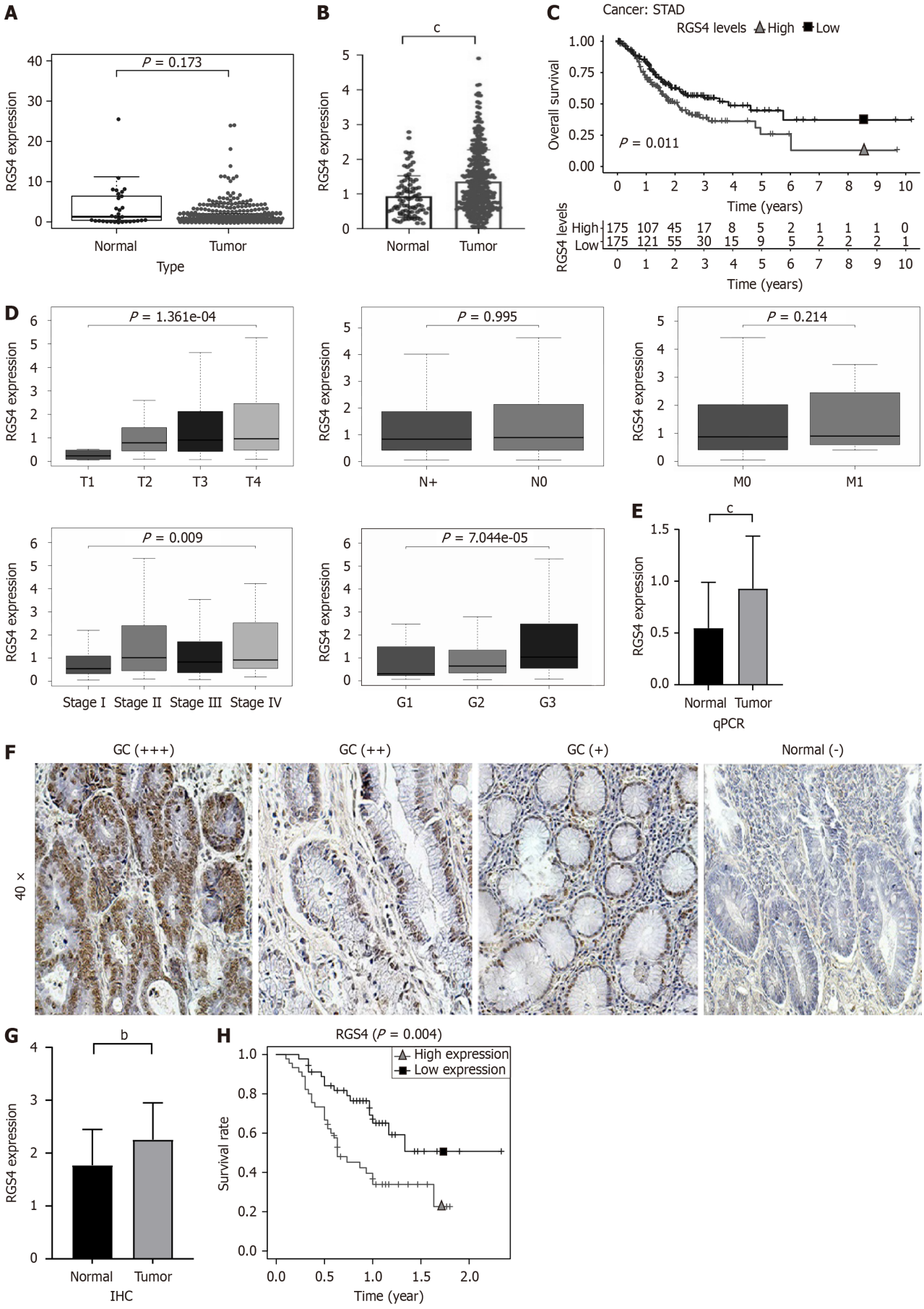
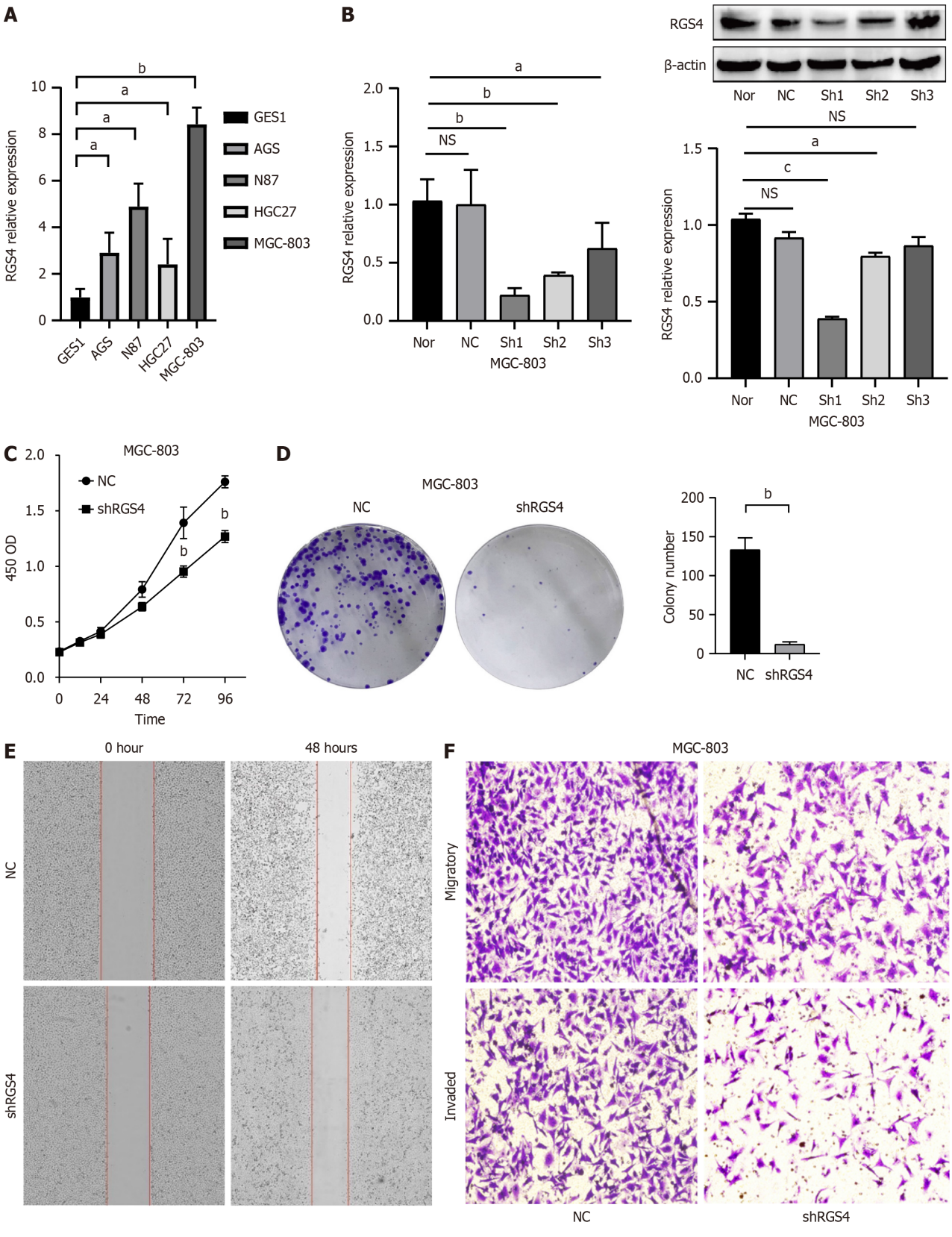


Figure 1 High expression of regulator of G protein signaling 4 was associated with poor prognosis in patients with gastric cancer. A: Differential expression of regulator of G protein signaling 4 (*RGS4*) in The Cancer Genome Atlas Stomach Cancer dataset (TCGA-STAD); B: Differential expression

of *RGS4* in the integrated analysis of TCGA-STAD and the Genotype-Tissue Expression dataset; C: High expression of *RGS4* was associated with lower overall survival in TCGA-STAD dataset; D: The correlation between *RGS4* and T stage, N stage, M stage, tumor-node-metastasis stage and tumor grade of gastric cancer (GC); E: Differential expression of *RGS4* in GC and para-carcinoma normal tissues of our center's patients; F: Representative immunohistochemistry images showing the expression of *RGS4* in GC and para-carcinoma normal tissues (strong staining: +++; moderate staining: ++; weak staining: +; negative staining: -); G: Differences in protein expression levels of *RGS4* between GC and para-carcinoma normal tissues; H: High expression of *RGS4* was associated with lower overall survival in GC patients in our center. $^{b}P < 0.01$, $^{c}P < 0.001$. RGS4: Regulator of G protein signaling 4; GC: Gastric cancer.



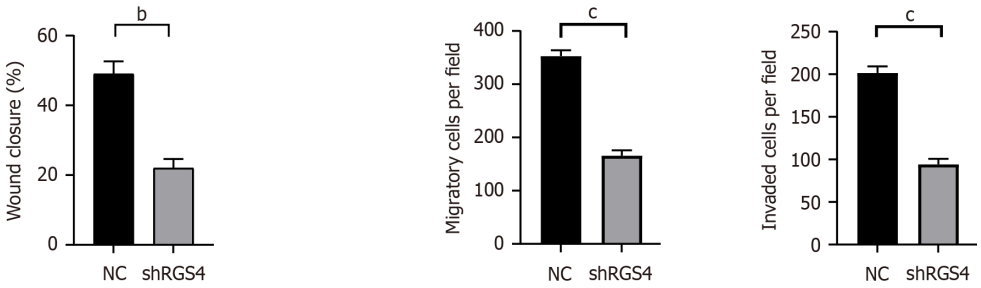
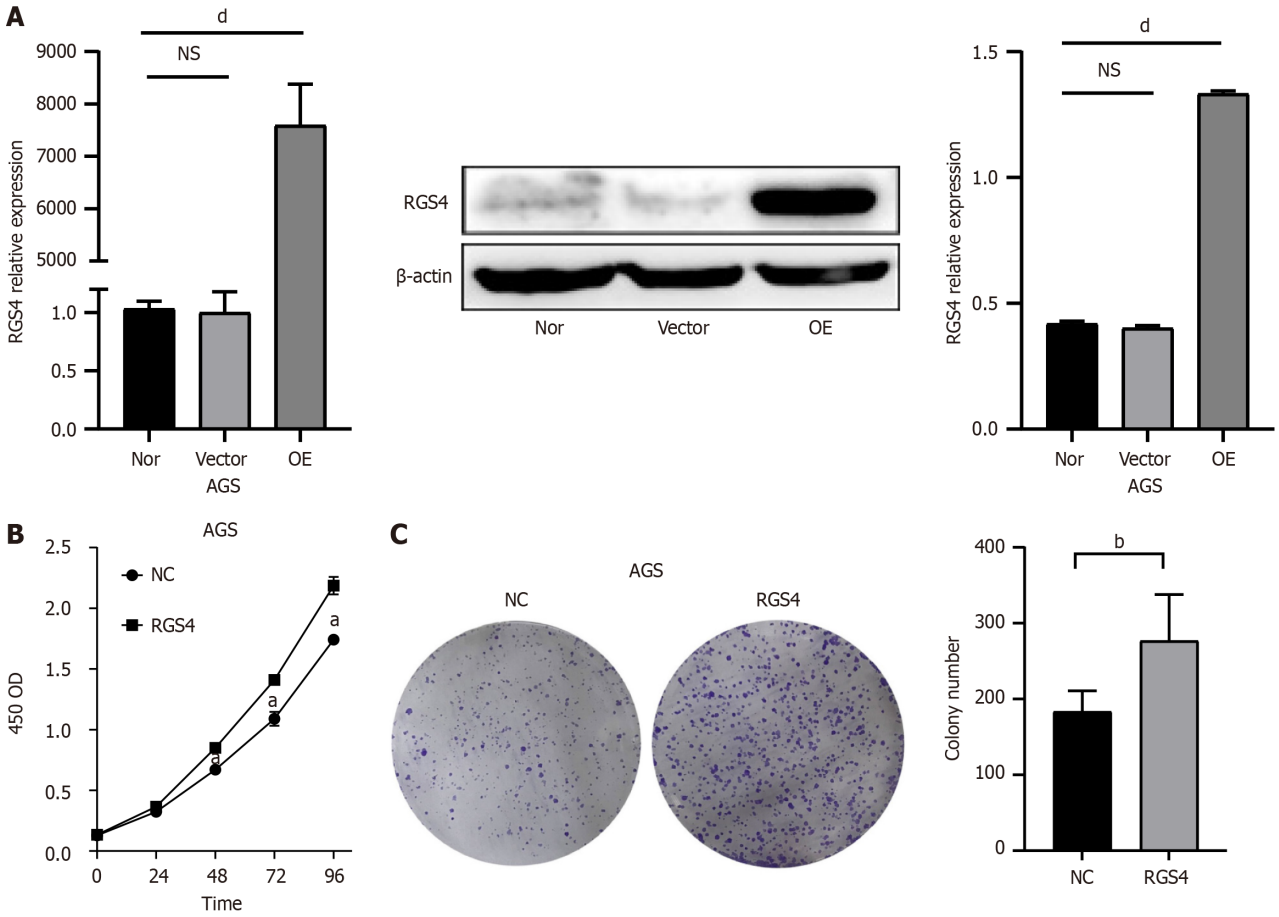


Figure 2 Regulator of G protein signaling 4 knockdown inhibited the proliferation, migration, and invasion of gastric cancer cells *in vitro*. A: The expression levels of regulator of G protein signaling 4 (*RGS4*) in different gastric cancer (GC) cell lines were detected by real-time quantitative polymerase chain reaction; B: The interference efficiency of the lentiviral human *RGS4*-targeting short hairpin RNA was validated by real-time quantitative polymerase chain reaction (left) and western blot (right) analysis; C: The proliferation of MGC-803 cells with *RGS4* knockdown was determined by cell counting kit-8 assays; D: The effects of *RGS4* knockdown on the colony formation of MGC-803 cells; E: The migration ability of MGC-803 cells was measured by wound healing; F: The invasion of MGC-803 cells with *RGS4* knockdown was determined by transwell assays. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, NS: No significance. RGS4: Regulator of G protein signaling 4.

RGS4 overexpression promoted proliferation, migration, and invasion in GC cells

To further elucidate the effect of *RGS4* overexpression on cell behavior, AGS cell lines stably overexpressing *RGS4* were established and their transfection efficiency was verified. Compared with the vector and NC groups, *RGS4* overexpression significantly increased protein and mRNA levels (Figure 3A). CCK-8 proliferation assays (Figure 3B) showed that AGS cells overexpressing *RGS4* exhibited enhanced proliferative capabilities (1.741 ± 0.037 *vs* 2.187 ± 0.073, $P < 0.05$). Colony formation assays (Figure 3C) showed that *RGS4* overexpression significantly promoted colony formation in AGS cells (183.3 ± 27.4 *vs* 276.7 ± 61.2, $P < 0.05$). Additionally, wound healing (40% ± 2.65% *vs* 63.33% ± 2.52%, $P < 0.05$) and transwell assays (migration: 238.7 ± 17.8 *vs* 639.7 ± 65.3, $P < 0.05$; invasion: 175.7 ± 10.1 *vs* 405.3 ± 23.4, $P < 0.05$) demonstrated that *RGS4* overexpression significantly increased cell migration and invasion (Figure 3D and E).



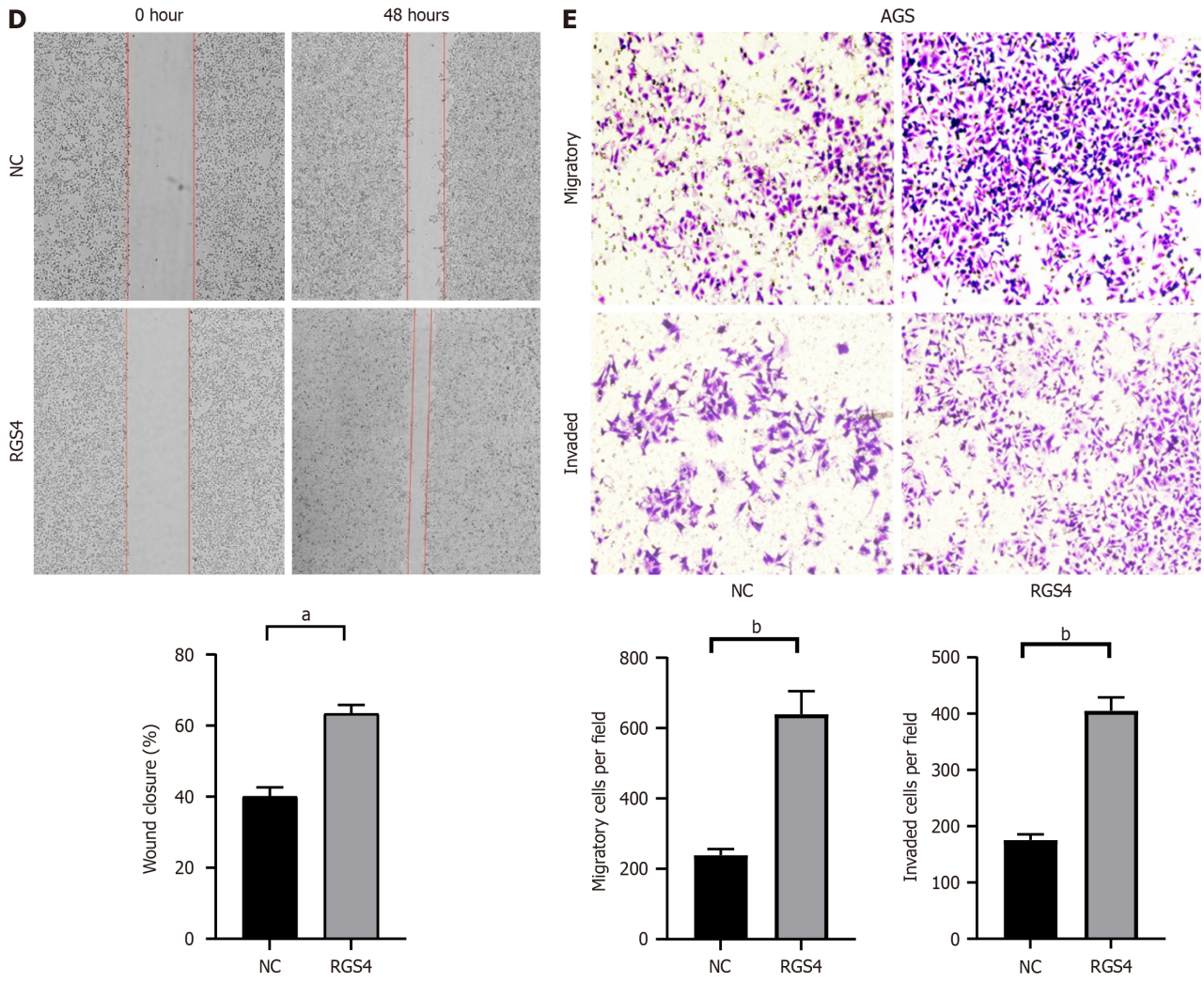


Figure 3 Overexpression of regulator of G protein signaling 4 promoted the proliferation, migration, and invasion of gastric cancer cells *in vitro*. A: The interference efficiency of the lentiviral carrying regulator of G protein signaling 4 (*RGS4*) was validated by real-time quantitative polymerase chain reaction (left) and western blot (right) analysis (overexpressing *RGS4*); B: The proliferation of AGS cells with RGS4 overexpression was determined by cell counting kit-8 assays; C: The effects of *RGS4* overexpression on the colony formation of AGS cells; D: The migration ability of AGS cells was measured by wound healing; E: The invasion of AGS cells with *RGS4* overexpression was determined by transwell assays. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{d}P < 0.0001$, NS: No significance. RGS4: Regulator of G protein signaling 4.

RGS4 potentially influenced GC cell behavior via the FAK/PI3K/AKT pathway and epithelial-mesenchymal transition

We analyzed the potential mechanism of *RGS4* affecting GC cells through GESA. The results of GSEA were showed in Figure 4A. Notably, extracellular matrix (ECM) receptor interactions and focal adhesion pathways exhibited the highest enrichment score. FAK was a key kinase of the focal adhesion pathway and FAK phosphorylation can promote cell proliferation by activating the PI3K/AKT pathway[18-20]. FAK also mediates various cellular functions, including epithelial-mesenchymal transition (EMT), which is necessary for tumor cells to migrate to and invade the ECM[20]. Additionally, the ECM receptor interaction pathway is closely related to EMT[21,22]. Accordingly, we hypothesized that *RGS4* activated the FAK/PI3K/AKT pathway and EMT, thereby promoting GC progression. This hypothesis was corroborated. *RGS4* knockdown reduced the ratios of p-FAK/FAK, p-PI3K/PI3K, and p-AKT/AKT ($P < 0.05$, Figure 4B), and up-regulated the level of epithelial marker E-cadherin ($P < 0.05$) and down-regulated mesenchymal markers N-cadherin and vimentin ($P < 0.05$, Figure 4C). In contrast, overexpressing *RGS4* exhibited the opposite effect (Figure 4D and E). These findings supported the hypothesis that *RGS4* enhances malignant behavior of GC cell by modulating the FAK/PI3K/AKT pathway and EMT.

RGS4 knockdown in GC inhibited tumor growth, while RGS4 overexpression promoted GC growth in vivo

A subcutaneous tumorigenesis experiment was conducted using nude mice for investigating the effect of *RGS4* on GC *in vivo*. The rate and volume of tumor growth significantly decreased in the MGC-803 cell line with *RGS4* knockdown compared with the control group (1043.3 ± 146.1 *vs* 384.6 ± 145.1 mm^3, $P < 0.05$, Figure 5A and B). Compared with the blank control group, the *RGS4* knockdown group had a lower tumor weight at endpoint (582.0 ± 61.2 *vs* 344.8 ± 101.6 g, $P < 0.05$, Figure 5C). On the contrary (Figure 5D-F), compared with the control tumors, overexpressing *RGS4* in AGS cells resulted in enhanced tumor growth *in vivo*, as evidenced by increased tumor volume (352.8 ± 137.6 *vs* 1035.3 ± 153.2 mm^3,

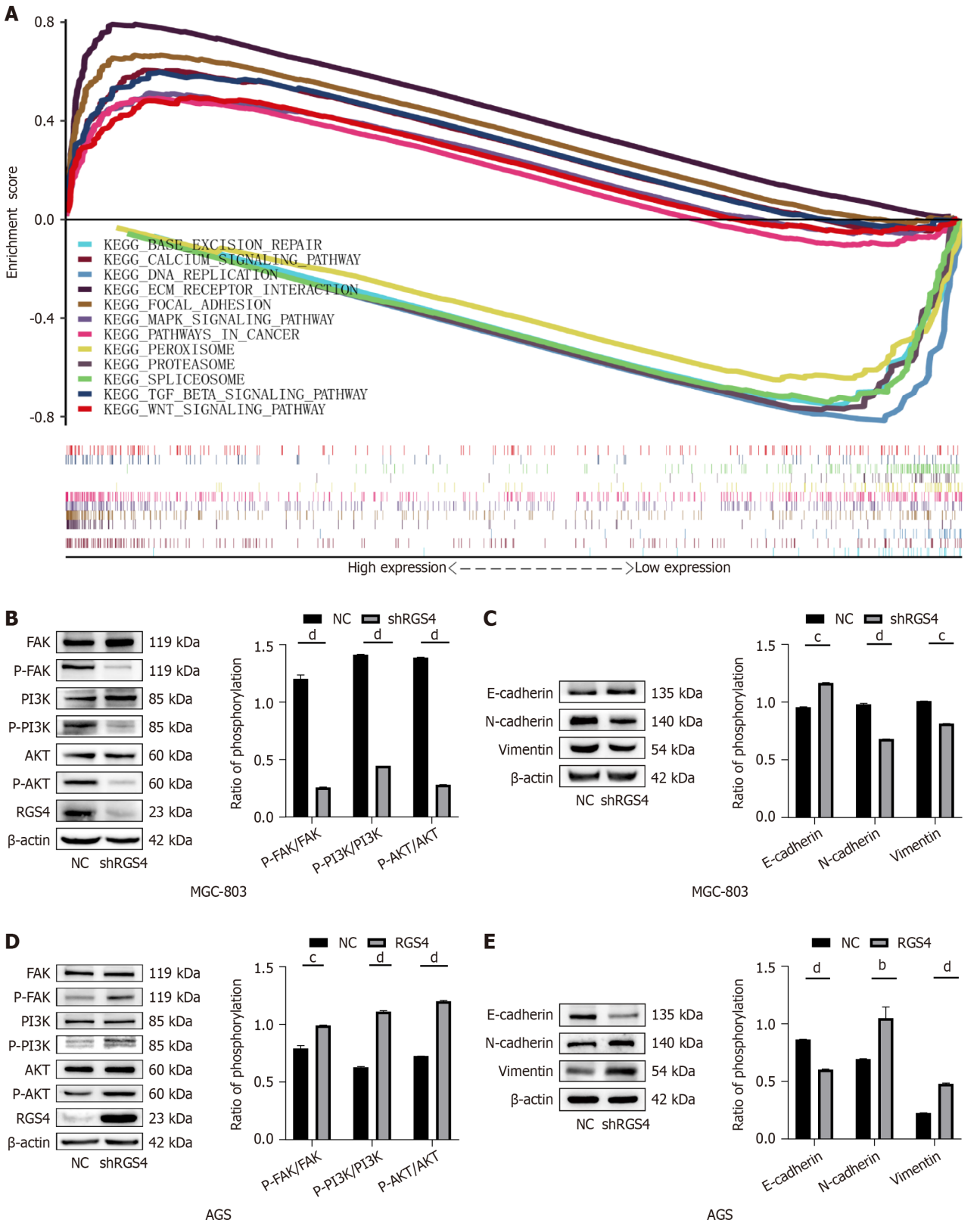
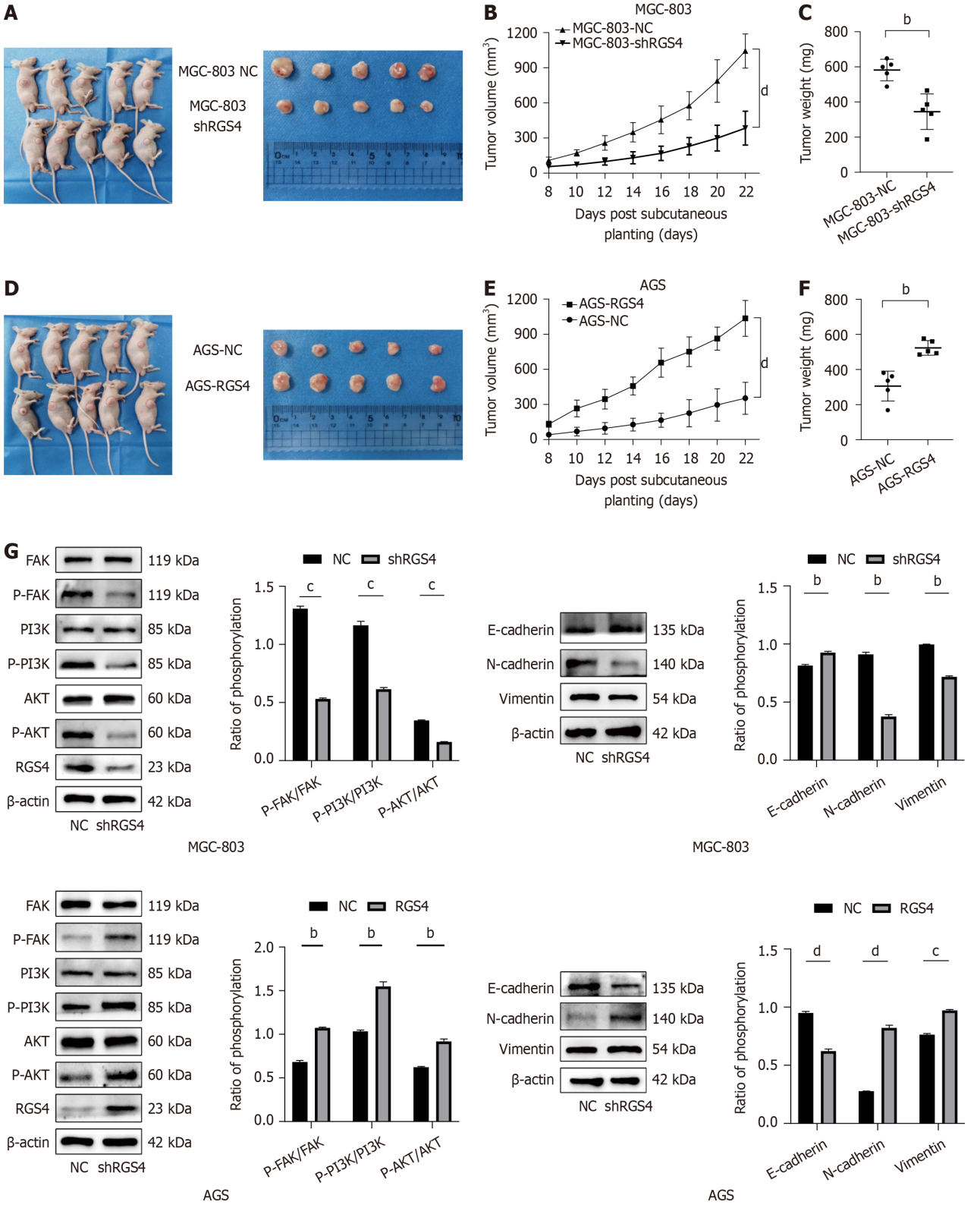


Figure 4 The expression level of regulator of G protein signaling 4 affected the focal adhesion kinase/phosphatidyl-inositol-3-kinase/protein kinase B pathway and epithelial mesenchymal transition in gastric cancer cells. A: Gene Set Enrichment Analysis results showed the relevant pathways that regulator of G protein signaling 4 may affected in The Cancer Genome Atlas Stomach Cancer dataset; B-E: The expression levels of focal adhesion kinase signaling pathway and epithelial mesenchymal transition related protein of MGC-803 cells and AGS cells were determined by western blot analysis. β-actin was used as a loading control. $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$. FAK: Focal adhesion kinase; PI3K: Phosphatidyl-inositol-3-kinase; AKT: Protein kinase B.

$P < 0.05$) and weight (305.6 ± 85.1 *vs* 523.4 ± 41.7 g, $P < 0.05$). Western blot analyses confirmed that *RGS4* knockdown had a significant effect on signaling pathways, leading to decreased ratios of p-FAK/FAK, p-PI3K/PI3K, and p-AKT/AKT; up-regulated level of E-cadherin and down-regulated level of vimentin and N-cadherin ($P < 0.05$). Conversely, *RGS4* overexpression notably increased these ratios and altered protein expression in a manner consistent with tumor progression ($P < 0.05$, Figure 5G). IHC staining further supported these findings, showing that *RGS4* modulation influenced key markers of cell proliferation and signaling, with knockdown reduction and overexpression increasing the expression of Ki67 and phosphorylated AKT (Figure 5H).



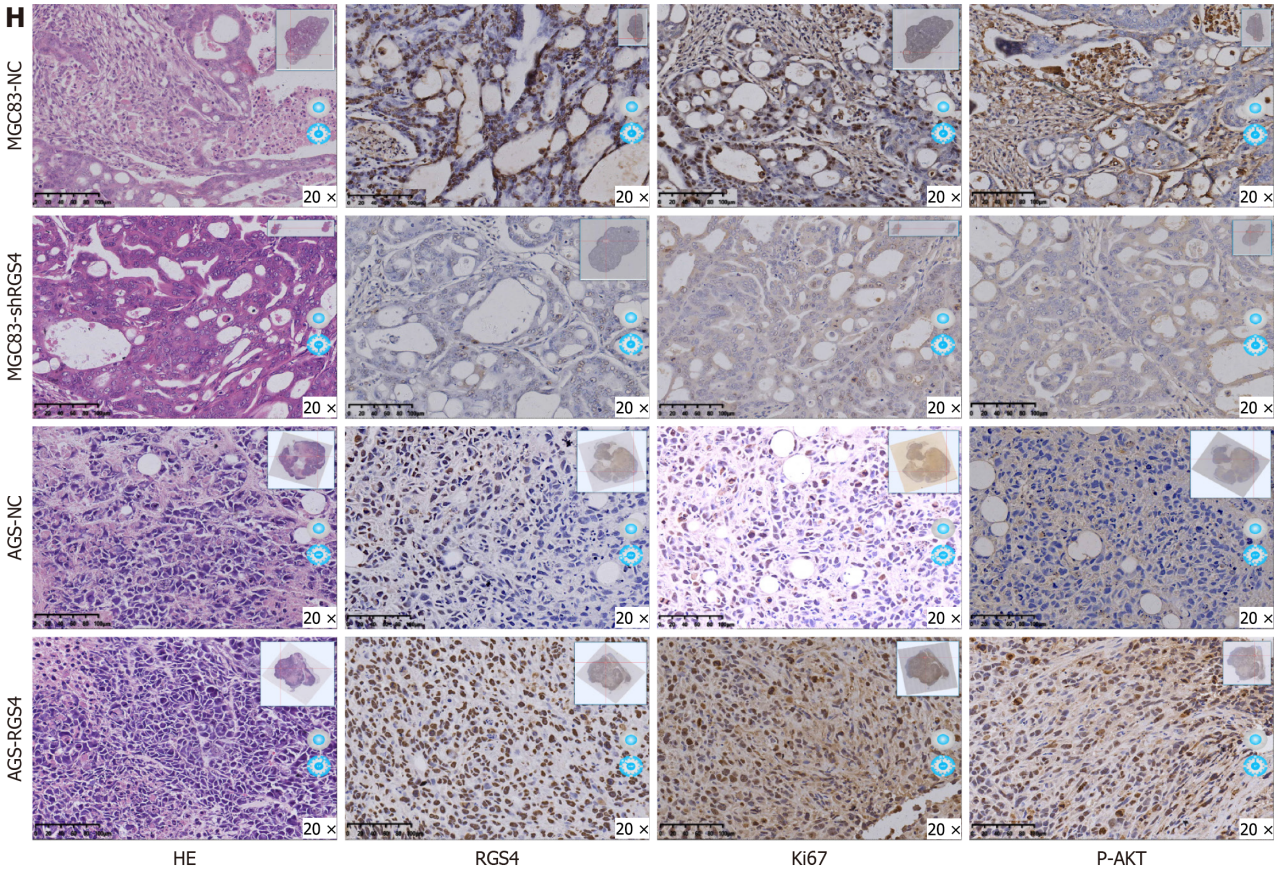


Figure 5 Knocking down regulator of G protein signaling 4 in gastric cancer cells inhibited tumor growth *in vivo*. A-F: Tumors from MGC-803 cells with regulator of G protein signaling 4 (*RGS4*) knockdown and AGS cells with *RGS4* overexpression and their controls (n = 5/groups) (A and D), tumor growth curves (B and E), tumor weight of each group (C and F); G: The expression of focal adhesion kinase/phosphatidyl-inositol-3-kinase/protein kinase B pathway and epithelial mesenchymal transition related proteins in MGC-803 cells and AGS cells *in vivo* were validated by western blot. β-actin was used as a loading control; H: The expression of *RGS4*, Ki67 and p-AKT in these xenografts *via* immunohistochemistry staining; I: Schematic diagram of the mechanism of *RGS4* promoting gastric cancer progression. $^{b}P < 0.01$, $^{c}P < 0.001$, $^{d}P < 0.0001$. FAK: Focal adhesion kinase; PI3K: Phosphatidyl-inositol-3-kinase; AKT: Protein kinase B; RGS4: Regulator of G protein signaling 4; PIP: Prolactin-induced protein; mTOR: Mechanistic target of rapamycin.

DISCUSSION

GC is a highly malignant gastrointestinal tumor and main treatment method is still surgical resection. However, many patients have poor prognosis due to local recurrence or distant metastasis[23]. Therefore, we need to improve the prognosis of patients by identifying potential therapeutic targets for GC. GPCRs are the largest family of cell surface receptors and dysregulation of their signaling pathways is related to the development of tumor. Certain receptors such as protease-activated receptors (PARs) have been confirmed to be downstream signaling factors mediated by RGS[24]. Therefore, it is important to investigate the role of RGS in carcinogenesis.

Table 1 Association between regulator of G protein signaling 4 expression and clinicopathological characteristics of patients with gastric cancer

Characteristics	RGS4 expression		*P* value
	Low group (*n* = 45)	High group (*n* = 45)	
Age, median (IQR)	64 (60, 68)	66 (61, 70)	0.199
Gender, *n* (%)			0.806
Female	12 (13.3)	10 (11.1)	
Male	33 (36.7)	35 (38.9)	
T stage, *n* (%)			0.011
T1	9 (10)	1 (1.1)	
T2	9 (10)	4 (4.4)	
T3	7 (7.8)	10 (11.1)	
T4	20 (22.2)	30 (33.3)	
N stage, *n* (%)			0.038
N0	21 (23.3)	11 (12.2)	
N1	10 (11.1)	9 (10)	
N2	6 (6.7)	5 (5.6)	
N3	8 (8.9)	20 (22.2)	
M stage, *n* (%)			1.000
M0	43 (47.8)	42 (46.7)	
M1	2 (2.2)	3 (3.3)	
Grade, *n* (%)			0.002
G1	16 (17.8)	6 (6.7)	
G2	18 (20)	12 (13.3)	
G3	11 (12.2)	27 (30)	
TNM stage, *n* (%)			0.011
Stage I	15 (16.7)	3 (3.3)	
Stage II	11 (12.2)	12 (13.3)	
Stage III	17 (18.9)	27 (30)	
Stage IV	2 (2.2)	3 (3.3)	
OS (month)			
Median (range)	28 (7-70)	19 (3-56)	0.004

RGS4: Regulator of G protein signaling 4; IQR: Interquartile range; TNM: Tumor-node-metastasis; OS: Overall survival.

RGS has been proved to be related to the development of many tumors, including lung cancer, breast cancer, osteosarcoma and bladder cancer[25]. Previous research by our team revealed that *RGS1* restrained the differentiation of GC cells, ultimately enhancing the malignancy of tumor. These findings indicate a potentially crucial role for the *RGS* gene in the advancement of GC. Therefore, we tried to investigate the effects of other *RGS* gene family members on GC. By mining public databases, we preliminarily identified that *RGS4* was highly expressed in GC and contributed to worse prognosis in patients of GC. Subsequent patient validation at our center further confirmed that patients with higher *RGS4* levels exhibited shorter OS and elevated T stage, N stage, TNM stage, and tumor grade. These results suggest that we can predict the prognosis of GC patients based on *RGS4* expression. As a member of the G protein-coupled receptor family, PAR4 is associated with more aggressive forms of GC[26]. *RGS4* formed a complex with the receptor and Gα in cells, inhibiting PAR4-mediated signaling[24]. Therefore, *RGS4* may contribute to the malignant progression of GC by hindering PAR4-related signaling, leading to poor prognosis and advanced TNM stage in patients. In addition to their conventional role in GTPase-activating protein activity, RGS proteins can also influence other non-canonical functions in cancer. For instance, *RGS5* and *RGS16* have been shown to facilitate tumor invasion and metastasis through EMT[27,28]. Belonging to the B/R4 *RGS* gene family, *RGS4*, *RGS5*, and *RGS16* exhibit similar patterns of cell growth and migration

regulation[25]. It is plausible that *RGS4* enhances metastasis in patients with GC by promoting EMT.

In this study, we conducted knockdown and overexpression experiments of the *RGS4* gene to further elucidate its mechanism of action. Our results indicated that overexpression of *RGS4* promoted malignant behaviors in GC cells, while knockdown of *RGS4* reversed these effects. This suggests that *RGS4* functions as a tumor-promoting factor in GC. Similarly, *RGS4* also promotes the progression of glioblastoma[29], non-small cell lung cancer[30] and osteosarcoma[13]. GSEA results demonstrated that *RGS4* expression was significantly associated with FAK signaling. Subsequent experiments confirmed that the overexpression of *RGS4* notably enhanced the activation of the FAK/PI3K/AKT pathway, whereas knockdown inhibited its activation. The FAK/PI3K/AKT pathway was essential in cell proliferation, metastasis, and survival[31,32]. It is well known that the activation of the FAK/PI3K/AKT pathway promotes malignant behavior in GC cells[33-35]. Additionally, studies have shown that reducing PAR4 expression enhances FAK transcription, thereby influencing cell survival, growth, and apoptosis[36]. *RGS4* inhibits PAR4-mediated signaling[24]. Accordingly, *RGS4* may activate the FAK/PI3K/AKT pathway by inhibiting PAR4, thereby promoting the malignant behavior of GC cells. Tumor development is often accompanied by EMT and it endows tumor cells with the ability to metastasize by promoting cell migration, invasiveness, and resistance to apoptosis[37]. In this process, the expression of epithelial markers decreases and mesenchymal markers increases, which leads to enhanced cell adhesion to the surrounding stroma and contributes to increased invasiveness of tumor cells during metastasis[34]. The findings of this research suggested that *RGS4* could promote EMT and lead to the migration of GC cells by enhancing N-cadherin and vimentin expression and reducing E-cadherin expression.

In summary, this study found that *RGS4* may promote the progression of GC *via* the FAK/PI3K/AKT pathway and EMT (Figure 5I). Inevitably, this study has several limitations. First, more clinical samples are necessary to confirm the prognostic significance of *RGS4* in GC. Second, whether *RGS4* affects FAK through GPCRs requires further clarification. Future investigations will involve additional experiments to better understand detailed mechanisms of RGS4-mediated oncogenesis in GC.

CONCLUSION

RGS4 is highly expressed in GC and can be regarded as a predictor to predict the prognosis of GC patients. *RGS4* contributes to the progression of GC *via* the FAK/PI3K/AKT pathway and EMT and may be a potential therapeutic target for GC.

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LETTER TO THE EDITOR

Advances and challenges in diagnosing and managing adult autoimmune enteropathy

Grigorios Christodoulidis, Sara E Agko, Marina N Kouliou, Konstantinos E Koumarelas, Dimitris Zacharoulis

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Abstract

Autoimmune enteropathy (AIE) is a rare immune mediated disorder primarily affecting children, characterized by chronic diarrhea, malabsorption, vomiting, weight loss and villous atrophy. It has also been observed in adults presenting diagnostic and treatment challenges due to its overlap with other gastrointestinal disorders such as celiac disease. Initial diagnostic criteria for AIE include small bowel villous atrophy, lack of response to dietary restrictions, presence of anti-enterocyte antibodies, and predisposition to autoimmunity without severe immunodeficiency. Refined criteria emphasize characteristic histological findings and exclusion of other causes of villous atrophy. AIE is associated with various autoimmune disorders and can present with overlapping features with Celiac disease, including villous atrophy but without significant intraepithelial lymphocytosis. Treatment primarily involves immunosuppression using corticosteroids, calcineurin inhibitors, and anti-tumor necrosis factor therapy, alongside nutritional support. Despite the challenges, understanding AIE's diverse manifestations and improving diagnostic criteria are essential for effective management and improved patient outcome. Further research is needed to elucidate the pathogenesis, disease progression and long-term outcomes of AIE.

Key Words: Autoimmune enteropathy; Diagnosis; Prognosis; Adult autoimmune enteropathy and challenges; Autoimmune enteropathy and treatment; Autoimmune enteropathy and diagnostic criteria; Pathophysiology of autoimmune enteropathy

Core Tip: Autoimmune enteropathy (AIE) is a rare, immune-mediated disorder predominantly affecting children, characterized by chronic diarrhea, malabsorption, vomiting, weight loss, and villous atrophy. Adults can also be affected, complicating diagnosis and treatment due to symptom overlap with other gastrointestinal disorders. Key diagnostic criteria include small bowel villous atrophy, lack of response to dietary restrictions, presence of anti-enterocyte antibodies, and a predisposition to autoimmunity without severe immunodeficiency. Further research is needed to elucidate the pathogenesis, disease progression and long-term outcomes of AIE.

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TO THE EDITOR

In this letter, we comment on the article published by Li *et al*[1]. Autoimmune enteropathy (AIE) is a rare immune-mediated disorder that primarily affects children, presenting with chronic diarrhea, malabsorption, vomiting, weight loss and villous atrophy[2,3]. This condition was identified by Unsworth and Walker Smith[2]. It is a rare immune intestinal disorder rarely observed in adults and is characterized by uncontrollable diarrhea leading to malabsorption[4]. Immune dysregulation, X-linked (IPEX) syndrome, enteropathy, polyendocrinopathy and mutations in *FOXP3* which is a master gene of regulatory T cells, can lead to AIE. Recent study has demonstrated that mutations in other T cells associated genes, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and cluster of differentiation 25 (CD25), can result in an IPEX-like phenotype[5]. Genetic studies have identified several monogenic immune disorders that can present as AIED in adulthood. These include lipopolysaccharide responsive beige-like anchor protein deficiency, CTLA4 haploinsufficiency, and nuclear factor kappa B subunit 1 mutation, all of which have been linked to adult-onset AIE[6].

The initial prognosed diagnostic criteria for AIE also include lack of response to dietary restriction, small bowel villous atrophy, predisposition to autoimmunity and/or presence of anti-enterocyte antibodies, with no evidence of deficiency of the immune system, such as hypogammaglobulinemia or common variable disorders of the immune system leading to immunodeficiency. Updated criteria have been emphasizing specific histological findings such as abnormal apoptosis, villous blunting, minimal intraepithelial lymphocytosis, and, deep crypt lymphocytosis, along with the exclusion of different reasons of villous atrophy. The presence of gut-specific antibodies is no longer required for diagnosis[7].

Lymphocyte deposits are present on the mucous membrane, and most affected individuals have anti-enterocyte or anti-goblet cell antibodies. Immunosuppressive drugs have been used with varying degrees of success. The prognosis of AIE depends on the severity of the intestinal mucosal damage and any associated extraintestinal symptoms and diseases [8].

The diagnosis of AIE is based on a characteristic combination of clinical signs, laboratory findings and histological features seen in small intestine biopsies. AIE often occurs with many other conditions and syndromes, especially notably IPEX syndrome and autoimmune polyglandular syndrome type 1. Diagnosing and managing AIE remains complex, and further research is needed to understand its underlying mechanism, disease progression and outcomes that will occur in the future[9].

The clinicopathologic manifestations of AIE are linked to a diverse group of disorders. The exact mechanisms underlying the AIE are still not fully elucidated. It appears to result from disrupted immune regulation of intestinal immunity alongside immunodeficiency. The overarching histopathologic changes seen in AIE include mucosal inflammation and damage to the epithelial layer, although these can manifest in various patterns[10]. Although AIE affects the entire gastrointestinal tract, nearly all cases show altered duodenal histology that follows known patterns with or without visible changes[4].

Clinical manifestations-diagnosis and long-term prognosis

AIE is a rare disorder marked by an abnormal immune response in the intestines, typically manifesting as severe diarrhea within the 1st 6 months of life. AIE is currently classified into five subcategories, the main AIE, syndromic (pediatric) AIE, primary (sporadic) AIE over 18-years-old, secondary (iatrogenic-driven) AIE in people over 18-years-old, and paraneoplastic AIE. Endoscopic findings in AIE can vary and include mucosal hyperemia, scalloping, ulcerations, and a mosaic appearance[7,11].

The underlying mechanisms of AIE are not completely understood, but evidence points to an overactive immune response caused by a defect in regulatory T-cell homeostasis[11,12]. It is a result from an imbalance in humoral and cellular immune function, driven by a defect in the regulatory T-cell system[2,12]. AIE has been incorporated in the IPEX syndromes (polyendocrinopathy, immunodysregulation, enteropathy X-linked) because it is frequently associated with other autoimmune disorders. For patients that have this condition, it is possible to have other autoantibodies, including liver/kidney microsomal antibodies, antinuclear antibodies, and anti-smooth muscle antibodies[12].

The following criteria exist to diagnose AIE such as the protracted diarrhea, failure to respond to an exclusion diet, absence of severe immunodeficiency and evidence of autoimmunity[13]. It has also been proposed as a morphological classification as it relates to duodenal mucosa, identifying four main histological patterns: (1) Active duodenitis; (2) Celiac disease (CD)-like pattern; (3) Acute graft versus host-like disease; and (4) Mixed pattern[11].

On the other hand, the adult AIE is defined by the following criteria such as the adult-onset protracted diarrhea (more than 6 weeks) that does not respond to any form of dietary exclusion. Also, the characterizing small-intestine histology, including villous atrophy, minimal intraepithelial lymphocytes with increased crypt apoptotic bodies, on absence of goblet or Paneth cells. Last but not least, the thorough elimination of other potential causes of villous atrophy, including CD or refractory CD, determined through combination of human leukocyte antigen (HLA) typing, celiac serology, histopathology features and response to a gluten free diet and enteropathy associated with common variable immunodeficiency determined by total immunoglobulin levels and histopathology[12,13].

Iaquinto *et al*[3] studied a case repost of a 73-year-old woman with a history of antinuclear antibodies, autoimmune hepatitis, and positive anti-enterocyte antibodies. She experienced 2 months of persistent diarrhea, nausea, loss of appetite and significant weight loss. Histological analysis of the duodenal mucosa showed severe villous shortening and flattening, leading to mucosal atrophy. An immunohistochemical study revealed a polymorphic lymphoid population, with B cells present in follicles and T cells in the diffuse component of the lamina propria. Remarkably, the patient had a complete recovery after 2 weeks of prednisolone treatment and following a gluten rich diet.

In another study, Chong *et al*[4] studied a case of seronegative AIE with atypical duodenal manifestations in a female (43-years-old). The diagnosis of AIE was confirmed by identifying mononuclear inflammation of the lamina propria, crypt epithelial apoptosis, and goblet cell loss in the intestinal mucosa beyond the duodenum. When colonic histological changes consistent with AIE are present, along with diarrhea and malnutrition despite sparing of the duodenum, it should raise suspicion for AIE due to the disease's pan-enteric nature.

Clinicians should consider AIE in their differential diagnosis for patients diagnosed with CD who do not respond to a strict gluten free diet. Studies describe cases where patients with AIE have both positive gluten sensitivity antibodies and celiac-associated HLA markers. There is also documented overlap in small intestinal biopsy findings between AIE and CD patients. While villous atrophy is seen in both CD and AIE, AIE is characterized by the absence of significant intraepithelial lymphocytosis and mucosal injury in other locations outside the small bowel, including esophagus, stomach and colon. Additionally, AIE is well documented to be associated with other autoimmune diseases[2]. AIE and CD differ in terms of their demographics, clinical presentation and response to treatments. As regard to the patient demographics, the men are affected at a higher proportion (60%) with AIE compared to CD and the average age pf patients with AIE is younger. In CD patients, chronic diarrhea is less frequent (71%) than in AIE patients (100%)[11].

The treatment of AIE is challenging and primarily relies on immunosuppression. This includes the use of corticosteroids, calcineurin inhibitors, and anti-tumor necrosis factor therapy. Nutritional support is also a crucial component of the treatment regimen[12]. A study presents a case of AIE with severe diarrhea, leading to multiple hospitalizations and parenteral nutrition. This patient is successfully managed with Ustekinumab. This suggests that Ustekinumab warrants further investigation as a potential treatment option[14]. Another study showed that treatment with corticosteroid had a good response but required total parenteral nutrition during her stay in the hospital. That was studied in an 82-year-old woman with a history of autoimmune thyroiditis with significant weight loss and diarrhea[15]. The treatment involves immunosuppression to eliminate inflammation in the small intestine. Prednisolone and budesonide combined together, have been used with varying degrees of success. When these treatments are ineffective, potentially harmful agents such as adalimumab, infliximab, or tacrolimus are often considered, though their success rates can also vary. Long-term use of these agents may increase the risk of systemic adverse effects[13].

CONCLUSION

In conclusion, AIE is a rare and challenging condition to diagnose and treat due to its varied manifestations and overlap with other gastrointestinal disorders. Our case studies highlight the importance of considering AIE in patients with persistent gastrointestinal symptoms unresponsive to conventional treatments, even when typical histologic changes are absent in the duodenum. The identification of specific histological features, such as crypt epithelial apoptosis, mononuclear inflammation, and goblet cell loss in the intestinal mucosa beyond the duodenum, can be crucial for diagnosis. The effectiveness of immunosuppressive therapy, particularly with prednisolone and budesonide, underscores the potential for restoring immune equilibrium and achieving long-term remission. Continued research and awareness are essential for improving diagnosis, treatment and long-term outcomes.

FOOTNOTES

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LETTER TO THE EDITOR

Prospects of elafibranor in treating alcohol-associated liver diseases

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Abstract

Alcohol-related liver disease (ALD), which is induced by excessive alcohol consumption, is a leading cause of liver-related morbidity and mortality. ALD patients exhibit a spectrum of liver injuries, including hepatic steatosis, inflammation, and fibrosis, similar to symptoms of nonalcohol-associated liver diseases such as primary biliary cholangitis, metabolic dysfunction-associated steatotic liver disease, and nonalcoholic steatohepatitis. Elafibranor has been approved for the treatment of primary biliary cholangitis and has been shown to improve symptoms in both animal models and *in vitro* cell models of metabolic dysfunction-associated steatotic liver disease and nonalcoholic steatohepatitis. However, the efficacy of elafibranor in treating ALD remains unclear. In this article, we comment on the recent publication by Koizumi *et al* that evaluated the effects of elafibranor on liver fibrosis and gut barrier function in an ALD mouse model. Their findings indicate the potential of elafibranor for ALD treatment, but further experimental investigations and clinical trials are warranted.

Key Words: Elafibranor; Alcohol-associated liver diseases; Peroxisome proliferator-activated receptor; Lipid; Apoptosis; Steatosis; Inflammation; Fibrosis

Core Tip: Elafibranor is an oral dual peroxisome proliferator-activated receptor α/δ agonist that has demonstrated efficacy in improving hepatic steatosis and inhibiting inflammation and fibrosis associated with nonalcoholic liver diseases. Alcohol-related liver disease (ALD), resulting from excessive alcohol consumption, also presents symptoms such as hepatic steatosis, inflammation, and fibrosis. However, the effectiveness of elafibranor in treating ALD remains unclear. A recent study by Koizumi *et al* revealed that elafibranor significantly reduced hepatic steatosis, apoptosis, and fibrosis in a mouse model of ALD. Despite the potential of elafibranor for ALD treatment appears promising, further experimental investigations and clinical trials are warranted.

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TO THE EDITOR

Elafibranor is an oral dual peroxisome proliferator-activated receptor (PPAR) α/δ agonist that mediates many physiological processes, such as lipid metabolism, glucose metabolism balance, and inflammatory responses, by activating PPARα and PPARδ[1]. Elafibranor has shown great potential in the treatment of liver disease and has been approved for the treatment of primary biliary cholangitis (PBC) in the United States[2]. Several studies have revealed that elafibranor treatment ameliorates steatosis, inflammation, and fibrogenesis resulting from conditions such as metabolic dysfunction-associated steatotic liver disease (MASLD), also known as nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and chronic hepatic diseases[3-10], in mouse models and *in vitro* liver cell or slice models.

Alcohol is one of the most common causes of liver disease worldwide and induces a wide range of direct liver damage, including steatosis, alcoholic hepatitis, cirrhosis, and hepatocellular carcinoma[11-13]. Alcohol-related liver disease (ALD) also involves lipid metabolism disorders and inflammation[14,15], but whether elafibranor is effective in treating ALD remains unknown. The mechanisms by which elafibranor treats ALD have been explored by studies using animal models. Li *et al*[16] reported that elafibranor reduced intestinal epithelial destruction and liver inflammation/apoptosis/steatosis in a mouse model of alcoholic steatohepatitis, thereby reducing the severity of liver injury. Recent findings of Koizumi *et al*[17] demonstrated that elafibranor reduced ALD related fibrosis by inhibiting lipid accumulation and suppressed inflammatory responses by restoring intestinal barrier function in a mouse model of NASH. Moreover, several studies have shown that the application of PPARα or PPARδ agonists alone could improve symptoms of ALD[18-20]. For example, PPARα agonists alleviate steatohepatitis in ALD mouse models by increasing lipid oxidation, increasing the expression level of antioxidant enzymes, downregulating the expression of proinflammatory factors[18-20], and enhancing autophagy by directly increasing the expression of autophagy genes[21], whereas PPARδ agonists alleviate alcohol-induced liver injury in mice and improve intestinal barrier function[22]. These findings lay a foundation for the application of elafibranor in ALD.

EFFICACY OF ELAFIBRANOR IN THE TREATMENT OF NONALCOHOLIC LIVER DISEASE

In addition to PBC, elafibranor has also been used for the treatment of liver fibrosis, NAFLD, primary sclerosing cholangitis, dyslipidemia, abdominal obesity and other diseases, but many clinical trials have terminated in stages 2 or 3. In clinical trials to treat PBC, researchers have tested two doses of elafibranor (80 mg and 120 mg per day)[23,24]. Both doses significantly reduced the serum levels of alkaline phosphatase (ALP) and total bilirubin, which are biomarkers of PBC, but the 120 mg group showed little improvement compared with the 80 mg group[23,24]. Notably, 16-30 days after treatment with elafibranor, the ALP level of the PBC patients increased again to the pretreatment level[24], indicating that although elafibranor could improve the plasma biochemical parameters of PBC patients, it did not fundamentally solve the problem of cholestasis.

The slow pace of drug development for liver disease is partly due to the lack of adequate tools to evaluate the efficacy of potential new drug candidates; thus, animal and cellular models of liver disease are important tools for studying disease pathogenesis and evaluating drug candidates[25,26]. Perakakis *et al*[4] evaluated the effects of elafibranor on a mouse model of NAFLD and reported that elafibranor (30 mg/kg/day) had profound effects on the hepatic lipidome, manifested by a reduction in triglycerides, increases in phospholipids, and beneficial regulation of fatty acid oxidation, inflammation, and oxidative stress. Baandrup Kristiansen *et al*[27] reported that although elafibranor (30 mg/kg/day) could significantly reduce the liver fat content in a NASH mouse model, it tended to exacerbate hepatomegaly. Van den Hoek *et al*[6] reported that oral administration of elafibranor (15 mg/kg/day) significantly reduced hepatic steatosis and liver inflammation and prevented the progression of liver fibrosis in a NASH mouse model, which was consistent with the results obtained by Briand *et al*[28] with 20 mg/kg/day of elafibranor. Briand *et al*[7] also reported that 20 mg/kg/day elafibranor had liver-protective effects on NASH model hamsters, greatly improving liver lesions and reducing the expression of genes associated with inflammation (*e.g.*, tumor necrosis factor-α and interleukin-1β) and fibrosis (collagen 1 alpha 1).

Hammoutene *et al*[29] constructed a 3D model of MASLD using patient-derived precision cut liver slices (PCLS) and reported that aspartate aminotransferase and lactate dehydrogenase leakage in PCLS culture supernatants was not significantly affected by elafibranor treatment. Four clinical trials of elafibranor in the treatment of NAFLD patients also demonstrated that elafibranor significantly reduced serum ALT levels, but there was no significant change in aspartate aminotransferase[30], which was consistent with the results obtained *via* PCLS[29].

van Os *et al*[26] established functional multicellular liver spheroid (MCLS) cultures using primary mouse hepatocytes, hepatic stellate cells, liver sinusoidal endothelial cells, and Kupffer cells and reported that elafibranor (30 μmol/L) inhibited steatosis and liver fibrosis. Gore *et al*[31] reported that improved fibrosis and inflammation occurred only in NASH model mice treated with elafibranor (15 mg/kg, administered orally twice a day), but not *in vitro* in mouse-derived PCLS treated with 0.2 or 1 μmol/L elafibranor, which increased the expression of only PPARα-regulated genes. Elafibranor (10 μmol/L) also decreased triglycerides and reduced inflammation in MAFLD patient-derived PCLS but had no effect on the expression of liver fibrosis-related markers, such as alpha smooth muscle actin, collagen 1 alpha 1, and collagen 1 alpha 2[29]. These studies indicate that the responses of animal models and MCLS/PCLS to elafibranor are different, but the differences may be caused by the different doses of elafibranor used in these studies.

Boeckmans *et al*[25] established *in vitro* models of NASH using primary human hepatocytes (PHHs), HepaRG cells, and human skin stem cell-derived hepatic progenitor cells (hSKP-HPCs) and reported that the transcriptomes of these models were similar to those of NASH human livers. Transcriptome analysis revealed that 60 μmol/L elafibranor reduced the toll-like receptor-dependent inflammatory response in NASH models of PHHs and hSKP-HPCs, and three genes (*ANGPTL4*, *PDK4*, and *PLIN2*) that promote fat production and accumulation were strongly upregulated. Nevertheless, elafibranor did not increase the expression of these three genes in the clinical samples of NASH patients. Moreover, the transcriptomes of PHHs, HepaRG cells, and hSKP-HPCs overlapped 35% with the transcriptomes of liver sample from NASH patients. Other comparative transcriptomic studies revealed that, after activation by wy14643 (a PPARα agonist), only 20% of the upregulated and 12% of the downexpressed genes overlapped between humans and mice[32], with genes involved in glycolysis and gluconeogenesis pathways being specifically upregulated only in mice, whereas genes involved in heterologous metabolism and the apolipoprotein synthesis pathway were specifically upregulated only in human hepatocytes[32,33]. These findings indicate large differences in gene expression and regulation between animal models and humans and may explain the failure of elafibranor in many clinical trials.

DOSAGES OF ELAFIBRANOR AND ITS SAFETY

Doses of 80 mg or 120 mg elafibranor per person per day were adopted in clinical trials for MAFLD and NASH in both adults and children[5,23,24]. The common doses are 15, 20, or 30 mg/kg/day for animal models[4,6,7,27,28], and 0.2, 1.0, 10, 30, or 60 μmol/L for MCLS/PCLS and cell cultures[25,26,29,31]. Curiously, although the doses were quite different, the effects of elafibranor were similar in terms of reducing inflammation and improving lipid metabolism. Additionally, the great distinction in dosage, especially for MCLS/PCLS and cell cultures, reflects the strong tolerance of human, animal, and isolated cells to elafibranor. As a ligand, elafibranor binds to PPAR α/δ to function[34]. PPARα/δ, as receptors, are saturated when the level of elafibranor reaches a certain level. Therefore, the efficacy of elafibranor may be related to the expression levels of PPARα/δ in the liver, but research on this relationship is still lacking.

In clinical trials for the treatment of PBC, compared with the placebo group, the elafibranor group had higher rates of adverse events, including abdominal pain, diarrhea, nausea and vomiting[23,24]. Elafibranor, which is administered once daily, is well tolerated in children aged 8-17 years with NASH[5]. Hammoutene *et al*[29] reported that 10 μmol/L elafibranor had no toxic effect on PCLS derived from MAFLD patients. Therefore, elafibranor is generally safe although it may increase the rate of mild to moderate adverse events.

PROSPECTS OF ELAFIBRANOR IN THE TREATMENT OF ALD

According to the World Health Organization's Global Status Report on alcohol and health, the incidence of ALD is much higher than that of PBC[35]. The pathogenic mechanism of ALD differs from that of nonalcoholic liver diseases such as MASLD and NASH, but ALD also involves abnormal fat metabolism and inflammation[14,36]. Alcohol absorption and metabolism disrupt liver metabolic homeostasis and form the basis for ALD through a multifaceted mechanism. Alcohol metabolism results in the production of large amounts of reductive NADH, which induces lipid synthesis in the liver[37]. Sterol regulatory element-binding protein-1c is the main regulatory factor that regulates fatty acid synthesis[38]. Excessive alcohol consumption enhances the activity of sterol regulatory element-binding protein-1c and thus promotes lipid synthesis. PPARα regulates fatty acid degradation, but excessive drinking inhibits PPARα activity. Consequently, heavy drinkers accumulate fat in the liver, which leads to the formation of alcohol-related fatty liver and then progresses to ALD[37]. PPARα prevents ALD progression through hepatic lipid metabolism pathways, including fatty acid oxidation, elongation, desaturation, and thyroglobulin synthesis and breakdown[38], while PPARδ functions in lipid metabolism through autophagy-mediated fatty acid oxidation[39].

Although the therapeutic effect of elafibranor on ALD cannot be confirmed at present because of the lack of clinical studies on the effects of elafibranor on ALD, the effects of elafibranor on the PPAR signaling pathway in some cell and mouse models have been reported. In alcohol-stimulated HepG2 cells, elafibranor promotes lipolysis and β-oxidation through the activation of PPARα, whereas in alcohol-stimulated Caco-2 cells, elafibranor protects the intestinal barrier through PPARδ[17]. In metabolic dysfunction-associated steatohepatitis mouse models, elafibranor elevated S100A4

expression by activating PPARβ/δ[3]. S100A4 is known to promote mitochondrial metabolism and maintain high levels of fatty acid β-oxidation in tumor-associated macrophages[40]. However, it is not clear whether the expression of S100A4 is also elevated in ALD. When NASH mice were treated with GFT505 (an alternative name for elafibranor), upregulated genes were enriched in the PPAR signaling pathway and fatty acid degradation pathway, and *Ehhadh* and *Acaa2* were two of the upregulated genes involved in fatty acid degradation[41].

Despite considerable efforts in the field of ALD research, the pathogenesis of ALD is still unclear, and no Food and Drug Administration-approved ALD drugs are available[37,42]. Given the effectiveness of elafibranor in nonalcoholic liver diseases and the shared symptoms between alcoholic and nonalcoholic liver diseases, the prospects of elafibranor in ALD treatment are promising. The positive effect of elafibranor on ALD mouse models reported by Koizumi *et al*[17] may support further exploration of the use of elafibranor in treating ALD. However, extensive experimental studies are needed to elucidate the mechanism of efficacy of elafibranor and the key to achieving optimal efficacy. Just as the level of ALP in PBC patients increased again after elafibranor was stopped, although elafibranor may have certain therapeutic effects on ALD, alcohol cessation may be the cornerstone for the treatment of ALD.

CONCLUSION

Due to its ability to improve lipid metabolism, inflammatory responses, and hepatic fibrosis, elafibranor is a promising drug that may play an essential role in treating ALD. PPAR is a transcription factor and elafibranor binds to PPARα/δ to function. Nevertheless, the amount of elafibranor needed to effectively activate PPARα/δ to initiate downstream gene expression, as well as the expression levels of PPARα/δ in patients with liver disease, deserves further research. In addition, the differences in reactions to elafibranor between patients and animal/cell models are also key factors that need to be fully considered when elucidating the mechanisms of ALD and evaluating drug efficacy and safety.

FOOTNOTES

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LETTER TO THE EDITOR

Interplay between creeping fat and gut microbiota: A brand-new perspective on fecal microbiota transplantation in Crohn's disease

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Abstract

Inflammatory bowel disease, particularly Crohn's disease (CD), has been linked to modifications in mesenteric adipose tissue (MAT) and the phenomenon known as "creeping fat" (CrF). The presence of CrF is believed to serve as a predictor for early clinical recurrence following surgical intervention in patients with CD. Notably, the incorporation of the mesentery during ileocolic resection for CD has been correlated with a decrease in surgical recurrence, indicating the significant role of MAT in the pathogenesis of CD. While numerous studies have indicated that dysbiosis of the gut microbiota is a critical factor in the development of CD, the functional implications of translocated microbiota within the MAT of CD patients remain ambiguous. This manuscript commentary discusses a recent basic research conducted by Wu *et al*. In their study, intestinal bacteria from individuals were transplanted into CD model mice, revealing that fecal microbiota transplantation (FMT) from healthy donors alleviated CD symptoms, whereas FMT from CD patients exacerbated these symptoms. Importantly, FMT was found to affect intestinal permeability, barrier function, and the levels of proinflammatory factors and adipokines. Collectively, these findings suggest that targeting MAT and CrF may hold therapeutic potential for patients with CD. However, the study did not evaluate the composition of the intestinal microbiota of the donors or the subsequent alterations in the gut microbiota. Overall, the gut microbiota plays a crucial role in the histopathology of CD, and thus, targeting MAT and CrF may represent a promising avenue for treatment in this patient population.

Key Words: Crohn's disease; Mesenteric adipose tissue; Creeping fat; Fecal microbiota transplantation; Gut microbiota

Core Tip: Some studies have indicated that dysbiosis of the gut microbiota is a significant characteristic in the development of Crohn's disease (CD). However, the functional role of translocated microbiota in the mesenteric adipose tissue (MAT) of CD patients remains ambiguous. Evidence has shown that the translocation of viable microbiota into human MAT can polarize macrophages, leading to adipogenesis within the MAT and contributing to the formation of creeping fat (CrF) in individuals with CD. Nonetheless, it remains an important inquiry to elucidate the role of MAT-associated microbiota in the pathogenesis of CD. This manuscript aims to discuss the article by Wu *et al*, which explores the potential therapeutic value of fecal microbiota transplantation in the management of CD. Study by Wu *et al* suggested that the interactions among gut microbiota, MAT hypertrophy, and intestinal fibrosis may mutually reinforce one another in the pathogenesis of CD. Consequently, targeting MAT and CrF may hold promise as a therapeutic strategy for patients suffering from CD.

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TO THE EDITOR

Mesenteric adipose tissue (MAT) hypertrophy, commonly referred to as fat wrapping or creeping fat (CrF), is a characteristic feature of Crohn's disease (CD) and has been identified as a distinctive aspect of this condition[1]. The mesenteric mesoderm, which is encased by the intestinal endoderm, plays a crucial role in the development of the intestine through its contributions of cellular and connective tissue[2]. This pathobiological interaction persists into adulthood and is now recognized as a factor contributing to the pathological changes observed in CD. In cases of inflammation and intestinal strictures, CrF frequently envelops the affected intestine, and its presence correlates with the clinical activity and severity of inflammation associated with CD[3]. It is noteworthy that CrF predominantly occurs in the small intestine, particularly in the ileum, and is not observed in ulcerative colitis (UC), another type of inflammatory bowel disease[4]. Furthermore, CrF has been established as an anatomical marker that aids surgeons in delineating resection margins during surgical procedures. Additionally, it has been demonstrated that mesentery-based surgical approaches for CD are linked to enhanced long-term postoperative outcomes[5].

Numerous studies have indicated that dysbiosis of the gut microbiota is a significant characteristic in the development of CD[6]. However, the functional role of translocated microbiota in the MAT of CD patients remains ambiguous[7]. Additionally, human studies have shown a correlation between intestinal permeability and visceral adiposity[8], as well as metabolic syndrome in obese individuals[9]. A recent investigation has further substantiated the presence of bacteria and bacterial DNA in various MATs linked to the metabolic consequences of obesity[7]. Moreover, a study with a limited sample size identified a microbiotic signature at the phylogenetic level within the MAT of CD patients[10]. Notably, the translocation of viable microbiota into human MAT has been shown to polarize macrophages, which subsequently promotes adipogenesis in MAT and contributes to the development of CrF in CD patients[11]. Nonetheless, the precise role of MAT-associated microbiota in the pathogenesis of CD remains an important area for further exploration.

This manuscript examines the research conducted by Wu *et al*[12], focusing on the potential therapeutic benefits of fecal microbiota transplantation (FMT) in the management of CD. The findings of Wu *et al*[12] indicate that significant histopathological changes occur in the CrF and intestinal tissues of patients with CD. Additionally, there is a notable increase in the expression levels of pro-inflammatory cytokines within the CrF. Wu *et al*[12] suggested that the interplay between gut microbiota, MAT hypertrophy, and intestinal fibrosis might collectively contribute to the pathogenesis of CD. One method of modulating the microbiota is through FMT, which involves the transplantation of fecal microbiota from a healthy donor into the distal gastrointestinal tract of a patient. Notably, FMT was also employed by Wu *et al*[12], which demonstrated positive effects on body weight, colon length, and histopathological changes in mice treated with trinitrobenzenesulfonic acid. Additionally, FMT resulted in partial improvements in intestinal permeability, barrier function, serum levels of cytokines and adipokines, whereas FMT from CD patients exacerbated these parameters. FMT has been recognized as an effective therapeutic approach for *Clostridium difficile* infection[13] and is presently under investigation as a possible intervention for CD. In summary, Wu *et al*[12] emphasized the considerable promise of FMT in the management of CD.

MAT AND CRF IN CD

MAT and CrF have been implicated in the pathogenesis of CD, with studies indicating that the ratio of intra-abdominal fat to total abdominal fat is elevated in individuals with CD compared to healthy controls. Additionally, a higher proportion of visceral fat has been linked to an increased incidence of postoperative disease recurrence[14,15]. CrF, characterized by its pathological alterations, is frequently observed in proximity to inflamed intestinal regions and is

associated with heightened disease severity[16]. This type of fat exhibits distinct characteristics, such as increased size and enhanced immune cell infiltration, which differentiate it from normal mesenteric fat[17,18]. Moreover, MAT also functions as a reservoir for C-reactive protein and is susceptible to bacterial translocation, thereby providing further insight into the complex relationships between adipose tissue and inflammatory mechanisms in CD[19]. The recognition of this complex relationship has expanded the understanding of CD's pathogenesis, underscoring the significance of MAT and CrF in the context of inflammation, immune responses, and disease progression[20,21].

CRF AND INTESTINAL INFLAMMATION

The inflammatory profile associated with MAT in patients diagnosed with CD is characterized by elevated levels of various cytokines, including tumour necrosis factor-α, interleukin (IL)-1β, and IL-6[22,23]. These elevated concentrations contribute to the inflammatory processes within the intestinal tract, while the downregulation of the protective adipokine adiponectin is implicated in the pathogenesis of CD. CrF have been identified as significant sources of pro-inflammatory and pro-fibrotic cytokines[24]. In the context of CD, the integrity of the intestinal barrier is compromised, facilitating the translocation of bacterial antigens and subsequently eliciting Th17 and Th1 immune responses[25]. The Th1 response is particularly characteristic of CD, leading to the secretion of cytokines such as IL-22, IL-1, interferon-γ, and IL-2, among others[26]. Notably, Th1 cells are more prevalent in CrF compared to the mucosal environment, which exhibits a higher infiltration of Th17 cells in response to bacterial infections. Furthermore, the CrF contains a greater proportion of M2 macrophages relative to M1 macrophages, in contrast to the lamina propria, where M1 macrophages are more predominant[27,28]. This preferential polarization towards M2 macrophages may facilitate fibrosis in the affected intestinal tissue through the secretion of substantial amounts of pro-fibrotic cytokines[29]. The intricate immune microenvironment present in CrF, along with its interactions with the inflamed intestinal tissue, plays a critical role in the pathogenesis and progression of CD.

BACTERIA AND CRF FORMATION

The compromised integrity of the intestinal barrier facilitates the translocation of gut-derived bacteria (Figure 1). Research indicates that up to 27% of patients with CD experience bacterial translocation to mesenteric fat, in contrast to 13% of healthy controls. This phenomenon has also been observed in models of experimental colitis and ileitis[19]. CD is associated with a microbiome profile characterized by an increased presence of *Proteobacteria* and *Clostridium innocuum*, with the relative abundance of these bacteria being influenced by the clinical status of the disease[10,11]. Furthermore, lymphatic flow is crucial for the transport of bacterial antigens and immune cells[30]. One hypothesis regarding lymphatic vessels suggests that CrF may be exacerbated by the leakage of fatty chylomicrons into the mesentery, facilitated by highly permeable lymphatic vessels[31,32]. Improving the integrity and pumping function of lymphatic vessels could potentially mitigate inflammation in MAT, thereby supporting the notion that the leakage of antigens triggers inflammatory responses and adipogenesis in MAT[33]. Indeed, single-cell RNA sequencing has identified CrF as both pro-fibrotic and pro-adipogenic, characterized by a diverse array of activated immune cells responding to microbial stimuli[11].

FMT AND CD

The existing literature on FMT for CD is relatively limited compared to that for UC. Case reports have yielded mixed outcomes, with some indicating both clinical and endoscopic remission, while others reported no significant effects[34]. Notably, a case involving a patient with severe, complicated CD documented a successful response to FMT[35]. In a cohort study involving 30 patients with refractory mid-gut CD, a single FMT administered *via* the nasoduodenal route resulted in a 77% rate of clinical remission one month post-treatment[36]. Furthermore, FMT may represent a viable treatment option for pediatric CD, as a recent case series reported remission in 5 out of 9 patients (56%) following FMT, with 7 out of 9 patients (78%) exhibiting engraftment of donor microbiota[37]. Previous investigations into FMT for active CD have produced inconsistent results. Among 20 subjects enrolled in an FMT study, 19 provided complete follow-up data. Although most participants experienced improvement post-FMT, the clinical outcomes varied significantly, with one individual who had severe disease prior to FMT ultimately requiring colectomy afterward[38]. Conversely, a recent meta-analysis indicated that FMT significantly decreased CD activity index scores within 4 weeks to 8 weeks following the procedure[39]. Collectively, these findings suggest that FMT may hold potential as a therapeutic approach for CD. However, further research is essential to evaluate both its clinical efficacy and the associated alterations in the gut microbiota of affected patients. In the study conducted by Wu *et al*[12], the transplantation of intestinal bacteria from individuals into CD model mice revealed that FMT from healthy donors alleviated CD symptoms, whereas FMT from CD patients exacerbated these symptoms. Importantly, FMT was found to influence intestinal permeability, barrier function, and levels of pro-inflammatory factors and adipokines. These results imply that targeting MAT and CrF may provide promising avenues for therapeutic interventions in CD patients. Consequently, the gut microbiota appears to play a pivotal role in the histopathology of CD, and strategies aimed at targeting MAT and CrF may be beneficial in the treatment of CD.

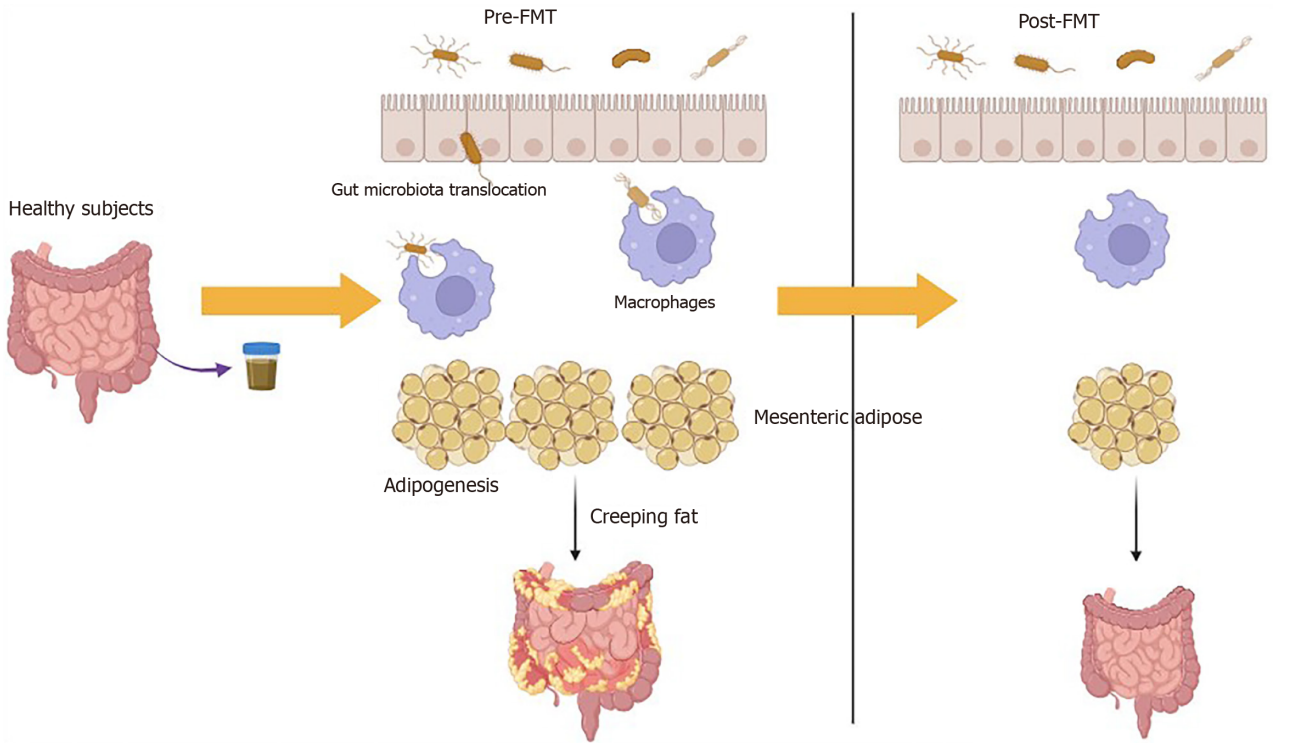

Figure 1 Interactions between gut microbiota, creeping fat, and fecal microbiota transplantation outcomes in Crohn's disease. FMT: Fecal microbiota transplantation.

GUT MICROBIOTA COMPOSITION CHANGES POST-FMT

Recent investigations have documented specific alterations in the gut microbiota of recipients following FMT. A study indicated a significant increase in alpha diversity post-FMT, in contrast to sham group. However, this enhancement was transient, with alpha diversity reverting to baseline levels 14 weeks after FMT[34]. Similarly, research conducted by Vaughn *et al*[38] suggested that a single colonoscopic FMT could induce short-term modifications in the fecal bacterial composition of patients with active CD, thereby increasing the diversity of the intestinal microbiota. Another study revealed that there was an increase in the abundance of *Collinsella* and several genera within the *Lachnospiraceae* family in FMT-responders. Nonetheless, certain genera from the *Ruminococcaceae* and *Lachnospiraceae* families remained underrepresented in post-FMT responders, and some members of the *Enterobacteriaceae* family persisted in two of the responders. Post-FMT, specific members of *Lachnospiraceae* were found to be more prevalent in responders, while *Ruminococcaceae* (*e.g.*, *Faecalibacterium*) and *Bacteroides* were predominantly more abundant in nonresponders[40]. It is important to note that the abundance of *Oscillibacter* has been negatively correlated with CD, as indicated by previous studies[41,42].

CONCLUSION

Overall, the gut microbiota plays a crucial role in the histopathology of CD, and thus, targeting MAT and CrF may represent a promising avenue for treatment in this patient population.

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LETTER TO THE EDITOR

Gastric polyps are not created equal: Know your enemy

Fady Daniel

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Abstract

Gastric polyps are commonly detected during upper gastrointestinal endoscopy. They are most often benign and rarely become malignant. Nevertheless, adequate knowledge, diagnostic modalities, and management strategies should be the endoscopist's readily available "weapons" to defeat the potentially malignant "enemies". This article sheds light on the valuable effort by Costa *et al* to generate a new classification system of gastric polyps as "good", "bad", and "ugly". This comprehensive overview provides clinicians with a simplified decision-making process.

Key Words: Gastric polyps; Polypoid subepithelial lesions; Early gastric cancer; Endoscopic ultrasonography; Electronic chromoendoscopy

Core Tip: Knowing and understanding the risks associated with gastric polyps (GPs) is an important step in diagnosing and managing them. Knowing "when to do", "what to do", and "how to do" is important for not missing any significant pre-malignant GPs. A good classification reinforces the clinical prerequisites for endoscopists when dealing with GPs during upper gastrointestinal endoscopy.

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TO THE EDITOR

Gastric polyps (GPs) are frequently detected during upper endoscopy to investigate

dyspepsia and acid-related disorders. Fundic GPs are the most frequently identified subtype, and their formation is linked to the wide use of proton pump inhibitors[1]. "If you know the enemy and know yourself, you need not fear the result of a hundred battles", this famous quote by Sun Tzu applies to GP management.

CHOOSING YOUR WEAPON

The term subepithelial lesion (SEL) is preferred over submucosal tumor to describe GPs, as the term submucosal tumor specifically describes lesions emerging from the submucosal layer[2]. Polypoid SELs in the stomach can arise from the muscularis mucosa, submucosa, or muscularis propria. To accurately evaluate these lesions, the initial step is histologic assessment through sampling biopsies or complete resection. However, standard endoscopic forceps biopsy of the mucosa overlying SELs often fails to obtain sufficient tumor tissue for pathology. Therefore, specialized techniques like bite-on-bite, jumbo, and snare biopsy have been developed. In addition, newer techniques, such as unroofing the SEL to expose its surface or the creation of a submucosal tunnel, allow for direct tumor biopsy. Third-space endoscopy techniques that rely on submucosal dissection have improved the quality of endoscopic sampling and facilitated the pathologist's task by providing better tissue specimens[3]. The European Society of Gastrointestinal Endoscopy recommends endoscopic ultrasonography (EUS) to characterize SEL features (size, location, originating layer, echogenicity, shape). For diagnosis of SELs measuring 20 mm or larger, EUS-guided fine-needle biopsy and mucosal incision-assisted biopsy are equally recommended[4,5]. Both techniques have been compared in three randomized controlled trials, and there were no significant differences found for complication rate or the diagnostic samples. Mucosal incision-assisted biopsy is more time-consuming and might make subsequent SEL resection more challenging *via* submucosal endoscopic tunneling. However, the European Society of Gastrointestinal Endoscopy recommends it as the first choice for SELs ≤ 20 mm, even though there is little supporting evidence[6-8].

KNOWING YOURSELF WELL

Diagnosis of GPs requires proper endoscopic characterization using Paris classification[9] and dye-based or electronic chromoendoscopy[10]. The presence of features suggestive of mucosal invasion in GP cases is a pivotal factor that warrants complementary EUS to determine invasion depth before considering endoscopic resection of a suspicious-looking GP. Once the pathology report is received, determining the need for surveillance and follow-up endoscopy timing is the next step. Guidelines addressing these issues are scarce in the medical literature[10]. In this issue, Costa *et al* [11] propose a simple but very practical classification system for GPs based on pathological type: The good, the bad, and the ugly. The authors are to be congratulated for their thoroughness in reviewing published evidence and detailing the best management for each GP. However, the classification relies primarily on polyp morphology and malignant potential evaluation to decide between resection or surveillance. Although advanced endoscopic resection techniques are becoming widespread in gastroenterological practice, they are associated with increased risks of complications compared to simple excisional biopsies. Thus, the only caveat of the proposed classification is the potential overuse of more invasive and riskier sampling techniques to ensure the most reliable sample for pathologic examination[12]. On the other hand, endoscopic surveillance impacts long-term healthcare expenditures, and a "watchful waiting" scenario of good/bad GPs might be associated with higher patient anxiety levels.

KNOWING YOUR BATTLEFIELD

Evaluation of the surrounding mucosa for atrophy, intestinal metaplasia, and dysplasia is essential and could result in a good GP being classified as "bad" and warrant earlier surveillance. This concept is well emphasized in the review. Finally, not all GPs or SELs are pathogenic. EUS features of SELs are pathognomonic for lipoma and varices. Fundic GPs are originally "good" in most cases, and syndromic ones are rarely encountered.

CONCLUSION

GPs can be complicated and difficult to classify or diagnose. A major difference exists between "good", "bad", and "ugly" GPs, though endoscopists should be aware of how to deal with each type. A good evaluation is possible with the foundation of good knowledge, and an adequate classification system would be effective in facilitating this. Such a model builds the cornerstone of future guidelines concerning the management of GPs. Future research should focus on comparing new endoscopic sampling techniques and their relative contribution to refining GP characterization. Long-term follow-up studies of "good" and "bad" GPs with a low to intermediate neoplastic transformation potential are warranted to define endoscopic surveillance intervals.

FOOTNOTES

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LETTER TO THE EDITOR

Exploring gut microbiota as a novel therapeutic target in Crohn's disease: Insights and emerging strategies

Tong Qiao, Xian-Hui Wen

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Abstract

Extensive research has investigated the etiology of Crohn's disease (CD), encompassing genetic predisposition, lifestyle factors, and environmental triggers. Recently, the gut microbiome, recognized as the human body's second-largest gene pool, has garnered significant attention for its crucial role in the pathogenesis of CD. This paper investigates the mechanisms underlying CD, focusing on the role of 'creeping fat' in disease progression and exploring emerging therapeutic strategies, including fecal microbiota transplantation, enteral nutrition, and therapeutic diets. Creeping fat has been identified as a unique pathological feature of CD and has recently been found to be associated with dysbiosis of the gut microbiome. We characterize this dysbiotic state by identi-fying key microbiome-bacteria, fungi, viruses, and archaea, and their contributions to CD pathogenesis. Additionally, this paper reviews contemporary therapies, emphasizing the potential of biological therapies like fecal microbiota transplantation and dietary interventions. By elucidating the complex interactions between host-microbiome dynamics and CD pathology, this article aims to advance our understanding of the disease and guide the development of more effective therapeutic strategies for managing CD.

Key Words: Crohn's disease; Gut microbiome; Dysbiosis; Creeping fat; Fecal microbiota transplantation

Core Tip: Crohn's disease (CD) represents a complex and challenging medical condition, characterized by the intricate interplay between genetic predisposition, gut microbiota dysbiosis, and immune dysregulation. This paper discusses the key effects of the gut microbiota on CD, as well as how they affect creeping fat. Furthermore, the paper highlights the urgent need to address gut microbiota imbalances, particularly focusing on pathogenic species and their mechanisms, as a critical therapeutic target. The findings underscore the importance of a personalized approach to CD management, emphasizing the need for continued research and innovation to address this complex disease.

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TO THE EDITOR

In this article, we review a recently published article by Wu *et al*[1] in the *World Journal of Gastroenterology*, titled "Role of gut microbiota in Crohn's disease pathogenesis: Insights from fecal microbiota transplantation in mouse model". The study delves into the disrupted characteristics of mucosal-associated lymphoid tissue (MAT) and intestinal tissues in Crohn's disease (CD) that were directly observed through hematoxylin and eosin (HE) staining, Masson's trichrome staining, and immunohistochemical staining. Furthermore, by establishing mouse models, they detected excessive expression of proinflammatory factors in creeping fat (CrF), which robustly validates the previous understanding of the role of CrF in the pathogenesis of CD. This paper outlines the critical contributions of gut microbiome studies to the pathogenesis of CD, with a particular focus on emerging therapies targeting the intestinal flora, notably fecal microbiota transplantation (FMT) and dietary interventions. Special emphasis is placed on the significance of gut microbiome research in elucidating disease mechanisms and providing novel perspectives and potential solutions for the treatment of CD.

CD AND ITS PATHOLOGICAL CHARACTERISTICS

CD

CD, one of the major entities within the spectrum of inflammatory bowel diseases (IBDs), commonly affecting the terminal ileum and proximal colon[2]. Most patients present with an inflammatory phenotype at diagnosis, but over time, complications such as fibrosis, strictures, fistulas, or abscesses may develop, often leading to the need for surgery[3].

Etiological analysis

Despite significant advancements in experimental techniques and models, the etiology of IBD remains unclear. Higher incidence rates of IBD observed in monozygotic and dizygotic twins, as well as among certain families, strengthen the case for a genetic influence on the disease. Additionally, researchers have identified hundreds of IBD-associated genetic polymorphisms and mutations through genome-wide association studies, further supporting the genetic underpinnings of IBD[4,5]. However, genetics alone is insufficient to account for the onset of the disease. With industrialization, changes in lifestyle, and urbanization in modern society, the incidence and prevalence of IBD have been on the rise[6]. Nonetheless, the timeframe of these developments is too short to be explained by genetic drift or natural selection. In this context, non-genetic factors such as environmental influences, higher ultra-processed food and lower unprocessed/minimally processed food intakes[7], smoking[8], and antibiotic use[9] all play a significant role in the pathogenesis of these diseases. In recent years, with the increasing depth of investigations into the gastrointestinal microbiota, more studies have indicated that dysbiosis of the gut microbiome is a significant factor in triggering and promoting the development of IBD.

CrF in CD

CrF is a hallmark of CD[10,11], characterized by the abnormal expansion of perienteric adipose tissue. This phenomenon involves the gradual encasement and infiltration of mesenteric fat around affected intestinal segments, resulting in a characteristic "creeping" appearance. A large number of cohort studies and clinical trials demonstrate that CrF exacerbates CD inflammation through various mechanisms[11-13] such as the release of pro-inflammatory molecules (leptin[14] and resistin[15]) and activation of immune cells (macrophages[16] and CD4+ T cells[17,18]). This is further supported by a few clinical trials that CrF promotes inflammation *via* SP/NKR1 activation and upregulating interleukin-17A (*IL-17A*) mRNA in preadipocytes and IL-17A receptor mRNA in colonic tissue[19,20].

It is worth noting that the gut microbiome, especially bacteria, may be a potential trigger for CrF. Emerging evidence suggests that CrF, the migration of MAT to sites of intestinal barrier dysfunction, may represent a protective response. This process aims to prevent systemic dissemination of potentially harmful bacteria that have translocated across the compromised intestinal epithelium. A study demonstrated that while bacterial translocation occurs in both normal MAT

and CD-associated CrF, the characteristics and metabolic functions of the gut microbiota differ significantly[21]. For instance, a specific population of translocated enteric bacteria, *i.e.*, *Clostridium innocuum*, is found in both the ileal mucosa and CrF. This bacterium promotes M2 macrophage polarization, stimulating adipose tissue remodeling and CrF formation[21]. Furthermore, adipocytes and preadipocytes within CrF express functional pattern recognition receptors, such as Toll-like receptors and nucleotide-binding oligomerization domain-containing protein 1[22]. These pattern recognition receptors recognize microbe-associated molecular patterns derived from translocated bacteria, triggering downstream signaling cascades that activate transcription factors like nuclear factor-κB (NF-κB) and induce the production of pro-inflammatory cytokines and chemokines[23]. Another study investigated whether microbial translocation in CD is a key factor in the progression of CrF[24]. The researchers identified a subset of mucosa-associated intestinal bacteria that consistently translocate during ileal resections in CD and remain viable in CrF; they pinpointed *Clostridium innocuum* as a marker of this population, noting strain variation between mucosal and adipose tissue isolates, which suggests a preference for lipid-rich environments[24]. Single-cell RNA sequencing revealed that CrF exhibits both pro-fibrotic and pro-adipogenic effects, along with an activated immune cell environment responsive to microbial stimuli. This was further confirmed in germ-free mice colonized with *Clostridium innocuum*[24].

Role and alterations of the gut microbiome in CD

The gut microbiota, including bacteria, fungi, viruses, and archaea, is closely related to the immune system and is of great importance to the normal physiological functions of the body[25,26]. Multiple studies have shown that gut microbiota dysbiosis is an important cause of the occurrence and aggravation of CD, and is closely related to the clinical manifestations of CD, such as inflammation and fibrosis. Wu *et al*[1] suggested a potential role of gut microbiota dysbiosis in the pathogenesis of CD, but their evidence was limited. Given the increasing focus on gut microbiota dysbiosis in CD research, we describe the impact of bacterial, fungal, viral, and archaeal dysbiosis, and their interactions, on CD development.

Intestinal bacterial microbiota in CD

The gut microbiome, especially bacteria, is the most extensively researched microbial community in patients with CD [27]. Studies using culture-independent methods (quantitative polymerase chain reaction, FISH analysis, 16S rRNA gene sequencing, shotgun-sequencing, and Illumina sequencing) on fecal samples consistently show that the observed decrease of some beneficial bacteria, such as *Bacteroides* and butyrate-producing *Firmicutes*, including *Faecalibacterium prausnitzii* and *Roseburia spp.*, alongside an increase in pathogenic bacteria, may significantly contribute to pro-inflammatory effects[28,29]. Such dysbiosis can lead to the disruption of certain metabolites produced by the microbiota, subsequently increasing the inflammatory response. For instance, short-chain fatty acids, particularly butyrate, play a crucial role as a histone deacetylase inhibitor, effectively suppressing the activation of NF-κB and downregulating pro-inflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α[29]. Secondary bile acids, which result from microbial metabolism, are critical in immune regulation, promoting regulatory T cell differentiation, and disruptions in bile acid metabolism worsen inflammation in IBD patients[30,31]. The reduced availability of tryptophan-derived metabolites, which modulate immune responses *via* aryl hydrocarbon receptors, correlates with higher disease susceptibility[32]. Sphingolipids, produced by both host and microbes, also regulate inflammation, with microbial sphingolipids like those from *Bacteroides* shown to protect against colitis by inhibiting natural killer T cell proliferation [33]. Furthermore, some clinical trials and relevant pre-clinical studies found that *Faecalibacterium prausnitzii* actively suppresses NF-κB activation and attenuates IL-8 production in intestinal epithelial cells *via* its secretory metabolites[28, 34]. In addition, CD is characterized by an increased abundance of *Ruminococcus gnavus* and mucosa-associated adherent-invasive *Escherichia coli* (AIEC), which bind to and invade intestinal epithelial cells through mechanisms involving microtubule polymerization and actin recruitment, inducing the secretion of inflammatory cytokines, particularly the transcriptional levels of interferon-γ and IL-8[35]. These cytokines are closely related to the immune response during CD, indicating the significant role of AIEC in perpetuating intestinal inflammation. When surviving and replicating in macrophages, AIEC can also induce TNF-α secretion, further contributing to the inflammatory milieu in CD[36]. A multicenter clinical study encompassing Spanish and Belgian CD cohorts investigated fecal microbiota composition using sample collection, genomic DNA extraction, high-throughput 16S rRNA gene sequencing, and bioinformatic analysis[37]. The results indicated that the depletion of beneficial bacteria, rather than an overabundance of pathogenic bacteria, is more strongly associated with CD[37]. These beneficial bacteria include butyrate-producing species such as *Faecalibacterium prausnitzii*, members of the *Lachnospiraceae* family (*e.g.*, *Roseburia* and *Ruminococcus*), and *Oscillospira*[37]. These findings support and extend previous reports of dysbiosis in CD[37].

Intestinal fungal microbiota in CD

Although the fungal community is far less abundant than bacteria, constituting only 0.1% of the gut microbiota[38], many studies have shown that fungi also play an important role in the pathogenesis of CD[39,40]. The abundance of *Candida albicans*, *Malassezia restricta,* and *Debaryomyces hansenii*, which are the most commonly detected fungal species in stool samples of CD subjects[41-43], shows significant changes in patients with CD. Recent studies have revealed that *Malassezia restricta* worsens colitis in mice through a caspase-recruitment domain 9-dependent mechanism. Upon *Malassezia restricta* recognition by C-type lectin receptors on immune cells, caspase-recruitment domain 9 is recruited to form a complex with B-cell lymphoma/leukemia 10 and mucosa-associated lymphoid tissue lymphoma translocation protein 1, activating the NF-κB pathway. This triggers production of TNF-α, IL-8, and other pro-inflammatory mediators, amplifying immune cell recruitment and inflammation[42]. *Debaryomyces*, specifically *Debaryomyces hansenii*, has been identified as being enriched in inflamed intestinal tissues of patients with CD and impairs colonic healing by modulating

the myeloid cell-interferon γ-C-C chemokine ligand 5 signaling axis[43]. Compared to healthy individuals, patients with CD exhibit an elevated proportion of *Candida albicans* and a reduced proportion of *Saccharomyces cerevisiae* in their gut. *Candida albicans*, originally a symbiotic bacterium in the gut, can delay intestinal mucosal healing and increase the secretion of IL-17 and IL-23 in diseases such as CD, thereby exacerbating inflammation[44]. *Saccharomyces cerevisiae*, a common probiotic in the gut, can strongly inhibit the adhesion of AIEC to the brush border of intestinal epithelial cells in CD. Furthermore, it restores barrier function by blocking AIEC-induced expression of Claudin-2, which forms tight junctions in pores on the plasma membrane of intestinal epithelial cells. These effects are accompanied by a reduction in the release of pro-inflammatory cytokines IL-6, IL-1β, and KC from the intestinal mucosa[45]. Additionally, several studies have indeed found that high serum titers of anti-*Saccharomyces cerevisiae* antibodies (ASCA) [immunoglobulin G (IgG) and IgA], which are directed against yeast cell wall-associated mannan, serve as a clinical biomarker for CD. The IgG ASCA-positive rate is 60%-70% in patients with CD[46].

Fungal-bacterial interactions

With the deepening of research on the intestinal microbiota in recent years, the interaction between different species has gradually attracted attention. The coexistence of fungi and some specific intestinal bacteria may be crucial for the development or improvement of intestinal diseases. Recently, Seelbinder *et al*[47] confirmed that antibiotic treatment significantly altered the composition of fungi, demonstrating that bacterial dysbiosis may lead to fungal dysbiosis. This study also showed that the use of *Candida albicans* aggravated the severity of the disease, while the use of *Saccharomyces boulardii* alleviated the symptoms of the disease. A cohort study, including patients from Northern France-Belgium, found a positive correlation between the abundance of *Candida tropicalis* and both *Streptococcus mitis* and *Escherichia coli*[48]. Additionally, the study showed an association between the levels of ASCA, a known biomarker for CD, and the abundance of *Candida tropicalis*[48].

Intestinal viruses and archaea in CD

The role of virome in CD may promote intestinal inflammation. CD is associated with an increase in temperate phages, critical for gut bacterial diversity and fitness, impacting microbial balance and CD progression. The "core phage" comprises double-stranded DNA *Caudovirales* (*Myoviridae*, *Podoviridae*, and *Siphoviridae*) and single-strand DNA *Microviridae*. CD patients exhibit altered viromes, with decreased *Microviridae* and *Virgaviridae*, and increased *Caudovirales*, *Alteronomonadales*, and *Clostridiales phages*. However, the specific virus-CD relationship remains elusive[49,50]. There is a paucity of relevant research in this area, and *in vitro* studies have demonstrated that bacteriophages can stimulate macrophages to induce MyD88-dependent pro-inflammatory cytokine production[51]. Besides, bacteriophages may also play a therapeutic role in CD treatment. A cocktail of three bacteriophages was demonstrated to reduce symptoms and significantly reduce AIEC in dextran sodium sulfate-induced colitis in mice[52]. Currently, research on archaea in CD is limited, with the majority of studies focusing on methanogens, which constitute approximately 10% of the intestinal anaerobic microbiota[53]. For instance, the presence of methanogens and the role of methane as a signaling molecule have been shown to prolong the contact time between the mucosa and toxic metabolic byproducts such as hydrogen sulfide. This extended exposure can enhance their absorption by the intestinal epithelium or lead to damage to the intestinal epithelial barrier, thereby increasing the risk of pathogen translocation[54]. *Methanobrevibacter smithii* is the most abundant species among methanogens, which accounts for approximately 94% of them, and recent studies have revealed that *Methanobrevibacter smithii* has been associated with increased acetate production[54,55] and acetate may exert a proinflammatory effect[56]. Additionally, the release of single-strand DNA from *Methanosphaera stadtmanae*, recognized as a microbe-associated molecular pattern, has been shown to stimulate the secretion of significant amounts of proinflammatory cytokines, such as IL-1β, TNF-α, and type-I and type-III interferons, through the activation of Toll-like receptor 8 [57].

Virus-bacterial interactions

The trans-cohort study by Cao *et al*[58] describes the cross-relationship between 50 types of bacteriophages and 30 species of bacteria, revealing a significant dysbiosis in the bacteriophage-bacteria ecological network of the small intestinal mucosa in patients with CD. Specifically, the phage-bacteria interaction network, which is typically centered around *Bifidobacterium* and *Lachnospiraceae* in healthy individuals, is significantly weakened, while the phage-bacteria interaction network centered around *Prevotella* is markedly strengthened in CD. Another study has focused on the virus-bacteria interaction network, identifying a positive correlation between DNA bacteriophages and certain bacteria (such as *Enterobacter cloacae* and *Escherichia coli*), while showing a negative correlation with *Faecalibacterium prausnitzii*[59]. Furthermore, these studies have noted distinct changes in virus-bacteria associations under different disease states, including a close correlation of torquetenovirus, *Bacteriophage spp*., and *Bacteroides phage B124-14* with fecal bacteria during active disease periods, which significantly diminishes during remission.

MICROBIOTA TRANSPLANTATION AND NUTRITIONAL THERAPY IN CD TREATMENT EFFICACY AND MECHANISMS

For the intractable chronic inflammation in CD, as well as severe complications such as intestinal stenosis and fistulas, various targeted surgical options and biological agents have been developed so far. With the updating of the concept of targeted drugs in recent years, some targeted therapies with theoretically better therapeutic effects and fewer side effects,

such as FMT and dietary interventions, have gradually been developed and put into clinical use, providing more options for the treatment of CD from a new perspective.

Conventional therapy

Approximately 15%-50% of patients with CD will require surgery within ten years following the diagnosis[60], including fistula repair, colectomy and ileostomy, proctectomy, and strictureplasty[2,61,62]. Notably, there is evidence indicating that biological therapy is more effective if introduced earlier in the disease course, preparing for early intervention[2]. The selection of medical therapy is determined by the patient's risk profile and the severity of the disease. Mild-to-moderate disease can be treated by oral mesalamine, immunomodulators like thiopurines (6-mercaptopurine and azathioprine), methotrexate, and steroids[63]. Moderate-to-severe disease (including fistulizing disease) is best treated with combined immunomodulators and biologics or biologics alone, including budesonide (corticosteroids), azathioprine, and mercaptopurine (immunomodulators)[64]. Anti-TNF agents (vedolizumab, ustekinumab, and infliximab) block the downstream effects of the TNF inflammatory cascade. These agents are efficacious in steroid-resistant or immunomodulator-refractory CD[65-67]. Notably, combination therapy with an immunomodulator and anti-TNF is more effective than monotherapy with either medication. Anti-IL-12/23 agents (ustekinumab and risankizumab) are efficacious in patients who failed prior corticosteroid, immunomodulator, or anti-TNF treatment.

Emerging biological therapies

Despite demonstrating efficacy in managing CD, conventional therapeutic interventions are correlated with a myriad of adverse reactions, encompassing metabolic disorders, serious infections, and dermatitis, *etc.*[68,69]. For instance, corticosteroids have the risk of causing cystoid appearance and hepatic steatosis, while anti-tumor necrosis factor therapy may cause severe bacterial infections in the lungs and a psoriatic eczema-like skin reaction. Initial analysis of early treatment regimens showed that some of these treatments disrupted the host gut microbiome, an occurrence linked to increased pathogenesis of CD[70-72]. Thus, introducing an adjuvant or supporting therapy that can modulate the gut microbiota and prevent gut microbiome dysbiosis may be a novel approach to supporting the management of CD. Not only does this approach have the potential of restoring the microbiome balance and it may also help achieve better response to the currently used medications[73,74].

Dietary therapy for maintenance of remission in CD

As the pathogenesis of CD becomes clearer, dietary factors are increasingly recognized for their role in disease management. Exclusive enteral nutrition (EEN) is well-established as an effective therapy for inducing remission in pediatric CD, with success rates of 60% to 80%[75]. EEN not only avoids the adverse effects of steroids but also improves nutritional status, promotes mucosal healing, and manages complications like strictures and fistulae[76-79], potentially reducing the need for surgery. In adult CD patients, corticosteroids and biologics are typically first-line treatments. However, a high-quality study has proposed EEN as an alternative therapy for adults, aiming to mitigate or avoid the side effects of conventional treatments and offering a standardized protocol for future research[80]. Despite its benefits, EEN has limitations, including its limited long-term effects on the gut microbiota and poor palatability, which hinder widespread adoption[81]. To address these limitations, alternative dietary strategies, such as partial enteral nutrition (PEN), low FODMAP diets, the specific carbohydrate diet, and Mediterranean diets, have been explored[82-84]. The efficacy of these interventions remains debated, and they are rarely used as standalone treatments[85].

The CD exclusion diet (CDED), characterized by low fat and animal protein and high carbohydrates and dietary fiber, has emerged as a promising alternative to EEN[86-88]. Clinical trials in pediatric CD patients have shown that CDED, combined with PEN, achieves remission rates comparable to EEN[89-91]. Recent studies indicate that CDED, alone or with PEN, effectively induces remission, reduces inflammation, and produces lasting changes in the gut microbiota[88,92]. Specifically, the combination of PEN and CDED reduces *Actinobacteria* and *Proteobacteria* while increasing beneficial *Clostridia* species after six weeks. Unlike EEN, which often sees microbiota reverting to baseline after remission, CDED-induced changes persist for 12 weeks post-therapy, suggesting a more sustainable approach to gut microbiota modulation and CD management[86]. It is noteworthy that the 2024 European Crohn's and Colitis Organization Guidelines on pharmacological treatment of CD have emphasized the importance of dietary therapy, offering a highly valuable option for personalized treatment of CD.

FMT

FMT involves the processing of stool from a healthy donor into a therapeutic formulation that is then administered to individuals with gut microbiota dysbiosis, with the aim of ameliorating the imbalance in the patient's intestinal microbiota. FMT has shown remarkable efficacy in treating refractory *Clostridioides difficile* infection[93-96], with a 90% success rate in the treatment of antibiotic recurrence cases. Given the critical impact of gut microbiota dysbiosis on the pathogenesis of CD, FMT is currently being explored as a therapeutic option for IBD.

A retrospective study integrated 11 high-quality CD reports involving a total of 106 CD patients and conducted a meta-analysis. The pooled clinical remission rate with a random effects model across these 11 reports was 0.62 (95% confidence interval: 0.48-0.81). Among them, seven studies reported a combined proportion of 0.79 for CD patients achieving clinical response after FMT treatment, with low heterogeneity (I^2 = 43%). Additionally, metagenomic analysis revealed that pre-FMT patients displayed reduced species diversity and significant disparities in microbiome composition when compared to their respective donors. Notably, following FMT, clinical responders, in contrast to non-responders, developed significantly elevated species diversity, more closely aligning with the donor's microbiome configuration[97]. Several additional meta-analyses also support this viewpoint. Gutin *et al*[98] reported that in FMT for CD patients, responders

had a more abundant presence of *Enterobacteriaceae* and *Bifidobacteriaceae* members, while non-responders had a higher relative abundance of *Lachnospiraceae* and *Ruminococcaceae* members[98]. Kao *et al*[99] reported that several groups of bacteria disappeared after FMT (*Enterococcus*, *Lactobacillus*, *Streptococcus*, *Burkholderia* group, and *Erysipelothrix* group), while the abundance of strains beneficial for CD (*Faecalibacterium* and *Roseburia*) increased. This indicates that the overall efficacy of FMT in CD patients is relatively good based on these studies. Additionally, the postoperative complications including abdominal pain, bloating, diarrhea and constipation typically following FMT are mild. And Gutin *et al*[98] and Sokol *et al*[100] further verified that the adverse reactions observed in patients were not associated with FMT. This validates the favorable safety characteristics of FMT as a treatment modality. Wu *et al*[26] have validated in mice models that FMT from healthy donors can improve CD, whereas FMT from CD patients exacerbates the condition. These findings strongly support the aforementioned viewpoint.

FUTURE PERSPECTIVES

Future research should focus on the extensive coverage of diverse populations across different regions, including germ-free animal models and genetically edited animal samples, while integrating cross-sectional and longitudinal cohort studies to ensure sample diversity and representativeness. The identification of key microbial strains and the development of related research methodologies should be expanded, emphasizing the application of metagenomic single nucleotide polymorphism/single-nucleotide variation analysis workflows, such as GT-Pro, and the utilization of AI algorithms to construct multidimensional diagnostic models linking species, genes, and single-nucleotide variations. The research should incorporate culture-based omics, bacterial gene editing technologies, and germ-free animal models to minimize and control the impact of confounding variables in experiments. By systematically summarizing research findings and validating causal relationships in animal models, further clinical trials can be conducted. Based on these results, treatment protocols for celiac disease should be optimized to achieve more precise early diagnosis, complemented by advanced imaging techniques to expedite disease assessment and promote the development of personalized therapeutic strategies. Microbiome-based therapeutic approaches, such as FMT and dietary interventions, have already been implemented in clinical settings and have shown promising therapeutic outcomes. These strategies aim to more effectively alleviate disease symptoms, reduce adverse events during treatment, and enhance the quality of life for patients.

CONCLUSION

CD, a complex chronic inflammatory bowel disorder, is characterized by its unique symptom of CrF. Dysbiosis of the gut microbiome exerts profound influence on the progression of the disease. Traditional therapies often exacerbate microbial imbalance and are accompanied by numerous adverse effects. Microbiome-based targeted therapies, with their unique efficacy and safety profiles, have paved a new avenue for personalized treatment of CD.

FOOTNOTES

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LETTER TO THE EDITOR

Human leukocyte antigen and donor-specific antibodies in liver transplantation

Qimudesiren, Sha-Na Chen, Li-Ren Qian

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Abstract

In this article, we comment on an article published in a recent issue of the *World Journal of Gastroenterology*. We specifically focus on the roles of human leukocyte antigen (HLA) and donor-specific antibodies (DSAs) in pediatric liver transplantation (LT), as well as the relationship between immune rejection after LT and DSA. Currently, LT remains the standard of care for pediatric patients with end-stage liver disease or severe acute liver failure. However, acute and chronic rejection continues to be a significant cause of graft dysfunction and loss. HLA mismatch significantly reduces graft survival and increases the risk of acute rejection. Among them, D→R one-way mismatch at three loci was significantly related to graft-versus-host disease incidence after LT. The adverse impact of HLA-DSAs on LT recipients is already established. Therefore, the evaluation of HLA and DSA is crucial in pediatric LT.

Key Words: Liver transplantation; Human leukocyte antigen; Donor-specific antibodies; *De novo* donor-specific antibody; Antibody-mediated rejection

Core Tip: Donor-specific antibodies (DSAs), particularly those against human leukocyte antigen (HLA) DQ loci, significantly affect rejection risk and graft survival in pediatric liver transplantation. The presence of DSAs is linked to increased rates of both acute and chronic rejection, which impacts long-term graft viability. These findings highlight the importance of improved HLA and DSA monitoring and management to improve long-term outcomes in pediatric liver transplant recipients.

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TO THE EDITOR

The human major histocompatibility complex is a family of genes that encode human leukocyte antigens (HLAs), vital for immunological surveillance of malignancies and resistance against external infections. HLA molecules are polymorphic antigens that constitute an immunodominant alloreactive trigger for the immune response, leading to rejection following transplantation[1]. In organ transplantation, HLA matching offers several advantages, such as improved graft function, fewer rejection events, extended graft life, and the potential for less immunosuppression. More frequent episodes of rejection accompanying mismatches necessitate heightened immunosuppression, raising the risk of infection and cancer [2]. Donor-specific antibody (DSA) has emerged as an essential biomarker of allograft injury[3]. These antibodies bind to donor HLA molecules, leading to graft damage. DSA can be categorized into preformed DSAs, which develop prior to transplantation due to previous exposure to non-self HLAs, and *de novo* DSA (dn-DSAs), which form post-transplantation response to the donor's HLA[4]. Preformed DSAs reflect alloimmune memory, while dn-DSAs indicate a primary immune response[5]. Studies have shown that preformed DSAs increase the risk of acute rejection and reduce graft survival, which is why they are frequently regarded as a transplant contraindication. Additionally, dn-DSA is linked to an increased risk of rejection, chronic or acute antibody-mediated rejection (AMR), and most likely mixed rejection[6]. According to a meta-analysis, there was acceptable heterogeneity and no discernible publication bias in the high correlation between dn-DSA and increased liver transplantation (LT) rejection risk. In the long-term follow-up, the rejection rates for dn-DSA-positive patients were almost seven times greater than those for dn-DSA-negative individuals. Additionally, dn-DSAs continued to significantly impact allograft outcomes in pediatric LT patients, increasing these patients by around ten times compared to patients with negative dn-DSA[7]. Moreover, DSA is associated with long-term allograft fibrosis after LT[6]. Therefore, progressive fibrosis after LT in pediatric recipients may reflect ongoing silent AMR. In particular, patients who do not take the medications as prescribed, have consistently low immunosuppression levels, or are candidates for immunosuppression withdrawal. Regular liver tests in the presence of progressive inflammation and fibrosis may suggest the need for protocol liver biopsies in addition to routine DSA and C4d analysis. The laxity of chronic AMR may obscure its actual effect on allograft health despite acutely increased prominence of AMR due to its evident and abrupt path to allograft failure. Chronic AMR fibrosis can lead to allograft cirrhosis, and many of these individuals may eventually develop concurrent chronic ductopenic rejection. The possibility of fibrosis reversibility with an increase in immunosuppression is unknown[8].

HLA and DSA in LT

As part of the immune recognition process, which is essential to the adaptive immune response, HLAs, which are cell surface proteins, present peptides to T cells, with thousands of alleles identified for the HLA loci, the HLA genes are the most polymorphic in the human genome[9]. HLA mismatches play a significant role in LT. This retrospective observational analysis included 22702 liver transplant recipients from the large-scale multi-center database UNOS/OPTN. Patients were divided into two groups according to the number of HLA mismatches (0–3 *vs* 4–6 mismatches), and the results were further subdivided by donor status and indication. The degree of HLA mismatch was linked to both the risk of acute rejection and allograft survival, and this relationship remained in patients who received transplants for congenital indications, metabolic disorders, hepatitis, and medications[10]. These findings underscore the importance of HLA matching in LT, suggesting that minimizing mismatches could improve graft longevity and reduce rejection risks across diverse patient groups.

Sensitization, the presence of anti-HLA antibodies, develops after exposure to non-self HLA through pregnancy, blood transfusion, or a previous organ transplant. Anti-HLA antibodies against the allograft can bring on both early AMR, which can occur days to weeks after transplantation, and hyperacute rejection, which can occur within minutes to hours of reperfusion. Allograft malfunction and failure may result from these types of rejection[9]. For a long time, the liver was thought to be less vulnerable to AMR than other organs. However, research has shown that anti-HLA antibodies, particularly DSAs, are essential for graft survival during pediatric LT[11]. Children are more likely than adults to experience DSAs following LT. DSAs target HLA class II molecules more frequently, primarily DQ. Such anti-class II DSAs (DQ/DR), particularly those of the complement-binding IgG3 subclass, may be linked to fibrosis, inflammation, endothelial damage, and T-cell-mediated rejection[12]. In the study by Melere *et al*[13], 67 transplanted youngsters were examined; 61 received grafts from living donors, and 85% were related to the recipients. Of these patients, 28.3% had pre-transplant

DSA (class I or II) and 48.4% had dn-DSA. Patients with and without dn-DSA anti-DQ had rejection-free survival rates of 76% *vs* 100% and 58% *vs* 95% at 12 and 24 months, respectively. Consequently, the results emphasize the importance of including DSA evaluation in protocols for pediatric LT recipients both before and after transplantation. Based on this analysis in pediatric LT recipients, future implications may include immunosuppression minimization strategies. Liu *et al* [14] showed that DSAs against HLA class II antigens accounted for 90% of all DSAs and were more prevalent than those against class I antigens in a retrospective cohort analysis including 48 juvenile liver transplant recipients. The HLA-DQ site dominates the HLA-II antigen. In the study by Miyagawa-Hayashino *et al*[15] 79 pediatric patients with satisfactory graft function who had a protocol liver biopsy performed more than five years following transplantation (median = 11 years, range = 5-20 years) were the subjects of a retrospective analysis. The findings demonstrated that 67 patients had DSAs: 32 patients (48%) had DSAs identified; these were often against HLA class II (30 cases), but were infrequently against class I (2 cases). Bridging fibrosis or cirrhosis was more common in these individuals (28/32 or 88%) than in DSA-negative patients (6/35 or 17%, $P < 0.001$). The study demonstrated that the presence of DSAs in pediatric patients more than 5 years post-transplant is associated with a higher incidence of fibrosis or cirrhosis, suggesting that DSAs may serve as an essential marker for long-term transplant complications.

Improvements in post-transplant survival can essentially be due to more targeted and less toxic immunosuppressive regimens. Liver grafts have been observed to stimulate less rejection compared with other organs. By causing peripheral microchimerism and a degree of tolerance, they may offer protection for other organs transplanted simultaneously. However, immunosuppression is still a barrier to long-term graft survival. Current regimens control various pathways, from calcineurin and mTOR inhibitors to antimetabolites and several antibody treatments, providing a chance to adapt each recipient's regimen. Targeting distinct immune system pathways also reduces dosage and drug toxicity without raising the risk of neoplasms, opportunistic infections, or rejection[16]. Vionnet *et al*[17] conducted a cross-sectional multicenter study of 190 adult LT recipients. The findings demonstrated the usefulness of liver stiffness measurement, class II DSAs, and alanine aminotransferase in noninvasively identifying stable LT recipients who may benefit from immunosuppressive minimization but do not have considerable underlying alloimmunity[18]. These tools can help identify stable patients who may not require intense immunosuppression; however, further research is needed to assess their applicability across different populations. Additionally, strict adherence to the treatment plan, lifelong immunosuppressive medication use, and vigilant medical monitoring are critical to long-term results following LT. In children who undergo LT, nonadherence is the most frequent cause of late acute rejection, with an estimated 35% to 50% of adolescents experiencing nonadherence[19].

CONCLUSION

Although AMR after LT has received increasing attention, there is still little information on LT consequences in pediatric patients. Nevertheless, the role of HLA mismatches and DSAs in pediatric LT has been identified as a critical determinant of graft survival and immune rejection. Current studies demonstrate the significant impact of HLA mismatches on acute and chronic rejection, with a strong association between DSAs, particularly those targeting HLA class II, and long-term graft outcomes. While advancements in immunosuppressive regimens have improved post-transplant survival, the presence of DSAs, especially in pediatric populations, continues to pose risks for graft dysfunction and fibrosis. Pre- and post-transplant DSA monitoring is essential for identifying high-risk patients and optimizing immunosuppressive strategies. Future research should prioritize standardizing DSA detection protocols and optimizing immunosuppressive strategies to improve graft survival. Additionally, developing long-term follow-up and management plans, particularly addressing the unique needs of pediatric patients, is critical to ensuring the ongoing health and functionality of the transplanted organ. Despite advances in understanding the impact of DSAs on pediatric LT, continued research is crucial to ensure the long-term success of these procedures.

FOOTNOTES

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