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REVIEW

Crohn's disease: Why the ileum?

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Abstract

Crohn's disease (CD) is an inflammatory bowel disease characterized by immune-mediated flares affecting any region of the intestine alternating with remission periods. In CD, the ileum is frequently affected and about one third of patients presents with a pure ileal type. Moreover, the ileal type of CD presents epidemiological specificities like a younger age at onset and often a strong link with smoking and genetic susceptibility genes. Most of these genes are associated with Paneth cell dysfunction, a cell type found in the intestinal crypts of the ileum. Besides, a Western-type diet is associated in epidemiological studies with CD onset and increasing evidence shows that diet can modulate the composition of bile acids and gut microbiota, which in turn modulates the susceptibility of the ileum to inflammation. Thus, the interplay between environmental factors and the histological and anatomical features of the ileum is thought to explain the specific transcriptome profile observed in CD ileitis. Indeed, both immune response and cellular healing processes harbour differences between ileal and non-ileal CD. Taken together, these findings advocate for a dedicated therapeutic approach to managing ileal CD. Currently, interventional pharmacological studies have failed to clearly demonstrate distinct response profiles according to disease site. However, the high rate of stricturing disease in ileal CD requires the identification of new therapeutic targets to significantly change the natural history of this debilitating disease.

Key Words: Ileum; Crohn's disease; Bile acids; Paneth cells; Diet; Genetics; Strictures

Core Tip: The ileum is most frequently affected by Crohn's disease (CD). Ileal CD differs from other CD types in its epidemiology and natural history. Anatomical and histological features of the ileum provide the keys to understanding this distinct phenotype. Moreover, we discuss herein the crosstalk that occurs in the ileum between an individual and her/his environment and the clinical significance.

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INTRODUCTION

Crohn's disease (CD) is an inflammatory bowel disease (IBD) characterized by repetitive inflammatory flares, and often chronicity. Unlike ulcerative colitis (UC), the other main subtype of IBD, CD can affect any part of the digestive tract. The site of the disease is a critical biological aspect of CD whereas inflammatory, stricturing or penetrating behaviour is thought to be a reflection of disease progression[1].

Data on the epidemiology of IBD are provided by population-based studies showing an increasing incidence and prevalence of IBD in the West over the last 50 years[2,3]. In a systematic review pooling all epidemiological studies on IBD worldwide since 1990, the global prevalence of IBD is higher in Western countries (322 per 100000 in Germany) than in newly industrialized countries[4]. In newly industrialized countries like Asia and the Middle East, epidemiological studies report a rising incidence of CD[4,5]. The Montreal classification distinguishes CD involving the ileum, the colon and both the colon and the ileum[6]. About one third of patients with CD presents a disease involvement limited to the ileum and this proportion does not vary between 'Western' and newly industrialized countries[3,5,7]. Once the diagnosis of ileal CD is made, less than one fifth of patients will present colonic lesions over time[3]. In addition, ileal CD occurs in younger patients than colonic CD[1]. These epidemiological observations have led some experts to plead for personalized approaches to therapy based on the disease site.

Even though CD pathogenesis remains elusive, current consensus considers CD a result of genetic, immunological and environmental factors[2]. Of relevance, the ileum is the site for the crosstalk of these multiple etiological factors in CD. In this review, we will depict the different physiopathological aspects of ileal CD and their clinical impact (Figure 1).

GENETIC SUSCEPTIBILITY

Genetic factors are involved in IBD physiopathology and genome-wide association studies have linked several genes with the site of the disease. In a large epidemiological study performed in more than 34000 patients with IBD across Europe, North America and Australia, susceptibility genes were determinants of the site of the disease whereas inherited genes showed a loose link with the inflammatory, penetrating or stricturing behaviour of the disease[1]. The genetic variants presented herein sum up the current state-of-the-art but new techniques such as genomic DNA are likely to provide new insights in the next few years.

NOD2

NOD2 is a sensor of the innate immune system, able to detect bacterial fragments, specifically muramyl dipeptide[8]. During *in vitro* differentiation of intestinal epithelial cells into Paneth cells, NOD2 signalling can modulate the expression of enteric antimicrobial peptides[9]. Even though intracellular pattern recognition receptor gene *NOD2* is widely associated with CD risk[2], mutations in the *NOD2* gene are strongly associated with ileal CD and are correlated with a younger age at diagnosis[1,10,11]. The specific association between *NOD2* mutations and ileal CD is partially explained by its distribution along the gastrointestinal tract. Histologically, *NOD2* is overexpressed in ileal crypts compared to colonic crypts[12].

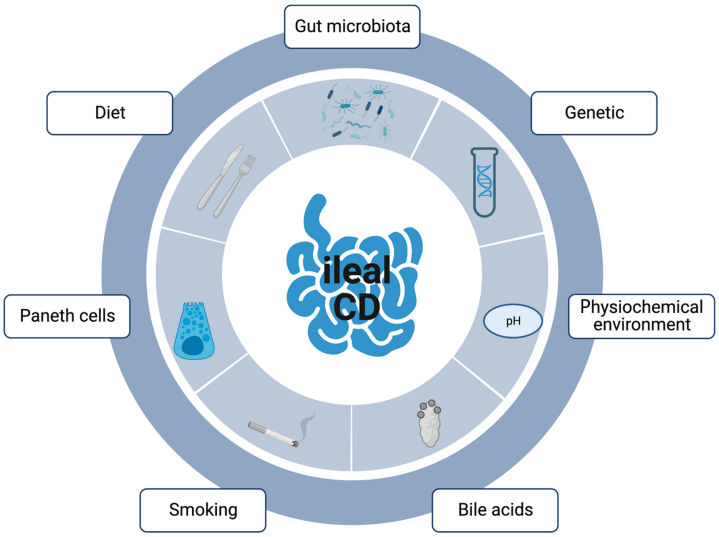

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Figure 1 Potential factors involved in ileal Crohn's disease. While Crohn's disease (CD) occurs in any part of the gastrointestinal tract, the ileum is frequently involved. Potential factors involved in ileal CD are genetic susceptibility genes and most of these genes are associated with Paneth cell dysfunction. Environmental factors may also be involved such as diet, gut microbiota and smoking. Diet can modulate the composition of bile acids and gut microbiota, which in turn affect the susceptibility of the ileum to inflammation. Created with BioRender.com. CD: Crohn's disease.

LRRK2

Like *NOD2*, the *LRRK2* gene is expressed in Paneth cells. *LRRK2* gene is implicated in vesicular trafficking, cytoskeleton homeostasis and consequently in inflammation and immune response[13]. More specifically, LRRK2 is overexpressed in Paneth cells and its deficiency causes deprivation of lysozyme in Paneth cells[14]. In a case-control study, mutations of *LRRK2* were associated with ileitis and an early onset of CD[15].

Major histocompatibility complex

The expression of other genes involved in immune response is likewise implicated in ileal CD. The major histocompatibility complex is involved in the presentation of antigen in a large variety of cell types including T-cells. In a recent meta-analysis, several mutations of these genes were associated with CD especially in the Korean population but also in the European population[16]. Of note, single nucleotide polymorphisms of these genes were more common in patients with ileal CD compared to patients with colonic CD[1].

ATG16L1

Among susceptibility genes identified in CD, the allele ATG16L1^{T300A} was associated with impaired autophagy[17,18]. The expression of *ATG16L1* was decreased in CD patients[19]. In 9-10-month-old mice, the specific deletion of *Atg16L1* allele in intestinal epithelium cells (IEC) led to a transmural CD-like ileitis associated with endoplasmic reticulum stress[20]. Interestingly, *NOD2* mutations are also associated with autophagy defects.

Tcf-4

In IBD patients, the reduction of the Wnt-signalling pathway transcription factor Tcf-4 was associated with a predisposition for ileal CD[21,22]. A reduced expression of Tcf-4 resulted in a reduced expression of Paneth cell defensins[21]. In a murine Tcf-4 knockout model, a decreased expression of alpha-defensin levels and a reduced capacity of bacterial killing were observed[21].

KCNN4

Finally, in the array of genes implicated in the immune response mediated by Paneth cells, the conductance calcium-activated potassium channel protein (KCNN4) is part of the potassium pump in the human intestine[23]. The blocking of this calcium-activated potassium channel protein reduced mouse Paneth cell secretion in response to bacterial stimulation[24]. In human, mutation of the *KCNN4* gene was associated with ileal CD in the Australian and New Zealand population[25].

In the susceptibility genes mentioned above, most genes are associated with Paneth cell dysfunction as illustrated in Figure 2. We will detail below the potential involvement of Paneth cells in ileal CD.

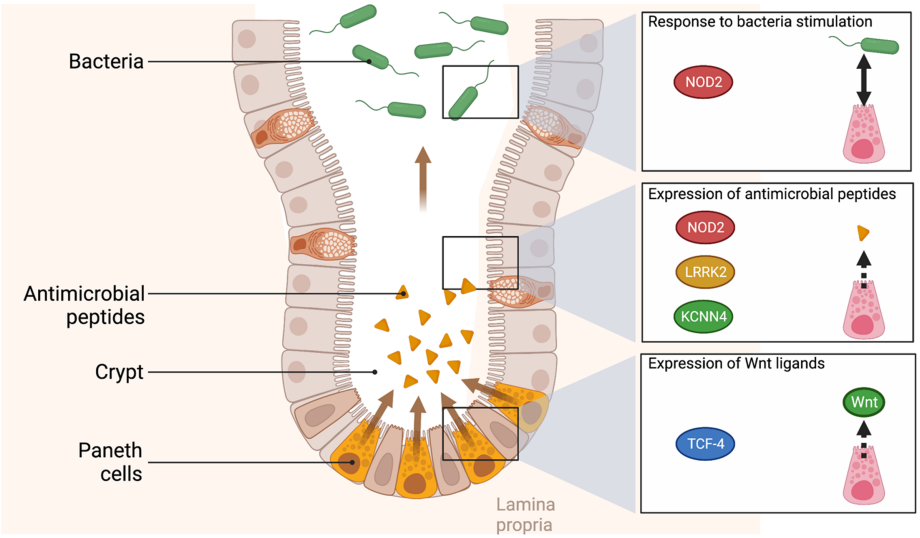

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Figure 2 Putative role of gene susceptibility in Paneth cell dysfunction. Paneth cells are secretory epithelial cells located in the intestinal crypts. Paneth cells produce antimicrobial peptides in response to bacterial components and support stem cell function through Wnt signalling. Most of the susceptibility genes associated with ileal Crohn's disease (CD) involve Paneth cell dysfunction. NOD2 and *LRRK2* genes are expressed in Paneth cells and their deficiency in ileal CD modulates the expression of antimicrobial peptides such as alpha-defensins or lysozyme. Similarly, the reduction of the Wnt-signalling pathway transcription factor Tcf-4 is associated with a predisposition for ileal CD and leads to a reduced expression of Paneth cell defensins. The blocking of calcium-activated potassium channel protein (KCNN4) inhibits mouse Paneth cell secretion in response to bacterial stimulation. Created with BioRender.com.

THE ILEUM, A SPECIFIC PART OF THE GASTROINTESTINAL TRACT

Beyond genetic factors, the histological and anatomical features of the ileum itself may partially explain the propensity of this specific part of the gastrointestinal tract to be affected by CD.

Physicochemical environment

The ileum presents a unique chemical microenvironment. The intraluminal pH in the ileum is 7.4, the highest of the human digestive tract as a result of small bowel mucosal bicarbonate secretion[26]. Comparatively, the intraluminal pH in the caecum is lower, 6.5, due to the bacterial production of short fatty acids by colonic bacteria[26,27]. The functional characteristics of the digestive tract microbiota are modulated by pH level. In the environment of the ileum (pH = 7.4), short chain fatty acids increase the growth and the motility of pathobionts whereas, in the colonic environment (pH = 6.5), short chain fatty acids downregulate the virulence of gene expression of these strains[28].

Histological changes in ileal CD

From a clinical point of view, most of histological features encountered in ileal CD were also found in other diseases as backwash ileitis in UC for instance[29]. Although epithelioid granuloma is considered as the histological hallmark for the diagnosis of ileal CD, it is not a mandatory prerequisite[30]. In about one quarter of patients with ileal CD, pyloric gland metaplasia resulting from the expression of mucin genes normally specific to the stomach (*MUC5AC* and *MUC6*) were noted[30,31]. Although numerous other histological features are described in ileal CD, focal crypt irregularities are considered by expert consensus as one of the most reliable signs of CD[30].

From a biological point of view, the most noteworthy change is found in Peyer's patches. Peyer's patches are ileal immune structures characterized by a B-cell germinal centre surrounded by a T-cell interfollicular region. These mucosal-associated lymphoid tissues can act as "gateways" of the intestine. The epithelium and the underlying lymphoid follicle differ from the surrounding villus epithelium of the ileum. Indeed, the function of this follicle-associated epithelium consists in sampling and transporting luminal antigens through M cells and dendritic cells to CD^{4+} cells[32]. Early histological changes in Peyer's patches were reported in ileal CD such as an increase in mast cells or erosive epithelial lesions[33,34]. Further, the increased number of glial cells in the Peyer's patches of patients with ileal CD resulted in an enhanced intestinal permeability[34]. Together these phenomena may explain the increased vulnerability of the ileal mucosa to bacterial invasion in CD patients[35].

Enteric nervous system

As mentioned in the previous section, the histological changes observed in ileal CD include glial cells and the enteric nervous system (ENS). In patients with ileal CD, both the submucous and the myenteric plexus present an overall increase in the number of neuronal cell bodies, enteroglia and interstitial cells

of Cajal associated with an upregulation of apoptosis in enteric neurons and enteric glial cells[36,37]. To that extent, although functional evidence is lacking in the literature to fully support this hypothesis, the increased transit time observed in CD patients could be seen as a consequence of ultrastructural injury to interstitial cells of Cajal in the myenteric plexus[38,39].

Beyond the role of the ENS in intestinal mobility, the density of enteric glial cells conveys a higher risk of ileal CD recurrence after surgery. Thus, after ileocolonic resection for CD, inflammation in or around nerve bundles or enteric ganglia was reported in several clinical studies as a risk factor for CD recurrence[40-43]. In the uninflamed section from ileocolonic samples, the number of S100-positive enteric glial cells was enhanced in patients with relapsing disease unlike vasoactive intestinal polypeptide or substance P positive cells[44]. Furthermore, the ileum of CD patients harbours a different distribution of enteric glial cells with a higher density of these cells around Peyer's patches. In parallel, the mediators of enteric glial cell increased the permeability of the ileal mucosa in CD patients whereas they decreased the permeability of the mucosa in non-IBD patients[34]. The importance of these findings on the natural history of CD remains to be determined. In particular, the effect of the modulation of the ENS in neuro-immune interplay needs to be investigated.

Paneth cells

Paneth cells are mostly located in the ileum and nearly absent from the colon[45]. This cellular type is found between intestinal stem cells in the small intestinal crypts. Paneth cells produce not only antimicrobial peptides that regulate host-microbe interplay but also factors such as Wnt ligands modulating the activity of intestinal stem cells. Many of the ileal CD-associated mutations discussed before involve cellular pathways of Paneth cells.

Paneth cells are rich in mitochondria to sustain their energy-expending secretory functions. In *SAMP1* mice, mice genetically predisposed to CD-like ileitis, the number of Paneth cells was decreased and abnormal Paneth cells were associated with disease progression[46,47]. Likewise, the number of Paneth cells is decreased in the small intestine of CD patients. This observation was made in several ethnic populations and particularly in paediatric cohorts[19,48,49]. Mucosal biopsies from adult CD showed ultrastructural abnormalities in mitochondria, especially in CD patients with inflammation (73.3%) but also in inactive CD patients (20.3%) which was not the case for goblet cells and enterocytes[50]. In patients with ileal CD, the count of abnormal Paneth cells correlated with disease activity and was predictive of recurrence after surgery[51]. Some authors hypothesised that this dysfunction in Paneth cells could result from mitochondrial impairment[50-52]. Besides, in mice, Paneth cell defects triggered by loss of prohibitin 1, a major component protein of the inner mitochondrial membrane implicated in cell respiration, caused ileitis[52]. This effect is mediated by oxidative stress. Furthermore, the use of a specific mitochondrial-targeted antioxidant (Mito-Tempo) ameliorates ileitis in mice. The use of the same mitochondrial-targeted antioxidant (Mito-Tempo) in human ileal biopsies of CD patients normalized the expression of 25% of altered CD genes, including genes implicated in antigen processing, lipid metabolism, apoptosis, and interleukin (IL)-17/IL-23 signalling[50].

Immune response

As highlighted by research in immunological processes, Paneth cells are part of the crosstalk with the immune system to maintain intestinal microbial homeostasis and intestinal barrier[53,54]. When biopsies from CD patients were compared according to the site of inflammation in the gastrointestinal tract, differences in neutrophil activities were observed. For instance, matrix metalloproteinase-9 and myeloperoxidase were relatively less increased in ulcer edges of ileal CD compared to colonic CD, suggesting less neutrophilic degranulation in ileal CD[55].

Likewise, innate lymphoid cells (ILCs) are gaining interest as components of the immune system. Three groups have been individualized according to their properties: ILC1, ILC2 and ILC3. A switch was noted in the inflamed ileal mucosa of CD patients, from an ILC3 phenotype limiting commensal bacteria specific $CD4^+$ T cell response to an ILC1 phenotype associated with interferon (IFN)-γ production[56]. The aryl hydrocarbon receptor (AhR), a ligand-dependent transcription factor, was involved in this process and downregulated in inflamed mucosa of IBD patients[57,58]. Pharmacological or genetic activation of the AhR enhanced ILC3 maintenance conferring a protection against pathogenic bacteria in mice and downregulated ILC2 maintenance implicated in immune response in worm[59]. AhR agonists arise from the environment, commensal flora and tryptophan metabolism[60]. In patients with ileal CD, impaired tryptophan metabolism was observed with decreased levels of kynurenine and expression of kynureninase[61].

Concordantly with a typical cellular immune response, ileal CD instigates a specific cytokine profile. Interferon lambda (IFNL) is secreted in response to microbial stimulation or to T-cell-mediated mucosal inflammation. IFNL was upregulated in the ileal mucosa of patients with CD and triggered ileitis in a murine model and induced Paneth cell depletion independently of tumour necrosis factor (TNF)[62]. The effect of IFNL in the ileum is mediated by the JAK-STAT pathway which represents a promising therapeutic target already being used in the clinic[62].

Besides the role played by IFNL, IL-22 contributes to the pathogenesis of ileal CD. IL-22 is produced by T-cells and ICL3 during IBD flares[63,64]. IL-22 is thought to assume an immunoregulative role as its inhibition by the IL-22 binding protein induced a severe inflammation in a rodent colitis model[64]. In

the ileum, interestingly, the levels of IL-22 binding protein were specifically high in comparison with the colon[65]. This heterogenous distribution along the gastrointestinal tract may result from the infiltration of eosinophils in the ileum and the production of IL-22 binding protein[64,65].

The ileum as an endocrine organ

If a high proportion of eosinophil cells was present in the ileum, an enhanced enteroendocrine cell activity was also found[66]. Additionally, terminal ileal chromogranin A cells and glucagon-like peptide 1 (GLP-1) positive L-cells were increased in ileal CD specifically[66]. However, studies on the serum levels of GLP-1 are rare and include small cohorts (< 20 patients). Data were inconclusive and showed similar serum levels of GLP-1 between patients with ileocolic CD and colonic CD but a higher serum level of GLP-1 in IBD compared to healthy controls[67,68]. Thus, no firm conclusion can be drawn regarding the nutritional and immune impact of this discovery.

Besides harbouring enteroendocrine cells, the ileum harbours oestrogen receptor subtype β, whether the ileum is affected by CD or not. The expression of subtype β was associated with a milder disease course in a cohort of 37 patients with ileal CD. Accordingly, the inflammation score was inversely correlated with the expression of oestrogen receptor β. Similarly, a higher expression of oestrogen receptor β was found in patients with non-stricturing non-penetrating disease[69]. In a chemical colitis model in rats, the activation of oestrogen receptor b reduced inflammation score as well as inflammatory pain and inhibited the ionotropic P2X3 receptor[70]. Notwithstanding this observation, female sex was not independently associated with ileal CD[1,71].

Myofibroblasts

While ileitis is often studied from an inflammation perspective, little is published about cell repair. The gene encoding tumour progression locus-2 kinase, a proinflammatory enzyme, was associated in a genetically invalidated murine model with a surprising homeostatic role, modulating the effect of chemically induced colitis. Mechanistically, this gene does not directly impact the immune response but plays a critical role in intestinal myofibroblasts which contribute to the healing of intestinal epithelium. In response to inflammatory signals from the microenvironment, intestinal myofibroblasts trigger compensatory epithelial proliferation in the intestinal crypts[72].

Transcriptome studies

The integration of the different pathways mentioned above in the pathogenesis of ileal CD is facilitated by multi-omic studies. Thus, differences in pathogenesis between the ileum and the colon are highlighted by these techniques. For instance, in CD patients, the metabolomic study of non-inflamed ileum and non-inflamed colon biopsies distinguish clearly different profiles. It is noteworthy that these differences were blurred in inflamed ileum and colon samples[73].

Although the physiopathology of ileal CD has been studied as a unique phenomenon, emerging evidence advocates for a more heterogeneous process in which physiopathological pathways differ from one patient to the other. Thus, transcriptomic data analysis from CD ileal tissue sample was able to identify subgroups of patients with distinct recurrence rates after surgery[74].

In a recent transcriptomic study of ileal mucosa samples, inflammatory genes (IL-6, IL-8, IL-1β) were upregulated whereas metabolic process genes were downregulated in ileal samples from CD patients compared to controls. Early post-operative recurrence of CD was associated with an overexpression of TNF-α, IFN-γ, IL-23A and IL-17A upregulation. In addition, using a regression model to predict post-operative recurrence of CD, mitochondrial dysfunction and JAK/STAT upregulation in the ileum were independently associated with post-operative recurrence[75]. Transcriptomic studies also provide new insights into cell population specificities in ileal CD. When mapping the cell type of the ileal mucosa of CD patients, tuft and $BEST^{4+}$ cells were the cell types associated with CD, irrespectively of the treatment status[76]. Intestinal tuft cells are a rare cell type implicated in the defence against helminthic and protozoan infections[77]. $BEST^{4+}$ cells represent about 1% of ileal epithelial cells and plausibly contribute to the mucus secretion of goblet cells[78]. Surprisingly in this ileal cell type study, immune compartment was slightly affected by CD despite the fact that treatment status in CD patients modified the epithelial and immune compartment[76].

Among the differentially expressed genes in ileal inflammatory response, activating transcription factor 4 (ATF4) is a transcription factor widely expressed in the human body, including the ileum. ATF4 downregulation in intestinal epithelial cells has been observed in active CD in patients and causes spontaneous enterocolitis in mice while altering ileal Paneth cell function. Furthermore, murine ATF4 deletion impaired the glutamine uptake of the intestinal epithelium and concordantly glutamine supplementation restored Paneth cell function and decreased intestinal inflammation[79]. A few years earlier, the inhibition of the ATF4 pathway demonstrated an altered autophagy in human intestinal epithelium in the presence of adherent-invasive Escherichia coli (AIEC). This pathological response resulted in the intracellular bacterial replication of AIEC and consequently in pro-inflammatory patterns[80].

BILE ACIDS

The role of bile acids is largely reported in liver disease in which bile acids are inflammatory cues and treatment targets[81]. The involvement of the gut in the pathogenesis of inflammatory liver diseases and similarities in etiological factors has led to consideration of the role of bile acids in IBD[82].

Primary bile acids are produced in the liver and secreted through the biliary tree into the gastrointestinal tract. Most primary bile acids are reabsorbed by the ileum and hence recycled several times. A minority of primary bile acids are transformed into secondary bile acids by a narrow range of gut bacteria. The pool of bile acids influenced the composition of gut microbiota which in turn modulated the composition of the bile acid pool[83]. Thus, impaired bile acid pools in relation to impaired microbiota enzymatic activities have been described as contributing to the inflammatory loop of IBD[84]. Bile acid composition in the lumen of the ileum differs between CD and non-CD patients with a relative decrease of primary bile acids in the ileum of CD patients[85]. Nevertheless, this finding should be interpreted with caution as malabsorption of bile acids was documented as a consequence of ileal CD especially in patients with a history of ileal resection[86,87]. Moreover, in a cohort of 166 patients, enhanced primary biliary level in the stool was independently associated with ileitis[88]. In a multi-omics approach based on a stool collection of 200 IBD patients, ileal CD profile was characterized by increased primary and secondary bile acid levels and shifts in taxa in favour of bacteria associated with bile acid-rich environments (*Gammaproteobacteria* and *Blautia sp*.)[89]. Further, the level of secondary bile acids in patients with inflammation limited to the ileum tended to increase after biological treatment reaching a similar level with control subjects[90].

In addition, bile acids exert inflammatory modulating properties through the stimulation of farnesoid X receptor (FXR). In a murine model of chemically induced inflammation, the activation of FXR demonstrated anti-inflammatory effects through a reduction of epithelial hyperpermeability and of proinflammatory cytokine production[91]. Furthermore, the obstruction of bile flow in mice induced mucosal ileal injuries reversed by administration of bile acids. In detail, this result may be explained by the activation of FXR by bile acids which promoted enteroprotective genes and limited ileal bacterial overgrowth[92]. Furthermore, bile acid pool modulation directly affects the ileum and Paneth cells. According to a recent article, Paneth cell number was linked to diet and to microbiota by bile acid in obese CD and non-CD patients irrespectively of other risk alleles (ATG16L1 and NOD2)[93]. In fact, in mice fed with a high fat diet, a similar phenomenon was observed and notably, high fat diet alone in germ-free mice as well as microbiome transfer alone in mice fed with standard diet were unable to induce an alteration in Paneth cells. The reason for this phenomenon was also dependent on FXR activation by the bile acid pool. Thus, the conjunction of a high fat diet and *Clostridium*-mediated production of secondary bile acids may explain the role of both diet and microbiome in this mechanism [93].

Lastly, bile acids promoted the expression of long polar fimbriae favouring the interplay of these strains with Peyer's patches and bacterial translocations[94]. For example, primary bile acid level was inversely correlated with the abundance of *Faecalibacterium prausnitzii* (*F. prausnitzii*) and its acetate and L-methionine producing enzyme[88]. In patients treated by surgery for ileal CD, bile acid metabolism specificities were associated with an ileal recurrence of CD[89].

Nevertheless, there is only limited evidence on the therapeutic role of bile acids in ileal CD. While several authors reported the protective role of ursodeoxycholic acid and of its precursor, the lithocholic acid, against chemically induced colitis in mice[95-97], there is a lack of data about the effect of oral supplementation with ursodeoxycholic acid in patients with IBD. Thus, only one small single-centre trial examined the effect of ursodeoxycholic acid in UC and none was performed in patients with CD [98].

MICROBIOTA

An increasing number of authors have investigated the link between gut microbiota and CD. However, many of these studies focused on the analysis of DNA extracted from stool. This methodology shed light on the colonic microbiota of the colonic lumen but was unable to draw any conclusions on the microbiota associated specifically with the ileum. Moreover, in a systematic review, the study mucosa-associated microbiota was regarded as more relevant in the understanding of CD pathogenesis[99]. In addition, dysbiosis was described in some mice models as a by-stander of ileitis. For example, in genetically predisposed mice Atg16L1$^{\Delta IEC}$, dysbiosis was observed but litter cross-fostering predisposed to colitis but not ileitis[20].

Host/microbiota interplay

Microbiota homeostasis plays a critical role in CD physiopathology[2]. Thus, mucosal immunity regulator molecules such as vitamin D receptors (VDR) have been associated with a susceptibility to bacterial and chemical colitis. The genetic inhibition of VDR in the Paneth cells of VDR$^{\Delta PC}$ mice resulted in a lower expression of lysozymes in Paneth cells[19]. Inhibition of VDR influences the response to

pathogenic bacteria. VDR$^{\Delta IPC}$ mice are not only more sensitive to bacterial infection but also to chemical damage. Conversely, this susceptibility in VDR$^{\Delta IPC}$ mice was reduced in case of co-housing with non-VDR$^{\Delta IPC}$ mice, indirectly suggesting a protective role of the microbiome[19]. In SAMP1/YitFcsJ (SAMP1) mice which develop spontaneous terminal ileitis, dysbiosis occurred during disease progression with a decrease in *Lachnospiraceae* and in *Bacteroides*. In the same animal model, α-defensins misfolding was associated with dysbiosis and even induced dysbiosis in wild-type mice[47].

In this regard, the barrier function of the ileum is also a major feature in the understanding of host-microbiota interplay. Accordingly, human β-defensin 3 peptide was decreased and redistributed to the basolateral surface of the ileal epithelium[100]. In parallel, increased enzyme indoleamine 2,3-dioxygenase 1 (IDO1) was found in patients with active CD. This enzyme is the first enzyme in tryptophan metabolism on the kynurenine pathway and is responsible for mucus layer thickening and mucus-associated modulation of microbiota. In a murine enterocolitis model, IDO1 upregulation reduced the abundance of enteropathogenic *E. coli* in the ileum. Likewise, IDO1 downregulated inflammation in response to chemical colitis in mice and augmented *A. muciniphila* and *M. schaedleri* abundance[101].

AIEC

In the nineties, a French team identified a strain of *Escherichia coli*, AIEC, which was adherent to the ileum without harbouring virulence factor-encoding genes[102]. AIEC was shown to be associated with ileal CD in further studies and to be able to invade epithelial cells[102,103]. Interestingly, AIEC was able to induce granulomas *in vitro*, which is one of the main histologic features of CD[2,104]. AIEC is classically identified on ileal biopsy but a dedicated serology could also be informative and less invasive [105]. The invasive property of AIEC is favoured by the overexpression of the glycoprotein *CEACAM6* in the ileal epithelium. The interaction of this glycoprotein with the bacterial adhesive factor FimH promoted AIEC-enterocyte interplay in the ileum[106]. Using this receptor, AIEC modulated the metabolism of the ileal epithelium and induced strong gut inflammation[107]. AIEC induced the expression of hypoxia inducible factor, overexpressed in the ileum of CD patients and promoted barrier defects in the intestinal epithelium paving the way for the onset of inflammation[108].

The relapse of CD in patients after surgical treatment may be used to understand the early steps in CD onset. In a recent prospective, multicentric cohort of patients with ileal resection, AIEC in the remaining ileum was associated with an early ileal lesion of CD recurrence. The presence of AIEC within the surgical ileal specimen was predictive of the endoscopic recurrence of CD[109].

As highlighted in a recently published article, factors influencing ileal susceptibility to AIEC were numerous and among them epigenetic regulators had a modulating effect on a large range of proteins [110]. For this reason, before considering a clinical application in ileal CD, further studies are needed to prevent possible adverse effects induced by the modulation of these epigenetic targets or to identify more specific genes associated with AIEC colonisation. Another promising approach would be the use of bacteriophages to target specifically AIEC in ileal disease[111]. To date, no bacteriophage has been approved for intestinal therapeutic use in human, either in the European Union or in the United States, despite numerous studies that have reported encouraging results *in vivo*. Therefore, data about the effect of bacteriophages on human microbiota are needed in the future[111].

F. prausnitzii

In a stool based multi-omics analysis of 200 IBD patients, the abundance of a member of Firmicutes, *F. prausnitzii,* was highly discriminant of ileal CD compared to colonic CD[89]. Even in non-inflamed ileal samples of CD patients, the commensal bacterium *F. prausnitzii* was decreased in comparison with healthy controls which suggests a relevant causal link between this bacteria and CD onset[112]. Accordingly, a low level of *F. prausnitzii* in the ileal mucosa-associated microbiota was associated with a higher recurrence rate of CD after ileal resection, corroborating the putative role of *F. prausnitzii* in the pathogenesis of ileal CD[113]. In addition, a recent study confirmed the role of *F. prausnitzii* in ileitis after ileocolectomy, pointing to a possible association with a bile salt profile. Thus, elevated levels of primary bile acids were associated with a decreased abundance of *F. prausnitzii*. These two parameters were the only factors associated with ileitis in this cohort of 166 patients[88]. The administration of *F. prausnitzii* or of its supernatant *in vitro* and *in vivo* counterbalanced gut inflammation by blocking nuclear factor kB activation and IL-8 production[113]. Interestingly, in CD patients, *F. prausnitzii* was associated specifically with ileitis, irrespectively of their genetic background[89,114]. In an original work conducted in twins in whom biopsies were performed in the lower gastrointestinal tract, the abundance of *F. prausnitzii* was specifically decreased in ileal CD, compared to colonic CD or in healthy twins[114].

Mycobiota

The study of microbiota in IBD is not limited to bacteria but also includes fungi. Fungal microbiota was suspected to be strongly involved in CD pathogenesis in particular because of the diagnosis value of anti-Saccharomyces cerevisiae antibodies in CD[2]. In a study of 168 ileal biopsies, mycobiota was modified in CD patients with an increased abundance of *Malassezia* and a decreased abundance of Saccharomyces. The increase of Malassezia was notably associated with a severe evolution of the disease

during follow-up[115].

The fungus *Debaryomyces hansenii* was increased in CD patients with inflamed ileum compared to non-inflamed ileum. Moreover, oral gavage with *Debaryomyces hansenii* impaired crypt regeneration and wound healing after biopsy injury[116].

ENVIRONMENTAL FACTORS

The microbiota bridges the gap between host susceptibility factors and its environment. Most environmental factors are identified by epidemiological studies and associated with the onset of CD or its recurrence after surgery. Few of them though can impede the natural history of CD once the disease is established.

Smoking

Cigarette smoking is a well-established risk factor for developing CD[1-3]. In patients with CD, the relative statistical weight of smoking was larger than the relative weight of the genetic variants presented in the previous section[1]. Besides, cannabis is frequently used as a symptomatic treatment by patients with CD involving the ileum and is associated with tobacco[117]. Regarding cannabis use, in a double-blind, randomized, placebo-controlled trial, cannabis oil induced clinical improvement without any endoscopic change[118].

Regarding tobacco, in mice, cigarette smoke extract induced intestinal inflammation and morphometric changes in the ileal epithelium regardless of the route of administration (intragastric or intraperitoneal), advocating for a systemic effect[119]. Moreover, mice exposed to cigarette smoking were more likely to develop pathological inflammation in response to bacterial inflammation which could be the hallmark of an impaired expression of antimicrobial peptides[119]. Besides, smoking was associated with a reduced number of normal Paneth cells in the ileum, both in patients presenting a CD susceptibility allele ($ATG16L1^{T300A}$) as well as in mice presenting this genetic susceptibility. This defect in the ileum was mediated by the Paneth cell apoptosis driven by the activation of peroxisome proliferator-activated receptor gamma (PPARγ) and prevented in mice by the use of anti-TNF-α drugs[120].

Moreover, smoking is associated with an upregulation of angiogenesis in smokers with CD compared to their non-smoking counterparts. Importantly, mice exposed to cigarette smoke for 8 wk presented mucosal tissue hypoxia associated with an increased expression of pro-inflammatory cytokines and of angiogenic factors whereas smoking cessation reversed this process in the ileal mucosa. In addition, cigarette smoke exposure was associated with an increased sensitivity to chemically induced colitis [121]. Although genes of hypoxia-inducible factor were overexpressed in the inflamed ileum and in the adjacent mesenteric tissue of CD patients, hypoxia in mice did not impact experimental ileitis[122-124]. All together, these results advocate for a specific impact of tobacco smoking in the pathogenesis of ileitis.

Evidence for other environmental factor is rare in the literature. Recently, serum levels of bisphenol A, a component used in the manufacture of various plastics, have been linked to inflammatory status in CD patients. Furthermore, patients with bacterial DNA translocation presented a higher serum level of bisphenol A associated with a reduced expression of tight junction genes[125].

Diet

Western diet is characterized by a low intake of fibres contrasting with a high intake of refined sugar, animal protein and total fat. Numerous links between diet and IBD have been established[126]. To that extent, diet modulates the microbiota associated with the ileal epithelium. Thus, AIEC was associated with hyperacetylated histone H3 in the ileal epithelium and consequently histone deacetylase enzymes controlled the entry of AIEC in IECs in a murine model. Interestingly, a high fat diet enhanced histone acetylation in mice compared to a standard diet[110]. Overweight (body mass index > 25 kg/m^2) in IBD and in non-IBD patients was associated with Paneth cell defects in the ileal epithelium. This defect can be induced by Western diet in mice[93].

Regarding fat intake, the ratio between n-3 and n-6 polyunsaturated fatty acids (PUFAs) is unbalanced in favour of an excessive intake of n-6 PUFAs in Western diet. Oral feeding with n-3 PUFAs in SAMP1/Yit mice ameliorated the histological features of ileitis, and decreased addressin molecule expression (MAdCAM-1) as well as lymphocyte infiltration in the ileum[127]. To that extent, oral supplementation with linseed oil rich in α-linolenic acid (n-3 PUFAs), in a physically active murine model, reduced inflammation after oral challenge with AIEC[128]. However, oral n-3 PUFAs supplementation has not shown definitive results in patients with CD[129]. In all likelihood, n-3 PUFAs alone are unable to stop the inflammatory loop once started but remain putative candidates to prevent the onset of ileal CD.

Fibre intake was identified as a protective factor against CD onset but not UC[130]. Although epidemiological data were based on a 40-year-old population, fibre intake and more accurately inulin supplementation modulated PPARγ signalling pathway in pigs[131].

Vitamin D deficiency in IBD patients has been reported in numerous studies reviewed elsewhere as an explanation of the North-South gradient of IBD prevalence[132]. In mice fed with a vitamin D deficient diet, miR-142-3p expression was upregulated, culminating in a reduction of ATG16L1 and autophagy specifically in ileal Paneth cells. In a paediatric cohort of IBD patients, colonic samples displayed likewise an enhanced expression of miR-142-3p statistically associated with low serum vitamin D levels[133]. As discussed previously, VDR were overexpressed in the ileum and were involved in the adequate response to enteropathogens[19]. When associated with a high fat diet, vitamin D deficiency and genetic VDR inhibition led to defective Paneth cell defensins secretion and gut permeability driving endotoxemia and systemic inflammation[134].

Last, Western diet is also characterized by a high intake of dietary additives related to the consumption of ultra-processed food. To that extent, dietary emulsifiers carboxymethylcellulose and polysorbate-80 were associated with an increased virulence and enrichment of ileal pathobionts[135].

More generally speaking, diet in patients with ileal CD should be based on the guidelines of the European Society of Nutrition. The findings discussed above and others have led to recommendations for a balanced diet rich in fruit, vegetables and n-3 fatty acids, avoiding a restrictive diet[136]. As highlighted in this European consensus, good quality data regarding the effects of experimental diets are rare in the literature. In particular, there is a lack of randomized control trials to recommend a more specific diet in patients with active ileal CD[136].

CLINICAL IMPLICATIONS

The factors summarised in the previous paragraphs give rise to a distinctive natural history of ileal CD with specific clinical consequences and interventions necessary.

Natural history of ileal CD

In CD, the shortest time from diagnosis to first surgery is in ileal CD compared to other disease sites. Thus, the median time to surgery in ileal CD is about 6 years. Thirty years after being diagnosed with ileal CD, almost all patients had undergone at least one surgery according to a broad international study [1]. At 12 mo after surgery, a recurrence was observed on colonoscopy, at and above this anastomosis in 73% of patients in absence of any treatment[137]. These data regarding recurrence rates published 30 years ago led to the development of preventive strategies after surgery.

In terms of behaviour, ileal CD was more likely than colonic CD to be complicated by penetrating or stricturing lesions[3]. These severe manifestations contribute to the high rate of surgery. Accordingly, strictures are challenging complications that are usually unresponsive to medical therapy in the absence of surgery[3]. Strictures result from the accumulation of fibrotic protein in the extracellular matrix produced by fibroblasts which are partially derived from epithelial cells *via* epithelial-mesenchymal transition (EMT)[138]. Accordingly, based on a comparative study of ileal *versus* colonic ulcers, EMT appeared to be a highly noticeable feature in ileal ulcers of CD unlike colonic ulcers of CD[55]. Ileal strictures were likely to present mesenteric fat wrapped around the stricture, known as creeping fat [139], which harbours viable bacterial translocation and leads to a pro-fibrosis M2-type microenvironment[140-143]. Remarkably, creeping fat associated with the ileum presented a 10-fold higher concentration of T-cells than colonic fat[144]. In addition, adipocyte hyperplasia was observed in ileal fat unlike colonic fat[144].

Beyond the ileum

Ileal crypts have been reported in the colon of CD patients[12,45]. Recently, the presence of ectopic ileal crypts discriminated IBD subtype in patients with undetermined (unclassified) colitis. The presence of ileal metaplasia in the colon was strongly associated with a final diagnosis of colonic CD[145]. This interesting discovery could enhance understanding of ileal physiopathology, pivotal to understand CD.

In a specific statistical model based on HLA types and single nucleotide polymorphism, colonic CD appeared to be an intermediate between UC and ileal CD[1]. Consecutively, knowledge of ileal CD may pave the way to understanding other phenotypes of CD.

A dedicated treatment for ileal CD?

Logically, the specificities of ileal CD discussed in this review should lead to a dedicated treatment strategy. Nevertheless, current ECCO guidelines on the medical management of CD do not advocate for a specific treatment in ileal CD[146]. Indeed, studies are controversial. Some studies reported a lower rate of response to infliximab, an anti-TNF treatment[147,148]. Conversely, numerous other studies did not describe such a difference in response rates with anti-TNF[149]. This discrepancy may result from a bias such as the extent of the disease irrespectively of the site of the disease. Interventional trials specifically dedicated to the study of ileal CD treatment are henceforth required. In the literature, colonic CD is more likely to respond to treatment compared to ileocolonic CD and mucosal healing may be more difficult to achieve in ileal disease. Likewise, ileal stricturing CD may have lower response rates [149].

As ileal stricture may develop in spite ofCD treatment, an American team sought to determine the gene pattern associated with ileal stricturing in a paediatric CD cohort. This analysis identified a long-chain fatty acid, the eicosatetranoic acid as a possible antifibrotic tool[150]. In a follow-up study, the same team showed a decrease in fibrosis and an improvement in stiffness in human intestinal organoids exposed to eicosatetraynoic acid[151]. In parallel, butyrate, a short-chain fatty acid, also downregulated fibrosis according to the same protocol[151].

CONCLUSION

The histological and anatomical features of the ileum are direct answers to the question "why the ileum?". Furthermore, the recent works presented in this review highlight the specific interplay between environmental factors, like smoking or diet, and the ileum. Currently, the prevailing requirement for surgery in a significant proportion of patients with ileal CD testifies to the urgent need for a dedicated pharmacological approach according to disease site. As the ileum is the site of interplay between host characteristics and environmental influences, a full understanding of the molecular crosstalk that occurs in the ileum is crucial to identify new therapeutic targets to significantly change the natural history of this debilitating disease.

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REVIEW

Emerging role of the gut microbiome in post-infectious irritable bowel syndrome: A literature review

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Abstract

Post-infectious irritable bowel syndrome (PI-IBS) is a particular type of IBS, with symptom onset after an acute episode of infectious gastroenteritis. Despite infectious disease resolution and clearance of the inciting pathogen agent, 10% of patients will develop PI-IBS. In susceptible individuals, the exposure to pathogenic organisms leads to a marked shift in the gut microbiota with prolonged changes in host-microbiota interactions. These changes can affect the gut-brain axis and the visceral sensitivity, disrupting the intestinal barrier, altering neuromuscular function, triggering persistent low inflammation, and sustaining the onset of IBS symptoms. There is no specific treatment strategy for PI-IBS. Different drug classes can be used to treat PI-IBS similar to patients with IBS in general, guided by their clinical symptoms. This review summarizes the current evidence for microbial dysbiosis in PI-IBS and analyzes the available data regarding the role of the microbiome in mediating the central and peripheral dysfunctions that lead to IBS symptoms. It also discusses the current state of evidence on therapies targeting the microbiome in the management of PI-IBS. The results of microbial modulation strategies used in relieving IBS symptomatology are encouraging. Several studies on PI-IBS animal models reported promising results. However, published data that describe the efficacy and safety of microbial targeted therapy in PI-IBS patients are scarce. Future research is required.

Key Words: Gut microbiome; Infectious gastroenteritis; Irritable bowel syndrome; Post infection syndrome; Pathophysiology; Inflammation

Core Tip: Acute infectious gastroenteritis can trigger the onset of irritable bowel syndrome (IBS), leading to the development of post-infectious IBS (PI-IBS). PI-IBS is a clinical entity associated with gut dysbiosis. Alterations in the gut microbiome can affect the gut-brain axis, visceral sensitivity, intestinal barrier, intestinal secretion, gut motility, and immune activation, which in turn can cause IBS symptoms. A better understanding of PI-IBS is necessary to develop more targeted and effective treatments. Therapies targeting the microbiome, such as probiotics, antibiotics, diet, and fecal microbiota transplants, improve IBS symptoms. There is a lack of evidence of their efficiency in PI-IBS.

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INTRODUCTION

Irritable bowel syndrome (IBS) is the most frequently encountered disorder of gut-brain interactions[1], with a worldwide prevalence ranging between 7% and 15% of the general population[2,3]. According to the Rome IV criteria, it is characterized by mild to severe recurrent abdominal pain and bloating associated with alterations in bowel habits in the absence of organic disease or biochemical abnormalities[4]. Due to its symptoms, IBS is thought to be a disabling disease. It generates significant healthcare costs, reduces work productivity and school attendance, and decreases the health-related quality of life of the affected individuals[5,6]. Despite being a frequent entity in current gastroenterology practice, the physiopathology of IBS is not fully understood. It is considered to be a complex multifactorial disorder affected by several factors such as age, sex, genetics, diet, psychosocial status, altered microbiota, subclinical inflammation, and hypersensitivity of the neural network[7,8]. In recent years, accumulating evidence has suggested that the alteration of the gut microbiota plays an important role in the pathophysiology of IBS, as gut microbes exert effects on the host immune system, on gut barrier function, and on the brain-gut axis[9,10].

Acute infectious gastroenteritis [bacterial-*Campylobacter* species[11], *Salmonella* species[12,13], *Escherichia coli* (*E. coli*)[14], *Shigella*[15], *Clostridium difficile* (*C. difficile*)[16], viral-norovirus[17], rotavirus [18], severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[19], and protozoal-*Giardia*[20]] has been shown to be one of the strongest risk factors for the development of post-infectious IBS (PI-IBS), a distinct condition in which IBS diagnostic criteria are met[21,22]. PI-IBS occurs after the resolution of a gastrointestinal-related infection and the clearance of the inciting pathogen, in a patient without a prior history of IBS symptoms[22,23]. IBS clinical features usually develop 6-18 mo after the infectious gastroenteritis episode[24]. PI-IBS management strategies involve nonpharmacologic and pharmacologic therapies. Current guidance relies on IBS treatment experience, as there is a lack of evidence-based recommendations for PI-IBS treatment[1]. The aim of this review is to analyze the current literature and to describe the role of the human gut microbiota on PI-IBS physiopathology. The main questions to be answered include which long-term consequences of acute enteric infections may serve as triggers to PI-IBS and whether the acute enteric infection associated dysbiosis and its recovery can be used to predict PI-IBS development. Additionally, under discussion is whether there is a specific microbial signature associated with PI-IBS, as most studies of PI-IBS combine patients infected by varying pathogens, thus generating a considerable variability of outcomes. The gut microbiota modulation and its potential therapeutic implications in PI-IBS in terms of efficacy and safety continue to be a subject of debate and highlight the need for specific treatment protocols. A better characterization of the relationship between gut-associated dysbiosis and PI-IBS progression will lead the way to personalized medicine and the individualized management of each patient.

PI-IBS GENERAL DATA

A globally accepted definition of PI-IBS is not currently available in the literature. Most authors consider

PI-IBS as a form of IBS for which the symptoms, as specified in the Rome IV criteria, appear after the resolution of an acute gastroenteritis episode in patients without any IBS symptomatology before the gastrointestinal infection[1,4]. In 2019, the Rome Foundation Working Group proposed symptomatic diagnostic criteria for PI-IBS based on the Rome IV criteria (Figure 1), as there are no sensitive and specific diagnostic markers available yet[25]. Considering the predominant bowel pattern graded on the Bristol stool scale, PI-IBS can be classified as diarrhea-predominant IBS (IBS-D), constipation-predominant (IBS-C), mixed bowel habits (IBS-M), and unclassified[1,26]. A bowel pattern is considered predominant when it is ≥ 25% of the time as either hard/lumpy (IBS-C), loose/watery (IBS-D), or both (IBS-M)[1]. PI-IBS patients are more likely than sporadic IBS patients to present a diarrhea-predominant phenotype. The association between each patient and a PI-IBS subtype is important for disease management and therapy[22].

While most pathogen agents cause self-limiting acute gastroenteritis, subsequent chronic alteration may persist in some genetically predisposed individuals[27]. According to several studies, about 1 in 10 individuals with an acute episode of gastroenteritis will develop PI-IBS[21,28]. A 2017 meta-analysis reported that the incidence of PI-IBS more than a year after the acute episode of gastroenteritis was 14.5%[29]. Epidemiologic data of the reported incidence and prevalence vary across the literature, in part due to the methodological heterogeneity, including the criteria used to define IBS (Rome criteria I, II, III, or IV)[3,16,22]. Moreover, there is evidence that the data might be underestimated due to the high incidence of infectious gastroenteritis and the poor recall of milder episodes, as the diagnosis relies on self-reported symptom clusters[2,21,30].

Female sex, a young age, certain psychological factors before or during acute infectious gastroenteritis (anxiety, depression, somatization, and/or neuroticism), and genetic predisposition (carriers of the *TLR9*, *CDH1*, and *IL6* genes) seem to increase the risk of developing PI-IBS[29,31]. The severity of the acute infectious episode also seems to increase the probability of developing PI-IBS after the gastroenteritis resolution[29]. The risk of PI-IBS is doubled when diarrhea lasts > 7 d and is tripled when diarrhea lasts > 21 d. Other symptoms such as abdominal cramps, weight loss, and bloody stools are also associated with an elevated risk, with abdominal cramps increasing the PI-IBS risk four times. Fever is not mentioned as a risk factor[32]. Moreover, it might have a protective action, representing the host's response to the infectious injury[33]. The risk of IP-IBS remains high for at least 2 to 3 years post infection[22].

Various pathogens have been reported as related to PI-IBS development. A systematic review evaluating the prevalence and risk factors of PI-IBS after acute gastroenteritis by specific pathogens revealed the fact that the evidence indicated a similar risk for bacterial pathogens, while for viral and parasitic gastroenteritis, the data were limited[34]. However, a recent study found the incidence of PI-IBS to be higher in patients with *Campylobacter jejuni* (*C. jejuni*) gastroenteritis, compared to other etiologic agents such as bacteria, viruses, or protozoa[35]. When comparing different pathogens, protozoal enteritis shows the highest risk for PI-IBS development, followed by bacterial and then viral [33,36]. Bacterial infections seem to generate more PI-IBS cases than viral gastroenteritis probably due to the fact that the mucosal damage and inflammation caused by bacteria is often greater than that caused by viral agents[37].

There is a strong association between travelers' diarrhea and PI-IBS. Self-reports of exposure seem to result in a higher PI-IBS occurrence than laboratory-confirmed cases of travelers' diarrhea, but further studies are needed to confirm this finding[38]. Culture-confirmed infections, either bacterial or viral, seem to present an equally increased risk of PI-IBS as nonspecific gastrointestinal infections[39]. However, viral gastroenteritis is more likely to develop transient forms of PI-IBS than bacterial episodes [40]. The significant decrease in the PI-IBS prevalence from 19% to 4% one year after a viral infection may be due to the less invasive nature of the pathogen, perhaps avoiding a stronger host response[29]. During the recent pandemic, the novel coronavirus SARS-CoV-2 displayed its potential to generate gastrointestinal manifestations[41,42]. It seems that the prevalence of gastrointestinal symptoms in coronavirus disease 2019 (COVID-19) patients is around 10%, and it is following an increasing trend[43, 44]. A multicenter study from 2020 reported that digestive symptoms such as diarrhea, vomiting, and abdominal pain were present in 50.5% of COVID-19 patients, while other studies concluded that the incidence of diarrhea in the same category of patients varied from 2% to 20%[41,45,46]. A recent study found an incidence of 11.6% of IBS, according to the Rome IV criteria, with symptom onset following COVID-19 infection[41]. Another group of researchers reported similar results, with 10% of the included patients with an acute episode of SARS-CoV-2 in their medical history meeting the Rome IV criteria for IBS 6 mo after the viral infection. Three percent of them had gastrointestinal symptoms during COVID-19[47]. Noviello *et al*[48] found an incidence of PI-IBS in 26.2% of patients. The risk of developing *de novo* IBS post-COVID-19 increases in patients with gastrointestinal symptoms present during the active viral infection. Similar to noninfectious IBS, the risk is higher in female patients. The presence of severe disease markers, such as an oxygen requirement and high procalcitonin levels, increases the risk of post-COVID-19 IBS[49]. Another similar study reported that patients with dyspnea at time of admission and a history of allergies and chronic treatment with proton pump inhibitors had an increased risk of developing post-COVID-19 IBS[50].

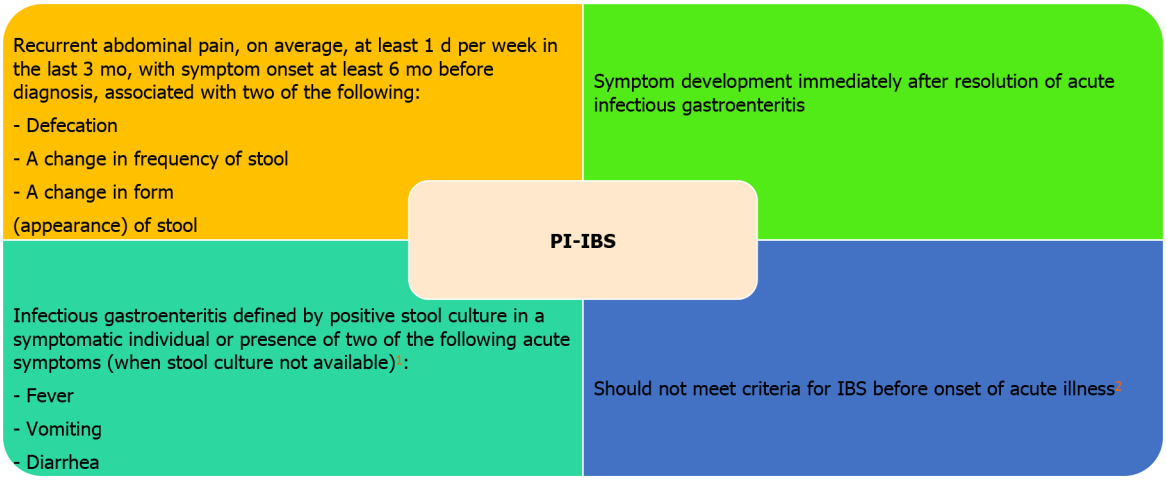

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Figure 1 Diagnostic criteria for post-infection irritable bowel syndrome (based on Rome IV criteria). [1]Mentioning the exact date of onset of irritable bowel syndrome (IBS) symptomatology can also be suggestive for post-infectious IBS; [2]Irregular bowel movements can be experienced even before the onset of the acute gastroenteritis episode (but not in association with frequent pain, as an IBS characteristic). IBS: Irritable bowel syndrome; PI-IBS: Post-infectious irritable bowel syndrome.

Compared to sporadic IBS, PI forms of the disease might have a better outcome[2]. The prognosis of PI-IBS appears favorable with the spontaneous and gradual resolution of symptoms in most patients [22]. However, one longitudinal follow-up study showed that 15% of patients with post-infection IBS remained symptomatic 8 years after disease onset[28].

PI-IBS PATHOPHYSIOLOGY

Available studies have failed to give a clear holistic picture on the underlying pathophysiology of PI-IBS. As IBS is a multifactorial disease, it has been hypothesized that the development of PI-IBS could result from the interplay of fecal microbiota, the immune response of the host, and the psychological factors[51], as illustrated in Figure 2. The current conceptual framework regarding the pathophysiologic mechanism for PI-BS suggests that the exposure to pathogenic organisms leads to the alteration of the gut microbiome. PI-IBS is associated with hyperplasia of enterochromaffin (EC) cells and increased counts of neutrophils, mast cells, and T cells in the colonic mucosa. It is believed that gastrointestinal infections stimulate the immune system causing low-grade inflammation leading to PI-IBS[52].

It was reported that patients with PI-IBS often present an increased visceral pain perception known as visceral hypersensitivity (VHS). The incomplete resolution of the immune response to acute infectious injury might facilitate the persistence of a microscopic inflammation of the bowel, activating and sensitizing pain-sensing nerves[53]. A persistent low-grade inflammation of the bowel is thought to trigger PI-IBS symptoms, by aberrant activation of intrinsic and extrinsic nerves. The increased numbers of immune cells and the enhanced cytokine signaling play an important role in the underlying mechanism of PI-IBS pathophysiology[54]. A group of researchers found an increased IL-1β mRNA expression in rectal tissue biopsies after an acute episode of infectious gastroenteritis. Patients with a *Campylobacter* enteritis were found to present increased EC cell numbers, intraepithelial lymphocytes, and intestinal permeability up to one year after the infectious episode[55], while patients with PI-IBS following a *Shigella* gastroenteritis had an increased number of mast cell in the terminal ileum[56]. These cells release mediators such as histamine, IL-1β, IL-6, and TNF-α, mediators that can stimulate or sensitize visceral nociceptors and possibly generate the abdominal pain in PI-IBS[54]. Excessive secretion of IL-8 is a hallmark of *Campylobacter* pathogenesis, being initiated by the host recognition of the pathogen-associated lipooligosaccharide[57,58]. Another study on PI-IBS patients, however, found no evidence of increased inflammatory gene expression or infiltrating inflammatory cells in biopsies and no increase in cytokine levels[54]. Similarly, no difference was reported in the number of T lymphocytes or proinflammatory cytokines between PI-IBS patients and infected healthy volunteers, 3 years after a *Salmonella* infection[59]. Another study on a large cohort found similar results, with no difference in serum cytokines and mucosal cytokine expression between PI-IBS patients and healthy volunteers[60]. γδT cells are a subset of T cells involved in initiating inflammatory responses and can acquire the capacity of inducing various cytokines. A recent study of Dong *et al*[61] found that the Vδ1 γδ T cells subset from PI-IBS patients remarkably proliferated, activated, and produced abundant IL-17. In these patients, the IFN-γ level remained unchanged, as proof of the fact that the local IL-17 could participate in the intestinal pathological disorder during PI-IBS, as the major proinflammatory cytokine

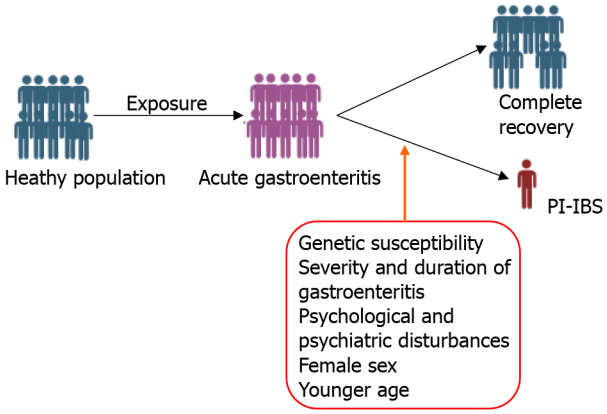

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Figure 2 Conceptual model for post-infectious irritable bowel syndrome. PI-IBS: Post-infectious irritable bowel syndrome.

[61]. Furthermore, a rodent study concluded that the upregulation of A2AR increased PI-IBS by promoting the T17 polarization of CD4+ T cells[62]. In their study, Balemans *et al*[54] reported that although initiated by inflammation, the pronociceptive changes seen in PI-IBS patients were mediated by the Hrh1 receptor sensitization of TRPV1 signaling, suggesting that similar to the "standard" IBS12, Hrh1 antagonism may represent an interesting new target for treatment of PI-IBS[54]. In conclusion, the presence of low-grade inflammation is an inconsistent finding in PI-IBS, as it might be an interim event following gastroenteritis that is superseded by another more persistent change in the gut microenvironment[54].

Increased intestinal permeability was shown to be an early event in PI-IBS physiopathology, associated with low-grade inflammation[1]. Patients with PI-IBS and high fecal proteolytic activity presented *in vivo* and *ex vivo* distal gut permeability that depended on the fecal level of proteolytic activity[63]. Following a mixed infection of enterohemorrhagic *E. coli* (EHEC) O157:H7 and *C. jejuni* during a waterborne outbreak of bacterial gastroenteritis, there was reported an increased intestinal permeability[58]. EHEC is known for its deleterious impact on the epithelial barrier[64]. *Giardia duodenalis* (*G. duodenalis*), a protozoan pathogen also implicated in promoting PI-IBS development, is well known to disturb the homeostatic barrier function through several mechanisms[65].

Viral gastroenteritis is a known risk factor for the PI onset of IBS. There are many studies describing the relation between norovirus infection and PI-IBS. As a conclusion to their study, Porter *et al*[66] suggested that dysmotility disorders may follow viral infections. A high incidence of functional gastrointestinal disorders was reported in patients who suffered from a norovirus gastroenteritis[66]. Similarly, another two studies reported an increased prevalence of PI-IBS in patients who experienced an acute episode of gastroenteritis during a confirmed norovirus outbreak[40,67]. It is thought that norovirus infection can cause epithelial barrier dysfunction, increased intestinal permeability, a reduction in the villous surface area and villous height, and a mucosal immune response with an increase in cytotoxic intraepithelial T cells, impairing the gut's sensory-motor function[67].

COVID-19, an immunologic and inflammatory response associated with low-grade inflammation and mucosal injury, caused the development of IBS features in genetically predisposed individuals. Patients with PI-IBS can present high levels of macrophages and T lymphocytes in intestinal samples, increased levels of calproctin and fecal cytokines, such as IL-8; all these were found in COVID-19 patients, as well as the presence of virus-specific IgA, together with increased blood levels of proinflammatory cytokines [68,69]. Similar to IBS, the altered intestinal permeability associated with gut dysbiosis and with an alteration of the neuromuscular function might also be involved in PI-IBS onset[67].

On the other hand, the pandemic led to a stressful situation, anxiety, and depression. These factors resulted in other diseases related to psychological stress and the nervous system similar to IBS[70,71]. Farsi *et al*[41] in their study found that psychological stress had no significant influence on COVID-19-induced IBS symptoms[41]. However, another similar study reported that COVID-19 had adverse effects on both GI and psychological symptoms among individuals with functional dyspepsia-IBS overlap syndrome[70]. Similarly, another study concluded that the COVID-19 pandemic increased psychosocial stress and gastrointestinal symptoms in patients already known to have IBS[72]. The interaction between the gut-brain axis disturbances and genetic and psychosocial factors can contribute to IBS development[41]. Psychological stress acts as a trigger in developing IBS through its adverse effects on intestinal permeability and motility and hypersensitivity to visceral pain. Acute and chronic stressful situations lead to cortico-tropin-releasing hormone secretion, activating the hypothalamic-pituitary-adrenal axis and creating new premises for IBS onset. Stress-induced dysbiosis can modulate the neuro-immune-endocrine systems and interfere with the brain-gut axis[73,74].

PI-IBS MICROBIOME ALTERATION

Due to the bioavailability of nutrients, the human gastrointestinal tract harbors the largest concentration and diversity of microbiota of the human body. Healthy adult gastrointestinal microbiota are represented by five primary bacteria phyla: *Firmicutes* (synonym *Bacillota*) and *Bacteroides* (synonym *Bacteroidota*) phylum predominate the microbiota, while *Actinobacteria* (synonym *Actinomycetota*), *Proteobacteria* (synonym *Pseudomonadota*), and *Verrucomicrobia* phylum are found in modest proportions[75]. The gut microbiota have a dynamic composition, influenced by many intrinsic and extrinsic factors, such as genetic inheritance, birth mode, breastfeeding duration, age, sex, diet, and drugs[75-77]. The alteration of the healthy microbial structure leads to dysbiosis, resulting in various gastrointestinal disorders, systemic metabolic diseases, and neurological impairments[75].

During an acute infectious gastroenteritis, there is a decline in the gut's microbial diversity[78]. There are several mechanisms explaining the disruption of the indigenous microbiota. One would be directly throughout pathogen agent-microbiota interaction. Secondly, the alteration of the microbiota might occur *via* the host's mucosal immune response, or there is the possibility of a combination of the two presented mechanisms[27,79]. In rodents, *Salmonella enterica* serovar typhimurium was shown to induce the loss of 95% of the total bacterial numbers of the intestinal tract, 7 d after the infectious episode[80]. *G. duodenalis* and *C. jejuni* can directly alter the composition of human gut microbiota[81]. Aside from the predominance of the etiologic bacterial pathogen, certain native taxa of the gut microbiome such as *Streptococci, Fusobacteria,* and *Campylobacter*, can also increase their numbers throughout the acute episode but also in the weeks following the bacterial infection[82,83].

Immediately after acute gastroenteritis with *Vibrio cholerae* (*V. cholerae*), increased levels of *V. cholerae, Streptococcus, Fusobacterium,* and *Campylobacter* species were found during a 16S rRNA gene polymerase chain reaction (PCR) analysis of the infected patients' stool samples. Two months after the first assessment, there was a decrease in *V. cholerae*, *Streptococcus, Fusobacterium,* and *Campylobacter* species, while *Ruminococcus obeum, Collinsella aerofasciens, Ruminococcus torques, Eubacterium rectale,* and *Faecalibacterium prausnitzii* increased their levels as a marker of recovery from the infection[82].

As for gastroenteritis triggered by viral agents, the alterations in the microbiota seem to be less consistent. The immune response to the viral injury is usually less harmful, resulting in a decreased diversity and the expansion of *Proteobacteria* species in some patients with viral gastroenteritis[83]. A study including patients with all-cause traveler's diarrhea investigated their gut microbial composition using fecal samples. In diarrheal patients, there was a decreased *Bacteroidota/Bacillota* ratio and changes in β-diversity, compared to healthy travelers who displayed an unexpected increased abundance of *Bacillota* phylum (*Streptococcus* and *Lactococcus* genera). Stool samples of patients with confirmed norovirus infection had an increased diversity of species characterizing their microbiota during the active infection, including *Clostridium XIVb, Bilophilia, Alistipes, Barnesiella,* and *Roseburia* species[84]. When assessing the gut microbiome correlation in symptomatic and asymptomatic patients infected with norovirus, Patin *et al*[85] found no significant difference between the alpha and beta diversity of the two groups studied. The symptomatic subjects presented relatively more species of *Bacillota* phylum, especially in the order *Clostridia,* while *Bacteroidota* phylum displayed fewer species than the asymptomatic subjects, particularly in the *Bacteroidia* order. In asymptomatic patients, three members of the genus *Parasutterella* and one in the *Nitrosomonadaceae* family were found in higher levels. The increased levels of *Bacteroidota* in the asymptomatic individuals suggest that its presence could improve the host's effort of resisting enteric viral infection or neutralizing its pathogenicity and symptoms[85]. Another study on Chinese patients with diarrhea of viral etiology reported a decreased diversity in the gut microbiota at stool sample examination, when compared to healthy individuals. *Bacillota* phylum with species of *Enterococcus, Peptostreptococcaceae, Incertae Sedi, Shigella, Weissella,* and *Clostridium* dominated the microbiota during the acute episode, while beneficial bacteria such as *Bacteroides vulgatus, Bifidobacterium,* and *Lactobacillus* species were found in decreased amounts[83]. Nelson *et al*[86] also tried to characterize the stool microbiota in norovirus-infected human patients. Their research found similarities between infected patients' and uninfected healthy individuals' microbiota. However, in a small number of infected patients there was found a significantly altered microbiome characterized by a reduced relative number of *Bacteriodota* and a corresponding increase in *Pseudomonadota.* Interestingly, the increased level of *Pseudomondota* phylum was due to a single operational taxonomic unit of *E. coli*[86]. This finding raises the concern that the alteration in gut microbiota during an acute viral gastroenteritis exposes the affected patients to some possible long term gastrointestinal complications.

SARS-CoV-2 infected patients seem to present an altered gut microbiota, characterized by the depletion of anti-inflammatory butyrate-producing bacteria and the enrichment of taxa with proinflammatory properties[87,88]. The Alpha and beta diversity index values appear to be significantly lower than in healthy individuals, all through the active infection and the recovery period[87,89]. Furthermore, an important reduction in the major bacterial phylum composition and diversity was also observed[90]. *Ruminococcusm gnavus* and *Bacteroides vulgatus* were found in increase amounts in post-acute COVID-19 patients' microbiomes, while *Faecalibacterium prausnitzii*, known for its anti-inflammatory proprieties, was characterized as decreased, when compared to healthy individuals[70].

Streptococcus, *Enterococcus,* and *Corynebacterium* species, well known as opportunistic pathogens, seem to be found in higher amounts in COVID-19 patients' stools than in healthy individuals[87]. *Fusicatenibacter*, *Romboutsia*, *Intestinibacter*, *Actinomyces,* and *Erysipelatoclostridium* species could actually be used as biomarkers in order to identify COVID-19 positive patients[91]. There is proof that the development of PI-IBS may be predicted by the composition of the salivary microbiome during acute SARS-CoV-2 infection[40,92]. Butyrate-producing bacteria showed an inverse correlation with IBS symptom onset 6 mo post-acute viral infection[93]. The gut microbiota composition was reported to be correlated with proinflammatory cytokine levels, as proof of its contribution to the immune response. The *Faecalibacterium* genus, belonging to the *Clostridia* class, was found to be decreased in COVID-19 patients during the acute episode and was inversely correlated with the IL-8 and IL-12 serum levels. The same study reported an enrichment of the *Actinomycetota* phylum and the *Propionibacteriaceae* family, which was positively correlated with the gp130/sIL-6Rb level[90]. The *Streptococcus* species was also associated with an increased expression of proinflammatory cytokines such as IL-18, TNF-α, and IFN-γ[87]. In contrast, IFN-gamma and IL-28A/IFN-12 levels were found to be negatively correlated to the class *Clostridia*, reduced in abundance in COVID-19 patients[90]. Changes in the microbiome composition were also identified in COVID-19 patients without antibiotic exposure. Depletion of beneficial commensal species and enrichment of opportunistic pathogenic bacteria, such as *Clostridium hathewayi*, *Actinomyces viscosus*, and *Bacteroides nordii,* were found in COVID-19 patients' stool samples. According to Zuo *et al*[94], the disease severity and the baseline abundance of certain genera and strains might be in close relationship, as the gut microbiota might actively influence the immune system response. Moreover, it appears that 50% of COVID-19 patients had an active intestinal infection, even in cases with no gastrointestinal complaints[94]. The deleterious effect of SARS-CoV-2 infection on beneficial gut microbiota seems to persist even after disease resolution, sustaining the possibility of the gut microbiome alteration's role in PI-IBS pathogenesis[95]. The intestinal infection persisted despite respiratory viral clearance[19,94]. There were cases described, where dysbiosis was persistent 30 d after the acute viral episode, suggesting the long-term influence of COVID-19 on gut microbiota[96]. Opportunistic pathogens such as *Collinsella aerofaciens*, *Collinsella tanakaei*, *Streptococcus infantis,* and *Morganella morganii* have been found in large amounts in stool samples with high SARS-CoV-2 infectivity. In stool samples with low-to-no viral infectivity, there were higher amounts of *Parabacteroides merdae*, *Bacteroides stercoris,* and *Lachnospiraceae bacterium 1_1_57FAA*, some of these within important role in augmenting host immunity[19,94]. Current evidence suggests that a high microbial exposure to Gram-negative bacteria could offer protective effects against COVID-19, possibly due to the increased interferon type I levels[43]. These findings sustain the idea that the gut homeostasis may suffer some alterations during the acute COVID-19 episode, independent from the presence of gastrointestinal symptoms, alterations that can persist beyond disease resolution[73]. There is evidence that COVID-19-induced impairment of the gut-lung axis might create predisposing factors for IBS development[43,97].

Moreover, there is evidence that microbiota composition prior to infection may also influence the possibility of developing an acute infection as well as PI-IBS[33]. Dicksved *et al*[98] reported that an increased abundance of *Bacteroidota* in pre-employment stool samples of abattoir workers increased the risk of developing a *C. jejuni* infection during the period of employment[98]. A *Clostridiales*-predominant microbiota type has a protective role, as an individual with such intestinal microbiota is more likely to return to a state of eubiosis after the remission of the infectious episode and pathogen clearance. A *Bacteroidota* predominant community, in contrast, may increase the risk of long-term dysbiosis after the acute episode's resolution[21].

Although the gut microbiota profile of IBS patients has been evaluated in several studies, there is not the same consistency of data regarding PI-IBS patients' gut microbiome alteration. In their study of the real-time PCR assay of rectal epithelium RNA expression, Jalanka-Tuovinen *et al*[51] concluded that the intestinal microbiota of PI-IBS patients were significantly different from healthy individuals but similar to patients with IBS-like symptoms (PI bowel disease, PI-IBS and IBS-D). They identified an "index of microbial dysbiosis" (IMD) that characterized the intestinal microbiota of PI-IBS. The IMD included 27 genus-like microbial groups including a twelvefold increased level of *Bacteroidota* phylum, including as *Bacteroides* and *Prevotella* species. The *Bacillota* phylum was less abundant, with decreased levels of various uncultured *Clostridiales* and *Clostridium* clusters. Moreover, dysbiosis was associated with gastrointestinal symptoms' severity and not with psychological symptoms. Dysbiosis was also associated with biopsy findings, such as increased levels of eotaxins, mast cells, and goblet cells and decreased EC cells[51]. Similar results were obtained by Sundin *et al*[99], when analyzing the mucosal and fecal microbiota of patients with PI-IBS. The fecal microbiota composition of PI-IBS patients was significantly different from the fecal microbiota of IBS patients and healthy individuals. Patients with PI-IBS had a reduced mucosal and fecal microbial diversity, with reduced levels of *Bacillota*, including *Clostridium* clusters IV and XIVa, and increased *Bacteroidota*, including *Bacteroides* species. The reduced diversity of the fecal microbiota was associated with increased activated lymphocytes in the lamina propria. At the level of major butyrate producer bacteria abundance, there was no difference identified between the PI-IBS patients and healthy individuals. Unlike Jalanka-Tuovinen *et al*[51], the reduced diversity of the microbiota was associated with the presence of psychological symptoms[99]. In PI-IBS patients, the *Bacteroidota* phylum seems to have a relatively greater abundance of microbes than in healthy individuals, while *Bacillota* phylum display a relative reduction in representative members in

the gut microbiota[78]. The main findings are summarized in Table 1.

MICROBIOME-DIRECTED THERAPY

Acute gastroenteritis, as mentioned above, can alter the gut microbiota, initiating the underlying mechanisms of PI-IBS. Modulation of the microbiota can be performed using probiotics, symbiotics, prebiotics, and antibiotics or by performing a fecal microbial transplantation, with the purpose of downregulating inflammation, improving barrier function, and reducing visceral sensitivity[100]. To date, there is no specific practice guideline or treatment strategies for PI-IBS. Different drug classes can be used for treating PI-IBS similar to patients with IBS in general[37].

Probiotics are known to be "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[101]. *Lactobacillus casei* DG (LC-DG) and its postbiotic were shown to attenuate the inflammatory mucosal response in an *ex vivo* organ culture model of PI-IBS-D[102]. Similarly, Hong *et al*[103] reported that probiotic administration (*Lactobacillus acidophilus* LA5, *Bifidobacterium animalis* subsp. *lactis* BB12, and *Saccharomyces cerevisiae* var. *boulardii*) decreased proinflammatory cytokine levels in both the control and PI-IBS induced mice[103].

Saccharomyces boulardii, given 750 mg/day for a period of 6 wk, was reported to improve the quality of life and the cytokine profile in PI-IBS patients[104]. *Bifidobacterium infantis,* M-63 1 × 109 cfu/sachet/day given daily for 3 mo, restored the normal composition of the gut microbiota and improved mental health in individuals with post-flood acquired IBS[105].

Given the current evidence that serotonin levels are increased in PI-IBS patients, serotonin-based therapy is a treatment option that deserves further study[106]. Cao *et al*[107] proved that an *Lactobacillus rhamnosus* supernatant had a positive effect on serotonin transporter (SERT) expression in colon tissues of rats with PI-IBS. By modulating the microbial composition, the serotonergic imbalance can be restored, followed by the improvement in IBS symptoms[107]. Another similar study on an animal model evaluated the efficacy of a *Bacillus subtilis* (*B. subtilis*), *Enterococcus faecium,* and *Enterococcus faecalis* (*E. faecalis*) supernatant, administrated in PI-IBS rats. The researchers reported that the supernatants could upregulate the expression level of SERT in intestinal cells, mentioning the fact that the combined supernatants of *B. subtilis* and *E. faecalis* had a superior effect to the administration of a single supernatant[108]. Studies regarding prebiotic and symbiotic use in PI-IBS patients are not available.

Fecal microbiota transplantation (FMT) is used in order to restore microbial dysbiosis by transferring a healthy microbiome to an individual with an alteration of microbial composition[109]. FMT has shown benefits in IBS patients. However, there is a lack of data on FMT use in PI-IBS. A recent randomized clinical trial assessed FMT's safety as well as its clinical and microbiological efficacy in patients with PI-IBS. The results demonstrated FMT's effectiveness compared to traditional pharmacotherapy, its safety, and tolerability[110].

However, FMT could be used in reestablishing the microbiota homeostasis following acute gastroenteritis, with the purpose of decreasing the risk of PI-IBS development. It is known that FMT is recommended for recurrent or refractory *C. difficile* enterocolitis, as a proved effective therapy[111]. There is evidence that FMT treatment can improve gut microbiota alteration in recovered COVID-19 patients, particularly in those who presented severe gastrointestinal symptomatology during the acute phase[112].

Jin *et al*[113] assessed the action of rifaximin on the VHS, barrier function, gut inflammation, and microbiota in a PI-IBS mouse model. Rifaximin administration improved the VHS, recovered the intestinal barrier function, and inhibited low-grade inflammation in the colon and ileum, without changing the composition and diversity of the gut microbiota[113]. However, Harris *et al*[114] and Tuteja *et al*[115] reported no benefit of rifaximin therapy on PI-IBS patients.

The results of mesalazine's efficacy in treating PI-IBS patients are contradictory. Lam *et al*[116] reported a significant improvement in symptoms such as abdominal pain, urgency, and stool consistency, when mesalazine was given to a small group of PI-IBS patients[116]. Bafutto *et al*[117] found that mesalazine administration in PI-IBS patients decreased the stool frequency and improved its form and consistency, after 30 d of treatment[117]. In contrast, another double-blind controlled trial including a small number of patients with diarrhea predominant PI-IBS reported no positive effect on clinical symptoms or quality of life[118]. However, mesalazine use during the acute infectious gastroenteritis may have a protective effect on PI-IBS development, as reported by a study on patients affected by hemorrhagic enterocolitis with *Shiga*-like toxin-producing *E. coli*[119]. Dunlop *et al*[120] found a significant reduction in the T- lymphocyte counts in the rectal tissue of PI-IBS patients treated with prednisone, although there was no positive effect on the EC cell count or symptom improvement[120].

Bile acid malabsorption may occur after an episode of acute gastroenteritis. It is confirmed that cholestyramine administration can alleviate symptoms in PI-IBS patients, especially with diarrhea symptoms[121]. The information regarding the therapeutic options in PI-IBS are summarized in Table 2.

Table 1 Alterations of the gut microbiota observed during acute gastroenteritis and during post-infectious irritable bowel syndrome

Ref.	Subjects/methods	Sample and techniques	Microbiota alterations	Other findings
Jalanka-Tuovinen *et al*[51], 2014	11 postinfection IBS, 11 postinfection bowel dysfunction, 12 postinfection without bowel dysfunction, 12 IBS-D, 11 healthy controls adults	16S rRNA gene phylogenetic microarray analysis with HITChip, 16S rRNA gene qPCR with group and species-specific primers of feacal sample	Index of microbial dysbiosis" comprised of 27 genus-like groups including: ↑ *Bacteroidota* including various *Bacteroides and Prevotella* species, ↓*Bacillota* including various uncultured *Clostridiales*, and *Clostridium* clusters	Dysbiosis was associated with bowel, not psychological symptoms; Dysbiosis associated biopsy findings: ↑eotaxin, mast cells, goblet cells, ↓enterochromaffin cells; Dysbiosis associated RNA expression pathways: ↑serotonin transport, condensed chromosome, B cell antigen receptor, ↓caspase
Hsiao *et al* [82], 2014	7 adults with *V. cholerae* AGE history, 50 healthy children, 12 healthy adults	16S rRNA gene PCR, V4 region analysis of faecal sample	One week after AGE: ↑*V. cholerae Streptococcus* spp *Fusobacterium* spp *Campylobacter* spp	Two months after AGE (recovery period): ↓*V. cholerae Streptococcus* spp *Fusobacterium* spp *Campylobacter* spp, ↑ species indicating recovery *Ruminococcus obeum*, *Collinsella aerofasciens Ruminococcus torques*, *Eubacterium rectale Faecalibacterium prausnitzii*
Ma *et al* [83], 2011	13 Adenovirus diarrhea, 13 Rotavirus diarrhea, 13 Astrovirus diarrhea, 13 Norvirus diarrhea, 6 control children	16S rRNA gene PCR, V3 region analysis of faecal sample	↓Diversity in diarrheal patients, ↑*Enterococcus*, *Peptostreptococcaceae*, *Incertae Sedi*, *Shigella*, *Weissella* spp	↓*Bacteroides vulgatus Bifidobacterium*, *Lactobacillus* spp
Youmans *et al*[84], 2015	111 all-cause traveler's diarrhea/12 healthy travelers	16S rRNA gene PCR, V3 and V5 regions analysis of faecal sample	↓*Bacteroidota: Bacillota* ratio in diarrheal patients; ↑Species diversity during norovirus infection; ↑*Clostridium XIVb Bilophilia Alistipes Barnesiella*, *Roseburia* spp during norovirus infection	↑*Bacillota* phylum *Streptococcus Lactococcus* spp in healthy travelers (unexpected)
Patin *et al* [88], 2020	4 symptomatic and 5 asymptomatic norovirus infected adults	16S rRNA gene analysis of faecal sample	Post norovirus challenge: ↑*Bacillota* phylum, particularly *Clostridia*, ↓*Bacteroidota Pseudomonadota*	Prior to norovirus challenge: Asymptomatic patients had ↑*Bacteroidota* phylum and ↓*Clostridia* compared to symptomatic
Nelson *et al*[86], 2012	38 norovirus infection, 22 healthy controls	16S rRNA gene 454 pyrosequencing, V3-V5 regions analysis of faecal sample	A subset (approximately 1/5) patients with norovirus had: ↓diversity, ↑*Pseudomonadota* phylum, *Enterobacteriaceae* family	*Escherichia coli* diversity and virulence was not associated with norovirus infection
Cheng *et al* [87], 2022	COVID-19 acute and recovery phase; Non COVID-19	Meta-analysis of 16S rRNA microbial data	↓Ruminococcus Faecalibacterium Roseburia, Coprococcus genus, ↑ Fusobacterium Streptococcus in recovery/post-recovery COVID-19 compared to non COVID-19	↓Clostridium clostridioforme, ↑ Bifidobacterium breve in COVID-19 compared to recovery/post-recovery COVID-19
Liu *et al* [93], 2022	68 COVID-19 patients, 68 non-COVID-19 patients	Shotgun metagenomic sequencing	At 6 mo follow up 76% developed PACS; Non-PACS showed recovered gut microbiome profile at 6 mo comparable to that of non-COVID-19 controls; ↑ *Ruminococcus gnavus*, *Bacteroides vulgatus* and ↓*Faecalibacterium prausnitzii* in PACS	Butyrate-producing bacteria, including *Bifidobacterium pseudocatenulatum* and *Faecalibacterium prausnitzii* showed the largest inverse correlation with PACS at 6 mo
Zuo *et al* [95], 2020	15 Acute COVID-patients, 6 community acquired pneumonia patients, 15 healthy controls	Shotgun metagenomic sequencing	Antibiotic naïve patients ↑*Clostridium hathewayi*, *Actinomyces viscosus*, and *Bacteroides nordii* compared with controls; COVID-19 with antibiotic use ↓ *Faecalibacterium prausnitzii*, *Lachnospiraceae bacterium 5_1_63FAA*, *Eubacterium rectale*, *Ruminococcus obeum*, and *Dorea formicigenerans* compared with COVID-19 naïve patients	Baseline abundance of *Coprobacillus*, *Clostridium ramosum*, and *Clostridium hathewayi* correlated with COVID-19 severity: There was an inverse correlation between abundance of *Faecalibacterium prausnitzii* and disease severity; Depletion of symbionts and enrichment of opportunistic pathogens persisted after clearance of SARS-CoV-2
Yeoh *et al* [96], 2021	100 COVID-19 patients, 78 non COVID-19 controls	Shotgun sequencing total DNA extraction from stool sample	Patients with COVID-19 were depleted in *Faecalibacterium prausnitzii*, *Eubacterium rectale* and several bifidobacterial species, which remain low up to 30 d from disease resolution	Composition of the gut microbiota in patients with COVID-19 is concordant with disease severity and magnitude of plasma concentrations of several inflammatory cytokines, chemokines and blood markers of tissue damage
Sundin *et al*[99], 2015	13 PI-IBS patients, 19 general IBS patients, 16 healthy controls	HITChip for mucosal and fecal microbiota	↓Mucosal and faecal diversity *Bacillota* phylum including *Clostridium* clusters IV and XIVa, ↑*Bacteroidota* phyum including *Bacteroides* spp	Reduced diversity was associated with psychological symptoms and increased activated lamina propria lymphocytes. Did not find a difference in major butyrate producer abundance

AGE: Acute gastroenteritis; COVID-19: Coronavirus disease 2019; IBS-D: Diarrhea-predominant irritable bowel syndrome; PACS: Post-acute coronavirus disease 2019 syndrome; PI-IBS: Post-infectious irritable bowel syndrome; qPCR: Quantitative polymerase chain reaction; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2.

Table 2 Post-infectious irritable bowel syndrome therapeutic options

Ref.	Therapeutic intervention	Outcome
Compare *et al* [102], 2017	*Lactobacillus casei* DG + postbiotic	↓The inflammatory mucosal response in an *ex vivo* organ culture model of PI-IBS-D
Hong *et al* [103], 2019	*Lactobacillus acidophilus* LA5, *Bifidobacterium animalis* subsp. *lactis* BB12 and *Saccharomyces cerevisiae* var. *boulardii*)	↓Pro-inflammatory cytokine levels in both the control and PI-IBS induced mice
Abbas *et al* [104], 2014	*Saccharomyces boulardii*	Improved the quality of life and the cytokine profile in PI-IBS patients
Lee *et al*[106], 2017	*Bifidobacterium infantis*	Restored the normal composition of gut microbiota and improved mental health among individuals with post-flood acquired IBS
Cao *et al* [107], 2018	*Lactobacillus rhamnosus* supernatant	Had a positive effect on SERT expression in colon tissues of rats with PI-IBS, improving IBS symptoms in PI-IBS rats
Chen *et al* [108], 2022	*Enterococcus faecium* and *Enterococcus faecalis* supernatant, in PI-IBS rats	The supernatants of B. subtilis, *Enterococcus faecium*, and *Enterococcus faecalis* can upregulate SERT expression in intestinal epithelial cells and the intestinal tissues in the rat model of PI-IBS
Tkach *et al* [110], 2022	RCT, low FODMAP diet + Otilonium Bromide + a multi-strain probiotic *vs* FMT procedure	FMT proved effectiveness in restoring normal gut microbiota and ameliorating PI-IBS symptoms, compared to traditional pharmacotherapy, as well as a high degree of safety and good tolerability
Liu *et al*[111], 2021	FMT procedure	FMT can partially restore the gut dysbiosis in COVID-19 patients by increasing the relative abundance of *Actinobacteria* (15.0%) and reducing *Proteobacteria* (2.8%) at the phylum level. At the genera level, *Bifidobacterium* and *Faecalibacterium* had significantly increased after FMT
Jin *et al*[113], 2017	Rifamixin in PI-IBS rats	Rifaximin alleviated visceral hypersensitivity, recoverd intestinal barrier function and inhibited low-grade inflammation in colon and ileum of PI-IBS rats. Exerts anti-inflammatory effects with only a minimal action on the overall composition and diversity of the gut microbiota
Harris *et al* [114], 2019	Rifamixin *vs* placebo in veterans with IBS	Rifaximin was not associated with signifcant improvement in global symptoms, abdominal pain, stool frequency, urgency, bloating, or stool consistency
Tuteja *et al* [115], 2019	Rifamixin *vs* placebo in veterans with IBS	Rifaximin was not effective in improving IBS symptoms and QOL in GW veterans with non-constipated IBS
Lam *et al* [116], 2016	Mesalazine *vs* placebo	Mesalazine was no better than placebo in relieving symptoms of abdominal discomfort or disturbed bowel habit. Mesalazine did not reduce mast cell percentage area stained. A subgroup of patients with postinfectious IBS may benefit from mesalazine
Bafutto *et al* [117], 2011	Mesalazine in PI-IBS patients compared to non-infective IBS patients	Mesalazine reduced key symptoms of postinfectious irritable bowel syndrome and noninfective irritable bowel syndrome with diarrhea patients, with no statistical difference between IBS and PI-IBS
Tuteja *et al* [118], 2012	Mesalazine *vs* placebo	There was no significant improvement in global symptoms or overall QOL with mesalazine in patients with PI-IBS
Andresen *et al*[119], 2016	Mesalazine during the AGE with STEC	Mesalazine administration during AGE with STEC might be a protective factor for PI-IBS
Dunlop *et al* [120], 2003	Prednisolone *vs* placebo	Prednisolone does not appear to reduce the number of enterochromaffin cells or cause an improvement in symptoms in PI-IBS

AGE: Acute gastroenteritis; FMT: Fecal microbial transplantation; IBS-D: Diarrhea-predominant irritable bowel syndrome; PI-IBS: Post-infectious irritable bowel syndrome; RCT: Randomised controlled trial; QOL: Quality of life; SERT: Serotonin transporter; STEC: Shiga-like toxin-producing *Escherichia coli*.

CONCLUSION

Acute gastroenteritis can significantly increase the risk of developing IBS, a chronic gastrointestinal pathology with high health-care utilization. Current studies in humans as well as animal models describe specific host-pathogen interactions that may lead to the onset of post-infection IBS symptoms. There is no curative treatment option for PI-IBS, and patients rely only on symptomatic therapy. There are numerous studies on IBS treatment options. However, there is a lack of data regarding PI-IBS therapeutic management, and there is a great need for evidence-based recommendations in post-acute

gastroenteritis IBS.

These advancements in understanding will be helpful in elaborating specific biomarkers used to identify patients with a high risk of developing IBS symptoms following an acute infectious gastroenteritis, as well as designing targeted pharmacotherapy. Microbial restoration, augmentation of barrier function, and targeting VHS remain the most promising areas for therapeutic interventions and represent a future research perspective.

FOOTNOTES

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Abstract

Biliary complications are the most common complications after liver transplantation. Computed tomography (CT) and magnetic resonance imaging (MRI) are cornerstones for timely diagnosis of biliary complications after liver transplantation. The diagnosis of these complications by CT and MRI requires expertise, mainly with respect to identifying subtle early signs to avoid missed or incorrect diagnoses. For example, biliary strictures may be misdiagnosed on MRI due to size mismatch of the common ducts of the donor and recipient, postoperative edema, pneumobilia, or susceptibility artifacts caused by surgical clips. Proper and prompt diagnosis of biliary complications after transplantation allows the timely initiation of appropriate management. The aim of this pictorial review is to illustrate various CT and MRI findings related to biliary complications after liver transplantation, based on time of presentation after surgery and frequency of occurrence.

Key Words: Liver transplantation; Biliary; Complications; Computed tomography; Magnetic resonance imaging; Hepatic imaging; Biliary tract; Cholangiopancreatography; Stricture

Core Tip: Biliary complications are the most common surgical complications after liver transplantation, and represent a major source of morbidity and mortality in liver transplant recipients. Magnetic resonance cholangiopancreatography is the gold standard for the non-invasive diagnosis of intra- and extrahepatic biliary complications. Computed tomography may also be helpful for the assessment of biliary complications, and it is often used due to its more widespread availability as compared to that of magnetic resonance imaging.

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INTRODUCTION

Computed tomography (CT) and magnetic resonance imaging (MRI) are cornerstones in the postoperative assessment of patients after liver transplantation[1]. Complications may be categorized by etiology, and include surgical, graft-related, immunologic, infectious, and neoplastic. Complications can also be classified based on their timing: Early (within 6 mo) or late (after 6 mo; Table 1). Surgical complications are typically categorized as vascular, biliary, or parenchymal. Biliary complications are the most common and represent a major source of morbidity and mortality in liver transplant recipients with an incidence of 10%-32%[2-4]. Biliary complications after liver transplantation include anastomotic stricture, non-anastomotic stricture, bile leak, bile cast, biloma, sphincter of Oddi dysfunction, and mucocele of the cystic duct remnant[4-9]. Biliary complications have a significant negative impact on patient survival and may lead to the need for re-transplantation[3,6]. Magnetic resonance cholangiopancreatography (MRCP) is currently the gold standard for the diagnosis of intra- and extrahepatic biliary complications, while invasive cholangiography should be restricted for therapeutic uses or when MRCP is equivocal[10].

The aim of this pictorial review is to illustrate CT and MRI findings of surgery-related biliary complications after liver transplantation, classified based on their usual timing of appearance and their frequency. The knowledge of surgical techniques is of key importance to understand postoperative anatomic changes and imaging evaluation. Therefore, we will first provide a short summary of the main techniques of liver transplantation with focus on biliary anastomosis. Then, we will discuss imaging tips and tricks for the prompt diagnosis of biliary complications on CT and MRI.

SURGICAL TECHNIQUE

Most liver transplantations are performed with orthotopic implantation of a deceased donor whole liver graft, and may be performed with a conventional or piggyback technique. Other surgical options include split or segmental liver transplantation (Figure 1)[7]. The split liver procedure may be performed by either of 2 approaches: In the most common approach, the liver is divided into a left lateral segment graft (II + III ± IV segments) if the recipient is a child or a right extended liver lobe graft (I + V-VIII ± IV segments) if the recipient is an adult; in the less common and more challenging variant of this procedure, the liver is split into 2 hemigrafts and the left side (I-IV) is transplanted to a small adult or a teenager and the right side (V-VIII) to a medium-sized adult. In patients with prior biliary disease or re-transplantation, a different biliary anastomosis technique may be performed[7]. Liver transplantation is a multi-step surgery. After skin preparation and incision, the surgeon checks if there is any undiagnosed malignancy or anatomic variant and then dissects the recipient's liver and gallbladder. The donor's liver, without the gallbladder, is then implanted into the recipient with the anastomoses between recipient and donor performed in the following order: (1) Systemic venous outflow (inferior cava vein-hepatic veins); (2) portal venous inflow; (3) hepatic arterial inflow; and (4) biliary anastomosis. The types of anastomosis depend on donor and recipient anatomy and surgeon preference. Finally, when the surgical field is dry, the abdomen is closed. Each of the above-mentioned steps is critical, and complications may be directly or indirectly related to failure of any of these steps [11,12].

Biliary anastomosis is known as the "Achilles tendon" of liver transplantation. The most common form of biliary reconstruction is choledochocholedochostomy (duct-to-duct anastomosis)[7], which may be performed in an end-to-end or end-to-side fashion. Choledochocholedochostomy can be performed either with a T-tube, which allows rapid decompression of the biliary tree if needed and reduces the risk of anastomotic stricture formation but may lead to biliary leakage and cholangitis at the time of

Table 1 Post-transplant complications with the relevance of computed tomography and magnetic resonance imaging in diagnosis

Time of onset	Type of complications	Relevance of CT/MRI for diagnosis
Early (< 6 mo)	Surgical	++++
	Graft-related	++
	Immunologic	+
	Infectious	++
Late (> 6 mo)	Surgical	++++
	Graft-related	++
	Immunologic	+
	Infectious	+++
	Neoplastic	++++
	Disease recurrence	++

++++: Highly relevant, often mandatory; +++: Very useful; ++: Useful; + sometimes helpful, but clinical diagnosis is usually very relevant. CT: Computed tomography; MRI: Magnetic resonance imaging.

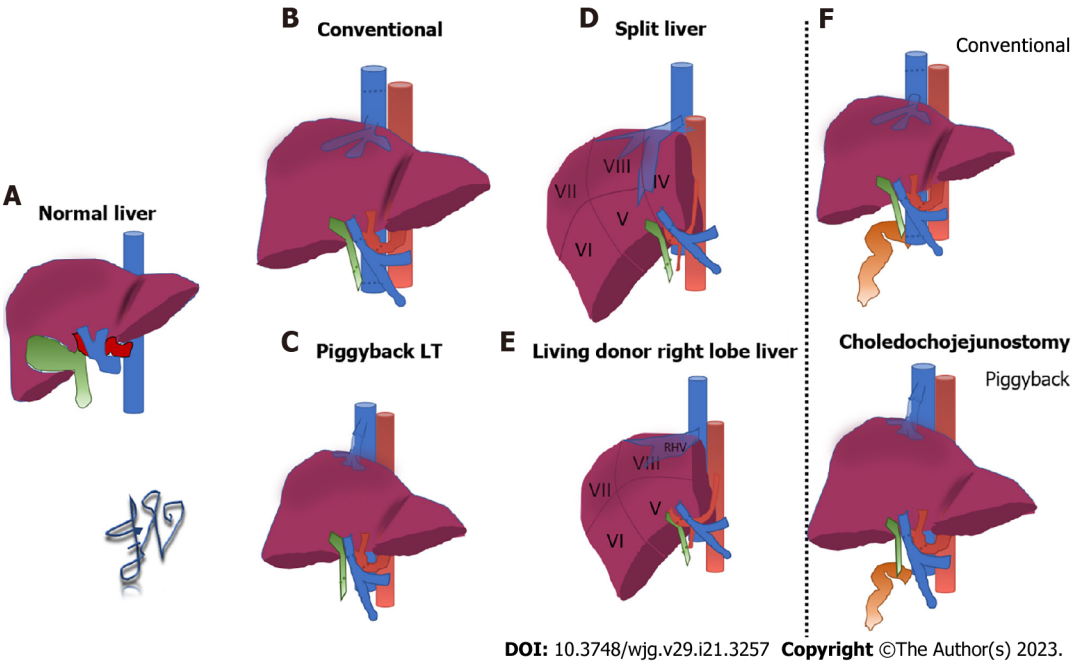

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Figure 1 Schematic representation of surgical techniques for liver transplantation. A: Normal anatomy of the liver; B: Conventional technique for liver transplantation; C: Piggyback technique; D: Split liver technique in adults; E: Living donor right lobe liver transplantation; F: Conventional (top row) and piggyback (bottom row) techniques with choledochojejunostomy.

removal, or without a T-tube[13]. Choledochojejunostomy to a Roux-en-Y defunctionalized intestinal loop (*i.e.* the connection of the bile duct to jejunum loop) (Figure 1F) is the second most common type of biliary reconstruction technique, usually preferred in patients with pre-existing biliary disease and in case of size mismatch between donor and recipient ducts, re-transplantation, or previous biliary surgery [7]. Potential complications of choledochojejunostomy include stricture, leakage, and bleeding at the jejuno-jejunostomy site. Choledochocholedochostomy is preferred over choledochojejunostomy due to shorter operation time, lower risk of septic complication, preserved sphincter of Oddi, better physiologic enteric function, and easier endoscopic access to the biliary tree for any future need.

CT/MRI OF BILIARY COMPLICATIONS

MRI has sensitivity and specificity of 98%-99% and 94%-96%, respectively, for the diagnosis of biliary

complications after liver transplantation[14,15]. MRI protocol includes 2D-MRCP and 3D-MRCP and an unenhanced T1-weighted sequence, while gadoxetate disodium hepatobiliary MRI is performed in selected cases[16]. Ultrasound is usually performed first, and may help identify any features that suggest the presence of complication. Despite not being as comprehensive as MRI, CT may also be helpful for the assessment of biliary complications and is often used due to its more widespread availability. Table 2 summarizes biliary complications related to surgery classified according to frequency, time of occurrence, and treatment[4-10].

Biliary strictures

Biliary strictures are distinguished as anastomotic or non-anastomotic. Anastomotic strictures (Figure 2) account for about 47% of biliary complications, being slightly more frequent after choledochocholedochostomy *vs* choledochojejunostomy, and may also occur after split liver donation[3]. Currently, percutaneous biliary techniques are considered effective treatment options with good outcomes in the setting of liver transplant with anastomotic biliary stricture[17]. Non-anastomotic strictures account for about 23% of all biliary complications, being slightly more frequent after choledochojejunostomy *vs* choledochocholedochostomy[3]. Non-anastomotic strictures (Figure 3) typically comprise ischemic-type biliary lesions in the early period after transplant, and are mostly related to recurrence of the primary biliary disease, chronic rejection, or secondary sclerosing cholangitis if occurring in the late postoperative period.

Biliary strictures are one of the most critical complications in ABO-incompatible living donor liver transplant recipients, and may occur as perihilar or diffuse, with the latter having worse clinical outcomes[18].

MRI demonstrates any stenosis at the level of the stricture as well as upstream irregular dilation of the biliary system; typically, the change in duct caliber at the level of the stricture is abrupt. Anastomotic strictures tend to be single, short in length, and occur at the level of anastomosis, usually in the late postoperative period. Non-anastomotic strictures are frequently multiple, long, hilar in location, and tend to occur early after transplantation and may result in graft loss. Radiologists should report the level of the biliary injury and the length of the obstruction. Although not routinely recommended for the diagnosis of biliary strictures, MRCP with hepatobiliary contrast may allow the assessment of the severity of bile duct obstruction based on the degree of hepatobiliary contrast filling distal to the stricture. Complete obstruction of the biliary tree is demonstrated in the case of absence of contrast distal to the stricture, while the obstruction is partial if there is limited passage of contrast beyond the stricture. In the case of complete obstruction, hepatic function may be impaired as evidenced by elevated bilirubin, which may hamper the excretion of hepatobiliary contrast[19]. Biliary strictures must be differentiated from their mimickers on MRI, which may include size mismatch of the donor and recipient common ducts (appearing as gradual tapering of the bile duct lumen at the anastomosis) and postoperative edema (can cause extrinsic compression at the level of the anastomosis and have a tapered "hour-glass" appearance). Other potential mimickers of biliary strictures on MRI include pneumobilia (which may occur normally if a choledochojejunostomy anastomosis has been performed) and MRI susceptibility artifacts caused by nearby surgical clips. CT may help in identifying the inadvertent placement of metallic surgical clips. In ABO-incompatible living donor liver transplant recipients, imaging and clinical follow-up is recommended if post-transplantation CT at 1 mo demonstrates subtle intrahepatic duct dilatation with perihilar abnormality to assess for the possible occurrence of diffuse intrahepatic duct dilatation stricture[18].

Biliary leak and biloma

Biliary leaks account for approximately 23% of all biliary complications[3]. They may be anastomotic or non-anastomotic (Figure 4) and are more common after choledochocholedochostomy *vs* choledochojejunostomy. Leaks at the biliary anastomosis are most common[20]. Non-anastomotic leaks may occur at the level of T-tube insertion, cystic duct, or the cut surface of a partial liver graft. The use of a T-tube may be a risk factor for biliary leak, most commonly after removal of the tube[21]. However, there are discordant data with respect to the causative mechanism of T-tube-related biliary leaks[22,23]. Non-anastomotic biliary leaks may be cut-surface leaks, such as those originating from small bile ducts that are transected perioperatively during hepatic resection, from the cystic duct stump, or may be caused by bile duct necrosis in patients with hepatic artery occlusion. Biliary leaks may result in the development of bilomas. Bilomas may be intra- or extrahepatic depending on the origin of the leak, although they most commonly occur in the perihepatic space. Bilomas may become infected and can potentially lead to sepsis. Another potential serious complication of biloma is erosion of the adjacent hepatic artery. Ultrasound (US) and CT are most commonly performed as first-line imaging techniques due to their wide availability; biliary leak or biloma are demonstrated as free fluid or fluid collection, usually in the perihepatic and subhepatic spaces, mostly anechoic on US and hypoattenuating with fluid density on CT. On MRI, biliary leaks and biloma are hypointense on T1-weighted sequence and hyperintense on T2-weighted sequence, with the former appearing as free fluid and the latter as a fluid collection (Figure 5). However, these findings are nonspecific, and biliary leaks and bilomas are virtually indistinguishable from other types of fluid collection and ascites. In the case of a biliary leak occurring after bile duct necrosis in the setting of hepatic artery occlusion, intrahepatic bilomas or bile lakes may develop in

Table 2 Post-transplant biliary complications related to surgery based on frequency, onset, and management

Type of complication	Frequency	Timing of onset	Common treatment
Biliary stricture	5%-15% (up to 30% in LDLT)	Early late	Refashioning after stenting
Biliary leak	2%-25%	Early	ERCP and stenting if anastomotic
Biloma/Biliary lake	2.6%-11.5%	Early	Percutaneous drainage and antibiotics if large
Bile duct filling defect	3%-6%	Early late	ERCP/percutaneous drainage
Sphincter of oddi dysfunction	2%-5%	Late	ERCP with sphincterotomy and consideration of stent placement
Redundant common bile duct	Rare	Late	Stent
Mucocele of bile duct remnant	Rare	Late	Surgery if causing compression

ERCP: Endoscopic retrograde cholangiopancreatography; LDLT: Living donor liver transplantation.

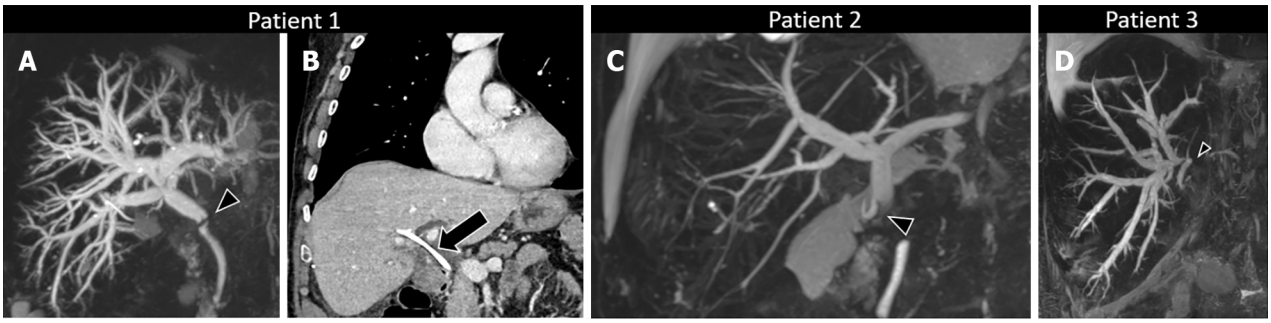

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Figure 2 Anastomotic biliary strictures. A: Anastomotic stricture of choledochocholedochostomy 3 mo after liver transplantation. Endoscopic retrograde cholangiopancreatography was performed with balloon dilatation of the stricture and stent positioning. Magnetic resonance cholangiopancreatography (MRCP) maximum intensity projection (MIP) demonstrates anastomotic biliary stricture (arrowhead) with marked upstream biliary dilatation; B: Contrast enhanced computed tomography in the coronal plane shows in the same patient the stent in the biliary tree (arrow) and normal biliary tree caliber; C: Anastomotic stricture of choledochojejunostomy 6 mo after liver transplantation. MRCP MIP demonstrates anastomotic biliary stricture (arrowhead) with marked upstream biliary dilatation; D: Anastomotic stricture of end-to-end biliary anastomosis after split liver transplantation with right split lobe. MRCP MIP demonstrates anastomotic biliary stricture (arrowhead) with marked upstream biliary dilatation of the right split transplanted lobe.

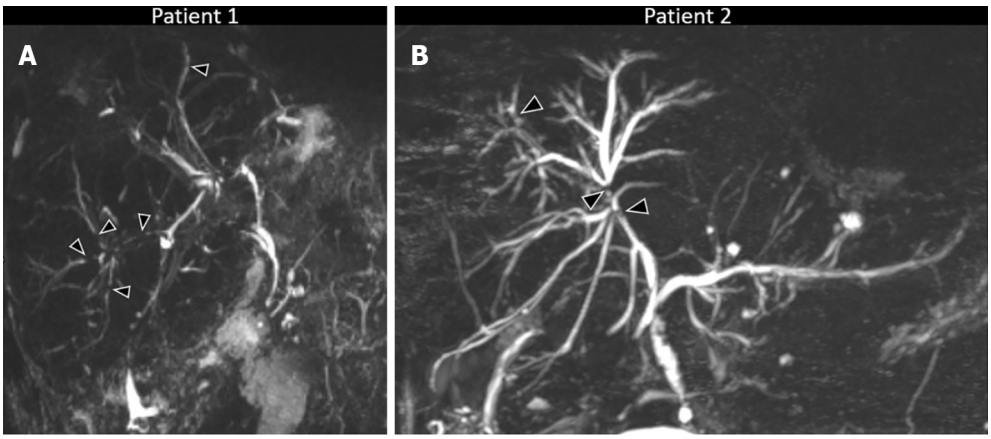

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Figure 3 Non-anastomotic biliary strictures. A: Non-anastomotic strictures in a patient with chronic rejection demonstrated at biopsy 11 y after transplant and then re-transplanted. Magnetic resonance cholangiopancreatography (MRCP) maximum intensity projection (MIP) demonstrates multiple non-anastomotic biliary strictures (arrowheads); B: Non-anastomotic strictures in a patient with recurrent secondary cholangitis 4 y after liver transplantation. MRCP MIP demonstrates multiple non-anastomotic biliary strictures (arrowheads) with upstream biliary dilatation.

the early postoperative period, with a characteristic appearance on imaging as cystic or linear dilatations of the intrahepatic bile ducts (Figure 6). MRCP with hepatobiliary contrast has 100% sensitivity and 98% specificity with respect to the diagnosis of bile leaks[24,25]. MRCP with hepatobiliary contrast allows to

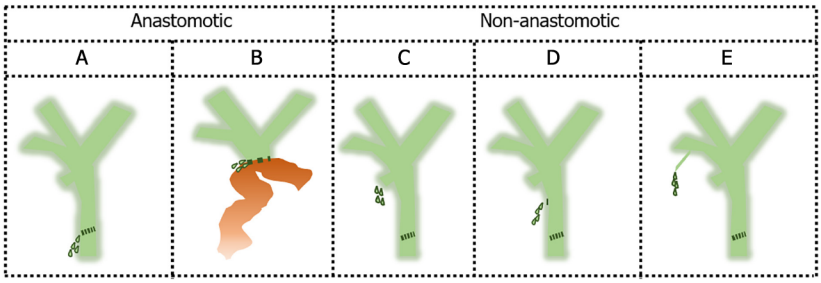

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Figure 4 Schematic representation of biliary leaks. A: Anastomotic leak at the level of choledochocholedochostomy; B: Anastomotic leak at the level of choledochojejunostomy; C: Non-anastomotic leak at the level of the cystic duct stump; D: Non-anastomotic leak at the level of T-tube removal; E: Non-anastomotic leak from small bile ducts that are transected perioperatively during hepatic resection.

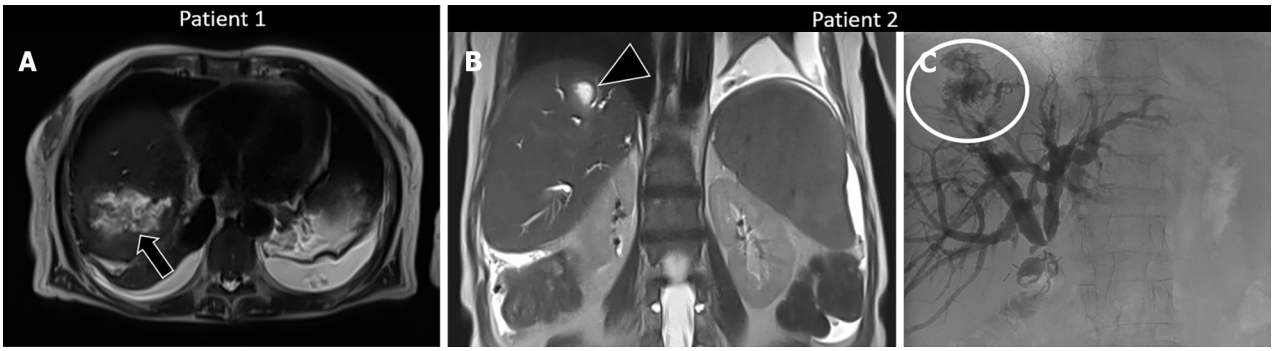

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Figure 5 Biloma and biliary leak after liver transplantation. A: Patient with biloma occurring 9 mo after liver transplantation. T2-weighted axial image shows a collection (arrow) in the right hepatic lobe with internal heterogenous signal intensities. Percutaneous drainage of the collection was performed demonstrating superinfected biloma; B: Patient with biliary leak occurring after liver transplantation. T2-weighted coronal magnetic resonance imaging shows an intrahepatic fluid collection (arrowhead) consistent with biloma; C: Cholangiographic image in the same patient demonstrated the biliary leak (circle) causing an intrahepatic biloma.

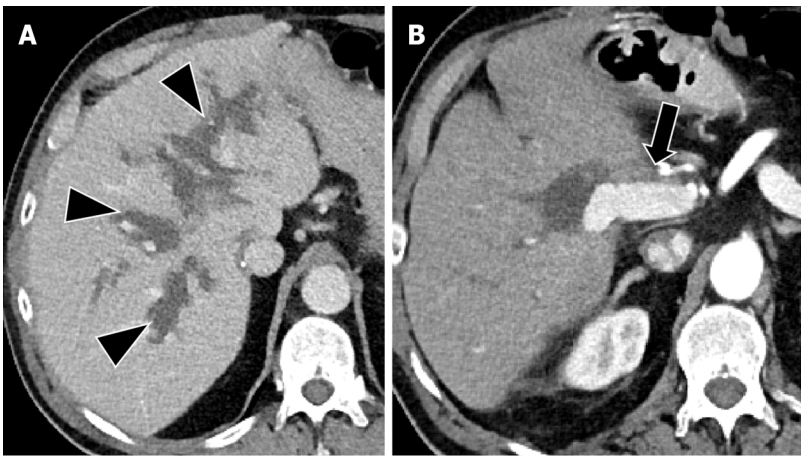

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Figure 6 Biliary lakes occurring after liver transplantation. A: Contrast-enhanced computed tomography (CT) in the axial plane in the portal venous phase demonstrates biliary lakes (arrowheads) adjacent to the portal vein branches; B: Contrast-enhanced CT in the arterial phase demonstrates lack of enhancement of the hepatic artery (arrow) caused by the adjacent surgical clip.

demonstrate the level and the entity of biliary leakage, showing contrast agent extravasation into bilomas in case of active leakage. However, small bilomas are often self-limiting, and active extravasation may not be demonstrated. The lack of active bile leak into a biloma as evidenced on imaging is highly clinically relevant, as it may help in choosing a conservative management. However, it is important to highlight that the diagnostic accuracy of MRCP with hepatobiliary contrast depends on the timing of acquisition of the hepatobiliary phase. When conventional acquisition at 20 min only is

adopted, sensitivity may be as low as 42.9%[26], while the acquisition at 60 min-90 min, 150 min-180 min, or even 210 min-240 min to 390 min increases the sensitivity[26,27]. The reason behind the lower sensitivity of the 20 min hepatobiliary phase compared to acquisitions at later times may be 2-fold. On one hand, the increased bilirubin in these patients may result in low uptake of hepatobiliary contrast by the hepatocytes at 20 min; indeed, bilirubin is taken up at the hepatocyte level by the same family of organic anion transport proteins of gadoxetate disodium (Figure 7). On the other hand, bile duct obstruction may result in the upregulation of a multidrug resistance protein, which could reduce the excretion of gadoxetate disodium, delaying or preventing the visualization of the bile ducts and any bile leak[28,29]. For this reason, based on consensus reports for liver MRI, an elevated bilirubin level is considered a relative contraindication to injection of gadoxetate disodium at some centers, with threshold bilirubin levels from 2.0 mg/dL-5.0 mg/dL. Overall, delayed acquisitions may prove to be helpful for the diagnosis of biliary leaks[29,30].

Biliary casts, stones and sludge

Biliary casts, stones, or sludge account for about 6% of all biliary complications[3], and usually complicate any biliary stricture present. Casts, stones, or sludge may occur at both the intra- and extrahepatic bile ducts as the consequence of bile stasis and may lead to cholangitis, graft failure, or the need for re-transplantation[31]. Biliary concretions after liver transplantation are related to a heterogenous group of lithogenic conditions mostly related to bile tract damage with a multifactorial, complex pathophysiology[32]. Biliary casts complicate up to 4.5% of liver transplantations, may recur, and may lead to biliary strictures in up to 85.0% of patients on follow-up[4]. Morphologically, biliary casts after liver transplantation may have a cordlike, columnar, or dendritic shape within the biliary tree[33]. The prompt identification of biliary casts is of utmost importance, as patients with biliary cast syndrome have lower overall and graft survival rates compared to patients with non-anastomotic and anastomotic strictures only[4]. MRCP has very good sensitivity in the identification of biliary concretions, which appear as filling defects surrounded by a thin film of hyperintense bile (Figure 8). Importantly, the sensitivity for biliary cast detection increases when using T1-weighted imaging compared to T2-weighted MRCP; unenhanced T1-weighted images show hyperintensities in the bile ducts (Figure 8C), leading to the correct diagnosis of biliary cast[34]. As recently pointed out, intraductal hyperintense filling material on T1-weighted MRI is a sensitive sign for biliary casts, and intraductal filling defect on T2-weighted MRI with the duct-in-a-duct feature is a specific sign and likely reflects biliary mucosal detachment[4].

Sphincter of Oddi dysfunction

Sphincter of Oddi dysfunction (SOD) comprises functional or mechanical obstruction of the sphincter of Oddi and involves the biliary sphincter and/or the pancreatic sphincter. Biliary and pancreatic SOD have each been subclassified into 3 types based on related symptoms, laboratory testing, and imaging (common bile duct diameter of at least 12 mm): Type I with biliary pain, abnormal liver enzymes and dilatation of the common bile duct; Type II with biliary pain and either abnormal liver enzymes or dilatation of the common bile duct; and Type III with biliary pain and no objective criteria[35,36]. More recently, the Rome IV consensus has proposed new classification, as most type I patients present with papillary stenosis rather than a functional disorder and have an excellent response after sphincterotomy; type II has now been renamed as suspected functional biliary sphincter disorder; and type III patients have no response to sphincterotomy[37,38]. SOD after liver transplantation has been reported in about 2%-5% of patients, with papillary stenosis (*i.e.* SOD type I) accounting for about 1% of cases and suspected functional biliary sphincter disorder (*i.e.* SOD type II) for about 1% as well[39]. The pathogenesis of biliary sphincter disorder in liver transplantation recipients is poorly understood; possible predisposing factors include the use of a T-tube, the presence of opportunistic infection, and postsurgical edema[40,41]. Patients with functional biliary sphincter disorder after liver transplantation may be asymptomatic due to hepatic denervation after the surgery and immunosuppression, thus making the diagnosis more difficult[41]. Therefore, suspicion of SOD after liver transplantation should be raised when cholestasis or dilation of bile ducts appears in the absence of bile stones or other structural abnormalities. Sphincter of Oddi manometry has been the gold standard for years, although it is invasive, patient- and operator-dependent, and may lead to post-procedure pancreatitis. Due to these factors, it is no longer routinely used in all patients with suspected SOD and its general utility has been questioned[39-41]. Hepatobiliary scintigraphy can demonstrate structural or functional partial biliary obstruction as evidenced by increased time to hepatic peak, delayed biliary visualization, delayed clearance of the radiotracer from the dilated bile ducts, and prolonged biliary to bowel transit[42-44]. MRCP may be used to exclude biliary lithiasis and other structural abnormalities, and may show an enlarged papilla in some cases of papillary stenosis (Figure 9). Secretin-MRCP may suggest the diagnosis of SOD, showing stenosis of the sphincter and lack of relaxation of the main pancreatic duct after secretin injection, increased prominence of pancreatic duct side branches, or acinarization[45]. Secretin-MRCP seems more useful for SOD type II, with a diagnostic accuracy of 73%, rather than for SOD type III for which accuracy drops to only 46%[46]. Given the low accuracy, the cost of secretin, and the acquisition time of at least 15 min, secretin-MRCP for SOD should be considered only in a few selected cases (*i.e.* noninvasive evaluation is preferred or when endoscopic evaluation is not available or

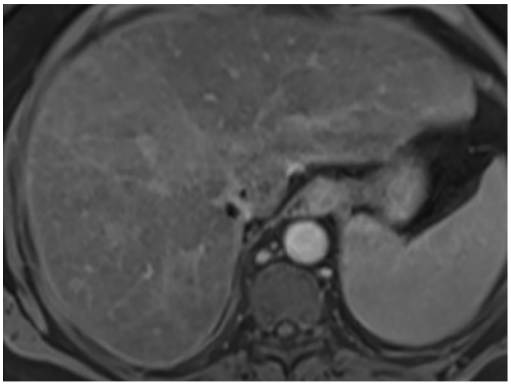

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Figure 7 Lack of excretion of hepatobiliary contrast after liver transplantation in a patient with increased serum bilirubin of 2.2 mg/dL. Gadoxetate disodium-enhanced magnetic resonance imaging in the hepatobiliary phase acquired at 20 min is inadequate as demonstrated by hypointensity of the liver parenchyma compared to that of hepatic vessels and lack of contrast in the biliary tree.

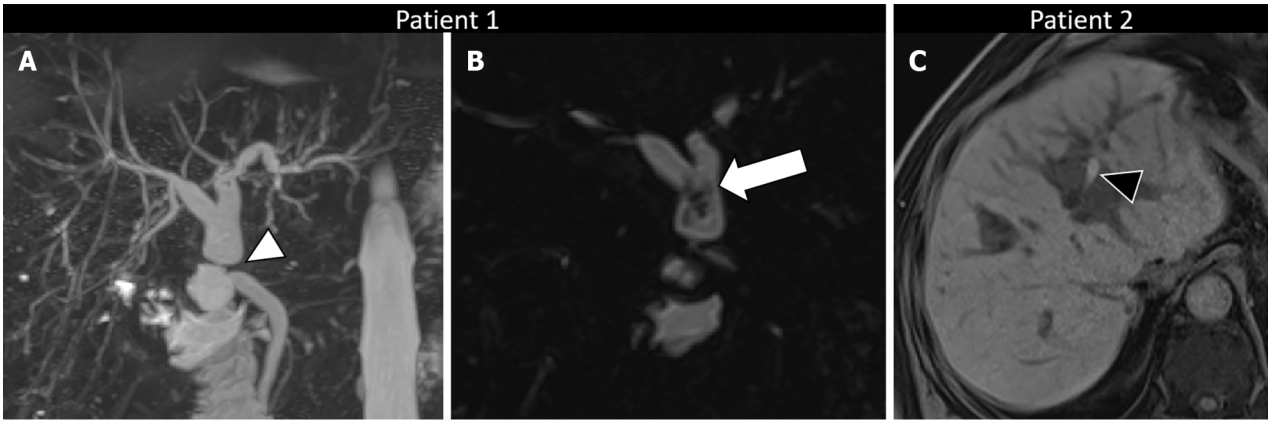

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Figure 8 Biliary sludge and biliary cast. A: Patient with biliary sludge and anastomotic stricture 3 mo after liver re-transplantation. Magnetic resonance cholangiopancreatography (MRCP) maximum intensity projection image demonstrates anastomotic biliary stricture (arrowhead) with marked upstream biliary dilatation; B: Three-dimensional MRCP in the coronal plane in the same patient demonstrates biliary sludge (arrow) in the dilated hepatic duct extending into the left and right ducts. C: Patient with biliary cast 2 y after liver transplantation. Unenhanced T1-weighted gradient-recalled image shows hyperintense content (arrowhead) in the left biliary duct, consistent with biliary cast.

impractical)[45]. Gadoxetate disodium-enhanced MRCP may help in ruling out SOD in the case of normal passage of hepatobiliary contrast in the duodenum at 20 min-30 min, and in suggesting the diagnosis in the case of delayed or no passage of bile through the ampulla of Vater after 30 min-1 h[47]. Interestingly, the diagnostic accuracy of gadoxetate disodium-enhanced MRCP for SOD has not yet been investigated; this represents an area of interest particularly when invasive evaluation is not indicated.

Mucocele of the cystic duct remnant

Mucocele of the cystic duct remnant, whether recipient or donor in origin, is an extremely rare complication after liver transplantation[48-51]. It is characterized by an abnormally dilated cystic duct remnant with flattening of the walls of the residual cystic duct to form a collection of mucus from cells lining the cystic duct remnant. The causative mechanism of the mucocele is still unclear. Lack of nervous regulation of the biliary tract after liver transplant may affect bile secretion and outflow. The differential diagnosis includes abscess, biloma, hemobilia, tumor, or aneurysm. If left untreated, the enlarged mucocele may cause chronic mechanical compression of the biliary system; however, it may also remain stable in size[50]. Ultrasound and CT demonstrate the presence of a collection at the level of the hepatic hilum. MRCP demonstrates a rounded and well-circumscribed collection adjacent to the common hepatic duct in the absence of other cause of obstruction (Figure 10).

Bile duct redundancy

Bile duct redundancy is described as a surgically reconstructed donor-recipient extrahepatic bile duct that creates a looped, sigmoid-shaped appearance in the absence of any anastomotic stricture[8]. A

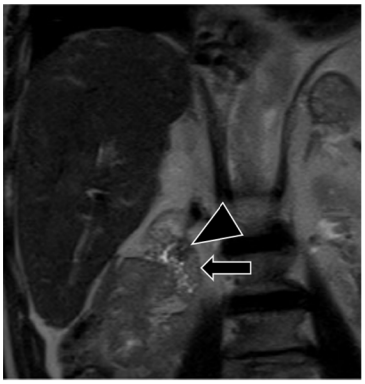

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Figure 9 Enlarged ampullary papilla occurring years after liver transplantation and causing minimal cholestasis. T2-weighted imaging in the coronal plane demonstrates an enlarged ampullary papilla (arrowhead) protruding in the duodenal lumen (arrow). Ultrasonography-endoscopy confirmed the enlarged ampullary papilla and biopsy was performed, which excluded malignancy and confirmed the diagnosis of papillary stenosis (*i.e.* sphincter of Oddi dysfunction); sphincterotomy was then performed.

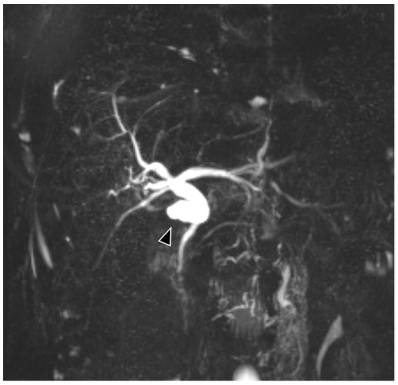

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Figure 10 Mucocele occurring years after liver transplantation. Magnetic resonance cholangiopancreatography maximum intensity projection shows a fluid collection in communication with biliary tree. Biliary content was confirmed with percutaneous drainage.

redundant bile duct occurs when the donor or the recipient bile duct is too long, and may lead to delayed bile flow into the duodenum, functionally translating into cholestasis, abnormal liver laboratory test results, and cholangitis; it may also predispose to kinking of the redundant bile duct with subsequent obstruction[8,52,53]. Two-dimensional and 3-dimensional MRCP may demonstrate the abnormal long-constructed donor-recipient extrahepatic bile duct shape as well as any kinking, if present.

Vanishing bile duct syndrome

Vanishing bile duct syndrome is a very rare biliary complication occurring after liver transplantation, characterized by progressive destruction and disappearance of the intrahepatic bile ducts in the portal area leading to cholestasis[54,55]. It is caused by an acute or chronic T-cell-mediated rejection of the allograft[54,55]. The diagnosis of vanishing bile duct syndrome is suspected in a patient with liver biochemical abnormalities consistent with cholestasis in the absence of other conditions associated with cholestasis[54-56]. Histologic examination through liver biopsy is needed for the diagnosis, and MRI may help in excluding other causes of cholestasis[54,56].

CONCLUSION

In conclusion, biliary complications represent a clinically relevant problem after liver transplantation and occur in up to 1/3 of liver transplant recipients. Radiologists need to be aware of surgical techniques and post-surgical anatomy as well as clinical information for comprehensive image interpretation. MRCP is an established non-invasive procedure for the diagnosis of post-transplantation biliary complications. In selected cases, gadoxetate disodium-enhanced MRCP is needed for improving diagnostic accuracy of biliary complications and the protocol for this technique must be tailored based

on the clinical suspicion.

FOOTNOTES

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ORIGINAL ARTICLE

Basic Study

Effects of ethanol and sex on propionate metabolism evaluated *via* a faster ^{13}C-propionate breath test in rats

Yosuke Sasaki, Naoyuki Kawagoe, Tsunehiko Imai, Yoshihisa Urita

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Abstract

BACKGROUND

Alcoholism is regarded as a risk factor for vitamin B_{12} (VB_{12}) deficiency. Because V B_{12} serves as a coenzyme of methylmalonyl-CoA mutase, a key enzyme in propionate metabolism, the ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for VB_{12} deficiency. However, the conventional PBT requires 2 h, which is inconvenient in clinical practice. We hypothesized that a faster PBT can be used to evaluate propionate metabolism and is more easily adaptable for clinical practice.

AIM

To evaluate a faster PBT for assessing the effects of long-term ethanol consumption on propionate metabolism in ethanol-fed rats (ERs).

METHODS

ERs were obtained by replacing standard drinking water (for control rats, CRs) with 16% ethanol solution in descendants of F344/DuCrj rats. Faster PBT was performed by administering ^{13}C-propionate aqueous solution to male and female ERs and CRs by inserting a metal tubule from the mouth to the stomach; exhaled gas was collected in a bag to measure its $^{13}CO_2/^{12}CO_2$ isotope ratio *via* infrared isotope spectrometry. Serum VB_{12} and alanine transaminase (ALT) levels were measured *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively. We evaluated statistical differences in mean body weight, change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), peak $\Delta^{13}CO_2$‰, and serum VB_{12} and ALT, between males and females and between ERs and CRs using the *t*-test and Mann-Whitney U test for normally and non-normally distributed variables, respectively.

RESULTS

Males weighed significantly more than females ($P < 0.001$); CRs weighed significantly more than ERs ($P < 0.008$). $\Delta^{13}CO_2$ reached a peak (C_{max}) at 20 min and

30 min in females and males, respectively, decreasing after 20-30 min without rebound in all groups. Males had significantly higher C_{max} and $\Delta^{13}CO_2$ at 15-45 min than females ($P < 0.05$; for all pairs). Propionate metabolism was enhanced in male ERs relative to male CRs, whereas metabolism did not differ markedly between ERs and CRs for females. Males had higher serum VB_{12} levels than females, without prominent differences between the ER and CR groups. Male CRs had notably higher ALT levels than male ERs. Thus, chronic ethanol consumption may trigger fatty acid production *via* intestinal bacteria and changes in gut microbiome composition.

CONCLUSION

Faster PBT shows that 16% ethanol consumption promotes propionate metabolism without inducing liver injury. This PBT may be used clinically to evaluate gut flora status.

Key Words: Alcoholism; Breath test; Carbon isotope; Gut flora; Propionate; Vitamin B12

Core Tip: Alcoholism is a risk factor for vitamin B_{12} (VB_{12}) deficiency. The ^{13}C-propionate breath test (PBT) is a diagnostic modality for VB_{12} deficiency, but requires 2 h for completion. We applied a faster PBT to evaluate propionate metabolism using an ethanol-fed rat model. After ^{13}C-propionate administration, the $^{13}CO_2/^{12}CO_2$ isotope ratio of gas collected every 5 min for 60 min was measured using infrared isotope spectrometry. The $\Delta^{13}CO_2$ peak occurred within 30 min. Ethanol-fed males showed marked propionate metabolism without associated liver injury. This study demonstrates the potential of the faster PBT to evaluate propionate metabolism under various clinical conditions.

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INTRODUCTION

Chronic alcoholism is a risk factor for vitamin B_{12} (VB_{12}) deficiency[1,2]. Because VB_{12} works as a coenzyme of methylmalonyl-CoA mutase, a key enzyme in propionate metabolism (Figure 1), the ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for VB_{12} deficiency, with favorable results[3,4]. For instance, Wagner *et al*[3] reported that the conventional PBT could reliably predict VB_{12} deficiency in humans, with an area under the curve of the receiver operating curve value of 0.88. Propionate is a ubiquitous short chain fatty acid produced by intestinal bacteria, such as *Phascolarctobacterium*[5]. Emerging evidence suggests that intestinal microbial flora have a healing influence on alcoholic liver damage[6], and propionate produced by intestinal bacteria has protective effects against alcoholic liver damage[7]. Thus, we believe that PBT may provide important information not only regarding VB_{12}deficiency, but also regarding alcohol metabolism and alcoholic liver damage.

However, the conventional PBT requires 2 h to complete, which can be highly inconvenient for patients in clinical settings. Thus, in the present study, we aimed to evaluate the potential of a faster PBT for assessing the effects of long-term ethanol consumption on propionate metabolism as well as VB_{12} deficiency using ethanol-fed rats (ERs) as an animal model of chronic alcoholism. As the protective effects of estrogen against VB_{12} deficiency have been reported[8], we also evaluated the effect of sex-related differences on propionate metabolism, as detected by the faster PBT.

MATERIALS AND METHODS

Animals and treatments

All animal experiments were performed with approval of the Toho University School of Medicine, No. 21-51-4960. Descendants of F344/DuCrj rats purchased from CLEA Japan Inc. (Tokyo, Japan) for our previous study[9] were used to establish the ER and control rat (CR) groups for this study. All rats used in the present study were 18th-generation descendants of the originally established ER and CR groups, maintaining the lines within treatments (*i.e.*, parents of ERs were ERs, parents of CRs were CRs).

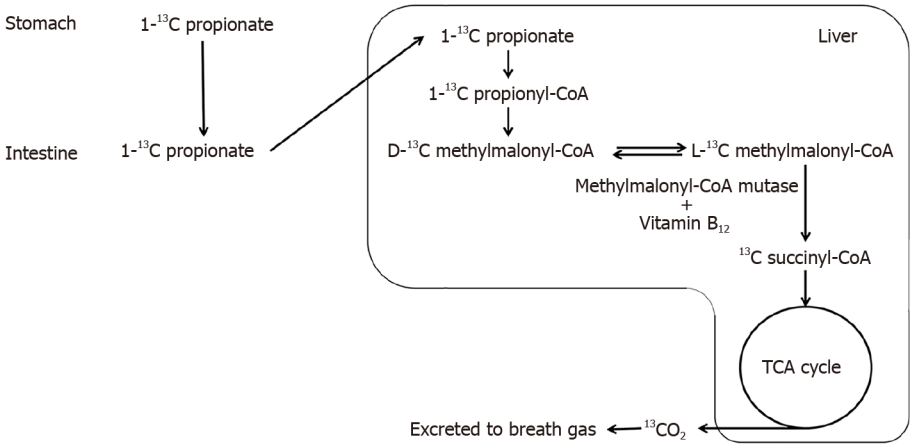

Figure 1 Propionate metabolism and the measuring principle of the propionate breath test. TCA: Tricarboxylic acid. Citation: Sasaki Y, Sato T, Maeda T, Komatsu F, Kawagoe N, Imai T, Shigeta T, Kashima N, Urita Y. [In Press] Evaluation of the One-Hour ^{13}C-Propionate Breath Test in 49 Patients from a Single Center in Japan to Detect Vitamin B_{12} Deficiency. *Med. Sci. Monit.* 2023 [DOI: 10.12659/MSM.940238]. Copyright © The Authors 2023. Published by Medical Science Monitor, International Scientific Information, Inc.

All rats were housed with their mothers until weaning at 4 wk of age. Subsequently, all rats were individually housed in a controlled environment (temperature, 23 ± 2 °C; humidity, 55% ± 5%) and provided a standard diet (CE-7; CLEA Japan Inc., Tokyo, Japan) and drinking liquid *ad libitum*. In the ER group, a 16% ethanol solution (Japanese Sake, Ozeki Corporation, Hyogo, Japan) was provided as a substitute for water by replacing the content of water bottles with ethanol solution in all cages of ERs.

A total of 16 ERs (8 males and 8 females) and 16 CRs (8 males and 8 females) aged 27-30 wk were used in the experiments; ERs continuously consumed alcohol for 23-27 wk. We used available descendants of F344/DuCrj rats that we had utilized in previous studies[9]. Therefore, we did not perform sample size calculation, randomization, or blinding.

Administration of ^{13}C-propionate and collection of exhaled gas

We purchased 1-^{13}C-sodium propionate from Cambridge Isotope Laboratories (Andover, MA, United States) and prepared a ^{13}C-propionate aqueous solution at 1 g/mL using distilled water immediately before administration. Body weight was measured immediately before administration. We performed gastrointestinal intubation in each rat and used a metal tubule, extending from the mouth to the stomach, to administer 0.1 mL/g of the ^{13}C-propionate solution. Immediately after administration, the rats were individually placed in the chambers of a dedicated exhaled-gas collection machine consisting of sealed chambers, pumps, and collecting bags, designed by Uchida *et al*[10]. We collected 100-200 mL of exhaled gas in the collecting bag for 90 s every 5 min for a total of 60 min.

Measurement of the ^{13}C recovery rate

Because ^{13}C-propionate is metabolized in the liver and exhaled as $^{13}CO_2$ (Figure 1), we measured the $^{13}CO_2/^{12}CO_2$ isotope ratio of the collected gas using infrared isotope spectrometry (POCone; Otsuka Electrics Co, Ltd., Hirakata, Japan) and monitored the change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), as reported previously[11].

Evaluation of serum VB_{12} and alanine transaminase levels

After collecting the exhaled gas for 60 min, the rats were immediately anesthetized *via* sevoflurane inhalation, and 5-10 mL of venous blood was collected from the inferior vena cava and the right atrium under laparotomy. After collecting sufficient blood samples, the animals were euthanized by rapid blood release. The blood was immediately centrifuged (relative centrifugal force: 1700 ×*g*) for 10 min, and the serum was collected. The serum was promptly frozen and submitted to FUJIFILM VET Systems Co. Ltd. (Tokyo, Japan) for measuring serum VB_{12} and ALT levels *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively.

Statistical analyses

We analyzed $\Delta^{13}CO_2$‰ measured every 5 min after ^{13}C-propionate administration for 60 min and serum VB_{12} levels, comparing the sexes and the ER and CR groups. The normality of the distribution of all variables was evaluated using the Kolmogorov-Smirnov test, and differences between groups were compared using the *t*-test and Mann-Whitney U test for normally and non-normally distributed variables, respectively. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using Stata/IC software (version 15.1; Stata Corp., College Station, TX, United States). We used R 4.2.0 for construction of graphics[12]. The statistical methods were reviewed by Yosuke Sasaki from the Toho

University School of Medicine (the first author). As Yosuke Sasaki has completed several certified biostatistics courses, we did not obtain additional biostatical review suggestions by external biomedical statisticians.

RESULTS

Influence of ethanol and sex on body weight

Body weight was significantly higher ($P < 0.0001$) in males (335.8 ± 37.0 g) than in females (176.6 ± 21.9 g). In addition, body weight was significantly higher in CRs than in ERs for both males and females ($P = 0.0082$ and $P = 0.005$, respectively, Table 1).

Sex-related difference in PBT results

The $\Delta^{13}CO_2$ reached its peak (C_{max}) at 20 min and 30 min in females and males, respectively. The $\Delta^{13}CO_2$ decreased after 20-30 min without rebound in both groups (Table 2 and Figure 2). Therefore, the overall trends in $\Delta^{13}CO_2$ over time were similar between males and females, although C_{max} was delayed in males and $\Delta^{13}CO_2$ was significantly higher in males at 30 min and thereafter (Table 2 and Figure 2). The C_{max} and $\Delta^{13}CO_2$ values between 15 and 45 min were significantly higher in males than in females ($P < 0.05$, Table 2). Considering these sex-based differences, we further compared the effects of ethanol in males and females separately.

Effects of ethanol on propionate metabolism

Propionate metabolism was accelerated in the ERs relative to that in the CRs in males (Figure 3), with $\Delta^{13}CO_2$ at 10 and 20 min being markedly higher in male ERs. However, $\Delta^{13}CO_2$ after 40 min was higher in the CR group (Table 3). The $\Delta^{13}CO_2$ reached C_{max} earlier in the ERs (at 20 min) than in the CRs (at 30 min). These findings suggest that ethanol promoted propionate metabolism in male rats. However, propionate metabolism was similar between the ER and CR groups among females, without any significant differences ($P > 0.110$ for all pairs, Table 3, Figure 4).

Effects of ethanol on serum VB_{12} and ALT levels

The serum VB_{12} levels were significantly higher in males than in females ($P = 0.0013$, Table 4); however, no significant differences were observed between the ER and CR groups for either sex ($P > 0.05$ for all pairs, Table 4). In contrast, serum ALT levels were significantly higher in male CRs than in male ERs ($P = 0.0347$, Table 5).

DISCUSSION

In this study, we compared propionate metabolism using a faster PBT in rats and compared serum VB_{12} and ALT levels between males and females and between ER and CR groups. Overall, our study demonstrates that (1) The faster PBT is useful for evaluating differences in propionate metabolism after administration of a ^{13}C-propionate solution; (2) Males show greater propionate metabolism, with higher serum VB_{12} levels, than females; (3) Ethanol consumption promotes propionate metabolism in male rats only; and (4) Ethanol consumption reduces body weight and serum ALT levels.

In the faster PBT, $\Delta^{13}CO_2$ peaked at 30 min, then decreased over time without rebound in all groups. Accordingly, we consider the faster PBT, which is completed within only 60 min after ^{13}C propionate administration, to be sufficiently sensitive to evaluate propionate metabolism, as a substitute for the conventional PBT that requires collecting exhaled gas for 2 h.

Using the PBT, our study showed a higher C_{max} and $\Delta^{13}CO_2$ between 15 and 45 min in male rats than in female rats, which suggests that male rats have stronger propionate metabolism. Suppression of carbohydrate metabolism and promotion of lipid metabolism by estrogen in females have been proposed as mechanisms contributing to lower carbohydrate metabolism in females than in males[13]. Furthermore, a protective effect of estrogen against VB_{12} deficiency in fertile females has been reported, along with higher susceptibility to VB_{12} deficiency in postmenopausal women[8,14]. Considering that VB_{12} works as a coenzyme of methylmalonyl-CoA mutase, and that serum VB_{12} levels were not pathologically low in the rats used in our study, we postulate that the lower propionate metabolism detected by the faster PBT and the lower serum VB_{12} levels in females than in males may reflect underlying physiological sex-related differences in carbohydrate metabolism associated with estrogen.

As we aimed to use the faster PBT to evaluate impaired propionate metabolism due to VB_{12} deficiency and liver disease caused by chronic alcohol consumption, we expected to find lower propionate metabolism and higher serum ALT levels in the ER group than in the CR group. However, we obtained contrasting results, with acceleration of propionate metabolism in the ER group and higher serum ALT levels in the CR group. Changes in the gut flora caused by chronic alcohol consumption may explain the promotion of propionate metabolism in the ER group. Using male marmosets, Zhu *et al*[15] reported

Table 1 Body weight (g) in the ethanol-fed rat and control rat groups

	ER (*n* = 16)	CR (*n* = 16)	*P* value
Male (*n* = 16)	313.0 (18.4)	358.5 (37.5)	0.0082[a]
Female (*n* = 16)	162.5 (14.9)	190.6 (18.7)	0.005[a]

[a]*P* < 0.05.

Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

Table 2 Propionate breath test results in male and female rats

	Male (*n* = 16)	Female (*n* = 16)	*P* value
C_{max}	1478.0	1302.3	0.039[a]
5 min	381.8	412.5	0.358
10 min	838.6	837.4	0.983
15 min	1259.2	1114.1	0.049[a]
20 min	1455.7	1238.2	0.011[a]
25 min	1471.3	1267.9	0.007[a]
30 min	1418.5	1248.2	0.008[a]
35 min	1330.6	1197.1	0.010[a]
40 min	1240.3	1136.5	0.013[a]
45 min	1134.5	1075.9	0.035[a]
50 min	1044.7	994.6	0.050
55 min	959.9	918.8	0.083
60 min	884.2	845.6	0.309

[a]*P* < 0.05.

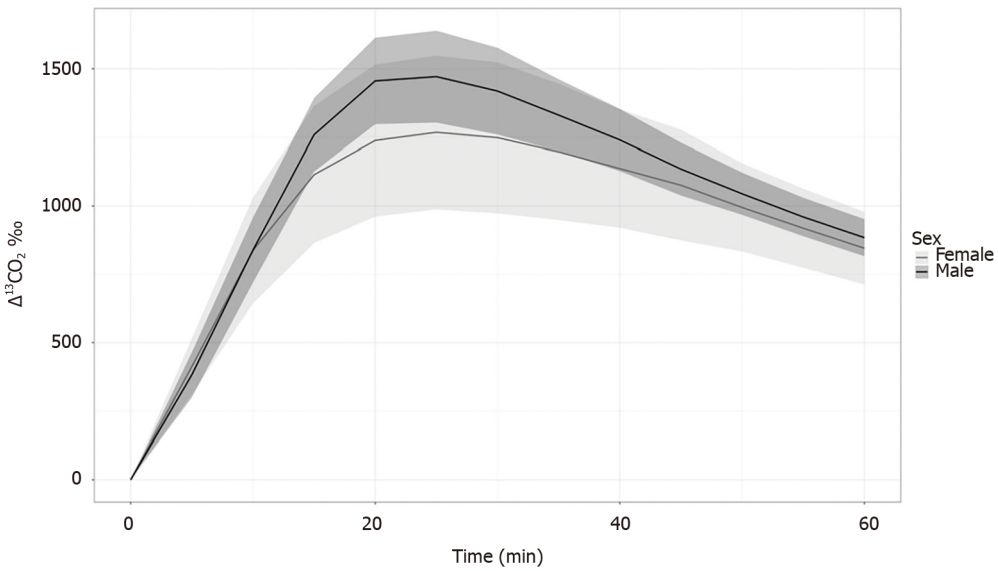

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Figure 2 Comparison of faster propionate breath test results between the male and female rats. Note: Tinted area around each line indicates the standard deviation range.

Table 3 Propionate breath test results in the ethanol-fed rat and control rat groups

	Male			Female		
	CR (*n* = 8)	ER (*n* = 8)	*P* value	CR (*n* = 8)	ER (*n* = 8)	*P* value
C_{max}	1422.8	1533.1	0.192	1366.1	1238.5	0.401
5 min	345.8	417.9	0.080	410.8	414.2	0.950
10 min	752.3	924.9	0.0008[a]	867.6	807.3	0.552
15 min	1186.1	1332.5	0.023[a]	1203.2	1025	0.160
20 min	1402.3	1509.1	0.186	1324.7	1151.7	0.223
25 min	1402.1	152.6	0.235	1334.5	1201.4	0.462
30 min	1379.9	1457	0.348	1296.8	1199.7	0.753
35 min	1313.9	46	0.629	1237.2	1157.1	0.753
40 min	1223.2	1257.3	0.565	1179.2	1093.8	0.529
45 min	1126.6	1142.3	0.756	1131.3	1020.6	0.208
50 min	1051.8	1037.5	0.724	1050.4	938.7	0.172
55 min	974.2	945.6	0.436	976.4	861.3	0.142
60 min	904.1	864.4	0.256	898.9	792.3	0.110

[a]$P < 0.05$.
CR: Control rat; ER: Ethanol-fed rat.

Table 4 Effects of ethanol and sex on serum vitamin B_{12} (pg/mL)

	Male (*n* = 16)	Female (*n* = 16)	*P* value[1]	Total (*n* = 32)
All (*n* = 32)	884.0 (124.8)	728.5 (123.3)	0.0013[a]	-
ER (*n* = 16)	878.0 (152.5)	696.3 (152.5)	0.0323[a]	787.1 (175.1)
CR (*n* = 16)	890.0 (100.2)	760.8 (81.7)	0.0134[a]	825.4 (110.7)
P value[2]	0.8551	0.3117	-	0.4658

[1]Comparison of males *vs* females.
[2]Comparison of Ethanol-fed rat *vs* Control rat.
[a]$P < 0.05$.
Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

Table 5 Serum alanine transaminase (IU/L) levels

	ER (*n* = 16)	CR (*n* = 16)	*P* value
Total (*n* = 32)	55.7 (16.1)	79.3 (39.5)	0.0347[a]
Male (*n* = 16)	65.0 (16.8)	110.3 (32.3)	0.0034[a]
Female (*n* = 16)	46.4 (8.5)	48.3 (9.9)	0.6898

[a]$P < 0.05$.
Data are presented as mean (SD). CR: Control rat; ER: Ethanol-fed rat.

that the concentrations of short-chain fatty acids, including propionate, depend on changes in intestinal bacteria, based on an observed reduction in fecal propionate levels along with a reduction in the relative abundance of *Phascolarctobacterium* in the gut. Moreover, Watanabe *et al*[5] reported that the substrates of short-chain fatty acids, including propionate, produced by intestinal bacteria depend not only on a single bacterial strain, but also on the specific composition of other bacteria present in the gut. According to these reports, ethanol can serve as both a potential substrate of fatty acid production by

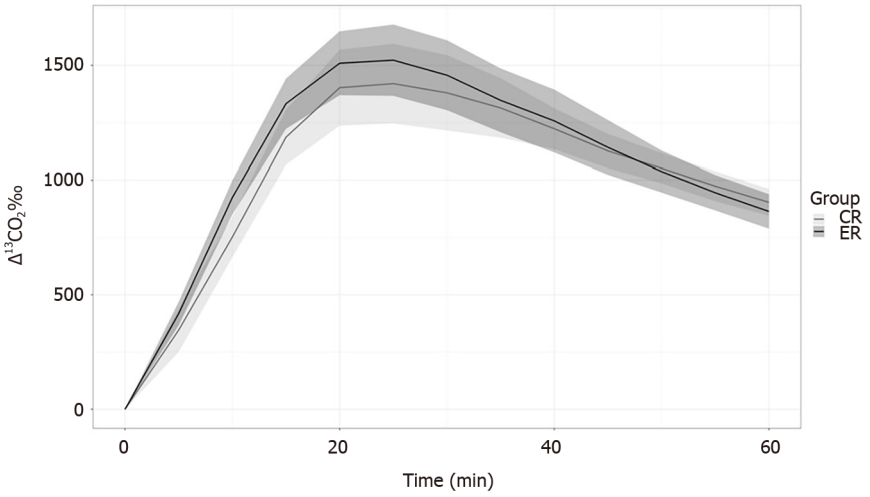

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Figure 3 Comparison of propionate breath test results between the male ethanol-fed rat and male control rat groups. Note: Tinted area around each line indicates the standard deviation range. CR: Control rat; ER: Ethanol-fed rat.

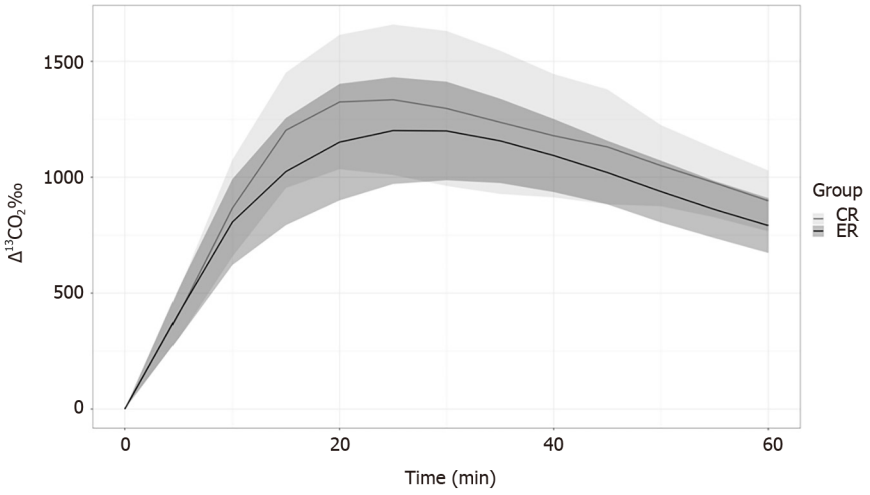

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Figure 4 Comparison of propionate breath test results between the female ethanol-fed rat and female control rat groups. Note: Tinted area around each line indicates the standard deviation range. CR: Control rat; ER: Ethanol-fed rat.

intestinal bacteria, such as *Phascolarctobacterium*, and as a trigger for changes in gut flora. Thus, we hypothesized that chronic alcohol consumption promotes propionate production both as a substrate for various fatty acids and as a trigger for changes in gut flora.

Alternatively, these observations may be due to the well-known difficulties in recapitulating the effects of chronic alcohol consumption in an animal model. We intended to establish a rat model of chronic alcoholism to evaluate the metabolic effect of ethanol consumption by oral administration of a 16% ethanol solution (corresponding to the level of alcohol commonly consumed by Japanese drinkers in the form of sake) for > 20 wk based on a previous study[16]. Therefore, we expected higher serum ALT levels in the ER group. Our contrasting result (lower serum ALT after 16% ethanol consumption) highlights the difficulty in the development of alcoholic animal models. A recent review on the utility of animal models for alcoholic liver disease mentioned that, in contrast to primates, rodent models fail to sufficiently display the full disease spectrum of alcoholic liver disease found in humans, despite many trials under various conditions[17]. The absence of craving in rats, owing to their natural aversion to ethanol[18,19], the faster ethanol catabolism in rodents than in humans[20], and differences in the innate immune systems of the species, have been proposed as the main factors contributing to the difficulty in establishing a useful rat model of human alcoholism[21]. It is therefore possible that our results also reflect failure to generate a chronic alcoholism rat model; thus, studies using primates or other small animals rather than rodents may be more appropriate. Considering the *ad libitum* diet administration, and the higher body weight in the CR group, fatty liver due to excessive dietary intake may explain the higher serum ALT levels in CRs. Because all of the rats consumed the same diet, it is possible that

consumption of 16% ethanol solution had a protective effect against liver damage. Given that propionate itself reportedly has protective effects against steatohepatitis[7], and that improvement of gut flora is an effective way to suppress liver damage[6], enhanced propionate metabolism and favorable changes in the gut flora might suppress liver damage in male ERs. As discussed earlier, the lack of acceleration in propionate metabolism in female ERs can be explained by sex-related differences in carbohydrate metabolism.

In addition to the lack of confirmation of the chronic alcoholism model, our study has other limitations. For instance, the serum methylmalonic acid (MMA) level, rather than the serum VB_{12} level, is required for the precise diagnosis of VB_{12} deficiency in humans[3]. However, we were not able to evaluate MMA levels because major domestic commercial laboratories no longer perform MMA testing of human serum or urine, and we could not find or access domestic laboratories measuring serum MMA in animal samples. Similarly, comparing the PBT results with biomarkers, such as aldehyde dehydrogenase and alcohol dehydrogenase, which sensitively and precisely reflect hepatic alcohol metabolism, may provide more information[22]. We believe that comparing levels of serum MMA and the markers evaluated using the faster PBT may provide further insight into the association between VB_{12} deficiency and alcoholism. Moreover, the present study only focused on the association between propionate metabolism and VB_{12} deficiency based on a previous study on PBT[3]. However, considering the complexity of intestinal propionate production due to the variety of propionate-producing bacteria, including *Clostridium* spp., *Veillonella* spp., *Fusobacterium* spp., *Salmonella ruminantium*, and *Propionibacterium* spp., and the complexity of substrates[23], the findings obtained herein, including the promoted propionate metabolism in male ERs and sex-related difference, may have potential clinical utility and provide a basis for future research into propionate metabolism and intestinal microbiota under various conditions. For instance, comparison of findings between faster PBT and the composition or changes in gut microbiota may provide interesting information on the association between gut microbiota and their products. Despite these limitations and lack of confirmation of VB_{12} deficiency under our experimental conditions, our study highlights the influence of ethanol and sex-related differences in propionate metabolism.

CONCLUSION

We evaluated a faster PBT in which C_{max} peaked within 30 min. This PBT could serve as a substitute for conventional PBT (which takes at least 2 h) for evaluating propionate metabolism and diagnosing VB_{12} deficiency. Although we could not evaluate the usefulness of faster PBT as a diagnostic modality for VB_{12} deficiency as initially intended because we failed to create a rat alcoholism model with VB_{12} deficiency, our study suggests that chronic consumption of 16% ethanol changed the composition of fatty acids produced by the intestinal flora, likely by changing the intestinal flora composition without causing corresponding liver injury. Considering the accumulating evidence of alteration of the gut flora as one of the mechanisms of alcoholism-related health impacts[24], our study demonstrates the potential utility of the faster PBT as a non-invasive and more convenient modality to evaluate changes in the gut flora associated with ethanol consumption and various other conditions.

ARTICLE HIGHLIGHTS

Research background

The ^{13}C-propionate breath test (PBT) has been studied as a non-invasive diagnostic modality for vitamin B_{12} (VB_{12}) deficiency by utilizing the role of VB_{12} as a coenzyme of methylmalonyl-CoA mutase in propionate metabolism. Although alcoholism has been regarded as a risk factor for deficiency, studies on propionate metabolism using the PBT in individuals with alcoholism is limited. Furthermore, conventional PBT requires up to 2 hours of breath collection time, which may undermine its clinical utility.

Research motivation

The scarcity of studies regarding the PBT in alcoholism, and the possibility of improving the clinical utility of the PBT by shortening the breath collection time, motivated us to perform this study.

Research objectives

The aim of this study was to evaluate the change in propionate metabolism due to long-term ethanol consumption in ethanol-fed rats (ERs) as an animal model of chronic alcoholism. We also aimed to evaluate the utility of a faster PBT that requires only 1 hour to collect breath.

Research methods

The ERs were 18th generation descendants of F344/DuCrj rats that had been bred by replacing standard drinking water with a 16% ethanol solution. A faster PBT was performed by injecting the ^{13}C-propionate aqueous solution from the mouth to the stomach of ERs and control rats (CRs); we collected exhaled gas in bags, and measured the $^{13}CO_2/^{12}CO_2$ isotope ratio using infrared isotope spectrometry. We measured serum VB_{12} and alanine transaminase (ALT) levels *via* chemiluminescence immunoassay and the lactate dehydrogenase-ultraviolet method, respectively. We evaluated statistical differences in mean body weight, change in $^{13}CO_2$ ($\Delta^{13}CO_2$‰), peak $\Delta^{13}CO_2$‰, and serum VB_{12} and ALT, between ERs and CRs, and males and females, respectively.

Research results

Besides male dominance of body weight ($P < 0.001$), CRs weighed significantly more than ERs ($P < 0.008$). The $\Delta^{13}CO_2$ reached a peak (C_{max}) within 30 min in both sex groups, while males had a significantly higher C_{max} and $\Delta^{13}CO_2$ at 15-45 min than females ($P < 0.05$; for all pairs). Enhanced propionate metabolism was observed in male ERs relative to male CRs, and although males had higher serum VB_{12} levels than females, no prominent differences were observed between the ER and CR groups. Male CRs had notably higher ALT levels than male ERs. These results suggest that chronic ethanol consumption may trigger fatty acid production *via* intestinal bacteria and changes in gut microbiome composition.

Research conclusions

We believe that a faster (1-h) PBT could serve as a substitute for the conventional PBT, as the $\Delta^{13}CO_2$ reached a peak (C_{max}) within 30 min in both sex groups. We failed to evaluate the usefulness of the faster PBT as a diagnostic modality for VB_{12} deficiency in the chronic alcoholism rat model; however, our study suggests that instead of inducing alcoholism, chronic consumption of 16% ethanol changed the composition of fatty acids produced by the intestinal flora.

Research perspectives

Our study demonstrates the potential utility of the faster PBT as a non-invasive and more convenient modality to evaluate changes in the gut flora associated with ethanol consumption and various other conditions *via* changes in propionate metabolism.

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FOOTNOTES

Author contributions: Sasaki Y and Urita Y coordinated the study; Sasaki Y, Kawagoe N, Imai T, and Urita Y performed the experiments; Sasaki Y and Urita Y acquired and analyzed the data; Sasaki Y and Urita Y interpreted the data; and Sasaki Y and Urita Y wrote the manuscript; All authors approved the final version of the article.

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ORIGINAL ARTICLE

Basic Study

Fibroblast growth factor 15, induced by elevated bile acids, mediates the improvement of hepatic glucose metabolism after sleeve gastrectomy

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Abstract

BACKGROUND

Fibroblast growth factor (FGF) 15/19, which is expressed in and secreted from the distal ileum, can regulate hepatic glucose metabolism in an endocrine manner. The levels of both bile acids (BAs) and FGF15/19 are elevated after bariatric surgery. However, it is unclear whether the increase in FGF15/19 is induced by BAs. Moreover, it remains to be understood whether FGF15/19 elevations contribute to improvements in hepatic glucose metabolism after bariatric surgery.

AIM

To investigate the mechanism of improvement of hepatic glucose metabolism by elevated BAs after sleeve gastrectomy (SG).

METHODS

By calculating and comparing the changes of body weight after SG with SHAM group, we examined the weight-loss effect of SG. The oral glucose tolerance test (OGTT) test and area under the curve of OGTT curves were used to assess the anti-diabetic effects of SG. By detecting the glycogen content, expression and activity of glycogen synthase as well as the glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (Pepck), we evaluated the hepatic glycogen content and gluconeogenesis activity. We examined the levels of total BA (TBA) together with the farnesoid X receptor (FXR)-agonistic BA subspecies in systemic serum and portal vein at week 12 post-surgery. Then the histological expression of ileal FXR and FGF15 and hepatic FGF receptor 4 (FGFR4) with its corresponding signal pathways involved in glucose metabolism were detected.

RESULTS

After surgery, food intake and body weight gain of SG group was decreased compare with the SHAM group. The hepatic glycogen content and glycogen synthase activity was significantly stimulated after SG, while the expression of the key enzyme for hepatic gluconeogenesis: G6Pase and Pepck, were depressed. TBA levels in serum and portal vein were both elevated after SG, the FXR-agonistic BA subspecies: Chenodeoxycholic acid (CDCA), lithocholic acid (LCA) in serum and CDCA, DCA, LCA in portal vein were all higher in SG group than that in SHAM group. Consequently, the ileal expression of FXR and FGF15 were also advanced in SG group. Moreover, the hepatic expression of FGFR4 was stimulated in SG-operated rats. As a result, the activity of its corresponding pathway for glycogen synthesis: FGFR4-Ras-extracellular signal regulated kinase pathway was stimulated, while the corresponding pathway for hepatic gluconeogenesis: FGFR4-cAMP regulatory element-binding protein- peroxisome proliferator-activated receptor γ coactivator-1α pathway was suppressed.

CONCLUSION

Elevated BAs after SG induced FGF15 expression in distal ileum by activating their receptor FXR. Furthermore, the promoted FGF15 partly mediated the improving effects on hepatic glucose metabolism of SG.

Key Words: Sleeve gastrectomy; Fibroblast growth factor 15; Bile acids; Hepatic glucose metabolism; Type 2 diabetes mellitus

Core Tip: Sleeve gastrectomy (SG) improves hepatic glucose metabolism and alleviates type 2 diabetes mellitus through the intestine-liver crosstalk mediated by fibroblast growth factor 15 (FGF15). Following SG, bile acids are elevated, inducing the expression and secretion of FGF15 *via* the activation of farnesoid X receptor in the ileum. FGF15 then acts as an endocrine factor to promote glycogen synthesis and inhibit gluconeogenesis in the liver by specifically stimulating hepatic FGF receptor 4 and its corresponding signaling pathways.

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INTRODUCTION

The incidence of obesity along with the relative metabolic abnormalities[1], especially type 2 diabetes mellitus (T2DM)[2], is on the rise globally. Bariatric surgery becomes the most efficient treatment option for obese patients with diabetes[3] due to its prominent weight reduction and durable metabolic improvement effect[4,5]. Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are the most commonly performed surgical procedures currently[3]. Moreover, SG has now exceeded RYGB as the preferred approach for most surgeons[6,7]. Still, the mechanisms underlying effects of bariatric surgery remain to be fully elucidated.

Bile acid (BA) has been considered as prospective factor for the metabolic actions of bariatric surgery [8] because of the considerable significance of its receptors, Takeda G protein-coupled receptor 5[9] and farnesoid X receptor (FXR)[10]. Through intestinal FXR, BAs could induce the expression and secretion of fibroblast growth factor (FGF) 15/19 in the distal ileum[11,12]. Recent studies illustrate that both BAs and FGF19 -the human ortholog of rodent FGF15[13]- are elevated after bariatric surgery[14-17]. Hence, the elevation of FGF19 after bariatric surgery probably results from the induction of FXR by elevated BAs, which needs further investigation. Moreover, FGF15/19 also plays a role in regulating the metabolic homeostasis, especially the hepatic glucose metabolism[18]. Similar to insulin's actions, FGF15/19 could act to lower the blood glucose level by repressing gluconeogenesis[19] and promoting glycogen synthesis[20] in the liver. Taken together, the ileal FXR-FGF15/19 pathway triggered by BAs might be another potential mechanism underlying the metabolic effects of bariatric surgery. However, to prove this, we need to confirm that the elevation of FGF15/19 results from the induction of FXR by elevated BAs and FGF15/19 participates in the improvement of hepatic glucose metabolism after

bariatric surgery.

As insulin and FGF15/19 can exert comparable metabolic actions on liver, only to detect the activities of glycogen synthesis and gluconeogenesis of liver could not distinguish the action of FGF15/19 from insulin. Previous studies demonstrated that FGF15/19 functions in an insulin-independent manner through activating FGF receptor 4 (FGFR4)[21] and its corresponding signal pathways. FGF15/19 could promote glycogen synthesis by activating the Ras- extracellular signal regulated kinase (ERK) pathway [20] and inhibit hepatic gluconeogenesis by suppressing the cAMP regulatory element-binding protein (CREB)- peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) pathway[19]. However, insulin induces these effects through phosphoinositide 3-kinase (PI3K)-Akt pathway. The signaling pathways engaged in regulation of hepatic glucose metabolism by FGF15/19 and insulin permit overlapping, but can distinct the biological effects of these two hormones. Hence, to detect the expression of FGFR4 and the activities of its corresponding signaling pathways after bariatric surgery would help to confirm the action of FGF15/19 on hepatic glucose metabolism.

We conducted SHAM and SG operations on an obese diabetic rat model induced by high-fat diet (HFD) and streptozotocin (STZ) in this study. BAs levels in serum and portal vein, and the ileal expression of FXR and FGF15 were detected to confirm the elevation of the FGF15 as well as the underlying mechanism after surgery. Moreover, we detected the hepatic expression of FGFR4 and the activity of its corresponding signaling pathways involved in glucose metabolism to investigate whether FGF15 mediates the improvement of hepatic glucose metabolic after SG.

MATERIALS AND METHODS

Animals

We used Wistar rats in this study, and the rats (8-wk-old, 200 g on average) were purchased and housed in Laboratory Animal Center of Shandong University (Jinan, Shandong Province, China). The obese diabetic rat model was induced as previously reported[22,23]. The rats received a HFD (40% fat, 42% carbohydrate, 18% protein, Huafukang Biotech, China) for 1 mo, followed by low dose intraperitoneal STZ injection (35 mg/kg, Sigma, United States). The glucose level of peripheral blood obtained from tail vein was tested using a glucometer (Roche Diagnostics, Germany). Diabetes induction was confirmed based on the Oral Glucose Tolerance Test (OGTT) results and blood glucose levels examined at random time-points (≥ 16.7 mmol/L). The diabetic rats then randomly divided into matched SHAM (n = 10) and SG (n = 10) group. All actions and interventions applied on rats were approved and guided by Laboratory Animal Ethical and Welfare Committee of Shandong University Cheeloo College of Medicine.

Before operation, rats were fasted for about 12 h after two-days feeding of 10% Ensure (Abbott, United States). Moreover, anesthesia for rats was induced by 10% chloral hydrate (3 mL/kg).

Surgical procedures

SG: First, the relevant vessels and gastro-epiploic structures were ligated and transected to externalize the greater curvature of the stomach. Then, we conducted gastrectomy of 80% of the whole stomach from greater curvature, including the whole gastric fundus. The residual stomach was closed and continuously sutured with a 5-0 silk suture (Ningbo medical, China). The cardia, pylorus of stomach and continuity of stomach with esophagus and duodenum were all remained unaffected.

SHAM: In order to minimize the concomitant influence and bias triggered by surgical operation and anesthesia, we set SHAM group as previously reported. First, we isolated the greater curvature of stomach similarly as what we did in SG group. Then we clamped the stomach along the greater curvature with a pair of blunt forceps between cardia and pylorus to exert a similar pressure on the stomach. The exposed area and time of surgical field were extended as that of SG group to eliminate the undeserving influence of operation and anesthesia.

Postsurgical care: After surgery, the rats were only supplied with some water during the first 24 h. Then following 3-d feeding of 10% Ensure, the rats resumed the HDF diet till the end of the study. We recorded the body weight and calorie intake of every rat in both groups every day. Antibiotics were unnecessary and all rats in both groups survived and were sacrificed until the end of the study (week 12 post-surgery).

OGTT

We conducted OGTTs preoperatively to confirm diabetes induction and to evaluate the anti-diabetic effects of SG at week 12 post-surgery. After 12-h fasting, the rats received 20% glucose (1 g/kg) chow *via* oral gavage. Blood glucose levels were measured at time point of 0 min, 10 min, 30 min, 60 min, and 120 min after administration.

Detection of BAs

At 12 wk after surgery, total BAs (TBA) levels were examined in both the systemic serum and portal vein. In addition, the specific levels of the following four FXR-agonist BA species were also examined: Chenodeoxycholic acid (CDCA), DCA, lithocholic acid (LCA), and cholic acid (CA). One hour before sacrificed, all rats were gavaged with 10% Ensure after 12-h fasting. When sacrificing the rats, we collected blood samples from the retrobulbar venous plexus and portal vein. Portal and systemic serum levels of TBA were detected on the Roche Cobas 8000 system using the enzyme cycling method. As previously described[22,23], the levels of BA species (CDCA, DCA, LCA, and CA) in these samples were evaluated using high-pressure liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). Standard substrates for these four BA species were purchased from Sigma Aldrich (United States).

Histological analysis of ileum and liver tissues

After sacrificing, ileum and liver tissues in both groups were divided into three parts. Some were fixed in 4% paraformaldehyde and then embedded in paraffin for Periodic Acid-Schiff (PAS) staining and immunohistochemistry staining; some were stored at -80 °C for western blotting and hepatic glycogen content detection; and the others were stored in RNA*later*™ Stabilization Solution (Thermo Fisher Scientific™) for real-time PCR.

Real-time PCR

The levels of FGF15, FXR in ileum and FGFR4, glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (Pepck) in liver were measured using RT-PCR. Total RNA was extracted from the tissue using TRIzol reagent (Invitrogen, United States) and then reversely transcribed to cDNA by the High Capacity cDNA Reverse Transcription Kit (TOYOBO, Japan). Subsequently, the relative level of mRNA amplification was determined through the SYBR Green Real-time PCR Master Mix Kit (TOYOBO, Japan) according to manufacturer's instructions. The following primers were used for analysis: FGF15: 5′-GCCATCAAGGACGTCAGCA-3′(F), 5′-CTTCCTCCGAGTAGCGAATCAG-3′ (R); FXR: 5′-TCCGGACATTCAACCATCAC-3′ (F), 5′-TCACTGCACATCCCAGATCTC-3′ (R); FGFR4: 5′-GGCCAGGTATACGGACATCA-3′ (F), 5′-GAGTCAGGCTGTCACATGTG-3′ (R); G6Pase: 5′-GTGGCAGTGGTCGGAGACT-3′ (F), 5′-ACGGGCGTTGTCCAAAC-3′ (R); Pepck: 5′-CACCATCACCTCCTGGAAGA-3′ (F), 5′-GGGTGCAGAATCTCGAGTTG-3′ (R).

Glycogen detecting and immunohistochemistry staining

We detected the hepatic glycogen content using the Glycogen Assay kit (ab65620, Abcam, United States) according to manufacturer's protocol. Sections of the paraffin-embedded tissues were prepared for PAS and immunohistochemistry staining. We stained the glycogen in hepatocytes with the PAS Stain Kit (Mucin Stain) (ab150680, Abcam, United States) according to the manufacturer's protocol. And we also examined the ileal expression of FGF15 and hepatic expression of FGFR4 by immunohistochemistry staining. The antibody for FGF15 (sc-16816) and FGFR4 (sc-136988) were both purchased from Santa Cruz Biotechnology (United States). Percentage of PAS- positive cells was calculated and quantification of immunoreactive signal was performed using Image J software.

Western blotting

We detected the hepatic expression of ERK1/2, p-ERK1/2, glycogen synthase (GS), p-GS, CREB, p-CREB and PGC-1α by western blotting. Anti-ERK1/2, anti-p-ERK1/2, anti-GS, anti-p-GS, anti-CREB, and anti-p-CREB for western blotting were purchased from Cell Signaling Technology (CST, United States), and anti-PGC-1α, anti-Actin were purchased from Abcam (United States).

Statistical analysis

All quantitative data are presented in the pattern of mean ± SEM. The area under the curve (AUC) was evaluated using trapezoidal integration. We used unpaired Student's *t* test to analyzed statistical significance, and $P < 0.05$ indicated statistical significance. All statistical analyses were performed by SPSS version 20.0.

RESULTS

Weight loss and anti-diabetic effects of SG

According to our previous studies, we evaluated the weight-loss and anti-diabetic effects of SG postoperatively. First, we assessed the weight-loss action by tracking the body weight and daily food intake of the rats in SHAM and SG group. After a sharp decrease during the first week after surgery, the body weight and food intake gradually increased in both groups. Since week 4 post-surgery, the increase of body weight and food intake in SG group became significantly slower than that in SHAM group (Figure 1A and B). And the body weight gain of SG group was correspondingly lower than that

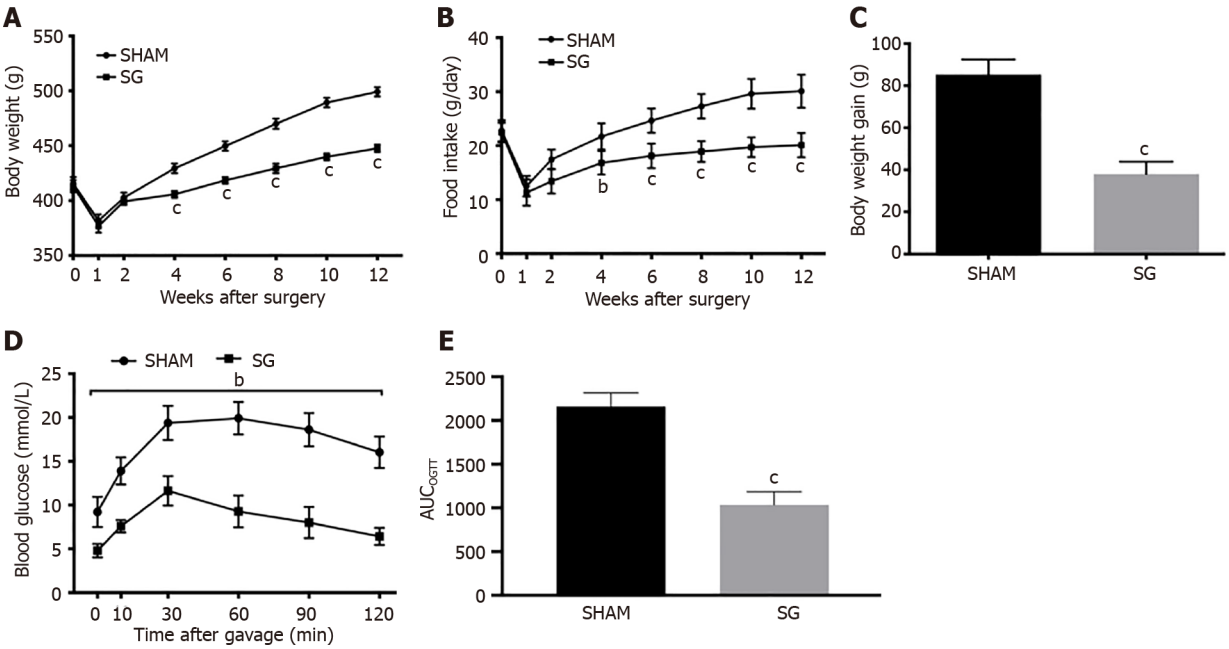

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Figure 1 The weight-loss and anti-diabetic effects of sleeve gastrectomy. A: Body weight; B: Food intake; C: Body weight gain after surgery; D: The oral glucose tolerance test (OGTT) curves; E: Area under the curve for OGTT curves of SHAM and sleeve gastrectomy (SG) group at week 12 postoperation. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$; SG *vs* SHAM group. SG: Sleeve gastrectomy.

in SHAM group till week 12 post-surgery (Figure 1C). Then, we conducted the OGTT in both groups to evaluate the anti-diabetic effects of SG. The OGTT curves and result of AUC$_{OGTT}$ showed much better glucose tolerance in SG group than SHAM group (Figure 1D and E), which indicated a considerable anti-diabetic effect of SG.

Effects of SG on BA levels and ileal FGF15, FXR expressions

We detected the TBA levels both in systemic serum and portal vein at week 12 post-surgery, together with four BA species: CDCA, DCA, LCA, and CA, which are all FXR agonists. TBA levels of SG group in serum and portal vein were significantly higher than that in SHAM group (Figure 2A and B). Both CDCA and LCA levels in these two kinds samples were elevated and the portal vein DCA level was also higher in SG group (Figure 2C and D).

Then, we detected the influence of SG on FGF15 and FXR expressions in distal ileum. Both mRNA and protein expression levels of FGF15 in SG group were significantly higher than that in SHAM group (Figure 3A and D; Figure 4). Also, FXR mRNA level was also considerably elevated after SG (Figure 4).

SG significantly improved the hepatic glucose metabolism

To evaluate the hepatic glucose metabolism, we detected the activities of hepatic glycogen synthesis and gluconeogenesis, together with the glycogen content in both groups after SG. The expression of glycogen synthase (GS) was significantly higher in the SG-operated rats, while the phosphorylation degree of GS (p-GS) was declined (Figure 5). In other words, the hepatic glycogen synthetic activity was significantly promoted after SG. Correspondingly, the glycogen content and the percentage of PAS-positive cells in liver were significantly higher in SG-operated rats (Figures 2A, 3B and E).

We then measured two key enzymes within hepatic gluconeogenesis, G6Pase and Pepck mRNA levels of both enzymes were significantly declined in SG group compared with SHAM group, which illustrated an inhibited gluconeogenesis process in SG-operated rats (Figure 4).

SG activated the receptor and regulated activities of the corresponding signal pathways of FGF15

FGF15/19 can activate its receptor, FGFR4 in the liver with unique specificity[25]. The expression of FGFR4 was significantly promoted at both mRNA and protein levels in SG group (Figure 4). FGF15-FGFR4 regulates hepatic glycogen synthesis and gluconeogenesis through Ras-ERK pathway and CREB-PGC-1α pathway, respectively. ERK1/2 regulates the phosphorylation degree of GS. We detected no difference in the expression of total ERK1/2 protein level. Whereas, the phosphorylation of ERK1/2 was increased in SG-operated rats, which indicates the activation of ERK1/2 pathway (Figure 5). On the contrary, the declined phosphorylation degree of CREB and decreased expression of PGC-1α (Figure 5) together with the inhibition of G6Pase and Pepck (G6Pase and Pepck are two target genes of PGC-1α) illustrated the inhibition of CREB-PGC-1α pathway in SG group.

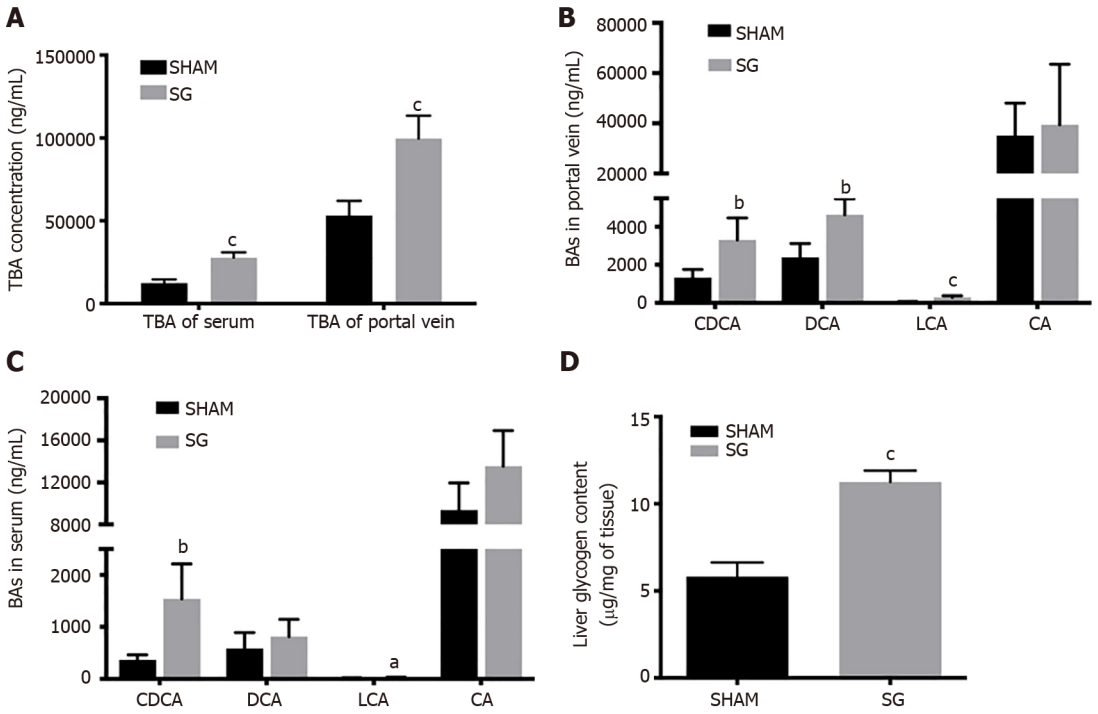

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Figure 2 Sleeve gastrectomy increased the bile acids levels and hepatic glycogen content. A: The concentration of total bile acid; B and C: The levels of chenodeoxycholic acid, DCA, lithocholic acid, and cholic acid in serum and portal vein, respectively; D: The glycogen content in liver at week 12 postoperation in SHAM and SG group. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$; SG *vs* SHAM group. BA: Bile acid; TBA: Total bile acid; SG: Sleeve gastrectomy; CDCA: Chenodeoxycholic acid; LCA: Lithocholic acid; CA: Cholic acid.

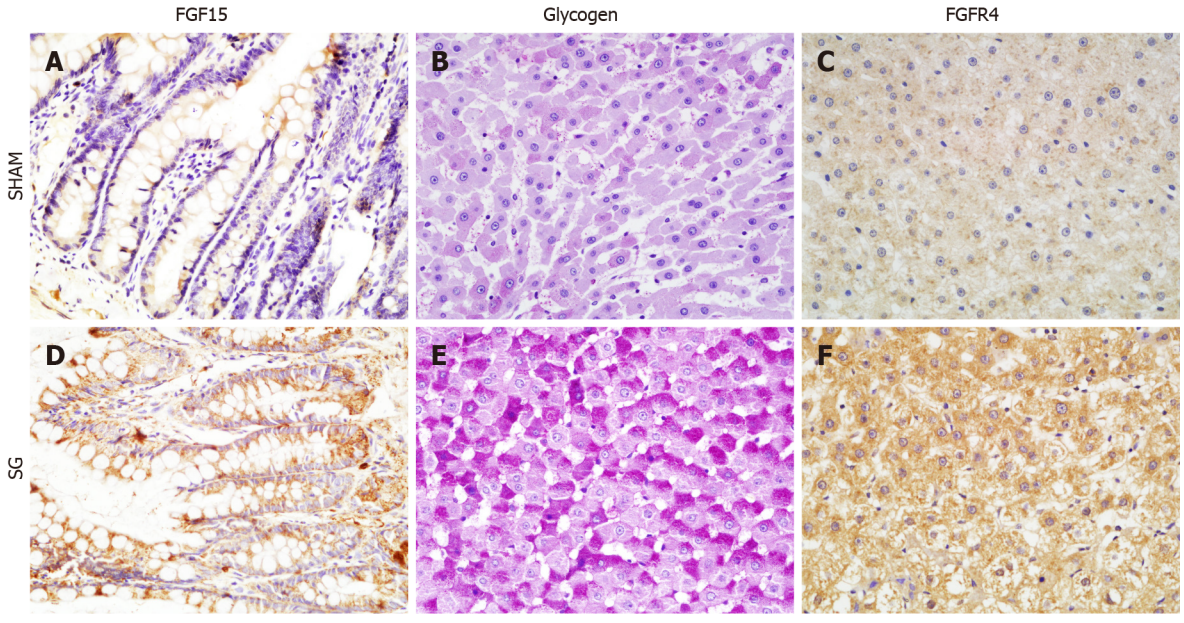

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Figure 3 Sleeve gastrectomy promoted fibroblast growth factor 15, fibroblast growth factor receptor 4 expression and hepatic glycogen synthesis. A and D: The result of immune-histochemistry staining for ileal fibroblast growth factor (FGF) 15; B and E: The result of Periodic Acid-Schiff staining for glycogen in hepatocytes; C and F: Immune-histochemistry staining of hepatic FGF receptor 4 in SHAM and sleeve gastrectomy group at week 12 postoperation. Scale bar: 100 μm. SG: Sleeve gastrectomy; FGF: Fibroblast growth factor; FGFR4: Fibroblast growth factor receptor 4.

DISCUSSION

In this study, SG induced considerable weight loss and significantly improved the glucose homeostasis, especially the hepatic glucose metabolism in an obese diabetic rat model. Levels of TBA and FXR-

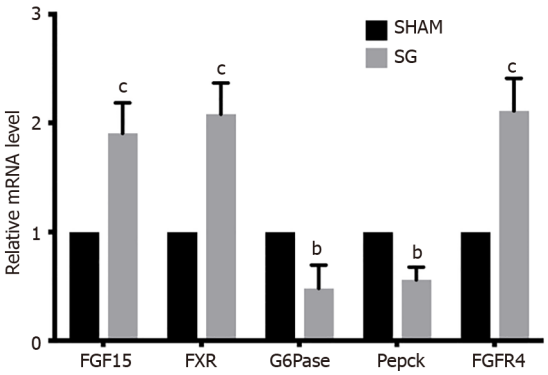

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Figure 4 Farnesoid X receptor- fibroblast growth factor 15-fibroblast growth factor receptor 4 were stimulated and hepatic gluconeogenesis were depressed after sleeve gastrectomy. The mRNA relative expression of ileal fibroblast growth factor (FGF) 15, farnesoid X receptor, and hepatic glucose-6-phosphatase, phosphoenolpyruvate carboxykinase and fibroblast growth factor receptor 4 in SHAM and sleeve gastrectomy (SG) group at week 12 postoperation. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$, SG *vs* SHAM group. SG: Sleeve gastrectomy; FXR: Farnesoid X receptor; FGF: Fibroblast growth factor; FGFR4: Fibroblast growth factor receptor 4; Pepck: Phosphoenolpyruvate carboxykinase; G6Pase: Glucose-6-phosphatase.

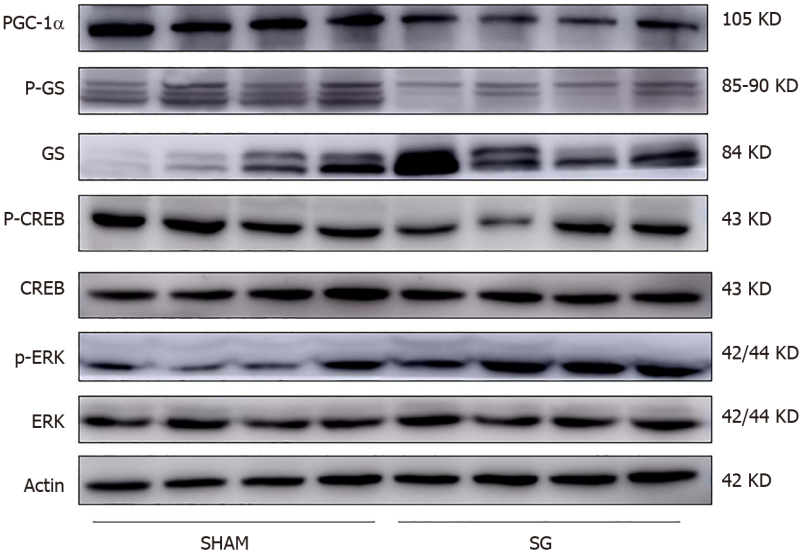

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Figure 5 Sleeve gastrectomy regulated activities of the corresponding pathways of fibroblast growth factor 15. Western blotting for expression of extracellular signal regulated kinase (ERK), p-ERK, cAMP regulatory element-binding protein (CREB), p-CREB, glycogen synthase (GS), p-GS, and proliferator-activated receptor γ coactivator-1α (PGC-1α) in the liver of both SHAM and sleeve gastrectomy group at week 12 postoperation. PGC-1α: Proliferator-activated receptor γ coactivator-1α; ERK: Extracellular signal regulated kinase; CREB: cAMP regulatory element-binding protein; GS: Glycogen synthase; SG: Sleeve gastrectomy.

agonistic BA subspecies were all increased in the serum of both peripheral circulation and portal vein. Besides, the expression of FGF15 which was induced by FXR activation in the ileum was increased after SG. And the FGF15 receptor FGFR4 in the liver together with its corresponding signal pathways involved in hepatic glycogen synthesis and gluconeogenesis were also activated.

Liver plays a key role in the metabolic regulation of the whole body, especially in maintaining homeostasis of blood glucose level through synthesis or breaking down of glycogen and the action of gluconeogenesis. One of the insulin's actions is to promote glycogen synthesis and inhibit hepatic gluconeogenesis which is impaired in diabetic patients. While, we found that SG improved the glucose tolerance of diabetic rats in this study, the hepatic glycogen content was elevated and the expression of two key enzymes[19] (G6pase and Pepck) responsible for hepatic gluconeogenesis were decreased in the SG-operated rats. In other words, SG increased the insulin sensitivity by promoting the synthesis of glycogen and inhibiting the hepatic gluconeogenesis. Moreover, recent studies found that FGF15/19 could also exert the similar effects as insulin on hepatic metabolism in an insulin-independent pattern [19,20]. Furthermore, the serum FGF19 Level of diabetic patients was elevated after bariatric surgery[14-17]. Therefore, except the improvement of insulin sensitivity, the improvement of glucose tolerance post-surgery may also be partly mediated by the elevated FGF15/19.

Consistent with previous findings of FGF 19 in human, the ileal expression of FGF15 was increased in SG-operated rats in our study and the TBA levels in systemic serum and portal vein were also increased after SG. This may inconsistent with previous reports that FGF15 acts as an endocrinal factor to repress BA synthesis in the liver[11]. However, our previous studies have already proved that the elevation of serum BA level was mainly a result of the promoted ileal reabsorption rather than hepatic synthesis[22,23]. According to the process of the enterohepatic circulation, systemic serum BAs is a combinational result of hepatic uptake and ileal reabsorption of BAs and portal vein BAs levels can reflect the ileal reabsorption and the BA concentration of distal ileum[26,27]. Hence, the elevation of TBAs in systemic serum and portal vein in this study must be a result of the promoted ileal reabsorption. Then, we also explored the cause for the elevated expression of FGF15 in our study. FGF15/19 is mainly expressed in distal ileum and can be induced by FXR[28,29]. The increased ileal mRNA level of FXR in our study should be responsible for the elevation of FGF15 after SG. Moreover, FXR can be activated by different BA subspecies, among which CDCA is the most efficacious[30]. Furthermore, oral or intraduodenal infusion of CDCA triggers increase of serum FGF19 Level in humans[31,32]. Consistent with our previous study[22,23], CDCA levels as well as other FXR agonists were elevated after SG both in systemic serum and portal vein. Hence, activation of ileal FXR in this study would result from the elevation of these agonists among the SG-operated rats. Taken together, enhanced reabsorption of BAs after SG activated FXR in the ileum, which then promoted the expression and secretion of FGF15.

Stimulated by ileal FXR, FGF15/19 is promoted and secreted into the enterohepatic circulation. In addition of the regulation of BA homeostasis, FGF15/19 also plays a role in the hepatic glucose metabolism. Evidence have shown that FGF 15 knockout mice showed impaired glucose tolerance with decreased hepatic glycogen storage and advanced hepatic gluconeogenesis[20]. Diet-induced obesity and insulin resistance was failed in FGF19 transgenic mice[33,34]. These effects of FGF15/19 are all mediated through FGFR[35-37], the receptor of FGF15/19 with an unique specificity[21,25]. Moreover, expression of FGFR4 in liver was decreased among STZ-induced diabetes[36]. FGFR4-deficient mice displayed glucose intolerance and insulin resistance as well as increased weight gain[35]. As described above, FGF15/19-FGFR4 plays an important role in hepatic glucose metabolism. Considering the elevation of FGF15, we then examined whether FGF15-FGFR4 functions in the improvement of hepatic glucose metabolism after SG. The hepatic expression of FGFR4 among the HFD and STZ-induced diabetic rats was elevated after SG in this study, which indicates that FGF15-FGFR4 was activated in SG-operated rats. However, we still can't conclude that FGF15-FGFR4 acts in improving the hepatic glucose metabolism because the regulation of BAs synthesis by FGF15 also needs activation of FGFR4.

To further confirm whether FGF15-FGFR4 contributes to the improvement of glucose metabolism in SG-operated rats, we detected the corresponding pathways of FGF15-FGFR4 involved in the hepatic glucose metabolism. Comparable to insulin, FGF15/19 could regulate hepatic glucose metabolism by promoting glycogen synthesis and inhibiting hepatic gluconeogenesis. But unlike the insulin-inducing PI3K-Akt patway, FGF15/19 acts through two different pathways. Moreover, FGF15/19 the inhibiting effect on BA synthesis of FGF15/19 was through a c-Jun N-terminal kinase-dependent pathway[12]. FGF15/19 promotes the hepatic glycogen synthesis by activating Ras-ERK pathway[20]. FGF15/19-FGFR4 triggers the phosphorylation of ERK1/2, which then increase the activity of GS by inhibiting the phosphorylation of this enzyme. Therefore, the increased phosphorylation of ERK1/2 and the declined phosphorylation of GS observed in this study indicated that FGF15-FGFR4-Ras-ERK pathway was activated in SG-operated rats. Together with the elevated glycogen content, we can conclude that the action of FGF15 (and its corresponding pathways) contributed to the glycogen synthesis promoting effect of SG. Moreover, FGF15/19 inhibits hepatic gluconeogenesis by supressing the CREB-PGC-1α pathway[19]. FGF15/19 could induce dephosphorylate and suppress the transcription factor CREB and correspondingly inhibited the expression of PGC-1α. Besides, G6Pase, and Pepck, the two key enzymes for hepatic gluconeogenesis, are two target genes of PGC-1α. So, integrating together the depressed phosphorylation of CREB, reduced expression of PGC-1α and the inhibited expression of G6Pase and Pepck, we could predicate that FGF15 inhibited the CREB-PGC-1α pathway and participated in suppressing the hepatic gluconeogenesis in SG-operated rats. Hence, the activated Ras-ERK1/2 pathway and inhibited CREB-PGC-1α pathway indicated that elevated FGF15 after SG played a role in regulating the hepatic glucose metabolism in parallel to insulin.

In this study, we confirmed the effect of elevated BAs and FGF15 on the hepatic glucose metabolism. FGF15, induced by elevated BAs in ileum, acts as an endocrine factor on the liver through portal vein. By specifically binding and activating the FGFR4 and its corresponding signal pathways, FGF15 improves the hepatic glucose metabolism through the intestine-liver crosstalk. Similarly, human FGF19, which is an ortholog of rodent FGF15, was also elevated after SG together with BAs. Besides, FGF19 can also bind and activate the FGFR 4 specifically. Thus, the results of this study presented basics for the research of human FGF19 after SG, and the FGF19 would be developed to be a target for clinical treatment of T2DM in the future.

Certainly, this study has several limitations. First, we did not directly detect the FGF15 Levels in serum and portal vein because no ELISA kits for FGF15 was available at that time. However, we detected the expression of FGF15 in the ileum at both mRNA and protein levels. As FGF15 is mainly expressed and secreted from the ileum into enterohepatic circulation, the ileal expression could reflect the FGF15 Levels in serum and portal vein. Second, the postprandial FGF15 Level varies with time, it is

better to trace the FGF15 Levels at different time points after gavage. However, we can only sacrifice the rat and detect the expression of FGF15 in the ileum once. And, as previously reported, the expression level of FGF15 mRNA in ileum and the phosphorylation degree of downstream hepatic ERK1/2 reach the peak 1h after feeding in rodent[19]. So, we sacrificed the rats 1h after gavage and the following results then may be more meaningful and advisable. Third, the improvement of hepatic glucose metabolism after SG should surely be mainly mediated by insulin. Though the action of FGF15 underlying the effects of SG was proved in our study, the proportion of these effects accounted by FGF15 still needs further evaluation. Finally, the data presented in this study only show associations, but no causal relationships between the alteration of FGF15 and improvement of hepatic glucose metabolism after SG, which would have to be further investigated by using KO models.

CONCLUSION

BAs levels were elevated in the SG-operated rats and induced FGF15 expression by activating their receptor, FXR in distal ileum. Moreover, the action of FGF15 on hepatic glycogen synthesis and gluconeogenesis further contributes to the anti-diabetic effects of SG.

ARTICLE HIGHLIGHTS

Research background

Bariatric surgery can significantly ameliorate type 2 diabetes mellitus (T2DM) through its rapid and durable weight-loss and hypoglycemic action. Both biles acids (BAs) and fibroblast growth factor (FGF) 15/19 are increased after surgery. Whether BAs and FGF15/19 participates in amelioration of T2DM after bariatric surgery and the underlying mechanism remain incompletely illuminated.

Research motivation

FGF15 which is induced by farnesoid X receptor (FXR) in ileum can improve the hepatic glucose metabolism through entero-hepatic circulation. Our previous study confirms that BA profiles within peripheral circulation and portal vein have changed after SG, with significant increase of FXR- activated BAs levels.

Research objectives

This study aimed to evaluate the effect of FGF15 on improvement of T2DM triggered by elevated BAs after sleeve gastrectomy (SG) and investigate the underlying mechanism.

Research methods

The weight-loss and hypoglycemic action of SG were detected in a diabetic rat model induced by High-fat diet and streptozotocin (STZ), as well as the hepatic glycogen content and gluconeogenesis activity. Total BA (TBA) together with the FXR-agonistic BA subspecies levels in systemic serum and portal vein were examined at week 12 post-surgery. Then the expression and activity of ileal FXR and FGF15 and hepatic FGFR4 with its corresponding signal pathways involved in glucose metabolism were detected.

Research results

Compared with SHAM group, SG induced sustained weight loss and improved the hepatic glucose metabolism by promoting hepatic glycogen synthesis and inhibiting the gluconeogenesis. TBA levels and the FXR-agonistic subspecies in serum and portal vein were elevated after SG. Consequently, the ileal expression of FXR and FGF15 were also advanced. Moreover, the hepatic expression of FGFR4 and the activities of its corresponding pathways were stimulated in SG-operated rats.

Research conclusions

FGF15, triggered by elevated BAs after SG, acts as an endocrine factor to induce the improvement of hepatic glucose metabolism through the intestine-liver crosstalk.

Research perspectives

FXR-agonistic BA subspecies and FGF15 participate in improvement of hepatic glucose metabolism, and may be developed as new targets for the treatment of T2DM.

FOOTNOTES

Author contributions: Wei M and Yu WB contributed to conception and design of research; Wei M, Sun DP, Yang WS, Cao WB, and Zhao RD contributed to performed experiments; Wei M, Liang YZ, and Cheng ZW analyzed data; Wei M, Sun DP, Liang YZ, and Ouyang J prepared figures; Wei M, Huang YD, and Sun DP drafted manuscript; Wei M, Liang YZ, and Yu WB edited and revised manuscript; Wei M, Sun DP, Cao WB, Zhao RD, Liang YZ, Huang YD, Cheng ZW, Ouyang J, Yang WS, and Yu WB approved final version of manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Ineffective esophageal motility is associated with acute rejection after lung transplantation independent of gastroesophageal reflux

Wai-Kit Lo, Brent Hiramoto, Hilary J Goldberg, Nirmal Sharma, Walter W Chan

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Abstract

BACKGROUND

Gastroesophageal reflux is associated with poorer outcomes after lung transplant, likely through recurrent aspiration and allograft injury. Although prior studies have demonstrated a relationship between impedance-pH results and transplant outcomes, the role of esophageal manometry in the assessment of lung transplant patients remains debated, and the impact of esophageal dysmotility on transplant outcomes is unclear. Of particular interest is ineffective esophageal motility (IEM) and its associated impact on esophageal clearance.

AIM

To assess the relationship between pre-transplant IEM diagnosis and acute rejection after lung transplantation.

METHODS

This was a retrospective cohort study of lung transplant recipients at a tertiary care center between 2007 and 2018. Patients with pre-transplant anti-reflux surgery were excluded. Manometric and reflux diagnoses were recorded from pre-transplant esophageal function testing. Time-to-event analysis using Cox proportional hazards model was applied to evaluate outcome of first episode of acute cellular rejection, defined histologically per International Society of Heart and Lung Transplantation guidelines. Subjects not meeting this endpoint were censored at time of post-transplant anti-reflux surgery, last clinic visit, or death. Fisher's exact test for binary variables and student's *t*-test for continuous variables were performed to assess for differences between groups.

RESULTS

Of 184 subjects (54% men, mean age: 58, follow-up: 443 person-years) met criteria

for inclusion. Interstitial pulmonary fibrosis represented the predominant pulmonary diagnosis (41%). During the follow-up period, 60 subjects (33.5%) developed acute rejection. The all-cause mortality was 16.3%. Time-to-event univariate analyses demonstrated significant association between IEM and acute rejection [hazard ratio (HR): 1.984, 95%CI: 1.03-3.30, P = 0.04], confirmed on Kaplan-Meier curve. On multivariable analysis, IEM remained independently associated with acute rejection, even after controlling for potential confounders such as the presence of acid and nonacid reflux (HR: 2.20, 95%CI: 1.18-4.11, P = 0.01). Nonacid reflux was also independently associated with acute rejection on both univariate (HR: 2.16, 95%CI: 1.26-3.72, P = 0.005) and multivariable analyses (HR: 2.10, 95%CI: 1.21-3.64, P = 0.009), adjusting for the presence of IEM.

CONCLUSION

Pre-transplant IEM was associated with acute rejection after transplantation, even after controlling for acid and nonacid reflux. Esophageal motility testing may be considered in lung transplant to predict outcomes.

Key Words: Ineffective esophageal motility; Esophageal hypomotility; Esophageal manometry; Gastroesophageal reflux disease; Lung transplantation; Acute rejection

Core Tip: While gastroesophageal reflux (GER) has been associated with poorer outcomes after lung transplant, the impact of esophageal dysmotility remains unclear. Our study found that ineffective esophageal motility identified on pre-transplant esophageal manometry was independently associated with increased acute allograft rejection, even after adjusting for GER. This suggests that esophageal hypomotility may increase the risk of poor lung transplant outcome independent of GER. Routine esophageal function assessment should be considered in the peri-transplant evaluation of lung transplant patients to identify, risk stratify, and more effectively manage esophageal dysfunction in such patients at risk of poorer outcomes.

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INTRODUCTION

Lung transplantation survival remains the lowest among solid organ transplants despite small gains over the past decade. Current 5-year survival rates are estimated at 59.2%[1]. Gastroesophageal reflux disease (GERD) and esophageal dysmotility are commonly found in patients undergoing lung transplant evaluation and have been associated with worsened transplant outcomes. For example, GERD has been implicated in the development of acute rejection and chronic lung allograft dysfunction (CLAD). Acute rejection is an early risk factor for CLAD, an important mediator of mortality after the first year post-transplant[2,3]. While esophageal dysmotility may be associated with worsened severity of GERD due to aberrant peristaltic clearance of refluxed gastroduodenal contents, less is known regarding its independent effects on lung transplant outcomes[4-6].

Acute lung rejection is common within the first year post-transplant with rates as high as > 50%[7]. Prior work from our group demonstrated an association between pre-transplant impedance measures of reflux and early allograft injury post-transplant as well as early hospital readmission[8,9]. These measures included prolonged bolus clearance, and increased total proximal and distal reflux episodes [10]. Studies in humans and mouse models have demonstrated that markers of refluxate such as pepsin and bile acids are also associated with allograft dysfunction, and result in impaired innate immune responses[11-14].

Despite the established connection between esophageal motility and reflux clearance, few studies have analyzed transplant outcomes in patients with esophageal dysmotility. Notably, the International Society of Heart and Lung Transplantation (ISHLT) guidelines consider severe esophageal dysmotility to be a risk factor associated with a substantially increased risk of a poor outcome[15]. A few single center studies to date have demonstrated disorders of esophageal motility impacting lung transplant outcomes like CLAD in esophagogastric junction outflow obstruction (EGJOO), as well as 1-, 3-, and 5-

year survival in the more severe phenotype of esophageal aperistalsis[16,17]. Thus far, limited data has impeded our understanding of less severe phenotypes of impaired peristalsis on lung transplant outcomes. The goal of our study is to determine the impact of pre-transplant esophageal dysmotility on lung transplant outcomes of acute rejection, specifically subjects with weak or impaired, but not fully absent, contractility, characterized as ineffective esophageal motility (IEM). We hypothesized that measures of esophageal dysmotility such as IEM are associated with increased rates of acute rejection in lung transplant patients.

MATERIALS AND METHODS

This was a retrospective cohort study of adult patients age > 18 who underwent lung transplantation between 2007 and 2018 at a tertiary care referral center. Patients with pre-transplant high-resolution manometry (HRM) and multichannel intraluminal impedance and pH (MII-pH) testing were included, and patients with a history of pre-transplant antireflux surgery were excluded. Study subjects meeting inclusion and exclusion criteria were reviewed for collection of baseline characteristics and outcomes data.

Baseline demographics included age at time of transplantation, sex, body mass index, primary pulmonary diagnosis, and pre-transplant cardiac ejection fraction. Covariates of interest included IEM on HRM, and presence of acid reflux and non-acid reflux on MII-pH study. The primary outcome of interest was development of first episode of acute cellular rejection. This was defined by clinical and histologic criteria per ISHLT guidelines[18]. Other measured outcomes included all-cause mortality during the study period, use of proton pump inhibitor (PPI) medication post-transplant, and development of pulmonary infection.

Pre-transplant HRM

All patients included in the study underwent HRM (Diversatek Healthcare, Milwaukee, WI, United States) prior to transplant. This system utilized a solid-state catheter with 32 circumferential pressure sensors spaced 1 cm apart. Transnasal catheter placement was performed with distal sensor placement directed into the proximal stomach, ensuring that the catheter is properly positioned across the lower esophageal sphincter (LES). After a brief accommodation period, patients were asked to perform ten 5-mL liquid swallows in the supine position. Results were analyzed utilizing a dedicated software package (BioView 5.6.3.0; Diversatek Healthcare, Milwaukee, WI, United States). IEM was defined by ≥ 50% weak or failed swallows using Chicago Classification v3.0 criteria[19]. Presence of IEM was classified as a dichotomous variable for data analysis.

Pre-transplant MII-pH Monitoring

All patients included in the study also MII-pH monitoring (Diversatek Healthcare, Milwaukee, WI, United States) off PPI prior to transplant. This system includes a portable data collection device, as well as the MII-pH catheter with two pH sensors (0, 15 cm) and eight impedance electrodes (-3, -1, 1, 3, 5, 9, 11, 13 cm). Transnasal catheter placement was performed and positioned with the distal pH sensor localized to 5 cm above the LES. Patients were asked to continue their normal daily activities during the 24-h study and to record meal periods, which were excluded from the analysis. MII-pH tracings were manually reviewed and analyzed utilizing a dedicated software package (BioView 5.6.3.0; Diversatek Healthcare, Milwaukee, WI, United States). Increased acid reflux was defined by acid exposure time (AET) > 4%, while increased non-acid reflux was defined as > 27 weakly acidic or alkaline (pH > 4) episodes per prior publications[20].

Post-transplant management and diagnosis of early allograft injury

Patients were placed on a standard immunosuppressive regimen consisting of azathioprine or mycophenolate, tacrolimus, and methylprednisolone. Surveillance bronchoscopy and biopsies were obtained according to standardized post-transplant protocol at 1, 3, 6, and 12 mo. Additional diagnostic bronchoscopies were triggered by development of clinical symptoms concerning for infection or rejection. Acute rejection was categorized according to ISHLT criteria. Minimal rejection grades of A1B0 were counted as acute rejection if the patient presented with suggestive clinical symptoms and received treatment with pulsed steroids, or had persistent grade A rejection on repeat bronchoscopy.

Statistical analysis

All statistical analyses were performed utilizing SAS 9.3 statistical package (SAS Institute Inc., Cary, NC, United States). Baseline characteristics were compared using student's *t*-test for continuous variables and Fisher's exact test for dichotomous variables. Time-to-event analysis using Cox proportional hazards model was utilized to analyze the primary outcome of first episode of acute rejection. Cox proportional hazards regression was used to adjust for baseline covariates in the final analysis.

RESULTS

Of the 181 patients met inclusion criteria for the study with a total of 439 person-years of follow-up. The mean age of the cohort was 58 with a slight male predominance (54%), and the most common pulmonary diagnosis was idiopathic pulmonary fibrosis which accounted for 41% of patients. Acute rejection was demonstrated in 59 patients (33.5% of those receiving at least one bronchoscopy with biopsy) during the follow-up period for this study. There were 30 deaths during the study period reflecting an all-cause mortality rate of 16.6%.

Pre-transplant HRM revealed normal esophageal motility in 130 patients (71.8%) and IEM in 31 patients (17.1%). The remaining 20 patients had abnormal manometry of other causes (7 distal esophageal spasm, 7 Jackhammer, 6 EGJOO). No patients had achalasia or absent contractility. The IEM group had slightly fewer Caucasian patients, but the remaining demographics were statistically similar compared to the normal group (Table 1). For the primary outcome on univariate analysis, IEM was associated with a decreased time to acute rejection [hazard ratio (HR): 1.984, 95%CI: 1.03-3.30, $P = 0.04$). The Kaplan-Meier survival curve trended toward significance with 40% of the IEM cohort developing acute rejection within approximately 250 d, compared to 500 d for the normal esophageal motility group (Figure 1). On multivariable analysis after adjusting for potential confounders including the presence of acid and nonacid reflux, IEM remained independently associated with acute rejection (HR: 2.20, 95%CI: 1.18-4.11, $P = 0.01$) (Table 2).

The presence of pathologic acid or nonacid reflux per MII-pH testing was also analyzed. Notably, pathologic acid reflux defined by AET > 4.2% was not associated with acute rejection on univariate (HR: 1.06, 95%CI: 0.63-1.76, $P = 0.83$) or multivariable analyses (HR: 0.92, 95%CI: 0.53-1.61, $P = 0.77$). On the other hand, increased non-acid reflux was associated with decreased time to acute rejection in the IEM group on univariate analysis (HR: 2.16, 95%CI: 1.26-3.72, $P = 0.005$) and multivariable analysis (HR: 2.10, 95%CI: 1.21-3.64, $P = 0.009$) (Table 2). This relationship occurred independent of the presence of IEM.

DISCUSSION

GERD and esophageal dysmotility are frequent comorbid conditions in patients with end-stage lung disease. There is increasing recognition of the role these esophageal dysfunctions play in the pathogenesis and clinical progression of specific etiologies of end-stage lung disease, as well as their role in the clinical outcomes of lung transplantation. Our study sought to determine the impact of esophageal hypomotility in the development of acute rejection after lung transplantation. We found that IEM demonstrated on pre-transplant testing was associated with increased risk of acute rejection after lung transplantation. This relationship remained after controlling for covariates including pre-transplant measures of acid and non-acid reflux. The magnitude of association was increased after controlling for these baseline factors on multivariate analysis. This suggests that esophageal motility may play a role in lung transplant outcomes that is independent of reflux-related allograft injury and rejection.

Acute rejection was demonstrated in 33.5% of the cohort in the study follow up. This is consistent with prior estimates which have ranged from 28% in the ISHLT registry to 53.3%[7,21]. Baseline demographics did not differ significantly between the IEM and control groups. Amongst the other covariates of interest, non-acid reflux was independently associated with decreased time to the development of acute rejection, and did not substantially alter the association between IEM and acute rejection.

Abnormal esophageal motility was found in 29.3% of our cohort. Of those with abnormal esophageal motility, 59% of these patients were classified as having IEM (or 17% of the total cohort). Prior studies have demonstrated esophageal dysmotility in as high as 78% of patients undergoing lung transplant evaluation, though it is important to note significant heterogeneity in how esophageal dysmotility has been categorized[22-25]. The prevalence in our cohort was consistent with another study that also categorized HRM diagnoses based on Chicago Classification v3.0, which found IEM in 32.7% of patients undergoing lung transplant evaluation[25], supporting the generalizability of our findings.

Recent studies have begun to characterize the impact of esophageal dysmotility on lung transplant outcomes. In a single center study of 31 patients with pre-transplant esophageal aperistalsis, defined as ≥ 90% failed swallows without any effective peristalsis on HRM, the 1-, 3-, and 5-year post-lung transplant survivals were lower than that of the control group with normal esophageal motility[17]. This study also demonstrated that recovery of peristaltic function post-transplant was associated with improved transplant survival outcomes matching that of the control group. Another study from the same group noted HRM diagnoses of esophageal dysmotility frequently changed post-lung transplant (51.4%) and that peristaltic vigor tends to increase, implicating a dynamic relationship between esophageal motility and pulmonary function[25]. These studies suggest that chronic lung diseases and the resultant altered respiratory mechanics may impact esophageal motility, most commonly associated with hypomotility that may improve with recovery of pulmonary function after transplantation. Two other single center studies utilizing post-lung transplant HRM also demonstrated associations between

Table 1 Baseline demographics between manometric diagnoses, mean ± SD

	Total (*n* = 181)	IEM (*n* = 31)	Normal motility (*n* = 130)	Other motility disorder (*n* = 20)
Follow-up (years)	2.43 ± 2.45	2.85 ± 2.77	2.43 ± 2.41	1.76 ± 2.11
Male sex	97 (53.6%)	20 (64.5%)	70 (53.8%)	7 (35.0%)
BMI	26.6 ± 4.37	25.8 ± 3.88	26.6 ± 4.55	28.0 ± 3.66
Age at transplant (years)	58.4 ± 10.2	57.9 ± 12.1	58.1 ± 10.2	61.6 ± 7.17
White race[1]	166 (92.2%)	24 (77.4%)	123 (94.6%)	20 (100%)
Pulmonary diagnosis				
ILD	99 (54.7%)	19 (61.3%)	69 (53.1%)	11 (55.0%)
IPF	74 (40.9%)	14 (45.2%)	51 (39.2%)	9 (45.0%)
COPD	57 (31.5%)	7 (22.6%)	43 (33.1%)	7 (35.0%)
CF	17 (9.39%)	4 (12.9%)	13 (10.0%)	0 (0%)
Cardiac function[2]				
LVEF (%)	61.7 ± 5.73	62.2 ± 5.79	61.4 ± 5.42	62.6 ± 7.55
PaP (mmHg)	27.7 ± 10.3	28.6 ± 11.0	27.3 ± 10.3	29.2 ± 8.91
PCWP (mmHg)	10.8 ± 4.96	9.06 ± 4.01	11.2 ± 5.15	11.5 ± 4.65
PVR (dynes/sec/cm^{-5})	252 ± 194	300 ± 238	233 ± 175	300 ± 217
Pulmonary function, baseline[2]				
FVC	1.93 ± 0.82	1.97 ± 0.92	1.94 ± 0.81	1.77 ± 0.78
FVC, %-pred	50.1 ± 47.0	49.2 ± 18.4	50.1 ± 19.0	51.3 ± 24.4
FEV1	1.24 ± 0.68	1.39 ± 0.74	1.22 ± 0.68	1.15 ± 0.57
FEV1, %-pred	40.5 ± 20.4	44.1 ± 21.1	39.4 ± 20.1	42.6 ± 21.6
FEV1/FVC	0.66 ± 0.25	0.72 ± 0.22	0.64 ± 0.25	0.67 ± 0.26
Manometry results				
Normal	130	0	130	0
IEM	31	31	0	0
DES	7	0	0	7
Jackhammer	7	0	0	7
EGJOO	6	0	0	6
Reflux monitoring				
Acid reflux	69 (38.1%)	13 (41.9%)	50 (38.5%)	6 (30.0%)
Nonacid reflux[2]	42 (26.6%)	11 (39.3%)	30 (26.8%)	1 (5.56%)
Lungs transplanted				
Unilateral	43 (23.8%)	6 (19.3%)	34 (26.1%)	3 (15.0%)
Bilateral	138 (76.2%)	25 (80.6%)	96 (73.8%)	17 (85.0%)
CMV mismatch	53 (29.3%)	7 (22.6%)	42 (32.3%)	4 (20.0%)
High-risk donor	69 (38.1%)	7 (22.6%)	52 (40.0%)	10 (50.0%)
Post-transplant PPI	128 (70.7%)	24 (77.4%)	92 (70.8%)	12 (60.0%)

[1]Indicates statistically significant difference between ineffective esophageal motility and normal, $P < 0.05$.
[2]Indicates subjects with available data.
BMI: Body mass index; ILD: Interstitial lung disease; IPF: Idiopathic pulmonary fibrosis; COPD: Chronic obstructive pulmonary disease; CF: Cystic fibrosis; LVEF: Left ventricular ejection fraction; PaP: Pulmonary arterial pressure; PCWP: Pulmonary capillary wedge pressure; PVR: Pulmonary vascular resistance; FVC: Forced vital capacity; FEV1: Forces expiratory volume in 1 second; IEM: Ineffective esophageal motility; DES: Distal esophageal spasm;

EGJOO: Esophagogastric junction outflow obstruction; CMV: Cytomegalovirus; PPI: Proton pump inhibitor.

Table 2 Cox univariate and multivariable analyses demonstrating the association between manometric diagnosis of ineffective esophageal motility as defined by CCv3.0 and acid reflux based on acid exposure time > 4.2%, and increased risk of acute rejection after lung transplantation

Covariate	Cox univariate analysis	*P* value	Cox multivariable analysis	*P* value
Ineffective esophageal motility	1.84 (1.03-3.30)	0.04	2.20 (1.18-4.11)	0.01
Nonacid reflux	2.16 (1.26-3.72)	0.005	2.10 (1.21-3.64)	0.009
Acid reflux	1.06 (0.63-1.76)	0.83	0.92 (0.53-1.61)	0.77
Body-mass Index	1.01 (0.95-1.06)	0.85	1.02 (0.96-1.09)	0.43
Age at transplant	1.00 (0.98-1.02)	0.94	1.00 (0.97-1.02)	0.93
Male gender	0.85 (0.51-1.41)	0.53	0.75 (0.44-1.29)	0.30

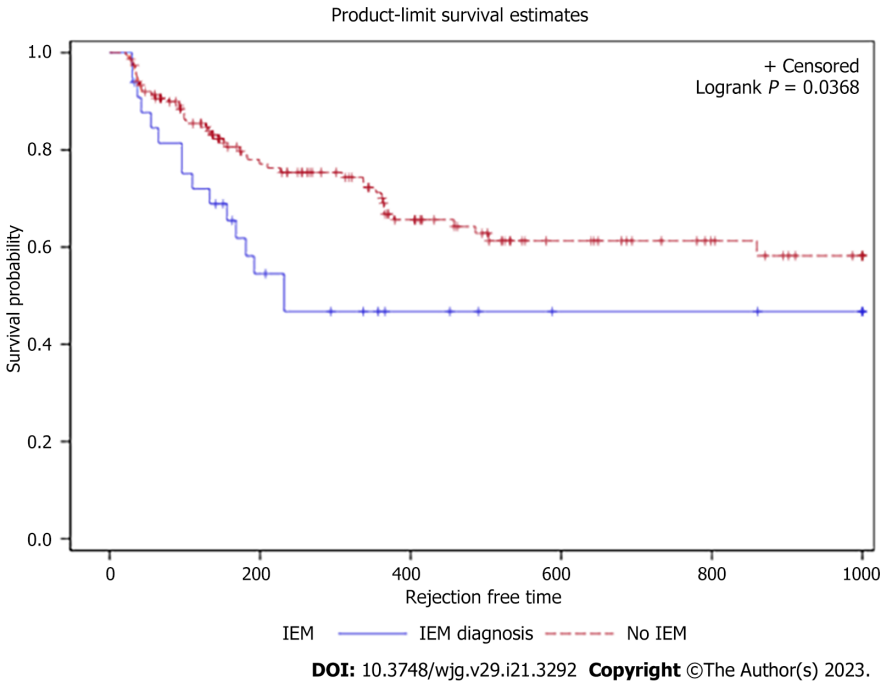

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Figure 1 Kaplan-Meier showing trend toward increased acute rejection in the ineffective esophageal motility compared to normal motility group. 40% developed acute rejection in approximately 250 d in the ineffective esophageal motility cohort *vs* 500 d in the normal cohort. IEM: Ineffective esophageal motility.

esophageal dysmotility and outcomes of acute and chronic rejection[16,26].

The mechanism through which esophageal dysmotility may impact lung transplantation outcomes is not completely clear, although it is speculated to largely be related to increased risk of microaspiration due to reduced esophageal clearance. Esophageal hypomotility may result in decreased clearance and increased proximal migration of gastric refluxate, thereby leading to higher risk for exposure to the airway. Reduced esophageal bolus transit and clearance may also be associated with elevated risk of esophago-pharyngeal reflux, with potential resultant injury to the lung allograft. On the other hand, abnormal reflux, which has already been previously linked with worse lung transplant outcomes, may also lead to esophageal hypomotility. However, our results suggested that esophageal hypomotility may be associated with higher risk of allograft rejection independent of reflux burden.

There remains significant heterogeneity in reflux and esophageal motility testing in the pre- and post-lung transplant settings. HRM is standardized within the pre-transplant evaluation at our institution. The results of this study indicate that results of pre-transplant HRM are informative for risk stratification and prognostication for lung transplant outcomes. This information, in turn, may also guide post-transplant care and monitoring for acute rejection.

There are several notable strengths to our study. Pre-transplant evaluation of esophageal motility on HRM and reflux measurements on MII-pH were standardized across all lung transplant candidates. Ascertainment bias for determination of acute rejection was minimized by surveillance bronchoscopy per standard protocol with biopsy at 1, 3, 6, and 12 months, though clinical symptoms in between these intervals could trigger additional diagnostic bronchoscopies. Baseline characteristics of the study cohort were consistent with previously published data for rates of acute rejection, prevalence of esophageal dysmotility during pre-transplant evaluation, and indication for lung transplantation. Lastly, distinct HRM diagnoses were categorized according to established classification criteria for analysis, and we were able to control for potential confounding of specific measures of reflux based on MII-pH monitoring results that were collected on all patients.

There are also several limitations to our study. This is a retrospective cohort study with results that are limited to a single academic institution with high volume of lung transplantation. The sample size is relatively limited within the IEM group, though consistent with prior studies published on the association between esophageal motility and lung transplant outcomes. While a small number of recent studies have suggested dynamic changes in esophageal motility post-transplant, post-transplant motility measurements were not obtained routinely as part of our study. Finally, due to the retrospective nature and inclusion period of our study cohort, Chicago classification v3.0 was used to define IEM. However, the most current Chicago classification v4.0 mainly further restricted the diagnosis of IEM with more stringent criteria than v3.0. Therefore, the use of Chicago classification v3.0 to define IEM would likely have biased our results towards the null, as some patients in our IEM group would have been classified as normal under v4.0. The fact that our results remained significant despite this potential bias would strengthen the observed relationship between IEM and acute allograft rejection.

CONCLUSION

In summary, our study demonstrated that IEM on pre-transplant esophageal motility testing was associated with decreased time to development of acute rejection after lung transplantation. Our study provides additional evidence for the association between esophageal dysmotility and poor lung transplant outcomes. It builds upon prior studies on esophageal aperistalsis and survival outcomes in lung transplantation by providing additional evidence for acute rejection in the less severe phenotype of IEM. It also suggests esophageal dysmotility may mediate long-term lung transplant outcomes through a pathway starting with acute rejection. Further studies are needed in delineating transplant outcomes by underlying pulmonary diagnosis, analyzing longer term outcomes such as chronic rejection and 3- and 5-year survival outcomes in the context of esophageal dysmotility, and comparing pre- and post-transplant esophageal function testing results on lung transplant outcomes.

ARTICLE HIGHLIGHTS

Research background

Gastroesophageal reflux is associated with poor outcomes after lung transplantation. However, the impact of esophageal dysmotility and role of esophageal manometry remains unclear. Ineffective esophageal motility (IEM) is a disorder of esophageal motility associated with decreased esophageal clearance that may worsen transplant outcomes.

Research motivation

Esophageal evaluation remains poorly standardized in lung transplantation, and this work suggests that routine esophageal motility testing to identify IEM may help identify patients at risk for acute rejection.

Research objectives

To evaluate the relationship between IEM and acute rejection after lung transplantation, controlling for confounders including coexisting pathologic acid and nonacid reflux.

Research methods

This was a retrospective cohort study of lung transplant recipients that underwent pre-transplant esophageal testing including manometry and pH at a tertiary referral center.

Research results

IEM on pre-transplant esophageal manometry was associated with higher risk of acute rejection on time-to-event analysis. On multivariable Cox regression analysis, IEM remains independently

associated with increased acute rejection, even after controlling for pathologic reflux. In addition, increased non-acid reflux was also an independent risk factor for acute rejection in the multivariable model.

Research conclusions

Lung transplant candidates with IEM had a greater risk of developing acute rejection, independent of pathologic acid and nonacid reflux. Additionally, nonacid reflux was independently associated with acute rejection. These findings suggest that IEM and other disorders affecting esophageal clearance may contribute to the pathophysiology of allograft injury, independent of a reflux-associated pathway.

Research perspectives

Future research should focus on the implementation of standardized esophageal motility testing in lung transplantation, investigation of the impact of IEM and other disorders of esophageal motility on longer term transplant outcomes including chronic rejection and survival, and assessment of changes in esophageal motility after transplant and its effect on transplant outcomes.

FOOTNOTES

Author contributions: Chan WW and Lo WK initiated study concepts and design; Lo WK, Goldberg HJ, and Chan WW contributed to acquisition of data; Chan WW, Lo WK, Hiramoto B, Goldberg HJ, and Sharma N performed analysis and interpretation of data; Lo WK, Hiramoto B, and Chan WW drafted the manuscript; Chan WW, Lo WK, Hiramoto B, Goldberg HJ, and Sharma N contributed to critical revision of manuscript for important intellectual content; Chan WW and Lo WK performed statistical analyses; Chan WW provided administrative support and overall study supervision.

Institutional review board statement: The study was reviewed and approved by the Mass General Brigham Healthcare Institutional Review Board, No. 2011P001563.

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: Lo WK, Hiramoto B, and Goldberg HJ, Sharma N-no relevant conflicts of interest for this article; Chan WW-Scientific Advisory Board (Takeda Pharmaceuticals, Phathom Pharmaceuticals, Sanofi Pharmaceuticals).

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ORIGINAL ARTICLE

Clinical Trials Study

Peutz-Jeghers syndrome without *STK11* mutation may correlate with less severe clinical manifestations in Chinese patients

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Abstract

BACKGROUND

Peutz-Jeghers syndrome (PJS) is an autosomal dominant genetic disease with skin mucosal pigment spots and gastrointestinal (GI) multiple hamartoma polyps as clinical characteristics. At present, it is considered that the germline mutation of *STK11* gene is the genetic cause of PJS. However, not all PJS patients can be detected *STK11* germline mutations. The specific clinical characteristics of these PJS patients without *STK11* mutation is an interesting clinical question. Or, like wild type GI stromal tumor, whether these PJS without *STK11* mutation are also called PJS is worth discussing. Therefore, we designed the study to understand the clinical characteristics of these PJS patients without *STK11* mutation.

AIM

To investigates whether PJS patients with known *STK11* mutations have a more severe spectrum of clinical phenotypes compared to those without.

METHODS

A total of 92 patients with PJS admitted to the Air Force Medical Center from 2010 to 2022 were randomly selected for study. Genomic DNA samples were extracted from peripheral blood samples, and pathogenic germline mutations of *STK11* were detected by high-throughput next-generation gene sequencing. Clinical-pathologic manifestations of patients with and without *STK11/LKB1* mutations

were compared.

RESULTS

STK11 germline mutations were observed in 73 patients with PJS. Among 19 patients with no detectable *STK11* mutations, six had no pathogenic germline mutations of other genes, while 13 had other genetic mutations. Compared with PJS patients with *STK11* mutations, those without tended to be older at the age of initial treatment, age of first intussusception and age of initial surgery. They also had a lower number of total hospitalizations relating to intussusception or intestinal obstruction, and a lower load of small intestine polyps.

CONCLUSION

PJS patients without *STK11* mutations might have less severe clinical-pathologic manifestations than those with.

Key Words: Peutz-Jeghers syndrome; *STK11*; Mutant type; Wild type

Core Tip: Peutz-Jeghers syndrome (PJS) is an autosomal dominant genetic disease with skin mucosal pigment spots and gastrointestinal (GI) multiple hamartoma polyps as clinical characteristics. At present, it is considered that the germline mutation of *STK11* gene is the genetic cause of PJS. However, not all PJS patients can be detected *STK11* germline mutations. The specific clinical characteristics of these PJS patients without *STK11* mutation is an interesting clinical question. Or, like wild type GI stromal tumor, whether these PJS without *STK11* mutation are also called PJS is worth discussing. Therefore, we designed the study to understand the clinical characteristics of these PJS patients without *STK11* mutation. Final results found that PJS patients without *STK11* mutations might have less severe clinical-pathologic manifestations than those with.

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INTRODUCTION

Peutz-Jeghers syndrome (PJS) is an autosomal dominant disorder which is mainly characterized by mucocutaneous pigmentation and hamartomatous polyps of the gastrointestinal (GI) tract[1,2]. While PJS is rare, with an estimated prevalence of 1:200000 births[3], the continuous growth of multiple GI polyps predisposes patients to serious complications including intussusception, intestinal obstruction, GI bleeding and malignancies. PJS patients also have a markedly increased risk of developing various neoplasms in extraintestinal sites such as the lungs, liver and breast[4].

Germline mutations in the *STK11* gene (also named *LKB1*), which is located on Chromosome 19p13.3 and encodes a serine/threonine protein kinase[5], have been identified as the major cause of PJS[6,7]. *STK11* is a tumor suppressor gene comprised of 433 amino acids with nine coding exons and one non-coding exon[8]. Depending on the screening method, *STK11* variants can be detected in over 80%-90% of PJS cases[8,9]. Previous studies have largely focused on exploring the phenotypic landscape of *STK11* variants based on their type or location. Due to the rare nature of PJS, very few studies have attempted to examine the correlations between *STK11* mutations and overall severity of PJS phenotype in terms of the earlier onset of GI pathology arising from the polyps, such as intussusception or earlier onset malignancy. Understanding the phenotypic differences between PJS patients with and without *STK11* variants could facilitate more personalized care for PJS patients and their families *via* appropriate counseling, risk stratification and targeted cancer screening[10].

A total of 92 PJS patients admitted to the Air Force Medical Center between February 2010 and February 2022 were randomly selected for inclusion in the study, and their peripheral venous blood was collected for high-throughput next-generation gene sequencing (NGS). 73 cases in which *STK11* gene mutations were detected were named mutant-type, and 19 cases in which no *STK11* gene mutations were detected were named wild-type. In this retrospective study, we aimed to investigate the differences in clinical phenotypes between wild-type and mutant-type *STK11* gene, and provide a theoretical basis for a more precise medical monitoring and follow-up strategy for different types of PJS

patients.

MATERIALS AND METHODS

Study participants

A total of 92 patients with PJS admitted to the Air Force Medical Center, PLA between February 2010 and February 2022 were randomly selected for inclusion in the study, and all patients met the diagnostic criteria for PJS recommended by the NCCN guidelines[11]. All enrolled patients and their guardians were aware of the purpose and process of the study, and had signed informed consent agreements. All data and information collection for this study followed the ethical principles of the Universal Declaration on the Human Genome and Human Rights, Declaration of Helsinki and Statement of the Human Genome Organisation Ethics Committee on DNA Sampling, Control and Access.

Inclusion and exclusion criteria

Inclusion criteria: All enrolled patients met the clinical diagnostic criteria of PJS[12-14] (in accordance with any of the following): (1) Two or more histologically confirmed PJS polyps; (2) Any number of PJS polyps in an individual with a family history of PJS in close relative(s); (3) Characteristic mucocutaneous pigmentation in an individual with a family history of PJS in close relative(s); and (4) Any number of PJS polyps in an individual with characteristic mucocutaneous pigmentation. Peripheral venous blood was retained and genomic DNA extracted, and the sequence of the coding region of the *STK11* gene was detected using polymerase chain reaction (PCR) amplification and NGS sequencing.

Exclusion criteria: Patients who could not meet both of the above two inclusion criteria, could not provide experimental specimens or did not agree to participate in this study.

Research methods

Observational index: The general information, diagnosis and treatment history, pathology, times of examination and other clinical data of the 92 enrolled PJS patients were collected for statistical analysis. The observed indices were as follows: (1) General patient information: Origin, gender, personal marital status, family history and ABO/RH blood groups; (2) History of diagnosis and treatment: Age of initial treatment, age of mucocutaneous pigmentation appearance, order of mucocutaneous pigmentation appearance, time interval from mucocutaneous pigmentation appearance to abdominal symptoms (abdominal pain, intestinal obstruction, GI bleeding, *etc.*), location of GI polyps, load and maximum diameter of GI polyps, pathology of polyps, carcinogenesis, total hospitalizations, number of operations and final age of follow-up; (3) Examinations: Endoscopic examinations and times of GI imaging examinations; and (4) Other: Comorbidities.

Genetic sequencing: EDTA anticoagulation tubes were used to extract 8 mL of peripheral venous blood from PJS patients. Leukocytes were isolated and DNA extracted, and the extracted DNA was interrupted by ultrasound using a Covaris M220 instrument to build a DNA library. The DNA library was then purified and hybridised by the probe library, which would bind specifically to the target DNA fragment through the principle of complementary binding of nucleic acid sequences. Magnetic beads conjugated with streptavidin were mixed with the hybridisation solution, and the streptavidin was tightly bound to the biotin. The captured exon target fragment was indirectly bound by the probe to the beads, which were adsorbed by a magnet, and the supernatant was discarded. The unbound DNA fragments were washed off and the desired DNA library eluted from the beads with the eluent. The eluted DNA library was then amplified using a PCR instrument. Lastly, NGS sequencing was used to detect full exons and associated single nucleotide polymorphism (SNP) and microsatellite instability sites for previously reported genes associated with hereditary GI tract tumors, including *STK11* (Table 1).

Statistical analysis: Statistical analysis was carried out using the SPSS 26.0 software package: (1) Cases and proportion of qualitative data were presented as percentages (%), and comparisons between groups were conducted using the *chi-square test* or *Fisher's exact test*; and (2) The *t*-test was used for quantitative data that matched the normal distribution with equal variance, the *t*-test was used for those with unequal variance and the rank sum test was used for skewed data; $P < 0.05$ was considered statistically significant.

RESULTS

Analysis of NGS test results

The 73 of 92 patients with PJS (79.3%) in this group had *STK11* gene mutations, of which 47 had *STK11* gene mutations in combination with other mutations. 19 of the 92 PJS patients (20.7%) had no *STK11*

Table 1 41 digestive tract tumour-associated genes

No.	Genes
1	*AKT1*
2	*BRAF*
3	*CYP2D6*
4	*GALNT12*
5	*MET*
6	*NRAS*
7	*POLD1*
8	*SDHC*
9	*UGT1A1*
10	*APC*
11	*BRCA1*
12	*DPYD*
13	*GREM1*
14	*MLH1*
15	*PDGFRA*
16	*POLE*
17	*SDHD*
18	*ATM*
19	*BRCA2*
20	*EGFR*
21	*HRAS*
22	*MSH2*
23	*PIK3CA*
24	*PTCH1*
25	*SMAD4*
26	*BLM*
27	*CDH1*
28	*EPCAM*
29	*KIT*
30	*MSH6*
31	*PMS1*
32	*PTEN*
33	*STK11*
34	*BMPRA*
35	*CHEK2*
36	*ERBB2*
37	*KRAS*
38	*MUTYH*
39	*PMS2*
40	*SDHB*
41	*TP53*

gene mutations, of which 6 (6.6%) had no other gene mutations and 13 (14.1%) had other gene mutations in the 41 genes group. By comparing the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php), dbSNP database (https://www.ncbi.nlm.nih.gov/snp/) and COSMIC database (https://cancer.sanger.ac.uk/cosmic/), a total of 582 *STK11* gene mutant sites were included in HGMD as of March 1, 2022. We identified 21 new mutant sites in other genes (Table 2) and 26 new *STK11* gene mutant sites (Table 3).

Gene detection results and pathogenicity analysis of PJS with mutant-type *STK11*: *STK11* gene detection results and pathogenicity analysis. *STK11* gene mutations were detected in 73 PJS patients in our group. By making comparisons with the dbSNP and COSMIC databases, 49 PJS-related gene mutations were included and 26 *STK11* gene mutation sites were newly identified by sequencing in this group (Table 3). Types of *STK11* gene mutations included premature termination codons (24), frame shift mutations (14), splice site variants (13), missense mutations (17), nonsense mutations (4), mutations in the 3' untranslated region (2) and gene fusion (1).

The pathogenicity of *STK11* gene mutations was determined by comparing the HGMD and ClinVar gene mutations in the disease database, and following the corresponding grading criteria (Table 3). By making conservative predictions of amino acid sequences, we made determinations about the pathogenicity of *STK11* gene mutations according to the corresponding grading criteria (Table 3): Premature termination codons, frame shift mutations, splice site variants, missense mutations, nonsense mutations and mutations in the 3' untranslated region were associated with pathogenicity. Premature termination codons accounted for 58.3% (14/24) that were clearly pathogenic and 41.7% (10/24) that were probably pathogenic. Frameshift mutations accounted for 35.7% (5/14) that were clearly pathogenic, 50.0% (7/14) that were probably pathogenic and 14.3% (2/14) that were of uncertain significance. Splice site mutations accounted for 23.1% (3/13) that were clearly pathogenic, 61.5% (8/13) that were probably pathogenic and 15.4% (2/13) that were of uncertain significance. Missense mutations accounted for 17.6% (3/17) that were clearly pathogenic, 41.2% (7/17) that were probably pathogenic and 41.2% (7/17) that were of uncertain significance. 3' untranslated region mutations accounted for 50.0% (1/2) that were clearly pathogenic and 50.0% (1/2) that were probably pathogenic. All four nonsense mutations were clearly pathogenic (100.0%).

Other gene detection results and pathogenicity analysis in PJS patients with mutant-type *STK11*: Among the 73 PJS patients with mutant-type *STK11*, 47 were combined with other gene mutations: Combined with *AKT1* mutation in 3 cases, *APC* mutation in 4 cases, *ATM* mutation in 5 cases, *BLM* mutation in 3 cases, *BRCA2* mutation in 4 cases, *CDH1* mutation in 3 cases, *CHEK2* mutation in 3 cases, *EGFR* mutation in 1 case, *EPCAM* mutation in 1 case, *GALNT12* mutation in 6 cases, *KIT* mutation in 3 cases, *MUTYH* mutation in 5 cases, *MSH6* mutation in 5 cases, *MSH2* mutation in 4 cases, *MLH1* mutation in 1 case, *PDGFRA* mutation in 5 cases, *PIK3CA* mutation in 1 case, *PMS1* mutation in 1 case, *PMS2* mutation in 1 case, *POLD1* mutation in 3 cases, *PTCH1* mutation in 4 cases, *POLE* mutation in 8 cases and *TP53* mutation in 2 cases.

Among the other gene mutations in PJS patients with mutant-type *STK11*, there were 52 missense mutations (68.4%), 5 intron mutations (6.6%), 11 mutations in the 3' untranslated region (14.5%), 3 splice site mutations (4.0%), 1 premature termination codon (1.3%), 1 frameshift deletion (1.3%), 1 mutation in the 5' untranslated region (1.3%), 1 upstream gene mutation (1.3%) and 1 case (1.3%) of stop gain. All mutations were of uncertain significance (100.0%) (Figure 1).

Gene detection results and pathogenicity analysis in PJS patients with wild-type *STK11*: Wild-type PJS gene mutations included *BLM* mutation in 2 cases, *BMPR1A* mutation in 1 case, *POLD1* mutation in 3 cases, *CHEK2* mutation in 4 cases, *MUTYH* mutation in 6 cases, *SDHC* mutation in 1 case, *POLE* mutation in 2 cases, *BRCA* mutation in 3 cases, *APC* mutation in 2 cases, *CDH1* mutation in 1 case, *ATM* mutation in 3 cases, *ERBB2* mutation in 1 case, *SMAD4* mutation in 1 case and *SBDS* mutation in 1 case.

Among the other gene mutations in wild-type PJS patients, there were 23 missense mutations (74.2%), 5 intron mutations (16.1%), 1 splice site mutation (3.2%), 1 frameshift deletion (3.2%) and 1 mutation in the 3' untranslated region (3.2%). All mutations were of uncertain significance (Table 4).

Comparison of general information, diagnosis and treatment, pathology and examinations

Through the comparison of related items between the two groups (Table 5), it can be found that there were significant differences between the two groups in the following items ($P < 0.05$). Such as Age of initial treatment, Total hospitalizations, Age of first intussusception, Frequency of intussusception, Age of initial surgery, Time interval from mucocutaneous pigmentation appearance to abdominal symptoms, Maximum diameter of gastric polyps, Load of duodenal intestine polyps, Distribution of colorectal polyps, Times of endoscopic examinations.

Exploration of PJS genotype-clinical phenotype linkage

Clinical phenotypic variations between PJS with mutant-type and wild-type *STK11*: (1) There were no statistical differences in gender, family history, ABO blood group or Rh blood group between PJS with mutant-type and wild-type *STK11*; (2) There were differences ($P < 0.05$) between wild-type and mutant-type in age of initial treatment, total hospitalizations, age of first intussusception, frequency of intussus-

Table 2 21 new mutant sites in other genes

Sample	Gene	Description	HGVSc	Mutation type
1	*AKT1*	p.E135G	c.404A>G	Missense variant
2	*APC*	p.A41T	c.121G>A	Missense variant
3	*APC*	p.C417G	c.1249T>G	Missense variant
4	*ATM*	p.L2750[1]	c.8249T>G	Stop gained
5	*ATM*	p.A84S	c.250G>T	Missense variant
6	*ATM*	p.I1332M	c.3996T>G	Missense variant
7	*BLM*	p.E1035G	c.3104A>G	Missense variant
8	*BRCA2*	p.D635E	c.1905T>A	Missense variant
9	*BRCA2*	p.T1346N	c.4037C>A	Missense variant
10	*CHEK2*	c.908+16T>G	c.908+16T>G	Intron variants
11	*CDH1*	c.47G>A[1]	c.47G>A[1]	3 prime UTR variant
12	*CDH1*	p.S145Y	c.434C>A	Missense variant
13	*CDH1*	p.883Yext?[1]	c.2649G>C	Stop lost
14	*GALNT12*	c.-6G>T	c.-6G>T	Upstream genetic variant
15	*KIT*	p.M289I	c.867G>C	Missense variant
16	*MLH1*	p.T451R	c.1352C>G	Missense variant
17	*PMS1*	p.D405E	c.1215T>A	Missense variant
18	*POLE*	p.R1556G	c.4666C>G	Missense variant
19	*POLD1*	p.K486del	c.1456_1458del	Conservative inframe deletion
20	*SDHC*	p.L106V	c.316C>G	Missense variant
21	*SMAD4*	p.A309V	c.926C>T	Missense variant

[1]Nonsense mutation leading to protein inactivation.

ception and age of initial surgery, with wild-type PJS having a much higher age of initial treatment, much smaller number of total hospitalizations, higher age of first intussusception and lower frequency of intussusception than mutant-type PJS. There was no statistical difference between wild-type and mutant-type PJS in number of operations; (3) There was no statistical difference between wild-type and mutant-type PJS in age or order of mucocutaneous pigmentation appearance, but there was a difference in time interval from mucocutaneous pigmentation appearance to abdominal symptoms ($P < 0.05$), which was longer in wild-type PJS than in the mutant-type PJS; (4) There was no statistical difference in distribution and load of gastric polyps between wild-type and mutant-type PJS, but the maximum diameter of gastric polyps was significantly lower in wild-type PJS than mutant-type PJS ($P < 0.05$). There was no statistical difference in distribution and maximum diameter of duodenal intestine polyps, but there was a difference in load of duodenal intestine polyps ($P < 0.05$), which was much lower in wild-type PJS than in mutant-type PJS. There was no statistical difference in load and maximum diameter of colorectal polyps, but distribution of colorectal polyps was significantly lower in wild-type PJS than in mutant-type PJS; and (5) There was no statistical difference in pathology or carcinogenesis of polyps between wild-type and mutant-type PJS. There was a difference in endoscopic examination between wild-type and mutant-type PJS ($P < 0.05$), with fewer times of endoscopic examination in wild-type than mutant-type PJS.

Clinical phenotypic differences between mutant-type PJS combined with other mutations and not combined with other mutations: (1) *MUYTH*: There was a difference in ABO blood group between mutant-type PJS with and without *MUYTH* mutations ($P < 0.05$); (2) *CHEK2:* There were differences in ABO blood group, total hospitalizations and number of operations between mutant-type PJS with and without *CHEK2* mutations ($P < 0.05$); (3) *APC:* There were differences in distribution, load and maximum diameter of gastric polyps between mutant-type PJS with and without *APC* mutations ($P < 0.05$); (4) *CDH1*: There were differences in load and maximum diameter of duodenal intestine polyps between mutant-type PJS with and without *CDH1* mutations ($P < 0.05$); (5) *GALNT12:* There were differences in time interval from mucocutaneous pigmentation appearance to abdominal symptoms and

Table 3 Characterization and pathogenicity of *STK11* mutations

Sample	Mutation_type	Description	HGVSc	dbSNP RS	COSM_ID	Classification
1	Stop gained	p.Y60[1]	c.180C>G	/	COSM20874	P
2	Splice acceptor variant	c.921-1G>A	c.921-1G>A	/	COSM49008	LP
3	Splice acceptor variant	c.921-1G>C	c.921-1G>C	rs398123406	/	P
4	Stop gained	p.K84[1]	c.250A>T	rs137853076	COSM3388586; COSM3388585	P
5	Splice acceptor variant	c.921-1G>C	c.921-1G>C	rs398123406	/	P
6	Stop gained	p.Q123[1]	c.367C>T	/	COSM5224269; COSM380443	P
7	Missense variant	p.W239C	c.717G>T	/	COSM333593; COSM4278104	LP
8	Missense variant	p.R297S	c.891G>T	rs730881984	/	P
9	Stop gained	p.Q100[1]	c.298C>T	/	/	LP
10	Stop gained	p.K84[1]	c.250A>T	rs137853076	COSM3388586; COSM3388585	P
11	Missense variant	p.R409W	c.1225C>T	rs368466538	COSM25854	VUS
12	Stop gained	p.Q112[1]	c.334C>T	/	COSM3528680; COSM3528681	LP
13	Missense variant	p.D176N	c.526G>A	rs730881979	COSM4827691; COSM4827690	P
14	Stop gained	p.K84[1]	c.250A>T	rs137853076	COSM3388586; COSM3388585	P
15	Missense variant	p.R304W	c.910C>T	rs786201090	COSM29468	LP
16	Stop gained	p.E120[1]	c.358G>T	rs775595174	COSM20875	P
17	Stop gained	p.K84[1]	c.250A>T	rs137853076	COSM3388586; COSM3388585	P
18	Conservative inframe deletion	p.Y60fs	c.179dup	rs876661012	COSM5219400; COSM1480565	P
19	Stop gained	p.R86[1]	c.256C>T	/	COSM4767773; COSM4767772	P
20	Stop gained	p.Q170[1]	c.508C>T	rs121913323	COSM20943	LP
21	Stop gained	p.Q170[1]	c.508C>T	rs121913323	COSM20943	LP
22	Missense variant	p.S240W	c.719C>G	rs730881976	/	VUS
23	Splice acceptor variant	c.921-2A>G	c.921-2A>G	/	/	LP
24	Splice acceptor variant	c.921-1G>C	c.921-1G>C	rs398123406	/	P
25	Conservative inframe deletion	p.P281fs	c.842del	rs121913321	COSM4336438; COSM20871	P
26	Splice acceptor variant	p.L245F	c.733C>T	/	COSM1523960; COSM4278108	VUS
27	Stop gained	p.Q137[1]	c.409C>T	rs730881970	COSM48901	P
28	Stop gained	p.Q137[1]	c.409C>T	rs730881970	COSM48901	P
29	Stop gained	p.Q123[1]	c.367C>T	/	COSM5224269; COSM380443	P
30	Missense variant	p.D194N	c.580G>A	rs121913315	COSM25847	VUS
31	3_prime_UTR_variant	c.201G>A[1]	c.201G>A[1]	rs528679025	/	P
32	Splice acceptor variant	c.598-2A>G	c.598-2A>G	/	/	LP
33	Stop gained	p.Q159[1]	c.475C>T	/	COSM5002233; COSM27316	LP
34	Gene fusion	STK11-MIDN	/	/	/	LP
35	Conservative inframe deletion	p.D53fs	c.157del	/	COSM27282; COSM6048514	VUS
36	Missense variant	p.D194N	c.580G>A	rs121913315	COSM25847	LP
37	Missense variant	p.P179Q	c.536C>A	/	COSM4822602; COSM4822601	LP
38	Stop gained	p.W308[1]	c.924G>A	/	/	LP
39	Stop gained	p.E65[1]	c.193G>T	/	COSM20876	P

40	Splice acceptor variant	c.920+1G>C	c.920+1G>C	/	COSM4412472; COSM4412473	LP
41	Conservative inframe deletion	p.C134fs	c.402_403del	rs587782424	COSM5508976; COSM5508975	P
42	Nonsense variant	p.Q220X	c.658C>T	/	COSM13480; COSM4278102	P
43	Nonsense variant	p.Y60X	c.180del	/	COSM20874; COSM27322; COSM48900; COSM5490514	P
44	Missense variant	p.D194N	c.580G>A	rs121913315	/	LP
45	Missense variant	p.R297K	c.890G>A	/	COSM401786; COSM6149636	LP
46	Splice acceptor variant	/	c.863-2A>G	/	/	LP
47	Nonsense variant	p.Q137X	c.409C>T	rs730881970	/	P
48	Splice acceptor variant	c.735-6_735-2del	c.735-6_735-2del	rs759090799	/	VUS
	Conservative inframe deletion[2]	p.L183fs	c.548del	/	/	LP
49	3 prime UTR variant	c.201G>A[1]	c.201G>A[1]	rs528679025	/	LP
	Conservative inframe deletion[2]	p.C158fs	c.472del	/	/	VUS
50	Stop gained[2]	p.K81[1]	c.241A>T	/	/	LP
51	Missense variant[2]	p.R304P	c.911G>C	/	/	P
52	Missense variant[2]	p.R297K	c.890G>A	/	/	LP
53	Conservative inframe deletion[2]	p.E145fs	c.426_448del	/	/	LP
54	Stop gained[2]	p.Y272[1]	c.816C>A	/	/	LP
55	Stop gained[2]	p.Q100[1]	c.298C>T	/	/	LP
56	Conservative inframe deletion[2]	p.K64fs	c.190_191del	/	/	LP
57	Splice acceptor variant[2]	c.921-2A>G	c.921-2A>G	/	/	LP
58	Stop gained[2]	p.Y292[1]	c.876C>G	/	/	LP
59	Conservative inframe deletion[2]	p.T212fs	c.634del	/	/	LP
60	Stop gained[2]	p.K97[1]	c.289A>T	/	/	P
61	Missense variant[2]	p.H154P	c.461A>C	/	/	VUS
62	Missense variant[2]	p.A153P	c.457G>C	/	/	VUS
63	Missense variant[2]	p.L140P	c.419T>C	/	/	VUS
64	Conservative inframe deletion[2]	p.F264fs	c.792del	/	/	P
65	Stop gained[2]	p.W308[1]	c.924G>A	/	/	P
66	Splice acceptor variant[2]	c.598-2A>G	c.598-2A>G	/	/	LP
67	Conservative inframe deletion[2]	p.F157fs	c.471_472del	/	/	LP
68	Conservative inframe deletion[2]	p.S193fs	c.577_578del	/	/	LP
69	Splice acceptor variant[2]	c.734+1G>A	c.734+1G>A	/	/	LP
70	Missense variant[2]	p.L290H	c.869T>A	/	COSM20944; COSM25847; COSM4278092	VUS
71	Nonsense variant[2]	p.Y60X	c.179dup	/	/	P
72	Conservative inframe deletion[2]	p.L282Afs	c.842dup	/	/	P
73	Conservative inframe deletion[2]	p.V77Rfs	c.228dup	/	COSM48901	LP

[1]Nonsense mutation leading to protein inactivationy.
[2]The table shows the 26 new *STK11* mutation sites.
P: Pathogenic; LP: Likely pathogenic; VUS: Uncertain significance; SNP: Single nucleotide polymorphism.

Table 4 Characterization and pathogenicity of mutations in wild-type Peutz-Jeghers syndrome patients

Sample	Gene	Description	HGVSc	Mutation_type	dbSNP RS	COSM_ID	Classification
1	*BLM*	p.I947V	c.2839A>G	Missense mutation	rs189925962	NA	VUS
	BMPR1A	p.A13T	c.37G>A	Missense mutation	rs200115604	NA	VUS
	POLD1	p.K486del	c.1456_1458del	Frameshift deletion	NA	NA	VUS
2	*CHEK2*	p.S252N	c.755G>A	Missense mutation	rs587781379	COSM6004987; COSM6004988	VUS
	MUTYH	c.36+11C>T	c.36+11C>T	Intron mutations	rs2275602	COSN17145138	VUS
	SDHC	p.L106V	c.316C>G	Missense mutation	NA	NA	VUS
3	*CHEK2*	p.R181H	c.542G>A	Missense mutation	rs121908701	NA	VUS
	MUTYH	c.37_39del[1]	c.37_39del[1]	Mutation in the 3' untranslated region	rs373507005	NA	VUS
	MUTYH	c.36+11C>T	c.36+11C>T	Intron mutations	rs2275602	COSN17145138	VUS
	MUTYH	p.G25D	c.74G>A	Missense mutation	rs75321043	NA	VUS
	MUTYH	p.P18L	c.53C>T	Missense mutation	rs79777494	NA	VUS
	POLE	c.3378+10A>G	c.3378+10A>G	Intron mutations	rs193075152	NA	VUS
4	*BLM*	p.M348I	c.1044G>A	Missense mutation	rs184657475	COSM1580597	VUS
5	*BRCA1*	p.P1192L	c.3575C>T	Missense mutation	NA	COSM4991001; COSM4991000	VUS
	BRCA2	p.F3328C	c.9983T>G	Missense mutation	rs770826575	NA	VUS
	CHEK2	p.H371Y	c.1111C>T	Missense mutation	rs531398630	COSM4002125	VUS
6	*APC*	p.I1524R	c.4571T>G	Missense mutation	rs200803739	NA	VUS
7	*CDH1*	p.S145Y	c.434C>A	Missense mutation	NA	NA	VUS
	POLE	c.3378+10A>G	c.3378+10A>G	Intron mutations	rs193075152	NA	VUS
8	*ATM*	c.3154-5C>T	c.3154-5C>T	Intron mutations	rs55719759	NA	VUS
	CHEK2	p.S252N	c.755G>A	Missense mutation	rs587781379	COSM6004987; COSM6004988	VUS
	ERBB2	p.V1253M	c.3757G>A	Missense mutation	rs36085723	NA	VUS
9	*ATM*	p.I1332M	c.3996T>G	Missense mutation	NA	NA	VUS
	POLD1	p.A532T	c.1594G>A	Missense mutation	rs765276497	NA	VUS
10	*MUTYH*	c.934-2A>G	c.934-2A>G	Splice receptor mutation	rs77542170	NA	VUS
	SMAD4	p.A309V	c.926C>T	Missense mutation	NA	NA	VUS
11	*APC*	p.A41T	c.121G>A	Missense mutation	NA	NA	VUS
	POLD1	p.R218H	c.653G>A	Missense mutation	rs150010804	NA	VUS
12	*SBDS*	p.K33R	c.98A>G	Missense mutation	rs373730800	COSM4826086	VUS
13	*ATM*	p.V519I	c.1555G>A	Missense mutation	NA	NA	VUS
	BRCA2	p.H523R	c.1568A>G	Missense mutation	rs80358443	NA	VUS

[1]Nonsense mutation leading to protein inactivation.
P: Pathogenic; LP: Likely pathogenic; VUS: Uncertain significance; NA: Not available.

Table 5 Comparison of clinicopathological parameters between two groups

Project	Wild-type (*n* = 19)	Mutant-type (*n* = 73)	*P* value
Gender			
Male	11	44	0.851
Female	8	29	
Family history			
No	13	51	0.903
Yes	6	22	
ABO blood group[1]			
A	7	23	0.964
B	6	21	
AB	4	18	
O	2	9	
Rh blood group[1]			
Negative	0	0	
Positive	19	71	
Age of initial treatment (years)	18.474 ± 8.8089	12.973 ± 8.3881	0.021
Final age of follow-up (years)	30.842 ± 11.3101	27.425 ± 9.7680	0.239
Total hospitalizations	3 (1, 4)	4 (3, 6)	0.003
Age of first intussusception (years)	22 (14, 27)	15 (9.25, 24)	0.025
Frequency of intussusception	1 (1, 2)	2 (1, 3)	0.006
Age of initial surgery (years)	19 (14, 25)	14 (8, 23.75)	0.007
Number of operations	1 (1, 2)	1 (1, 2)	0.924
Age of mucocutaneous pigmentation appearance (years)	3 (0, 5)	3 (0.5, 5)	0.811
Order of mucocutaneous pigmentation appearance			
Lips	2	17	0.213
Lips and limbs	1	46	
Lips to limbs	16	10	
Time interval from mucocutaneous pigmentation appearance to abdominal symptoms (years)	14.5 (8, 25.5)	10 (5, 15)	0.038
Distribution of gastric polyps			
Yes	16 (84.2%)	60 (82.2%)	1
No	3 (15.8%)	13 (17.8%)	
Load of gastric polyps	5 (5, 10)	5 (3.25, 10)	0.111
Maximum diameter of gastric polyps (mm)	7 (4.25, 10)	10 (6, 15)	0.012
Distribution of duodenal intestine polyps			
Yes	18 (94.7%)	71 (97.3%)	1
No	1 (5.3%)	2 (2.7%)	
Load of duodenal intestine polyps	3 (1, 6.5)	7 (4, 15.5)	0.013
Maximum diameter of duodenal intestine polyps (mm)	30 (15, 50)	48 (30, 60)	0.110
Distribution of colorectal polyps			
Yes	6 (31.6%)	52 (71.2%)	0.001
No	13 (68.4%)	21 (28.8%)	
Load of colorectal polyps	4 (1.5, 12)	3 (1, 10)	0.864

Maximum diameter of colorectal polyps (mm)	30 (15, 50)	25 (13.5, 40)	0.664
Carcinogenesis			
Yes	0 (0%)	9 (9.52%)	0.239
No	19 (100%)	64 (90.48%)	
Pathology of polyps			
Hamartoma	12	35	0.344
Adenoma	2	8	
Hamartoma + adenoma	2	4	
Carcinogenesis	0	9	
Deletion	3	17	
Times of endoscopic examinations	2 (1, 2)	2 (2, 4.75)	0.012

[1]A few cases did not undergo relevant laboratory tests.

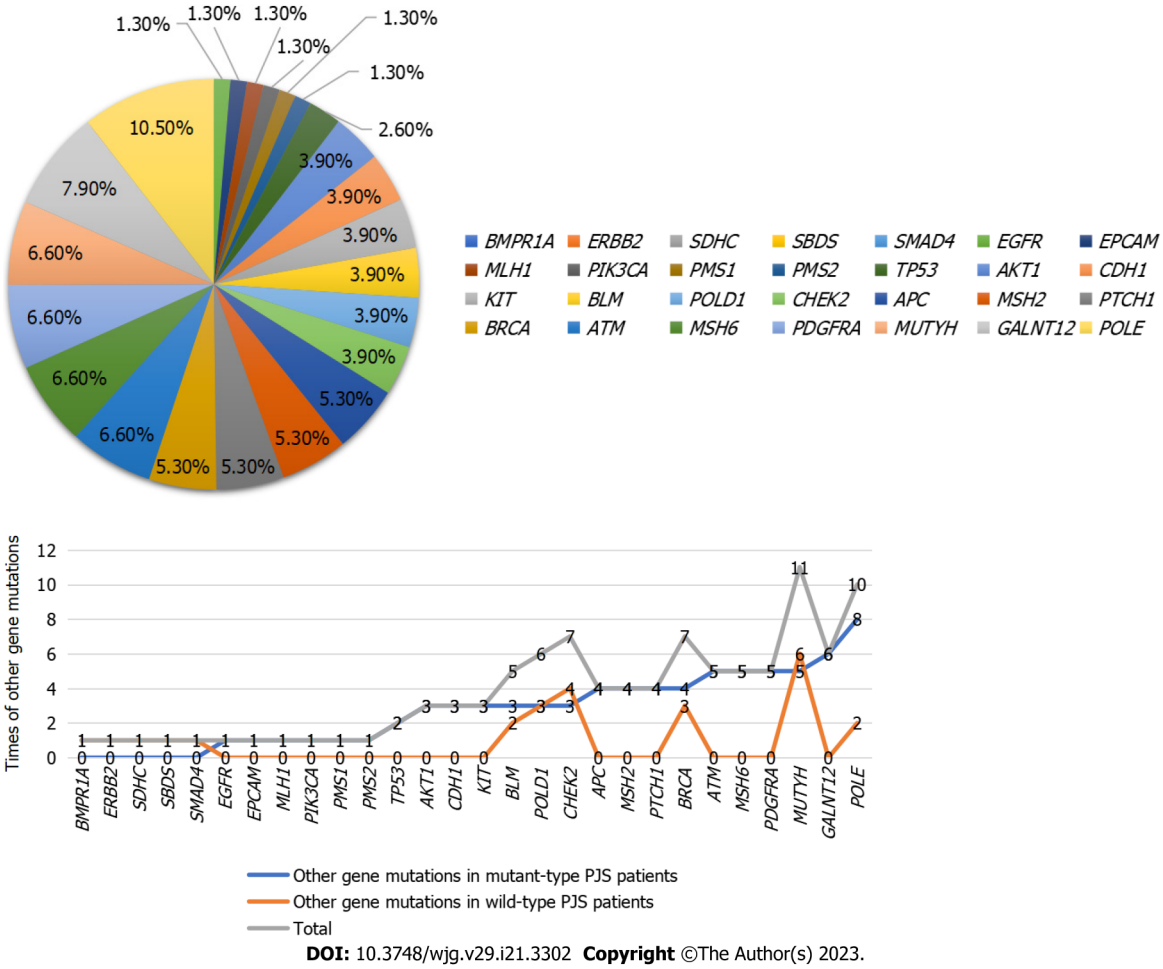

Figure 1 Comparisons of percentage of other gene mutations.

maximum diameter of duodenal intestine polyps between mutant-type PJS with and without *GALNT12* mutations ($P < 0.05$); (6) *BRCA*: There were differences in maximum diameter of duodenal intestine polyps between mutant-type PJS with and without *BRCA* mutations ($P < 0.05$); (7) *KIT:* There were differences in ABO blood group and age of first intussusception between mutant-type PJS with and without *KIT* mutations ($P < 0.05$); (8) *MSH:* There was a difference in distribution of colorectal polyps between mutant-type PJS with and without *MSH* mutations ($P < 0.05$); (9) *PTCH1:* There were differences in load of gastric polyps and maximum diameter of colorectal polyps between mutant-type

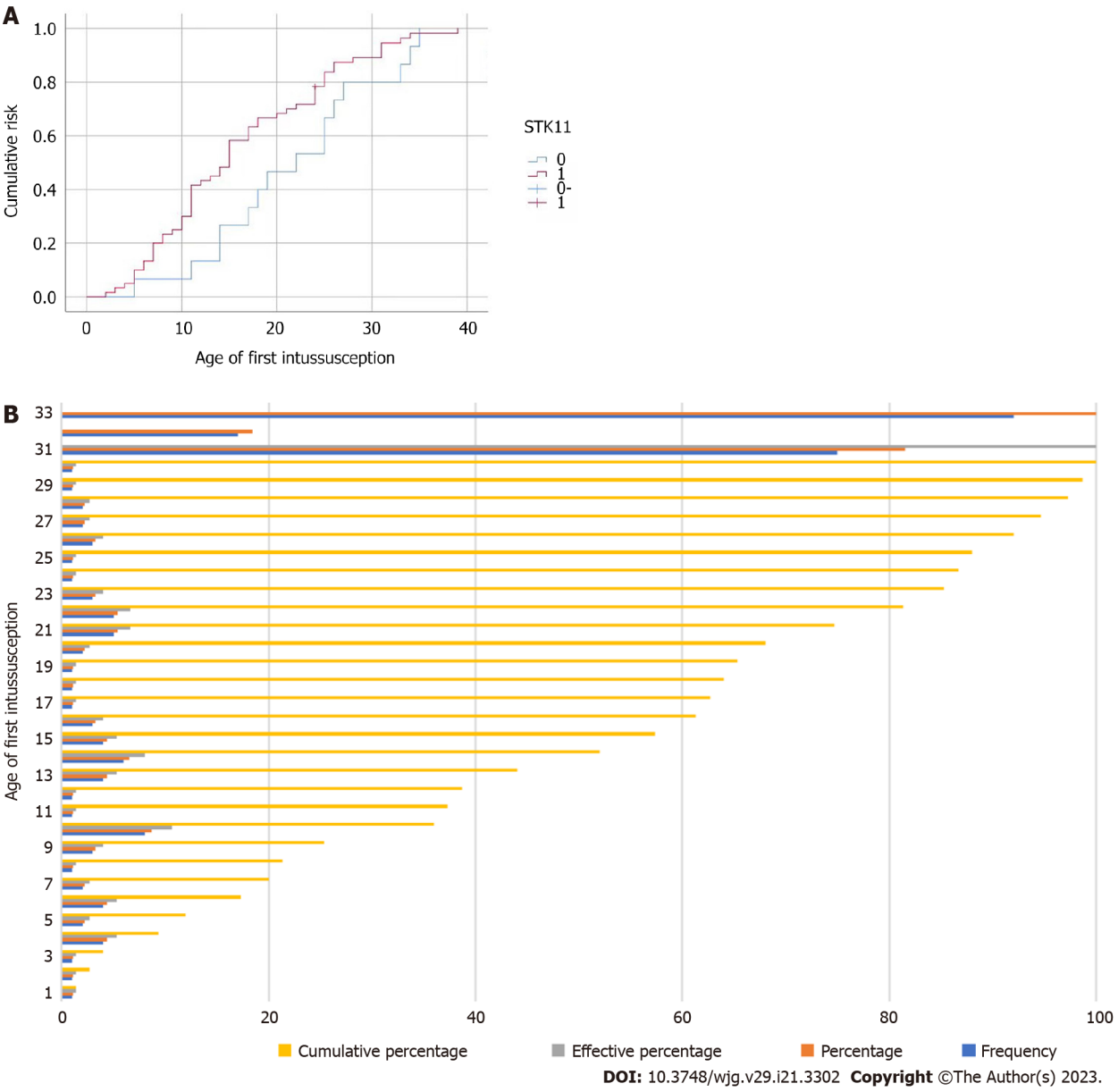

Figure 2 Intussusception. A: Cumulative risk function for intussusception; B: Cumulative percentage at age of first intussusception.

PJS with and without *PTCH1* mutations ($P < 0.05$); (10) *ATM:* There was a difference in age of mucocutaneous pigmentation appearance between mutant-type PJS with and without *ATM* mutations ($P < 0.05$); (11) *PDGFRA*: There was a difference in final age of follow-up between mutant-type PJS with and without *PDGFRA* mutations ($P < 0.05$); and (12) *POLD1:* There was a difference in age of first intussusception between mutant-type PJS with and without *POLD1* mutations ($P < 0.05$).

DISCUSSION

PJS is an autosomal dominant disorder with a prevalence of approximately 1 in 200000[15,16]. Although PJS is a rare disease, the large population of China and the prolonged course of PJS lead to the accumulation of a great number of PJS patients in Chinese society. Germline mutations in the *STK11* gene are recognized as the molecular genetic cause of PJS. The tumor suppressor gene *STK11* is involved in multiple processes such as embryonic development, cell polarity, cell cycle arrest, apoptosis and metabolism, and its mutations have been detected in a variety of disseminated cancers. A related study [17] demonstrated that the lack of the *STK11* gene resulted in a significant increase of intracellular reactive oxygen species levels and enhanced expression of phosphorylated histone γ-H2AX, which resulted in DNA damage, oxidative damage to the genome and an increased mutation rate. The rate of *STK11* germline mutations detected in this group of PJS patients was 79.35%, which is generally

Table 6 Recommended follow-up and intervention strategies for mutant-type and wild-type Peutz-Jeghers syndrome

Age (yr)	Mutant-type		Wild-type	
	Surveillance	Intervention	Surveillance	Intervention
< 7	Routine abdominal ultrasound surveillance is recommended every year	Removal of polyps	Abdominal ultrasound is recommended every 3-5 yr	Follow-up observation
8-11	Routine abdominal ultrasound surveillance is recommended every year. For symptomatic individuals with PJS, an abdominal ultrasound should be performed earlier	Removal of polyps	Abdominal ultrasound is recommended every 3-5 yr. For symptomatic individuals with PJS, an abdominal ultrasound should be performed earlier	Removal of polyps
8-18	Total GI surveillance every year (CT scan of small-bowel or enteroscopy/capsule endoscopy should be offered as options	Polyps > 10 mm should be removed	Total GI surveillance 2-3 yr	Removal of polyps
19-30	Routine total GI polyps surveillance every 2-3 yr and screening for systemic tumors	Removal of polyps and treatment of tumors	Routine total GI polyps surveillance every 2-3 yr	Removal of polyps
> 30	Focus on detection of tumors in digestive tract and other organs	Treatment of tumors	Focus on detection of tumors in digestive tract and other organs	Treatment of tumors

PJS: Peutz-Jeghers syndrome; GI: Gastrointestina; CT: Computed tomography.

consistent with the literature[18].

The target genome covered 41 pathogenic genes associated with digestive tract tumors, including *STK11*, and PJS patients were classified into wild-type or mutant-type based on the presence or absence of *STK11* mutations. The results of the clinical characteristic analysis showed that there were significant differences between wild-type and mutant-types PJS in age of initial treatment, age of first intussusception, frequency of intussusception, age of initial surgery, time interval from mucocutaneous pigmentation appearance to abdominal symptoms, maximum diameter of gastric polyps, load of duodenal intestine polyps, distribution of colorectal polyps, times of hospitalization and times of endoscopic examinations. Mutant-type PJS typically has an earlier age of initial treatment and a shorter time interval from mucocutaneous pigmentation appearance to abdominal symptoms than wild-type PJS. They are often first admitted to hospital for serious complications such as intussusception and intestinal obstruction. Our study found that the age of first intussusception was significantly younger in mutant-type PJS than in wild-type PJS, and its cumulative risk of intussusception at the age of 20 years was 68.3%, which was significantly higher than that of wild-type PJS (Figure 2). This is consistent with the findings of domestic and international studies[19,20]. As a result, complications such as intestinal obstruction and intussusception are more likely to occur in mutant-type PJS than in wild-type, and occur earlier. The earlier intervention of treatment for GI polyps in mutant-type PJS patients would be beneficial in reducing the occurrence of these complications. In addition, mutant-type PJS has a higher distribution of colorectal polyps and a larger maximum diameter of gastric polyps than wild-type PJS, requiring more frequent endoscopic examinations and treatment in hospital.

Overseas studies have shown that PJS patients have an increased risk of cancer at several sites, including the GI tract, breast, ovaries, testes and lungs[21]. PJS malignancy is a serious threat to the life and health of patients; studies have shown[21,22] that the death rate of PJS patients is as high as 32%, and malignancy is the main cause of death. The incidence of malignant tumors in PJS patients is 19%-32%, with an average age of 42-45 years, and a predominance of GI tract tumors (51%-69%), followed by gynecological tumors (22%-26%). The risk of GI malignancy is 50 times higher than that of the general population. The 19 cases of wild-type PJS in this study were not combined with malignant tumors. Among the 73 mutant-type patients, 8 had GI polyps and 4 had cancers of other sites: 1 adnexal cystadenocarcinoma, 1 ovarian mucinous tumor, 1 cervical adenocarcinoma and 1 nasopharyngeal carcinoma. It was found that patients with a detectable *STK11* truncating mutation tend to develop more polyps and cancers, and require more surgical intervention[23]. There was no statistically significant difference in the cancer rate between mutant-type and wild-type in this study, which may be related to the shorter follow-up period of the enrolled patients. Germline mutations of *STK11* are essential factors in GI tumourigenesis, and the cell types and signal pathways that lead to the malignant transformation of polyps are still unclear. A foreign study showed[24] that after the knockout of *STK11* in mice, IL-11 inflammatory mediators mediated the activation of the JAK/STAT3 pathway due to *STK11* deficiency in mouse stromal cells, which eventually resulted in the formation of polyp malignancy in mice, and the treatment of *STK11*-deficient mice with JAK1/2 inhibitors significantly reduced the occurrence of polyps. We can block the development and malignancy of polyps at the root by blocking the associated inflammatory mediators or transduction pathways, which provides a new idea for us in studying the pharmacological treatment of PJS patients. Previous studies have shown that PJS patients have a progression pathway of hamartoma-(adenoma)-carcinoma[25], which is corrob-

orated by the presence in our study of PJS patients with malformation combined with adenomatous polyps and with adenoma combined with polyp carcinoma. Accordingly, the detection of *STK11* mutations can be useful for guiding the assessment of polyp carcinogenesis risk in PJS patients and their relatives.

PJS patients have a prolonged disease course, with GI polyps growing larger with age and recurring more easily, and can result in intestinal obstruction, intussusception or even cancer, but the characteristics and severity of the disease vary significantly among PJS patients. The clinical presentation is distinctly heterogeneous. Clinical data and epidemiological data collected from more than 500 PJS patients at our center suggests that polyps grow fastest in adolescence, leading to serious complications such as intussusception, and the age of first surgery is significantly younger in mutant-type PJS than in wild-type PJS. Meanwhile, the incidence of tumors in the digestive tract and other organs of the body is significantly higher in middle-aged PJS patients.

CONCLUSION

Therefore, in the individualized treatment of PJS patients, we recommend that they should have the *STK11* gene tested, and the genotypes classified into mutant-type and wild-type. Patients with mutant-type PJS should be strictly controlled in terms of treatment and follow-up strategies, while patients with wild-type PJS can be treated with relaxed treatment conditions and follow-up years. For the monitoring and treatment of PJS, we refer to the different follow-up strategies by age group proposed by a domestic study[26], and further refine the treatment and follow-up strategies for PJS based on this treatment strategy as follows (Table 6).

ARTICLE HIGHLIGHTS

Research background

Peutz-Jeghers syndrome (PJS) is an autosomal dominant genetic disease with skin mucosal pigment spots and gastrointestinal multiple hamartoma polyps as clinical characteristics. At present, it is considered that the germline mutation of *STK11* gene is the genetic cause of PJS. However, not all PJS patients can be detected *STK11* germline mutations.

Research motivation

The specific clinical characteristics of these PJS patients without *STK11* mutation is an interesting clinical question. Or, like wild type gastrointestinal stromal tumor (GIST), whether these PJS without *STK11* mutation are also called PJS is worth discussing. Therefore, we designed the study to understand the clinical characteristics of these PJS patients without *STK11* mutation.

Research objectives

To investigates whether PJS patients with known *STK11* mutations have a more severe spectrum of clinical phenotypes compared to those without.

Research methods

The general information, diagnosis and treatment history, pathology, times of examination and other clinical data of the 92 enrolled PJS patients were collected for statistical analysis. Genomic DNA samples were extracted from peripheral blood samples, and pathogenic germline mutations of *STK11* were detected by high-throughput next-generation gene sequencing. Clinical-pathologic manifestations of patients with and without *STK11/LKB1* mutations were compared.

Research results

Compared with PJS patients with *STK11* mutations, those without tended to be older at the age of initial treatment, age of first intussusception and age of initial surgery. They also had a lower number of total hospitalizations relating to intussusception or intestinal obstruction, and a lower load of small intestine polyps. Final results found that PJS patients without *STK11* mutations might have less severe clinical-pathologic manifestations than those with.

Research conclusions

PJS patients without *STK11* mutations might have less severe clinical-pathologic manifestations than those with.

Research perspectives

At present, it is considered that the germline mutation of *STK11* gene is the genetic cause of PJS.

However, not all PJS patients can be detected *STK11* germline mutations. The specific clinical characteristics of these PJS patients without *STK11* mutation is an interesting clinical question. Or, like wild type GIST, whether these PJS without *STK11* mutation are also called PJS is worth discussing.

FOOTNOTES

Author contributions: Jiang LX, Chen YR, Xu ZX and Zhang YH contributed equally to this study; Gu GL designed the research; Jiang LX, Chen YR, Xu ZX, Zhang YH, Zhang Z, Yu PF, Dong ZW and Yang HR collected and analyzed the clinical data; Jiang LX, Chen YR and Xu ZX wrote the manuscript; Gu GL and Dong ZW revised the manuscript.

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

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Artifacts in two-dimensional shear wave elastography of liver

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Abstract

BACKGROUND

Artifacts are common when using two-dimensional shear wave elastography (2-D SWE) to measure liver stiffness (LS), but they are poorly recognized.

AIM

To investigate the presence and influence of artifacts in 2-D SWE of liver.

METHODS

We included 158 patients with chronic liver disease, who underwent 2-D SWE examination by a novice and an expert. A cross line at the center of the elastogram was drawn and was divided it into four locations: top-left, top-right, bottom-left, and bottom-right. The occurrence frequency of artifacts in different locations was compared. The influence of artifacts on the LS measurements was evaluated by comparing the elastogram with the most artifacts (EMA) and the elastogram with the least artifacts (ELA).

RESULTS

The percentage of elastograms with artifacts in the novice (51.7%) was significantly higher than that of the expert (19.6%) ($P < 0.001$). It was found that both operators had the highest frequency of artifacts at bottom-left, followed by top-left and bottom-right, and top-right had the lowest frequency. The LS values (LSVs) and standard deviation values of EMAs were significantly higher than those of ELAs for both operators. An intraclass correlation coefficient value of 0.96 was found in the LSVs of EMAs of the two operators, and it increased to 0.98 when the LSVs of the ELAs were used. Both operators had lower stability index values for EMAs than ELAs, but the difference was only statistically significant for the novice.

CONCLUSION

Artifacts are common when using 2-D SWE to measure LS, especially for the novice. Artifacts may lead to the overestimation of LS and reduce the repeatability and reliability of LS measurements.

Key Words: Ultrasound; Elastography; Artifact; Liver

Core Tip: Artifacts are common when using two-dimensional shear wave elastography (2-D SWE) to measure liver stiffness (LS), especially for the novice. We investigated the presence and influence of artifacts in 2-D SWE of liver. Our results showed artifacts were more likely to occur in the bottom-left corner of the elastogram. Artifacts may lead to the overestimation of LS and reduce the repeatability and reliability of LS measurements. For the elastograms with artifacts, we should place the Q-Box away from the artifacts.

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INTRODUCTION

Chronic liver disease is a growing problem worldwide. The main causes of chronic liver disease include hepatitis virus infection, alcoholic liver disease, and non-alcoholic fatty liver disease[1]. It mainly causes diffuse liver fibrosis, which in turn leads to liver cirrhosis. Some of them eventually develop hepatocellular carcinoma, portal hypertension, and hepatic encephalopathy[2,3]. Accurate assessment of liver fibrosis is important for treatment prioritization, surveillance, and determination of prognosis[4]. Moreover, liver biopsy allows the assessment of the degree of fibrosis[5]. However, liver biopsy is an expensive and invasive diagnostic tool. Its main complications are bleeding and pain[6,7], which limit its clinical application.

Recently, the application of ultrasound elastography in the diagnosis of non-invasive assessment of liver fibrosis has developed rapidly[8]. US elastography is mainly classified into two major types: Strain elastography and shear wave elastography[9,10]. Two-dimensional shear wave elastography (2-D SWE) is a type of shear wave elastography that uses acoustic radiation force to create shear waves. The velocity of the shear wave can be used to calculate the tissue stiffness by the formula $E = 3\rho c^2$, where E is tissue elasticity (Young's modulus, kPa), ρ is tissue density (kg/m^3), and c is shear wave velocity (m/s). The 2-D SWE is based on the quantification of the propagation speed of shear waves in the liver to create an elastogram. The elastogram is displayed using a color-coded map superimposed on a conventional B-mode image, where different colors represent different stiffness, allowing an assessment of homogeneity[10].

It has been reported that 2-D SWE has shown sufficient accuracy in evaluating the degree of liver fibrosis[11-13]. However, there was significant heterogeneity in the results of these studies. This heterogeneity may be caused by different patient populations, research designs and equipment used[14]. Another important reason may be that the presence of artifacts leads to inaccurate liver stiffness (LS) measurements. Bruce *et al*[15] reported that 2-D SWE artifacts resulted in a significant variability in the assessed LS.

Although 2-D SWE artifacts of the liver are common in clinical practice, they are poorly recognized, and there is even no clear definition. To the best of our knowledge, only a few review articles have been published[15,16]. Therefore, the purpose of this study was to investigate the presence and effects of artifacts in 2-D SWE of the liver. This is important to avoid artifacts and improve diagnostic performance in future operations.

MATERIALS AND METHODS

Patient selection

This prospective study was approved by the institutional ethical review board of our hospital. All patients signed a written informed consent document to participate in the study. We included 158 consecutive patients with chronic liver disease, who underwent 2-D SWE examination in our department. The study was conducted according to the principles reported in the Declaration of Helsinki and approved by the authors' institutional review board. The exclusion criterion was that no valid measurement was obtained by either operator. Seven patients were excluded because the novice operator did not obtain any valid measurements after five consecutive measurements. The baseline

characteristics of the patients were presented in Table 1.

2-D SWE examination

LS measurements were performed with an Aixplorer US system (SuperSonic Imagine, Aix-en-Provence, France) with a convex probe (SC6-1, 1-6 MHz). Patients fasted for more than 6 h and were examined in the supine position with the right arm in maximal abduction. The right anterior lobe of the liver was examined by intercostal scanning, and the SWE mode was started with neutral breathing during breath-holding. The upper limit of the color-coding scale was set to 70 kPa. The sampling frame was approximately 2.5 cm × 3.5 cm, placed at least 1 cm below the liver capsule, avoiding the large vascular structures. Image acquisition was performed after the elastography image was stable for 3-5 s. The quantitative analysis system (Q-Box) was then activated and placed at the center of the sampling frame. The Q-Box was 2 cm in diameter and the measurement depth was 3-5 cm. The LS measurement was considered invalid if there was no color-coding or the coded area was smaller than the Q-Box size[17]. When the area of color-coding is larger than the Q-Box size, the LS measurement was considered valid even if there are artifacts within it.

Each patient was continuously measured five times by an expert and a novice, respectively. The operators performed consecutive LS measurements in a randomized blinded manner. The median value of all valid measurements performed by the two operators represents the LS value (LSV) of the subject and was used for the correlation analysis with artifacts. The expert operator had 9 years of experience in the 2-D SWE examinations and had successfully performed approximately 15000 2D SWE examinations. The novice operator was trained by an expert operator and successfully performed 50 2-D SWE examinations.

Analysis of elastogram for artifacts

Artifacts were defined as the mottled area in the elastograms, and the area of the artifacts was measured using a tracing instrument attached to the device. We can manually trace the edge of the artifacts and automatically display the area and perimeter of the artifacts (Figure 1A). We drew a cross line at the center of the elastogram and divided it into four locations: top-left, top-right, bottom-left, and bottom-right. The location of the artifacts in each elastogram was recorded. The elastogram with the most artifacts (EMA) and the elastogram with the least artifacts (ELA) in each patient measured by the two operators were found by all authors. For the elastograms with artifacts, the Q-Box was placed in the center of the sampling frame (Figure 1B) and away from the artifacts for measurements (Figure 1C). The influence of artifacts on LS measurement was evaluated by comparing the differences in LSVs, standard deviation (SD) values and stability index (SI) values.

Statistical analysis

All quantitative data are expressed as mean ± SD (range), and qualitative variables are expressed as numbers (percentages). The Shapiro-Wilk test was used to test whether the numeric variables were normally distributed. Non-parametric tests with the Kruskal-Wallis method were used to compare the difference in numeric variables with a non-normal distribution. Differences between numeric variables with a normal distribution were assessed using a parametric test (*t*-test). The χ^2-test was used to compare the proportions expressed as percentages. Interobserver repeatability was evaluated using intraclass correlation coefficient (ICC). Relationships between various parameters were examined using Pearson's correlation test. Statistical significance was set at $P < 0.05$, and all P values were two-sided. Statistical analysis was performed using MedCalc software (MedCalc Software, version 17.4, Ostend, Belgium).

RESULTS

Among the 158 patients, 151 patients with valid measurements obtained by both operators were enrolled in this study. In theory, each operator should obtain 755 (151 × 5 = 755) elastography images. However, in the examination of 12 patients by the two operators, 35 elastography images were invalid and excluded. To ensure that the two operators had the same number of valid elastograms for each patient, valid measurements corresponding to the 35 invalid measurements were also excluded. Therefore, 720 elastography images from each operator were included (Figure 2).

The presence of artifacts

For the expert operator, the percentage of elastograms with artifacts was 19.6% (141/720), and the area of artifacts was 0.92 ± 0.68 cm^2. For the novice operator, the percentage of elastograms with artifacts was 51.7% (372/720), and the area of artifacts was 1.36 ± 0.87 cm^2. The percentage of elastograms with artifacts and the area of artifacts in the novice were significantly higher than those in the expert, and the difference were both statistically significant (both $P < 0.001$). We counted all the artifacts according to their locations, and the results are shown in Table 2. There were no significant differences in the frequency of the occurrence of artifacts between the two operators at the same location (all $P > 0.05$).

Table 1 Patient characteristics (*n* = 151)

Characteristic	Value
Age (yr)	46.2 ± 13.1 (19-75)
Liver stiffness value (kPa)	9.7 ± 7.8 (3.8-34.9)
Liver cirrhosis, *n* (%)	17 (11.3)
Subcutaneous fat thickness (cm)	0.4 ± 0.3 (0.1-2.4)
Sex, *n* (%)	
Male	72 (47.7)
Female	79 (52.3)
Body mass index (kg/m^2)	23.3 ± 3.4 (17.2-36.3)
Normal (< 25 kg/m^2), *n* (%)	101 (66.9)
Overweight (25-30 kg/m^2), *n* (%)	41 (27.2)
Obese (> 30 kg/m^2), *n* (%)	9 (5.9)
Etiology of chronic liver disease, *n* (%)	
Hepatitis B virus	122 (80.8)
Hepatitis C virus	8 (5.3)
Alcoholic liver disease	10 (6.6)
Nonalcoholic fatty liver disease	6 (4)
Autoimmune disease	5 (3.3)

Table 2 Artifacts at different locations of the two operators

Locations	Expert, *n* (%)	Novice, *n* (%)	*P* value
Top-left	40 (21.5)	120 (20.5)	0.769
Bottom-left	102 (54.8)	309 (52.8)	0.634
Top-right	7 (3.8)	42 (7.2)	0.098
Bottom-right	37 (19.9)	114 (19.5)	0.904

Qualitative data are expressed as *n* (%).

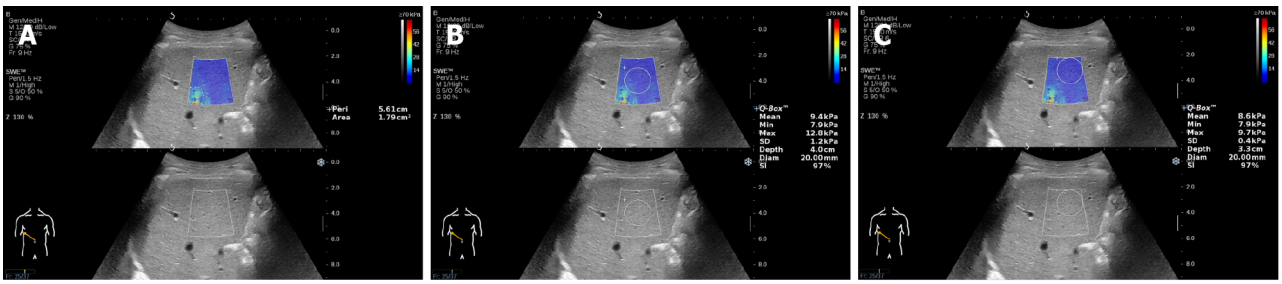

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Figure 1 Elastograms of a 60-year-old man with hepatitis B virus. A: Elastogram with artifacts at the bottom-left of the sampling frame, and the area of artifacts was 1.79 square centimeters; B: For the elastogram with artifacts, the Q-Box was placed in the center of the sampling frame [mean liver stiffness: 9.4 kPa, standard deviation (SD): 1.2 kPa, stability index (SI): 97%]; C: For the elastogram with artifacts, the Q-Box was placed away from the artifacts (mean liver stiffness: 8.6 kPa, SD: 0.4 kPa, SI: 97%). SD: Standard deviation; SI: Stability index.

Comparing the occurrence frequency of artifacts in all locations of the two operators, it was found that both operators had the highest frequency of bottom-left, followed by top-left and bottom-right, and top-right had the lowest frequency. No statistical difference was found between the frequency of top-left and bottom-right ($P > 0.05$), but the frequency among other locations was statistically different (all $P <$

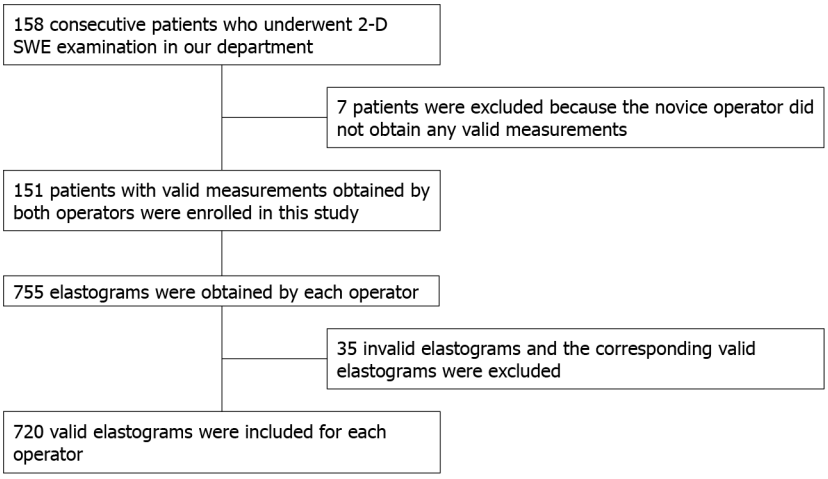

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Figure 2 Study flow diagram. 2-D SWE: Two-dimensional shear wave elastography.

0.001) (Table 3).

Influence of artifacts on LS measurements

The LSVs of EMAs were higher than those of ELAs for both operators, and the differences were statistically significant (both $P < 0.001$). There was a significant difference in the LSVs of the EMAs between the two operators ($P = 0.006$). However, there was no statistically significant difference in the LSVs of the ELAs between the two operators ($P = 0.051$) (Table 4).

The ICC values and 95%CIs were calculated by comparing the LSVs of the EMAs and ELAs of the two operators. An ICC value of 0.96 (95%CI: 0.94-0.98) was found in the LSVs of EMAs, and it increased to 0.98 (95%CI: 0.97-0.99) when the LSVs of the ELAs were used. The SD values of EMAs were higher than those of ELAs for both operators, and the differences were statistically significant (both $P < 0.001$). The SI values of the EMAs were lower than those of the ELAs for both operators. The difference was only statistically significant for the novice ($P = 0.002$), but not for the expert ($P = 0.135$) (Table 5).

For the elastograms with artifacts, the LSVs and SD values of the Q-Box placed in the center of the sampling frame were higher than those of the Q-Box placed away from the artifacts. The SI values of the Q-Box placed in the center of the sampling frame were lower than those of the Q-Box placed away from the artifacts. There were significant differences in LSVs, SD values and SI values between the Q-Box placed in the center of the sampling frame and away from the artifacts for both operators (all $P < 0.05$) (Table 6).

Patient characteristics and artifacts

The total number of elastograms with artifacts measured by the two operators was 513 (141 by the expert, 372 by the novice). The number of elastograms with artifacts in male subjects was 238 (46.4%), and that in female subjects was 275 (53.6%). There was no significant difference between the male and female subjects ($P = 0.378$). Pearson's correlation test showed that there was no significant linear correlation between age and the number of elastograms with artifacts ($r = 0.21$, $P = 0.126$). In the entire cohort, Pearson's correlation test showed that there was a positive correlation between LSV, body mass index (BMI), subcutaneous fat thickness and the number of elastograms with artifacts ($r = 0.47$, $P = 0.001$; $r = 0.41$, $P = 0.002$; and $r = 0.42$, $P = 0.002$, respectively).

DISCUSSION

When using 2-D SWE to measure LS in clinical practice, artifacts are commonly observed in elastograms [18]. It is difficult for some subjects to obtain satisfactory elastograms, such as obesity, poor acoustic window and inability of the subjects to hold their breath. Despite our best efforts to avoid artifacts, even operators with 9 years of operating experience still have a certain percentage of artifacts. In this study, we compared the difference in the frequency of occurrence artifacts between two different experienced operators. The results showed that the percentage of elastograms with artifacts and the area of artifacts in the novice were significantly higher than that of the expert. This may be because the expert operator can obtain high-quality B-mode imaging, which is required for accurately tracking shear waves[18]. Previous studies have shown that experts have better repeatability and reliability in measuring LS, which may have an important relationship with the fact that there were few artifacts in their elastograms[19,20]. Therefore, some studies have suggested that novices should perform at least 300

Table 3 Compare the percentage of artifacts at different locations

Locations of artifacts	P^1 value	P^2 value
Top-left *vs* bottom-left	< 0.001	< 0.001
Top-left *vs* top-right	< 0.001	< 0.001
Top-left *vs* bottom-right	0.703	0.669
Bottom-left *vs* top-right	< 0.001	< 0.001
Bottom-left *vs* bottom-right	< 0.001	< 0.001
Top-right *vs* bottom-right	< 0.001	< 0.001

[1]Compare the percentage of artifacts at different locations of the expert.
[2]Compare the percentage of artifacts at different locations of the novice.

Table 4 Comparison of liver stiffness values of elastograms with different area artifacts for two operators

	Expert	Novice	P^1 value
LSVs of EMAs (kPa)	10.2 ± 8.3	11.0 ± 8.7	0.006
LSVs of ELAs (kPa)	9.5 ± 7.4	9.8 ± 7.7	0.051
P^2 value	< 0.001	< 0.001	N/A

[1]Compare the liver stiffness values (LSVs) of the two operators.
[2]Compare the LSVs of elastograms with the most artifacts and elastograms with the least artifacts.
LSVs: Liver stiffness values; EMAs: Elastograms with the most artifacts; ELAs: Elastograms with the least artifacts; N/A: Not applicable.

Table 5 Standard deviation and stability index of the elastograms with different area artifacts for two operators

	SD of EMAs (kPa)	SD of ELAs (kPa)	P^1 value	SI of EMAs	SI of ELAs	P^2 value
Expert	1.2 ± 1.2	0.8 ± 0.6	< 0.001	92% ± 12%	95% ± 5%	0.135
Novice	2.1 ± 1.7	1.1 ± 1.1	< 0.001	89% ± 8%	93% ± 6%	0.002

[1]Compare the standard deviation values of elastograms with the most artifacts (EMAs) and elastograms with the least artifacts (ELAs).
[2]Compare the stability index values of EMAs and ELAs.
SD: Standard deviation; SI: Stability index; EMAs: Elastograms with the most artifacts; ELAs: Elastograms with the least artifacts.

Table 6 Comparison of Q-Box parameters measured at different positions of the elastograms with artifacts

	Number	Q-Box in the center of the sampling frame			Q-Box away from the artifacts		
		LSV	SD	SI	LSV	SD	SI
Expert	141	14.6 ± 9.5	1.7 ± 1.1	93% ± 6%	14.1 ± 9.3	0.8 ± 0.6	94% ± 7%
Novice	372	12.1 ± 9.5	1.9 ± 1.3	90% ± 7%	11.6 ± 9.4	0.9 ± 0.7	93% ± 6%

LSV: Liver stiffness value; SD: Standard deviation; SI: Stability index.

abdominal US scans or more than 50 supervised 2-D SWE examinations; however, this may not be sufficient[19,21]. A learning curve has been observed for 2-D SWE, a proportion of operator error would decrease over time[22].

We divided the elastogram into four locations and calculated the frequency of occurrence of artifacts at each location. The occurrence frequency of artifacts is arranged in descending order: bottom-left, top-left, bottom-right, and top-right. The two operators in this study had the same results, indicating that this difference may have certain regularity. The reason for this result may be that the aerated lung leads to a shadowing artifact on the left side of the B-mode image, which makes it impossible to form a well-defined push beam in this area[15,23]. On the other hand, to avoid liver capsule reverberation artifacts,

the depth of the sampling frame has increased, especially in obese or overweight patients. When the depth exceeds the penetration limit, attenuation artifacts and larger vessels may have more pulsatile artifacts at the bottom of the sampling frame[16,23]. We found the same phenomenon on another 2-D SWE ultrasound system (Aplio500, Canon, Tochigi, Japan). We found that artifacts were more likely to occur in the bottom-left corner of the elastogram, where distortion waves were noted in the propagation map of the corresponding site. The distribution of artifacts may also be applicable to other devices of 2-D SWE technology, because they have the same imaging principles.

Usually a color-coding scale of up to 30 kPa is sufficient, but in this study the upper limit of the color-coding scale was set to 70 kPa. The reason is that some patients have an LSV greater than 30 kPa, and a lower color-coding scale setting will make the elastogram appear only in red. At this time, it is impossible to distinguish whether there is an artifact or not. Although the color-coding scale was set to 70 kPa may ignore tiny artifacts, it is easier to show obvious artifacts.

The presence of artifacts affects the assessment of LS, but there is no detailed research report yet. This study showed that the LSVs of the EMAs were higher than those of the ELAs. This indicates that artifacts may lead to the overestimation of LS. This study compared the differences between the two operators in the LSVs of EMAs and ELAs. The results showed that in either the EMAs or ELAs, the LSVs of the novice were higher than that of the expert, which may be due to the higher proportion of artifacts in the elastograms measured by the novice. The ICC value between the two operators calculated with the LSVs of the EMAs was lower than that calculated with the LSVs of ELAs. This shows that artifacts can reduce inter-observer repeatability.

Although the degree of liver fibrosis in chronic liver disease will be slightly different, the color-coded LSmapping image will hardly show obvious mottled area. These mottled areas are considered as artifacts and belong to noise. Some studies use signal-to-noise ratio as the standard to evaluate image quality[24,25]. The new software version of the device provides SD and SI as indicators to evaluate the reliability of LS measurement[26-28]. The SD reflects the homogeneity of LSVs in the measurement area of the Q-Box. The higher the SD values, the greater heterogeneity of the LSVs in the measurement area. Thiele *et al*[29] reported that the diagnostic accuracy for cirrhosis by 2D SWE increased at SD < 1.75 kPa. The SI is an indicator of temporal stability of the measurement area, and the manufacturer recommends that a reliable LS measurement should have a SI greater than 90%. Our study showed that the SD values of the EMAs were much higher than those of the ELAs, which indicated that artifacts made the elastograms heterogeneous. The SI values of the EMAs were lower than those of the ELAs, which showed that artifacts may reduce the temporal stability of the elastograms. In short, artifacts can reduce the reliability of the LS measurements. For the elastograms with artifacts, we found that placing the Q-Box away from the artifacts can obtain more reliable LS measurements than placing it in the center of the sampling frame (generally the default measurement position of the equipment).

Furthermore, we investigated the relationship between patient characteristics and the occurrence of artifacts. We found that the occurrence of artifacts had no significant relationship with sex or age. However, we found that patients' BMI, subcutaneous fat thickness and LSVs were positively correlated with the occurrence of artifacts. Higher BMI and subcutaneous fat thickness usually indicate overweight or obesity with a thicker abdominal wall. Artifacts are prone to occur when measuring LS in overweight or obese subjects due to the combined effects of attenuation artifacts, reverberation artifacts, and vessels [16,30]. Previous studies have also shown that a high BMI is the main reason for measurement failure and unreliable assessment[17,31,32]. Patients with liver cirrhosis usually have higher LSVs, and they often have artifacts because of their shrunken liver volumes and poor sonic window. Other studies have demonstrated that severe liver fibrosis is a risk factor for unreliable LS measurements[17,33].

To the best of our knowledge, this is the first prospective study to analyze artifacts in 2-D SWE of the liver. This study analyzed the predilection sites and people for artifacts, and explored the effects of artifacts on LS measurements. Knowledge of the artifacts is essential to improve operation technology to obtain high-quality images. It is very important to obtain accurate measurements in an attempt to optimize its performance and application value. In addition, knowledge from this and other studies on artifacts can be used to investigate how training and education could reduce the occurrence of artifacts. Hopefully, engineers and researchers can improve the product design, provide quality indicators and other ways to avoid the acquisition of improper data due to artifacts.

Our study had several limitations. First, artifacts may be ignored when the color changes are inconspicuous. Second, only one device was tested in this study. Third, this study did not analyze the causes of artifacts, because it is sometimes difficult to accurately determine. Finally, we analyzed only a small sample of data from two operators. Therefore, a larger sample study involving more operators and devices needs to be conducted in future.

CONCLUSION

In conclusion, artifacts are common when using 2-D SWE to measure LS, especially for the novice. Artifacts may lead to the overestimation of LS and reduce the repeatability and reliability of LS measurements. For the elastograms with artifacts, we should place the Q-Box away from the artifacts.

ARTICLE HIGHLIGHTS

Research background

Chronic liver disease is a growing problem worldwide. Accurate assessment of liver fibrosis is important for treatment prioritization, surveillance, and determination of prognosis. Liver biopsy is still considered as the gold standard for staging liver fibrosis. However, liver biopsy is an expensive and invasive diagnostic tool. Its main complications are bleeding and pain, which limit its clinical application. Recently, the application of two-dimensional shear wave elastography (2-D SWE) in the diagnosis of non-invasive assessment of liver fibrosis has developed rapidly. However, the presence of artifacts leads to inaccurate liver stiffness (LS) measurements.

Research motivation

Although 2-D SWE artifacts of the liver are common in clinical practice, they are poorly recognized, and there is even no clear definition. To the best of our knowledge, only a few review articles have been published. Knowledge of the artifacts is essential to improve operation technology to obtain high-quality images. It is very important to obtain accurate measurements in an attempt to optimize its performance and application value.

Research objectives

We aim to investigate the presence and influence of artifacts in 2-D SWE of liver.

Research methods

In this study, we performed 2-D SWE examination in patients with chronic liver disease by a novice and an expert. The elastogram was divided into four locations: top-left, top-right, bottom-left, and bottom-right. The occurrence frequency of artifacts in different locations was compared. The effect of artifacts on the LS measurements was evaluated by comparing the elastogram with the most artifacts (EMA) and the elastogram with the least artifacts (ELA).

Research results

Each operator had 720 elastography images were included for analysis. The percentage of elastograms with artifacts and the area of artifacts in the novice were significantly higher than those in the expert (both $P < 0.001$). Comparing the occurrence frequency of artifacts in all locations of the two operators, it was found that both operators had the highest frequency of bottom-left, followed by top-left and bottom-right, and top-right had the lowest frequency. This study showed that the LS values and standard deviation values of the EMAs were higher than those of the ELAs. Both operators had lower stability index values and intraclass correlation coefficient values for EMAs than ELAs.

Research conclusions

Artifacts are common when using 2-D SWE to measure LS, especially for the novice. Our results showed artifacts were more likely to occur in the bottom-left corner of the elastogram. Artifacts may lead to the overestimation of LS and reduce the repeatability and reliability of LS measurements.

Research perspectives

In this study, we only analyzed a small sample of data from two operators of one device. Therefore, a larger sample study involving more operators and devices needs to be conducted in future studies.

FOOTNOTES

Author contributions: Wang HP and Sang L designed the research study; Wang HP and Zheng PC performed the research; Wang HP and Wang XM collected and analyzed the data; Wang HP and Sang L wrote the manuscript; all authors reviewed and approved the manuscript.

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ORIGINAL ARTICLE

Observational Study

Therapeutic anticoagulation for splanchnic vein thrombosis in acute pancreatitis: A national survey and case-vignette study

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Abstract

BACKGROUND

Splanchnic vein thrombosis (SVT) is a major complication of moderate and severe acute pancreatitis. There is no consensus on whether therapeutic anticoagulation should be started in patients with acute pancreatitis and SVT.

AIM

To gain insight into current opinions and clinical decision making of pancreatologists regarding SVT in acute pancreatitis.

METHODS

A total of 139 pancreatologists of the Dutch Pancreatitis Study Group and Dutch Pancreatic Cancer Group were approached to complete an online survey and case vignette survey. The threshold to assume group agreement was set at 75%.

RESULTS

The response rate was 67% (n = 93). Seventy-one pancreatologists (77%) regularly prescribed therapeutic anticoagulation in case of SVT, and 12 pancreatologists (13%) for narrowing of splanchnic vein lumen. The most common reason to treat SVT was to avoid complications (87%). Acute thrombosis was the most important factor to prescribe therapeutic anticoagulation (90%). Portal vein thrombosis was chosen as the most preferred location to initiate therapeutic anticoagulation (76%) and splenic vein thrombosis as the least preferred location (86%). The preferred initial agent was low molecular weight heparin (LMWH; 87%). In the case vignettes, therapeutic anticoagulation was prescribed for acute portal vein thrombosis, with or without suspected infected necrosis (82% and 90%), and thrombus progression (88%). Agreement was lacking regarding the selection and duration of long-term anticoagulation, the indication for thrombophilia testing and upper endoscopy, and about whether risk of bleeding is a major barrier for therapeutic anticoagulation.

CONCLUSION

In this national survey, the pancreatologists seemed to agree on the use of therapeutic anticoagulation, using LMWH in the acute phase, for acute portal thrombosis and in the case of thrombus progression, irrespective of the presence of infected necrosis.

Key Words: Acute pancreatitis; Splanchnic vein thrombosis; Therapeutic anticoagulation; Bleeding; Recanalization; Outcomes

Core Tip: Splanchnic vein thrombosis is a relatively common complication of moderate and severe acute pancreatitis, but there is still much debate about its treatment with therapeutic anticoagulation. This national survey and case vignette study among 93 pancreatologists demonstrates that the majority prescribe therapeutic anticoagulation for acute portal vein thrombosis and thrombus progression in patients with or without infected necrosis, despite the absence of evidence supporting its use. Whether this collective opinion is accurate needs to be confirmed in future (preferably prospective) studies.

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INTRODUCTION

Acute pancreatitis is an inflammatory disorder of the pancreas and is self-limiting in the majority of patients[1,2]. However, approximately 20% of patients develop a moderate or severe disease course, with (peri) pancreatic necrosis and collections[3,4]. Due to the combination of local inflammation and

mechanical compression, these complications may cause thrombus formation in the splanchnic circulation, including the portal, splenic and superior mesenteric vein[5,6]. The reported estimates on the incidence of splanchnic vein thrombosis (SVT) in acute pancreatitis range from 17% to 23%, and are even higher in complicated acute pancreatitis[7,8]. The clinical presentation of SVT varies between an asymptomatic thrombus to potential lethal complications, such as portal or left side hypertensive bleeding and small bowel ischemia[9-11]. For this reason, early treatment with therapeutic anticoagulation is recommended in patients with acute SVT[12-14]. However, consistent evidence to drive this decision in acute pancreatitis patients does not exist[15-18]. In fact, a recent meta-analysis from our study group showed that 53% of acute pancreatitis patients do not receive therapeutic anticoagulation [15]. This proportion of untreated patients is substantially higher than previously reported in other SVT populations[19], probably because of the fear of serious bleeding. Variation in clinical practice also became apparent in this meta-analysis[15], as anticoagulation use and the type of agent used were very heterogeneous between studies. Therefore, the aim of this survey was to gain more insight into current opinions of pancreatologists on anticoagulation therapy for SVT following acute pancreatitis.

MATERIALS AND METHODS

We conducted an online national survey and case vignette study among members of the Dutch Pancreatitis Study Group (DPSG) and the Dutch Pancreatic Cancer Group (DPCG). Members were excluded if they were not primary care-takers in the treatment of patients with AP (*e.g.*, radiologists, oncologists, basic scientists). The survey was built in Research Electronic Data Capture, and invitations to participate were sent by e-mail in November 2021, followed by four weekly reminders. Additionally, the survey was promoted through newsletters and during annual study group meetings of the DPSG and DPCG.

Survey design

The survey was developed by a multidisciplinary team of surgeons, gastroenterologists, and radiologists, and included 3 demographical questions, 17 general questions and 3 case vignettes (Supplementary material). Demographic information included the responders' specialty, type of hospital and working experience. The general questions focused on treatment of SVT and potential factors that may influence the responders' decision. The case-vignettes addressed the preferred treatment strategy in different clinical cases at different time points. All cases however, concerned a 50-year-old male patient with acute alcoholic necrotising pancreatitis, and can be summarized as follows.

Case vignette 1: A patient visited the emergency department, 5 d after onset of abdominal pain. Contrast-enhanced CT (CECT) showed necrotising pancreatitis with acute necrotic collection in the head of the pancreas (Figure 1A) and: A1: Luminal narrowing of the portal vein without the presence of collateral circulation (Figure 1B); A2: Intraluminal filling defect in the portal vein without the presence of collateral circulation; A3: Intraluminal filling defect in the portal vein without the presence of collateral circulation + a pseudoaneurysm in the proximal splenic artery (Figure 1C).

Case vignette 2: A patient admitted to the ward, 14 d after onset of abdominal pain. The patient showed signs of clinical detoriation with fever and rising inflammatory parameters. The CECT showed almost fully encapsulated pancreatic necrosis without gas configurations (Figure 1D) and a new intraluminal filling defect in the portal vein without the presence of collateral circulation (Figure 1E). The diagnosis of suspected infected pancreatic necrosis was made.

Case vignette 3: A homeless patient visited the emergency department, 30 d after onset of vague abdominal pain. CECT showed necrotising pancreatitis and: CA: Intraluminal filling defect in the portal vein with the presence of collateral circulation; CB: Thrombus progression and expansion of the collateral circulation (Figure 1F).

The threshold to assume group agreement was set at 75%. If a question ranged from always, usually, sometimes and never, agreement was defined when 75% of the pancreatologists rated it as always or usually (regularly), or sometimes and never (infrequently).

Definitions

SVT was predefined as an actual intraluminal filling defect on imaging of one or more of the splanchnic veins. The chronicity was divided into (sub)acute thrombosis or chronic thrombosis (with concomitant collaterals), anatomical location into portal, splenic and/or superior mesenteric vein, degree into a total or partial occlusion and extent into an isolated thrombus or a thrombus in several venous segments. Thrombus progression was defined as progression into other splanchnic vein(s), into total occlusion, or both.

Statistical analysis

Descriptive data are presented as counts with proportions for categorical data. All analyses were performed using IBM SPSS (20).

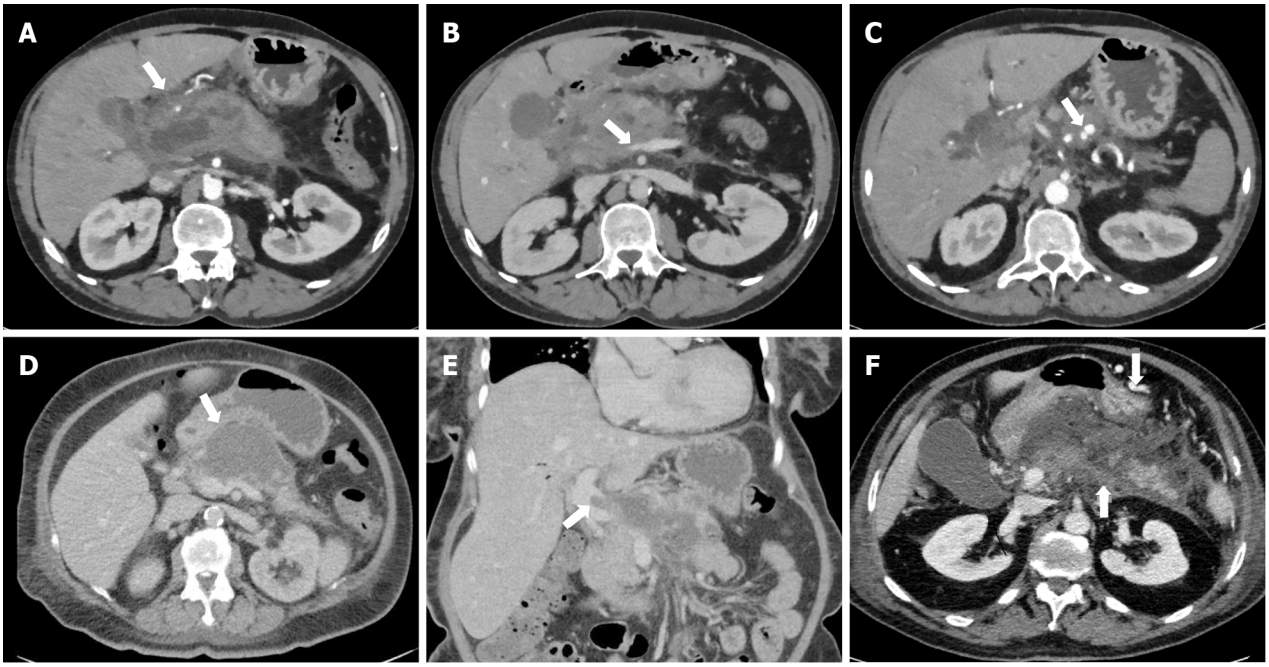

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Figure 1 Imaging findings of case vignette. A: Acute necrotic collection in the head of the pancreas in case vignette 1; B: Luminal narrowing of the portal vein without the presence of collateral circulation in case vignette 1; C: Pseudoaneurysm in the proximal splenic artery in case vignette 1; D: Almost fully encapsulated pancreatic necrosis without gas configurations in case vignette 2; E: Luminal filling defect in the portal vein without the presence of collateral circulation in case vignette 2; F: Extension of the thrombus to the splenic vein (arrow pointing upwards) and expansion of the collateral pathway in the gastroepiploic veins along the great curvature of the stomach (arrow pointing downwards) in case vignette 3.

RESULTS

A total of 93 of the 139 invited pancreatologists (67%) responded and participated in this survey and case vignette study; 67 gastroenterologists (72%), 25 surgeons (27%) and 1 intensivist (1%). The majority worked in a non-academic centre (70%) and had more than 10 years of experience in treating AP patients (60%). Demographic characteristics are presented in Table 1.

Indications for and details of treatment with therapeutic anticoagulation

Agreement was reached on whether therapeutic anticoagulation should be prescribed for SVT and luminal narrowing of one or more of the splanchnic veins in acute pancreatitis patients. For SVT, therapeutic anticoagulation was regularly prescribed by 71 (76%) and infrequently by 22 (24%) pancreatologists. In case of luminal narrowing, therapeutic anticoagulation was only regularly prescribed by 12 (13%) pancreatologists. Avoiding complications, such as portal hypertension and bowel ischemia, was the main reason for 81 pancreatologists (87%) to start therapeutic anticoagulation. Screening for an underlying prothrombotic disorder in patients diagnosed with SVT was regularly performed by 14 (15%) pancreatologists, only in patients with a history of one (or more) thrombotic events by 40 (43%), and infrequently by 39 (42%) pancreatologists. There was agreement on the preferred initial type of therapeutic anticoagulation for SVT 81 pancreatologists (87%) preferred subcutaneous low-molecular-weight heparin (LMWH)], but not on the preferred follow-up type. Imaging after the index admission was chosen as follow-up strategy by 79 pancreatologists (85%). Thirteen pancreatologists (13%) indicated that they usually stop anticoagulant therapy in case of achieved radiological recanalization, 35 (38%) after a period of 3 mo, 42 (45%) after 6 mo, and 3 (3%) after 12 mo. All details are provided in Table 2.

Determinants of prescribing therapeutic anticoagulation

Seventy-eight pancreatologists (84%) have chosen the time course of thrombosis as the most important factor supporting anticoagulant therapy; 84 pancreatologists (90%) prescribe therapeutic anticoagulation in case of a (sub)acute thrombosis *vs* 9 (10%) for both (sub)acute and chronic thrombosis. Moreover, 70 pancreatologists (76%) have chosen portal vein thrombosis as the most preferred location to initiate therapeutic anticoagulation, whereas splenic vein thrombosis was chosen as least preferred location by 80 pancreatologists (86%). The majority of pancreatologists (85%) treat both total and partial occlusive thrombosis. There was no agreement whether the risk of different types of bleeding should be considered as a major barrier to prescribe therapeutic anticoagulation. The need for invasive interventions for local complications of acute pancreatitis influenced the decision whether or not to

Table 1 Details of respondents, *n* (%)

Demographics	*n* = 93
Specialty	
Surgeon	25 (27)
Gastroenterologist	67 (72)
Intensivist	1 (1)
Type of hospital	
Academic	28 (30)
Non-academic, teaching hospital	60 (65)
Non-academic, non-teaching hospital	5 (5)
Experience in treating patients with acute pancreatitis	
0-5 years	10 (11)
5-10 years	27 (29)
10-15 years	17 (18)
15-20 years	23 (25)
> 20 years	16 (17)

initiate anticoagulation therapy in about half of pancreatologists (52%). All details are outlined in Table 3.

Statements on prognosis

An association between the presence of SVT and worse clinical outcomes in patients with acute pancreatitis was assumed by 67 pancreatologists (72%) (Figure 2). Moreover, the vast majority (88%) agreed that therapeutic anticoagulation for splanchnic vein thrombosis improves clinical outcomes in these patients. Insufficient evidence was the most frequently quoted reason among pancreatologists who disagreed with this second statement.

Case-vignettes

The results of the case vignettes are summarized in Figure 3. In the first case vignette (patient 1, day 5 of acute necrotising pancreatitis), 11 pancreatologists (12%) would prescribe a therapeutic dose anticoagulation if luminal narrowing without collateral circulation was detected in the portal vein. Of the 82 pancreatologists (88%) who opted for no therapeutic dose anticoagulation, 73 (89%) would change treatment strategy in case an actual filling defect in the portal vein was detected. In total, 84 pancreatologists (90%) would prescribe therapeutic dose anticoagulation to this patient with an actual portal vein thrombosis without collateral circulation. If a pseudoaneurysm was concomitantly present, 43 of those 84 pancreatologists (51%) who favoured a therapeutic dose anticoagulation would switch to a prophylactic dose anticoagulation (*n* = 28, 65%) or no anticoagulation at all (*n* = 15, 35%), leaving 41 pancreatologists (44%) in the therapeutic anticoagulation group.

In the second case vignette (patient 2, day 14 of suspected infected necrotising pancreatitis), 77 pancreatologists (82%) would prescribe therapeutic dose anticoagulation if a portal vein thrombosis without collateral circulation was detected. The presence of (suspected) infected pancreatic necrosis influenced the choice of anticoagulation agent in 49 pancreatologists (52%). Almost all of these pancreatologists pointed out that once infected pancreatic necrosis is suspected, they would choose an agent with a short half-life because of the potentially need of invasive intervention.

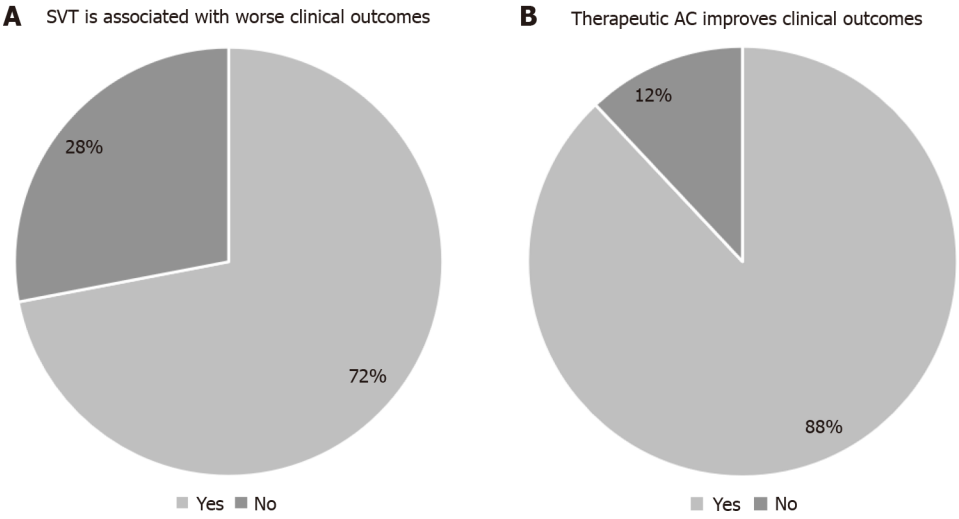
In the third case vignette (patient 3, day 30 of acute necrotising pancreatitis), 44 pancreatologists (47%) would prescribe a therapeutic dose anticoagulation if a portal vein thrombosis with collateral circulation was detected. Of these 44 pancreatologists, 19 (43%) would perform upper endoscopy to screen for and-if present-treat oesophageal varices before starting anticoagulation therapy. In case of thrombus progression (extension of the thrombus to the splenic vein and expansion of the collateral pathway), 11 pancreatologists (12%) would stay conservative (*i.e.*, no therapeutic dose of anticoagulation), 82 (88%) would start or continue a therapeutic dose anticoagulation and none would proceed to an intervention.

Table 2 Survey results: Indication for and details of treatment with therapeutic anticoagulation, *n* (%)

Item	Total (*n* = 93)
Do you prescribe therapeutic AC in case of detected thrombosis in one (or more) of the splanchnic veins?	
Always	23 (25)
Usually	48 (52)
Sometimes	21 (23)
Never	1 (1)
Do you prescribe therapeutic AC in case of detected luminal narrowing of one (or more) of the splanchnic veins?	
Always	3 (3)
Usually	9 (10)
Sometimes	29 (31)
Never	52 (56)
Main reason(s) to start therapeutic AC (multiple answers were possible)	
To achieve vessel recanalization	52 (56)
To avoid complications	81 (87)
To prevent formation of altered venous anatomy	31 (33)
To prevent recurrence of SVT	27 (29)
To prevent another venous thromboembolism	30 (32)
Other reason[1]	1 (1)
Do you screen for an underlying prothrombotic disorder?	
Always	2 (2)
Usually	12 (13)
Sometimes	25 (27)
Only in patients with a history of one (or more) thrombotic events	40 (43)
Never	14 (15)
Which initial type of therapeutic AC do you prefer?	
(Low molecular weight) heparin subcutaneous	81 (87)
Unfractionated heparin intravenous	4 (4)
Direct oral anticoagulation	3 (3)
Vitamin K antagonist	4 (4)
Platelet aggregation inhibitor	1 (1)
Urokinase/recombinant tissue plasminogen activator	0
Which follow-up type of therapeutic AC do you prefer?	
(Low molecular weight) heparin subcutaneous	9 (10)
Unfractionated heparin intravenous	0
Direct oral anticoagulation	53 (57)
Vitamin K antagonist	29 (31)
Platelet aggregation inhibitor	2 (2)
Urokinase/recombinant tissue plasminogen activator	0
Do you generally follow-up SVT after index admission?	
Yes, clinically only	5 (5)
Yes, with imaging	79 (85)
No	9 (10)

After how long do you usually stop the therapeutic AC?	
In case of achieved radiological recanalization	13 (14)
3 mo	35 (38)
6 mo	42 (45)
12 mo	3 (3)
Never	0

[1]In free text: expansion of thrombosis.
AC: Anticoagulation; SVT: Splanchnic vein thrombosis.



DOI: 10.3748/wjg.v29.i21.3328

Figure 2 Statements on prognosis. A: Splanchnic vein thrombosis is associated with worse clinical outcomes; B: Therapeutic anticoagulation improves clinical outcomes. AC: Anticoagulation; SVT: Splanchnic vein thrombosis.

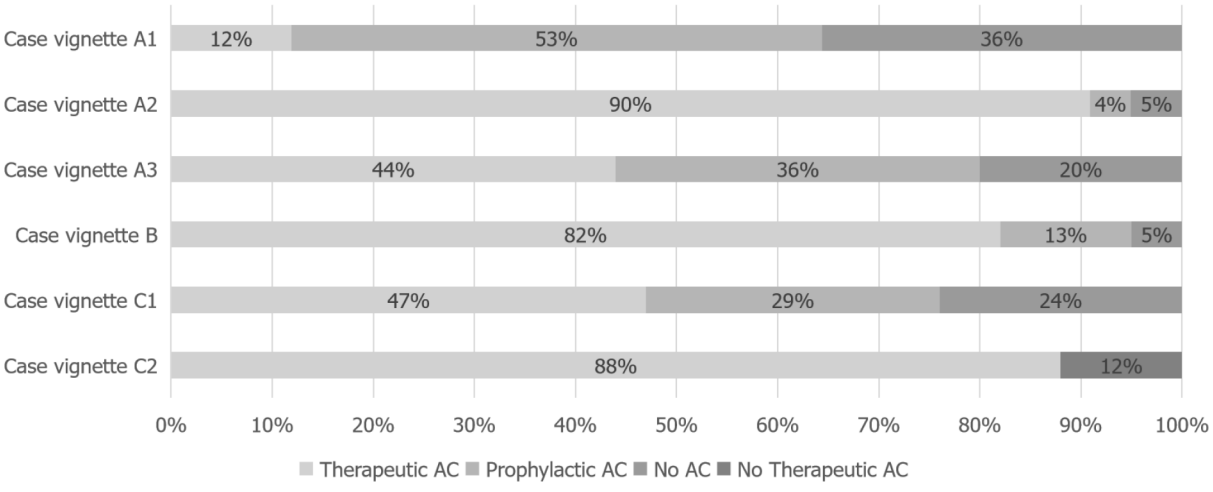


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Figure 3 Case vignettes results: Choice of treatment. AC: Anticoagulation.

DISCUSSION

This first nationwide survey and case vignette study gives insight into the clinical scenarios in which therapeutic anticoagulation is currently used, and not used to treat or prevent splanchnic vein thrombosis in acute pancreatitis patients. In an earlier study[15], we found 7 retrospective cohort studies evaluating therapeutic anticoagulation in this patient category with conflicting results in clinical

Table 3 Survey results: Determinants of prescribing therapeutic anticoagulation, *n* (%)

Item	Total (*n* = 93)
Do you consider of ... the thrombosis as an important factor to prescribe therapeutic AC? (multiple answers were possible)	
Age (acute or chronic)	78 (84)
Anatomical location (portal, splenic or superior mesenteric vein)	42 (45)
Degree (total or partial)	45 (48)
Extent (isolated thrombosis or thrombosis in several segments)	49 (53)
Progression (over time)	40 (43)
When do you prescribe therapeutic AC? In case of:	
(Sub)acute thrombosis	84 (90)
Chronic thrombosis	0
Both	9 (10)
Rank the anatomical location of the thrombosis from most likely to less likely to start therapeutic AC:	
Portal vein-splenic vein-superior mesenteric vein	9 (10)
Portal vein-superior mesenteric vein-splenic vein	61 (66)
Splenic vein-portal vein-superior mesenteric vein	0
Splenic vein-superior mesenteric vein-portal vein	1 (1)
Superior mesenteric vein-portal vein-splenic vein	19 (20)
Superior mesenteric vein-splenic vein-portal vein	3 (3)
When do you prescribe therapeutic AC? In case of:	
Total thrombosis	9 (10)
Partial thrombosis	5 (5)
Both	79 (85)
Do you consider the risk of ... as a major barrier to prescribe therapeutic AC? (multiple answers were possible)	
Bleeding in general	52 (56)
Bleeding related to portal hypertension	17 (18)
Bleeding related to pseudoaneurysm	49 (53)
Other risk[1]	1 (1)
Does the need for invasive interventions for local complications of acute pancreatitis influence your decision regarding AC therapy?	
Yes	48 (52)
No	45 (48)

[1]In free text: CVA bleeding history.
AC: Anticoagulation; SVT: Splanchnic vein thrombosis.

outcome[20-27]. These studies were of moderate quality and therefore the pancreatologist' preference and belief predominate in current decision making rather than scientific evidence.

An important finding of the current study was that more than 75% of pancreatologists regularly prescribe therapeutic anticoagulation for SVT, particularly for a thrombus that acutely developed. This is in line with recommendations from general guidelines for SVT management[12-14]. In the absence of a visualized thrombus, most pancreatologists indicated not to treat compressed veins with anticoagulation. Although wall shear stress in a compressed vessel may promote platelet activation, and subsequently thrombus formation[28], there is no data yet to question the opinion of the pancreatologists.

In this study, the most important reason to administer therapeutic anticoagulation was to avoid complications including bowel ischemia and portal hypertension. Bowel ischemia has been reported in up to 33% of acute pancreatitis patients treated with therapeutic anticoagulation *vs* 16% of untreated patients[22,24,25]. A potential explanation for this discrepancy could be that bowel ischemia was already present prior to the start of therapy, therefore being an indication for therapeutic anticoagu-

lation rather than a consequence. In addition, the presence of varices and other collaterals have been equally reported[15], and only one of the aforementioned studies described one case of bleeding from oesophageal varices in an anticoagulated patient[24]. Again, it is likely that a perceived bleeding risk influenced the decision whether or not to prescribe therapeutic anticoagulation. This confounding by indication clearly limits the interpretation of these retrospective studies.

Achieving vessel recanalization was chosen as the second goal. A recent meta-analysis showed that the pooled rate of recanalization of SVT was similar between treated (36%) and untreated patients (31%) [15]. However, there is reason to believe that the benefit of anticoagulation therapy may alter when considering the anatomical location of the thrombosis[21,29]. Patients with portal vein or superior mesenteric vein thrombosis may have an increased risk of complications, while having lower spontaneous recanalization rates. In particular, mortality rates of patients with superior mesenteric vein thrombosis are reported up to 50%[30,31]. On the other hand, splenic vein thrombosis, which is by far the most common site of thrombosis in acute pancreatitis patients, forms a less serious concern for gastrointestinal bleeding and insufficient recanalization[8,26,32]. A selective anticoagulation policy, in which therapeutic anticoagulation was reserved for portal- and superior mesenteric vein thrombosis, was recently assessed in a retrospective study[33]. This study showed a recanalization rate of 67% in portal- and superior mesenteric vein thrombosis, which is substantially higher than previously reported [15]. In addition, a recent practice guideline from the Pancreas study group, Chinese Society of Gastroenterology, recommends a selective anticoagulation policy[34]. In this survey, portal vein thrombosis, followed by superior mesenteric vein thrombosis, was also the pancreatologists' preferred location for prescribing therapeutic anticoagulation, while splenic vein was the least preferred location.

With respect to chronic SVT, the current guidelines do not recommend therapeutic anticoagulation [10]. This is in line with the reported use in case vignette 3, with the exception of the case of the patient with thrombus progression and expansion of the collateral circulation. In this scenario, 88% of pancreatologists would treat such patient with therapeutic anticoagulation. A recent multicentre randomised controlled trial comparing daily rivaroxaban 15 mg/d to no anticoagulation in patients with noncirrhotic chronic portal vein thrombosis[35], formally challenged the guideline recommendations. This study showed that rivaroxaban, even in prophylactic dose, reduced the incidence of venous thromboembolism; therefore, this study may initiate a shift towards a more frequent use of anticoagulation in chronic SVT. On that note, primary prophylaxis of portal hypertensive bleeding should be performed, as laid out by the BAVENO IV guideline[13]. In this survey, however, the minority of pancreatologists followed this recommendation. Improvements should also be made to distinguish acute from chronic SVT. Currently, no clear definition for chronic SVT exists other than a presumed time course of more than 6 mo or the presence of multiple small collaterals around the obstructed veins [10,36], which is not useful to diagnose a nonocclusive chronic thrombosis (*i.e.*, absence of collateral pathways). A promising invention to overcome this problem is magnetic resonance noncontrast thrombus imaging, though validation is still needed[37].

According to our survey, subcutaneous LMWH was the favoured initial type of therapeutic anticoagulation, while no agreement regarding the choice of long-term anticoagulation and its duration was found. In current guidelines, switching LMWH to a vitamin K antagonist once reaching the target range is the reported strategy for patients with SVT[12-14,38]. The use of direct oral anticoagulation (*i.e.*, apixaban) in acute pancreatitis patients with SVT is reported in two studies and showed comparable results[21,33]. However, in the case of (suspected) infected pancreatic necrosis, LMWH seems to be preferred by more than half of the pancreatologists, due to its short half-life and reversibility. Besides, many acute pancreatitis patients have reduced caloric intake limiting the absorption of DOACS. Therefore, it seems fair to advise LWMH, especially in the acute phase. Looking at the duration of anticoagulation therapy for provoked SVT in patients with a transient risk factor, such as acute pancreatitis, the suggested duration is 3 mo to 6 mo[12-14,38]. Consistently, 38% and 45% of the pancreatologists in our survey preferred 3 mo and 6 mo treatment duration, respectively.

Based on the available literature, it remains unclear whether therapeutic anticoagulation is associated with higher rates of bleeding. An increased bleeding risk with therapeutic anticoagulation has been reported up to 33% of patients[21,25,26], but there are also studies showing lower rates of bleeding[24]. The theory for this latter finding is that therapeutic anticoagulation prevents thrombus progression, therefore reducing the portal pressure and consequently the risk of bleeding[19]. In this study, the risk of bleeding was not identified as a significant discouraging factor, as only about half of the pancreatologists considered bleeding in general and bleeding related to pseudoaneurysm as a major barrier to prescribe therapeutic anticoagulation. Also, the possible need for invasive intervention, due to suspected infected necrosis, did not significantly influence the treatment strategy. Another critical question is whether SVT influences the disease course of acute pancreatitis patients, but again this remains unanswered[15]. In this survey, the majority of pancreatologists assumed that the occurrence of SVT is associated with worse clinical outcomes, and interestingly, even more pancreatologists were convinced that the use of therapeutic anticoagulation leads to improved patient outcomes.

A strength of this study is the response rate of 67%, which is relatively high compared to previous surveys among pancreatologists[39-41]. Furthermore, the ratio of 30:70 between academic and non-academic pancreatologists attributed to a valuable insight into the pancreatologists' opinions on the use of therapeutic anticoagulation. This study also has several limitations. First, the results may not directly

reflect the actual practice in other countries as only members of two Dutch associations of pancreatology were invited. This decision was made to avoid selection based on publication record, and consequently include pancreatologists who are not actively involved in the treatment of acute pancreatitis[40]. Another advantage of our method is that it allowed us to calculate the survey's response rate by bypassing the confidentiality of membership lists of international pancreatic associations. Second, the clinical presentation of SVT is very heterogeneous, as well as the patient characteristics and clinical disease course among acute pancreatitis patients, which influences current decision making. For this reason, it might have been difficult for pancreatologists to answer some of the general questions. Therefore, case vignettes were used to explore what considerations underpin their decisions. As the descriptions throughout the case vignettes were consistently formulated and only one clinical detail was changed at a time, treatment of patients with superior mesenteric vein and splenic vein thrombosis was not assessed in the case vignettes. Consequently, the pancreatologists' preference on this manifestation of SVT in acute pancreatitis remained unknown. Finally, the rationale behind the "nonprescribing trend" was not assessed adequately, which could be a focus for future research.

CONCLUSION

In conclusion, this national survey demonstrates the tendency of pancreatologists to prescribe therapeutic anticoagulation for acute thrombosis, in particular for acute portal vein thrombosis and in case of thrombus progression, irrespective of the presence of infected necrosis. With therapeutic anticoagulation, the majority of pancreatologists believed that the clinical outcomes of acute pancreatitis patients with splanchnic vein thrombosis will improve. Furthermore, this study reflects on several knowledge gaps in literature, and sets out clear points for future research. Specifically, a deeper understanding of the pathophysiology and natural course of splanchnic vein thrombosis secondary to acute pancreatitis would allow us to clarify the therapeutic role of anticoagulation.

ARTICLE HIGHLIGHTS

Research background

Splanchnic vein thrombosis (SVT) is a severe complication of acute pancreatitis that may cause portal hypertensive complications and bowel ischemia. To prevent such complications, therapeutic anticoagulation is recommended in the general population of patients with an acute SVT.

Research motivation

Evidence to support this recommendation in acute pancreatitis patients does however not exist and as a result, clinical decision-making is mostly based on the preferences and beliefs of the pancreatologists.

Research objectives

To gain insight into current opinions on the use of therapeutic anticoagulation for SVT in acute pancreatitis.

Research methods

An online survey was sent to 139 Dutch pancreatologists. The threshold to assume agreement was set at 75%.

Research results

The response rate was 67% (n = 93). Seventy-one pancreatologists (77%) regularly prescribed therapeutic anticoagulation for SVT, using LMWH in the acute phase (87%). The majority favored therapeutic anticoagulation for acute thrombosis (90%), portal vein thrombosis in patients with or without infected necrosis (82% and 90%) and in case of thrombus progression (88%). There was no agreement whether the risk of bleeding is a barrier for initiation of therapeutic anticoagulation.

Research conclusions

The pancreatologists reached agreement regarding the use of therapeutic anticoagulation for SVT, particularly in cases of acute thrombosis, portal vein thrombosis and thrombus progression.

Research perspectives

To get a better understanding of the therapeutic role of anticoagulation, it is crucial to conduct prospective studies targeting the pathophysiology and natural course of SVT in acute pancreatitis patients.

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FOOTNOTES

Author contributions: Sissingh NJ, Groen JV, and Mieog JSD designed the study; all authors critically assessed the study design; Boekestijn B and Bollen TL provided the radiological images; Sissingh NJ, van Hooft JE, Mieog JSD, and van Eijck CHJ sent or promoted the study; Sissingh NJ did the statistical analysis and wrote the initial draft of the manuscript; Groen JV, Timmerhuis HC, Besselink MG, Boekestijn B, Bollen TL, Bonsing BA, Klok FA, van Santvoort HC, Verdonk RC, van Eijck CHJ, van Hooft JE, and Mieog JSD critically assessed and edited the manuscript; Sissingh NJ coordinated the writing process and revised the manuscript; and all authors read and approved the final manuscript.

Institutional review board statement: This survey research is not subject to the Dutch Medical Research involving Human Subjects Acts (WMO) as participants are not subject to procedures or are required to follow rules of behavior. Consequently, this study does not need a full review by an accredited MREC or the CCMO.

Conflict-of-interest statement: All authors declare no conflict of interest.

Data sharing statement: Requests for data can be made to the corresponding author and will be discussed during a meeting of the Dutch Pancreatitis Study Group. After approval by the Dutch Pancreatitis Study Group, data that underlie the results reported in this study, will be shared.

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SYSTEMATIC REVIEWS

Lumen-apposing-metal stent misdeployment in endoscopic ultrasound-guided drainages: A systematic review focusing on issues and rescue management

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Abstract

BACKGROUND

The introduction of lumen-apposing metal stents (LAMS) for endoscopic ultrasound (EUS)-guided drainages has marked a turning point in the field of interventional ultrasound and it is gathering worldwide diffusion in different clinical settings. Nevertheless, the procedure may conceal unexpected pitfalls. LAMS misdeployment is the most frequent cause of technical failure and it can be considered a procedure-related adverse event when it hampers the conclusion of the planned procedure or results in significant clinical consequences. Stent misdeployment can be managed successfully by endoscopic rescue maneuvers to allow the completion of the procedure. To date, no standardized indication is available to guide an appropriate rescue strategy depending on the type of procedure or of misdeployment.

AIM

To evaluate the incidence of LAMS misdeployment during EUS-guided choledochoduodenostomy (EUS-CDS), gallbladder drainage (EUS-GBD) and pancreatic fluid collections drainage (EUS-PFC) and to describe the endoscopic rescue strategies adopted under the circumstance.

METHODS

We conducted a systematic review of the literature on PubMed by searching for

studies published up to October 2022. The search was carried out using the exploded medical subject heading terms "lumen apposing metal stent", "LAMS", "endoscopic ultrasound" and "choledochoduodenostomy" or "gallbladder" or "pancreatic fluid collections". We included in the review on-label EUS-guided procedures namely EUS-CDS, EUS-GBD and EUS-PFC. Only those publications reporting EUS-guided LAMS positioning were considered. The studies reporting a technical success rate of 100% and other procedure-related adverse events were considered to calculate the overall rate of LAMS misdeployment, while studies not reporting the causes of technical failure were excluded. Case reports were considered only for the extraction of data regarding the issues of misdeployment and rescue techniques. The following data were collected from each study: Author, year of publication, study design, study population, clinical indication, technical success, reported number of misdeployment, stent type and size, flange misdeployed and type of rescue strategy.

RESULTS

The overall technical success rate of EUS-CDS, EUS-GBD and EUS-PFC was 93.7%, 96.1%, and 98.1% respectively. Significant rates of LAMS misdeployment have been reported for EUS-CDS, EUS-GBD and EUS-PFC drainage, respectively 5.8%, 3.4%, and 2.0%. Endoscopic rescue treatment was feasible in 86.8%, 80%, and 96.8% of cases. Non endoscopic rescue strategies were required only in 10.3%, 16% and 3.2% for EUS-CDS, EUS-GBD, and EUS-PFC. The endoscopic rescue techniques described were over-the-wire deployment of a new stent through the created fistula tract in 44.1%, 8% and 64.5% and stent-in-stent in 23.5%, 60%, and 12.9%, respectively for EUS-CDS, EUS-GBD, and EUS-PFC. Further therapeutic option were endoscopic rendezvous in 11.8% of EUS-CDS and repeated procedure of EUS-guided drainage in 16.1% of EUS-PFC.

CONCLUSION

LAMS misdeployment is a relatively common adverse event in EUS-guided drainages. There is no consensus on the best rescue approach in these cases and the choice is often made by the endoscopist relying upon the clinical scenario, anatomical characteristics, and local expertise. In this review, we investigated the misdeployment of LAMS for each of the on-label indications focusing on the rescue therapies used, with the aim of providing useful data for endoscopists and to improve patient outcomes.

Key Words: Lams misdeployment; Endoscopic ultrasound-guided drainage; Lams maldeployment; Biliary drainage; Gallbladder drainage; Pancreatic fluid collections; Lumen-apposing metal stents

Core Tip: Scant data are available about rescue techniques in cases of lumen-apposing metal stents (LAMS) misdeployment which is the main cause of technical failure in endoscopic ultrasound-guided drainage procedures. We performed a systematic review of the literature about LAMS misdeployment and rescue techniques in the biliopancreatic setting, focusing on technical aspects and success rate of endoscopic maneuvers. In accordance with our results endoscopic rescue techniques are feasible in most cases (up to 96.8%). Three endoscopic rescue strategies have been identified. The choice of the endoscopic rescue maneuver is based on the clinical scenario, type of misdeployment and expertise of the endoscopic team.

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INTRODUCTION

The progress in interventional endoscopy, particularly in the field of endoscopic ultrasound (EUS), has changed the treatment algorithms for digestive and pancreaticobiliary diseases. The evolution of devices combined with improvements in endoscopic techniques, have allowed access to mini-invasive therapeutic solutions for complex diseases that affect areas beyond the gastrointestinal tract.

Nowadays, interventional EUS can manage local complications of acute pancreatitis[1], drain the biliary tree and gallbladder[2], establish gastrointestinal anastomoses[3], and treat tumors by radiofrequency ablation or injection of substances[4,5]. A paradigmatic example of this evolution is the approach to biliopancreatic drainage. The first EUS-guided transluminal drainage of the biliary tree was performed in 2001. Giovannini *et al*[6] used a 10-Fr plastic stent to achieve trans-duodenal biliary drainage (BD) under EUS guidance in a patient with a pancreatic head mass after failed endoscopic retrograde cholangiopancreatography (ERCP). Since then, the evolution of EUS-guided drainage has led to the continuous improvement of available devices and of endoscopic techniques. The introduction of self-expanding metal stents (SEMS) and lumen-apposing metal stents (LAMS) for EUS-guided drainage is a turning point in the field of endoscopic drainage. The first transluminal stenting between two nonadherent lumens of the digestive tract using a bi-flanged covered metal stent with lumen-to-lumen apposition property was described by Binmoeller and Shah[7] in an *ex vivo* model. Itoi *et al*[8] reported the first use of LAMS in humans, describing the successful treatment of 15 symptomatic pancreatic pseudocysts and five acute cholecystitis cases in patients unfit for surgery.

Itoi and Binmoeller[9] successfully performed the first EUS-guided choledochoduodenostomy (EUS-CDS) with LAMS in a patient with unresectable pancreatic cancer and obstructive jaundice. Prior to that, the technique of LAMS deployment was the same as that of tubular stents (plastic or metal stents), which is a multi-step procedure with device exchanges that are exposed to the risk of adverse events (*i.e.*, loss of the wire and/or scope position, biliary leak). To address this issue, a new LAMS delivery system with an electrocautery tip [electrocautery-enhanced (EC)-LAMS-Hot-Axios, Boston Scientific Corp., Marlborough, Massachusetts, United States] was developed, giving rise to a single-stage technique[10,11]. Presently, two LAMS in different diameters and lengths are commercially available: The Hot Axios stent (Boston Scientific, Marlborough, Mass, United States) and the Hot Spaxus stent (Taewoong Medical Co. Gimpo, Korea), and new LAMS types are on the way. Other fully covered (FC) metal stents are available for similar indications: Aixstent (Leufen Medical, Aachen, Germany), Hanarostent (Mi-TECH-Medical Co, Seoul, South Korea), and NAGI stents (Taewoong Medical Co., Ltd., Ilsan, Korea)[11,12]. These stents are non-cautery and require a multi-step procedure for their insertion. Anyway, LAMS loaded on an EC delivery system require precise execution of some sequential steps (puncture of the target lumen, opening and retraction of the distal flange to the adjacent cavity wall, deployment, and release of the proximal flange) to achieve technical success, which is defined as the correct placement of the stent across the newly created tract.

There are various issues that may occur during LAMS deployment, resulting in stent misdeployment. Unfavorable conditions range from unfamiliarity with the stent to patient movement, angled scope tip or confined space within the gastrointestinal cavity, small diameter of the target lumen[13], and target structure located at a distance of more than 15-20 mm[14]. Stent misdeployment usually results in a full-thickness defect of the gastrointestinal wall, possibly associated with the perforation of the target organ. Prompt identification of this complication is crucial to managing the perforation, possibly completing the procedure, and avoiding major consequences. This paper reviews EUS-guided drainage procedures using LAMS, with a focus on misdeployment and endoscopic rescue therapies.

MATERIALS AND METHODS

Data collection

This systematic review was performed in agreement with PRISMA guidelines. Two independent investigators (Cominardi A and Metelli F) performed a review on PubMed by searching for studies published up to October 2022. The search was limited to English-language articles and human studies, and it was carried out using the exploded medical subject heading terms "lumen apposing metal stent", "LAMS", "endoscopic ultrasound" and "choledochoduodenostomy" or "gallbladder" or "pancreatic fluid collections". We included in the review on-label EUS-guided procedures namely EUS-CDS, EUS-guided gallbladder drainage (EUS-GBD) and EUS-guided pancreatic fluid collections drainage (EUS-PFC). Boolean operators (NOT, AND, OR) were also used in succession to narrow and widen the search.

Both investigators used a standardized data collection form to increase uniformity and reduce bias in reporting. In the case of discrepancy, the investigators resolved the disagreement by discussion with a senior investigator (Armellini E). Only publications on EUS-guided LAMS positioning were considered, whereas studies on drainage with other metallic or plastic stents were excluded. Meta-analysis, review and drainage performed in non-human models were also excluded.

The studies reporting a technical success rate of 100% and other procedure-related adverse events were considered to calculate the overall rate of LAMS misdeployment, while studies not reporting the causes of technical failure were excluded. Case reports were considered only for the extraction of data regarding the issues of misdeployment and rescue techniques.

The full paper of each identified article was retrieved, and references were evaluated to search for potentially missed articles. Data were extracted independently and entered standardized Excel spreadsheet (Microsoft Inc. Redmond, Washington, United States). For EUS-CDS and EUS-PFC drainage the following data were extracted from each study: Year of publication, study design, study

population, clinical indication, technical success, reported number of misdeployment, LAMS type and size, flange misdeployed, and type of rescue strategy. For EUS-GBD the following data were collected from each study: Author, year of publication, study design, study population, clinical indication, access to GB (stomach or duodenum), technical success, reported number of misdeployment, LAMS type and size, flange misdeployed, and type of rescue strategy.

Data analysis

Baseline characteristics of study population, EUS-guided procedures, technical details, and procedure outcomes were summarized as means (SD) or medians (with interquartile range and range) for continuous data, and as frequencies and proportions for categorical data. Data were analyzed using the Statistical Package for Social Sciences (SPSS software v. 15.0, Chicago, Illinois, United States) for Windows.

RESULTS

EUS-CDS

Literature research identified 82 studies that were fully assessed for eligibility in this review. We excluded 57 studies since they did not meet our inclusion criteria. A total of 25 studies were included in our review[15-39]; in 20 studies LAMS misdeployment occurred[20-39] (study flow chart was shown in Figure 1).

A total of 1081 patients underwent EUS-CDS for malignant biliary obstruction (MBO), almost all after failed ERCP. The overall technical success rate of EUS-CDS was 93.7%. Excluding 5 case reports, a total of 63 LAMS misdeployments were reported, with a rate of 5.8%; the study detailed characteristics were summarized in Table 1. Including case reports, an Axios stent was employed in 61 (89.7%) cases of EUS-CDS. Spaxus stent was used in 3 (4.4%) cases. In 4 cases (5.9%) type of stent was not declared. The procedures were performed with a pre-loaded guidewire in 23 (33.8%) cases, without a pre-loaded guidewire in 12 (17.6%) cases, whereas this data was not available in most of cases (48.6%).

In 28 (41.2%) cases the misdeployment of the distal flange was reported, in 4 (5.9%) cases the misdeployment involved the proximal flange, in 3 (4.7%) EUS-CDS LAMS was entirely misdeployed inside the common bile duct (CBD) and in 2 (2.9%) patients no bile flow was observed after LAMS deployment despite no evidence of LAMS misdeployment. The type of misdeployment was not properly described in 31 (45.6%) EUS-CDS. Seven other causes of technical failure were reported in four studies[15,22,24,38]: 1 massive bleeding, 1 inability to puncture the bile duct, 2 duodenal perforations, 1 failure of fistula creation, 1 mechanical failure and 1 patient intolerance. In three studies[31,33,37], the effective rescue therapy after LAMS misdeployment was considered a technical success by the authors and in one study[15] duodenal perforation during dilation of the fistulous tract was the cause of technical failure.

LAMS misdeployment was managed by endoscopy in 86.8% (n = 59/68) cases, while 4.4% (n = 3/68) misdeployment cases were treated by percutaneous transhepatic BD (PTBD), 2.9% (n = 2/68) by rendezvous *via* PTBD and during surgery, and 2.9% (n = 2/68) by surgery. In 2 cases the procedure was abandoned in favor of supportive therapy. In 44.1% (n = 30/68) cases of LAMS misdeployment, the rescue strategy was LAMS removal followed by over-the-wire deployment of a new stent; in 7 (23.3%) cases a new LAMS was deployed and in 23 (76.7%) cases a SEMS was used.

The stent-in-stent technique was the treatment of choice in 16 (23.5%) cases of misdeployment; in 7 (43.7%) cases SEMS was used, in 1 (6.3%) a plastic stent and in 8 (50%) the stent type was not specified. In 8 (11.8%) cases rendezvous procedure was performed by EUS/endoscopic technique. EUS-CDS was repeated in 4 (5.9%) cases. In 1 (1.5%) case EUS-GBD was used as a rescue strategy (see Table 2).

EUS-GBD

We identified 213 studies using our search strategy; 185 studies were excluded since they did not meet inclusion criteria. A total of 28 studies reported cases of EUS-GBD[8,22,29,37,40-63] including 16 studies in which LAMS misdeployment occurred[22,29,50-63]. The study flow chart is shown in Figure 2.

A total of 667 patients underwent EUS-GBD for acute cholecystitis since they were unfit for surgery. Only in one study patients were treated by EUS-GBD for unsuccessful BD with ERCP[60]. Studies characteristics are summarized in Table 3. The overall technical success of EUS-GBD was 96.1%. Excluding two case reports, we identified 23 LAMS misdeployments among patients who underwent EUS-GBD, with a rate of 3.4%. In 19/25 (76%) cases an Axios stent was employed for EUS-GBD, while a Spaxus stent was used in the remaining 6/25 (24%) cases.

Only 8 studies reported if the GB was accessed from the stomach (n = 7/11, 63.6%) or duodenum (n = 4/11, 36.4%)[22,51-55,59,61]. The misdeployment of the proximal flange occurred in 9/25 (36%) cases, while the distal flange was misdeployed in 16/25 (64%) cases. The use of a guidewire was reported in 16/25 (64%) cases of misdeployment. Endoscopic management was the treatment of choice in 21/25 (84%) cases of LAMS misdeployment during EUS-CDS. In 15 of these 25 (60%) complicated EUS-GBD collected in our study, the initial failed LAMS deployment was overcome by reinsertion of a FC-SEMS

Table 1 Characteristics of studies reporting lumen-apposing metal stents misdeployment during endoscopic ultrasound-guided choledochoduodenostomy

Ref.	Study design	Study population (*n*)	Clinical indication	Technical success (%)	LAMS misdeployment (*n*)	LAMS type	Size of LAMS (mm)	Use of guidewire	LAMS flange misdeployed	Rescue therapy
Anderloni *et al*[20], 2019	Retrospective single center	46	MBO	93.5	3	Hot Axios	-	No	Distal (1/3), not specified (2/3)	Rendezvous technique with transpapillary placement of 10 mm × 40 mm FCSEMS after advancing a guidewire through the existing fistula into the bile duct and then across the papilla (1/3); 10 mm × 10 mm LAMS placement through the existing fistula (1/3); double-pigtail plastic stent placement across the LAMS (1/3)
Di Mitri R *et al*[21], 2022	Retrospective single center	31	MBO	80.6	7	Hot Axios	8 mm × 8 mm (6/7) 10 mm × 10 mm (1/7)	Yes	Distal	Over-the-wire FCSEMS placement (5/7); transpapillary percutaneous-transhepatic-endoscopic rendezvous (1/7); transpapillary laparoscopic-endoscopic rendezvous (1/7)
Rajadurai *et al* [22], 2022	Retrospective multicenter	66	MBO	90.9	6	Hot Axios	-	No (4/6). Yes (2/6)	Distal	Over-the-wire FCSEMS placement (2/6); laparotomy (2/6); EUS-GBD (1/6); palliation due to rapid deterioration (1/6)
Jacques *et al* [23], 2019	Retrospective multicenter	52	MBO	88.5	4	Hot Axios	-	-	Distal (1/4), proximal (1/4 intraperitoneal, 2/4 intraparietal)	Stent-in-stent strategy with SEMS (1/4); ERCP rendezvous (1/4); repeat classic EUS-CDS with SEMS (2/4)
Jacques *et al* [24], 2020	Retrospective multicenter	70	MBO	98.6	1	Hot Axios	-	-	No evidence of bile flow even if stent was correctly in situ	Stent-in-stent strategy
El Chafic *et al* [25], 2019	Retrospective multicenter	67	MBO	95.5	2	Hot Axios	-	Yes	-	Over the same guidewire FCSEMS placement (all)
Fugazza *et al* [26], 2022	Retrospective multicenter	256	MBO	93.3	17	Hot Axios	-	-	-	Over the guidewire SEMS placement (10/17); deployment of a second LAMS (4/17); EUS-guided rendezvous with subsequent placement of a transpapillary stent (3/17)
Hindryckx and Degroote [27], 2021	Retrospective single center	13	-	92.3	1	Hot Axios	8 mm × 6 mm	Yes	Distal	Clip closure of duodenal defect and new EUS-CDS with 8 mm × 6 mm LAMS
Armellini *et al* [28], 2023	Case report	1	Difficult biliary lithiasis	-	1	Hot Axios	8 mm × 8 mm	Yes	Distal	Rendezvous technique with transpapillary placement of FCSEMS after advancing a guidewire directly through the LAMS and choledochal breach into the bile duct and then across the papilla
Teoh *et al* [29], 2021	Prospective multicenter	26	MBO	88.5	3	Cold spaxus	-	Yes	Entirely into the bile duct	Over the guidewire SEMS placement
Fugazza *et al* [30], 2020	Case report	1	Pancreatic adenocarcinoma	-	1	Axios	6 mm × 8 mm	Yes	Proximal	Stent-in-stent strategy using SEMS
Brückner *et al*	Case series	5	MBO	80	1	Cold	6 mm × 8	Yes	Distal	Over the guidewire stent placement

[31], 2015						Axios	mm			
Vanella *et al* [32], 2023	Retrospective study of prospectively maintained databases	93	MBO	97.8	4	Hot Axios	-	No	Distal (2/4); misdeployments after both flanges release (2/4)	Repeat EUS-CDS (1/4); inserting a guidewire through the LAMS catheter followed by over the guidewire LAMS placement (1/4) (the effective rescue therapies were counted as technical success); PTBD (2/4)
de Benito Sanz *et al*[33], 2021	Retrospective single center	37	MBO	100	4	-	-	-	Distal (2/4); not specified (2/4)	Stent-in-stent strategy (the effective rescue therapies were counted as technical success)
Garcia-Sumalla *et al* [34], 2021	Retrospective multicenter	41	MBO	95.1	2	Hot Axios	-	-	No evidence of bile flow even if stent was correctly in situ; distal flange	Stent-in-stent strategy using SEMS; rendezvous technique with placement of a transpapillary FCSEMS
Sanchez-Ocana *et al* [35], 2022	Case report	1	Pancreatic adenocarcinoma	-	1	Axios	8 mm × 8 mm	Yes	Distal	EUS-guided gallbladder drainage as a portal for antegrade transcystic guidewire passage, followed by rendezvous ERCP with placement of a biliary metal stent and clips to seal the perforation
Graves *et al* [36], 2021	Case report	1	Pancreatic metastasis of renal cell carcinoma	-	1	Axios	10 mm × 10 mm	No	Distal	A bridging 10 mm × 8 mm FCSEMS was deployed over the guidewire and through theccessing LAMS
Chin *et al*[37], 2020	Retrospective analysis of a prospectively maintained database	56	MBO	100	1	Axios	-	-	-	Over the guidewire tubular biliary stent placement (the effective rescue therapy was counted as technical success)
On *et al*[38], 2022	Retrospective multicenter	120	MBO	90.8	7	Hot Axios	-	No (4/7). Yes (3/7)	-	Bridging stents (5/7), PTBD (1/7), conservative management (1/7)
Ligresti *et al* [39], 2018	Case report	1	Pancreatic adenocarcinoma	-	1	Axios	8 mm × 8 mm	Yes	Distal	Reinsertion of the delivery system over the guide wire and second deployment of distal flange into common bile duct under EUS guidance

MBO: Malignant biliary obstruction; FCSEMS: Fully covered self-expanding metal stent; SEMS: Self-expanding metal stent; LAMS: Lumen-apposing metal stent; EUS-GBD: Endoscopic ultrasound-guided gallbladder drainage; ERCP: Endoscopic retrograde cholangiopancreatography; EUS-CDS: Endoscopic ultrasound-guided choledochoduodenostomy; PTBD: Percutaneous transhepatic biliary drainage.

through the LAMS lumen, the so-called "stent-in-stent" strategy; only in 1/15 a double pigtail stent was inserted. In 2/25 (8%) cases, the misdeployed stent was removed and a new LAMS was re-deployed over the guidewire across the fistula created during the first attempt for EUS-GBD.

In 3/25 (12%) cases, the LAMS was endoscopically removed, and the gastrointestinal wall perforation was closed by endoscopic clipping followed by transpapillary stent placement in 1 case. No further endoscopic maneuvers were attempted in the remaining 2 cases in favour of supportive care. Surgical management was the therapeutic option in 3/25 (12%) cases of misdeployment. In 1 (4%) case, emergent percutaneous cholecystostomy was performed after unsuccessful stent-in-stent placement attempt. In 1 (4%) case, palliation was the preferred strategy (see Table 2).

Table 2 Summary of rescue strategies for lumen-apposing metal stent misdeployment

EUS-CDS	
Deployment of a new stent through the created fistula tract	44.1%
Stent-in-stent strategy	23.5%
Endoscopic rendezvous	11.8%
Non-endoscopic rescue strategies	10.3%
EUS-GBD	
Stent-in-stent strategy	60%
Clip closure of gastrointestinal wall defect	13%
Deployment of a new stent through the created fistula tract	8%
Non-endoscopic rescue strategies	16%
EUS-PFC	
Deployment of a new stent through the created fistula tract	64.5%
Repeated EUS-guided drainage	16.1%
Stent-in-stent strategy	12.9%
Non-endoscopic rescue strategies	3.2%

LAMS: Lumen-apposing metal stent; EUS-CDS: Endoscopic ultrasound-guided choledochoduodenostomy; EUS-GBD: Endoscopic ultrasound-guided gallbladder drainage; EUS-PFC: Endoscopic ultrasound-guided pancreatic fluid collections drainage.

EUS-PFC

We collected 48 studies from the literature that were fully assessed for eligibility in this review; 20 studies were excluded since they did not meet our inclusion criteria. A total of 28 studies were included in our review[10,12,57,64-88] including 15 studies in which LAMS misdeployment occurred[10,12,70,77-88] (Figure 3). The overall technical success of EUS-PFC drainage was 98.1%. The cause of technical failure corresponded to LAMS misdeployment in all the studies except in one reporting two cases of technical failure due to a difficult scope position that prevented the advancement of the EC-LAMS device outside the operative channel of the echoendoscope[57]. In 3 cases, the effective rescue therapy by re-insertion of the same LAMS after misdeployment was considered a technical success by the authors[78,79].

Excluding two case reports, we collected 1684 patients who underwent EUS-PFC drainage in which 34 LAMS misdeployments occurred, with a rate of 2.0%. All study characteristics were reported in Table 4. In 13 (36.1%) cases misdeployment of the distal flange occurred, in 2 (5.5%) cases the proximal flange was deployed and then migrated entirely into the PFC. In most cases included in our study (20/36; 55.5%), the issue of misdeployment was not clearly described. A case report described the misdeployment of the stent in a non-target organ. In 4 cases data regarding rescue strategy were not available and the procedure was abandoned in one case.

The LAMS misdeployment in EUS-PFC drainage was managed as following: In 20/31 (64.5%) cases an over-the-wire deployment of a new stent was performed (10/20 with LAMS, 2/20 with SEMS, 8/20 with plastic stents), in 5/31 (16.1%) cases the EUS-PFC drainage was repeated, in 1/31 (3.2%) case surgical drainage was performed. The stent-in-stent strategy was the rescue treatment in 4/31 (12.9%) cases of LAMS misdeployment; in 3 (75%) cases a LAMS-in-LAMS technique was performed and in 1 (25%) case a SEMS was deployed inside the misdeployed LAMS (see Table 2).

DISCUSSION

Technical success of EUS-drainage is defined as the correct deployment of the stent between the gastrointestinal wall and target organ with evidence of bile flow in patients who underwent EUS-BD[24,25,32] or content flow/established access to the cavity in EUS-PFC or EUS-GBD cases[52,57].

Actually, stent misdeployment emerges as the primary cause of technical failure in the procedure of EUS-guided drainage. Among the studies we collected, different terms were used to define this complication, including misdeployment, dislodgement, and flange migration. We adopted the term ‘misdeployment’, as reported in the European Society of Gastrointestinal Endoscopy guidelines for therapeutic EUS[14].

Table 3 Characteristics of studies reporting lumen-apposing metal stents misdeployment during endoscopic ultrasound-guided gallbladder drainage

Ref.	Study design	Study population (*n*)	Clinical indication	Access to GB	Technical success (%)	LAMS maldeployment (*n*)	LAMS type	Size of LAMS (mm)	LAMS flange misdeployed	Rescue therapy
Ngamruengphong *et al* [50], 2015	Case report	1	Cholecysto-choledocal lithiasis	Duodenum	-	1	Hot Axios	-	Proximal	Stent-in-stent strategy using SEMS
Rajadurai *et al*[22], 2022	Retrospective multicenter	49	-	Duodenum	95.7	2	Hot Axios	10 mm × 10 mm; 15 mm × 10 mm	Distal	Closure of the defect with clip; palliation (no further endoscopic treatment)
Cho *et al*[51], 2019	Prospective single center	22	Acute cholecystitis unfit for surgery	-	95.5	1	Spaxus	10 mm × 20 mm	Proximal	Stent-in-stent strategy using SEMS
Walter *et al*[52], 2016	Prospective multicenter	30	Acute cholecystitis unfit for surgery	Stomach	90	3	Hot Axios			Stent-in-stent strategy using SEMS
Irani *et al*[53], 2015	Retrospective multicenter	15	Acute cholecystitis unfit for surgery	Duodenum	93	1	Axios	10 mm × 10 mm	Distal	Stent-in-stent strategy using SEMS
de la Serna-Higuera *et al*[54], 2013	Prospective single center	13	Acute cholecystitis unfit for surgery	Stomach	84.6	1	Axios	10 mm × 10 mm	Distal	Closure of the defect with clip (no further endoscopic treatment)
Dollhopf *et al*[55], 2017	Retrospective multicenter	75	Acute cholecystitis unfit for surgery	Stomach	98.7	1	Hot-Axios	10 mm × 10 mm	Proximal	Surgery
Teoh *et al*[56], 2017	Retrospective multicenter	59	Acute cholecystitis unfit for surgery	-	96.6	1	Axios	10 mm × 10 mm	Distal	Surgery
Mangiavillano *et al* [57], 2021	Retrospective multicenter	18	Acute cholecystitis unfit for surgery	-	83.3	1	Spaxus		Distal	Closure of the defect with clip followed by transpapillary stent placment
Teoh *et al*[29], 2021	Retrospective multicenter	27	Acute cholecystitis unfit for surgery	-	88.9	2	Spaxus	10 mm × 10 mm; 16	Proximal	Stent-in-stent strategy using SEMS
Higa *et al*[58], 2019	Retrospective single center	40	Acute cholecystitis unfit for surgery	-	97.5	2	Hot Axios	10 mm × 10 mm; 15 mm × 10 mm	Distal	Redeployment of a new LAMS
Garg *et al*[59], 2018	Case report	1	Acute cholecystitis unfit for surgery	Stomach	-	1	Hot Axios	10 mm × 10 mm	Distal	Stent-in-stent strategy using SEMS
Torres Yuste *et al*[60], 2020	Retrospective single center	34	Acute cholecystitis unfit for surgery	-	97.1	3	Axios	10 mm × 10 mm; 15 mm × 10 mm	Distal	Stent-in-stent strategy using SEMS (2/3); double pig-tail plastic stent in LAMS (1/3)
		37		-	97.3	1	Axios	10 mm × 10 mm; 15 mm × 10 mm	Proximal	Stent-in-stent strategy using SEMS

James *et al*[61], 2019	Retrospective multicenter	15	Acute cholecystitis unfit for surgery	Stomach	93.3	1	Axios	10 mm × 10 mm; 15 mm × 10 mm	Proximal	Surgery
Irani *et al*[62],2017	Retrospective multicenter	45	Acute cholecystitis unfit for surgery	-	97.8	1	Axios	-	Distal	Stent-in-stent strategy using SEMS
Cho *et al*[63], 2020	Retrospective multicenter	36	Acute cholecystitis, advanced malignancy unfit for surgery	-	94.4	2	Spaxus	-	Proximal	Stent-in-stent strategy using SEMS, emergent PTC

SEMS: Self-expanding metal stent; LAMS: Lumen-apposing metal stent; PTC: Percutaneous cholecystostomy; GB: Gallbladder.

According to the American Society for Gastrointestinal Endoscopy lexicon for endoscopic adverse events, LAMS misdeployment can be considered a procedure-related adverse event when it hampers the completion of the planned procedure and/or results in significant clinical consequences (*i.e.*, prolongation of existing hospital stay and elicitation of the need for another procedure)[89]. Misdeployment can be defined as an incident if it does not interfere with the completion of the planned procedure or change the plan of care. Therefore, stent misdeployment can be managed successfully by endoscopic rescue maneuvers to allow the completion of the procedure; however, complications with different levels of severity can occur in some cases. In EUS-BD, stent misdeployment may be associated with spillage of bile and secretions into the peritoneal cavity or retroperitoneal space, resulting in peritonitis and pneumoretroperitoneum[90]. Recently Fabbri *et al*[91] proposed a classification of misdeployment types during EUS-guided gastroenterostomy as follows: Proximal flange misdeployment, distal flange misdeployment, stent misdeployment perforating other organs, and stent misdeployment into the peritoneum. This model considers which flange is misdeployed and the anatomical localization of the stent after misdeployment and can be supposedly adopted for all EUS-guided procedures involving the use of LAMS.

EUS-CDS

The rate of ERCP failure is 2%-10% and it is due to surgically altered anatomy, gastric outlet obstruction, duodenal and/or bile duct tumor infiltration, indwelling enteral stent, periampullary diverticula, impacted stones, and technical difficulties[92]. European guidelines suggest EUS-BD as the second-line treatment for patients with MBO. The optimal drainage strategy depends on the underlying disease (benign/malignant) and location of the obstruction (distal/hilar)[15].

EUS-BD proved to be equally effective with fewer adverse events and re-intervention compared to PTBD especially in gastric outlet obstructions[93]. In addition, EUS-BD is less invasive, leads to better nutrition, prevents electrolyte imbalances, and provides better quality of life[94]. As experience in EUS-BD continues to grow, comparative studies of EUS-CDS and ERCP have reported encouraging data in support of EUS-CDS as the primary treatment for distal MBO, challenging the role of ERCP[95].

In a meta-analysis, the technical and clinical success rates of EUS-CDS using LAMS were 93.6% and 94.8%, respectively, with pooled rate of overall adverse events of 17.1% and procedure-related adverse events of 6.2%[96]. In our research, LAMS misdeployment rate was 5.8%, and LAMS misdeployment represented the main cause of technical failure in EUS-CDS. Notably, technical failure was due to other

Table 4 Characteristics of studies reporting lumen-apposing metal stent misdeployment during endoscopic ultrasound-guided pancreatic fluid collections drainage

Ref.	Study design	Study population (*n*)	Clinical indication	Technical success (%)	LAMS misdeployment (*n*)	LAMS type	Size of LAMS (mm)	Use of guidewire	LAMS flange misdeployed	Rescue therapy
Venkatachalapathy *et al*[77], 2018	Retrospective multicenter	116	WON, PFC	99	1	Hot Axios	-	No	Distal	LAMS removal followed by over-the-wire deployment of a new LAMS
Khan *et al*[78], 2021	Retrospective multicenter	208	PFC	97.1	7	-	-	-	Distal	LAMS re-insertion (not counted as technical failure) (1/7); immediate repeat drainage (5/7); procedure abandoned (1/7)
Law *et al*[79], 2018	Retrospective single center	46	WON	93.5	5	Cold Axios/hot Axios	-	-	-	LAMS removal followed by over-the-wire deployment of a new LAMS (4/5; 2 LAMS re-insertion, not counted as technical failure) and of a FCSEMS 10 × 60 mm (1/5)
Walter *et al*[10], 2015	Prospective multicenter	61	PFC	98	1	Axios	-	Yes	Entirely inside the PFC	Placement of double pigtail stents
Siddiqui *et al*[80], 2016	Retrospective multicenter	82	PFC	97.5	2	Cold Axios	-	Yes	Distal	LAMS removal followed by over-the-wire deployment of a SEMS; surgical cystogastrostomy (difficulty to re-advance the guidewire into the PFC to perform an endoscopic rescue therapy)
Mendoza *et al*[81], 2020	Retrospective single center	21	WON	95	1	Hot Axios	-	Yes	Entirely inside the PFC	LAMS misdeployed was left inside the collection and a new one was then successfully placed through the original puncture site (both stents were removed 4 wk later)
Shah *et al*[82], 2015	Prospective multicenter	33	PFC	91	3	Cold Axios	-	Yes	-	Placement of double pigtail stents
Despott *et al*[83], 2020	Case report	1	WON	-	1	Hot Axios	20x10	-	Deployment in a non-target organ (colon)	LAMS removal and closure of both colonic and gastric defects with over-the-scope-clips
Rinninella *et al*[84], 2015	Retrospective multicenter	93	PFC	98.9	1	Hot Axios	-	Yes	Distal	Placement of double pigtail stents
Song *et al*[85], 2019	Prospective multicenter	34	PFC	97.1	1	Hot spaxus	-	Yes	Distal	LAMS in LAMS technique
Fugazza *et al*[86], 2020	Retrospective multicenter	328	PFC, WON	97.9	7	Hot Axios	-	-	-	New LAMS placement (4/7); placement of plastic stents (3/7)
Zhang *et al*[12], 2022	Retrospective multicenter	35	PFC	97	1	Hot Axios	15 mm × 15 mm	Yes	-	SEMS in LAMS technique
Yang *et al*[87], 2019	Retrospective multicenter	80	PFC	97.5	3	Cold Axios/hot Axios	-	-	-	-

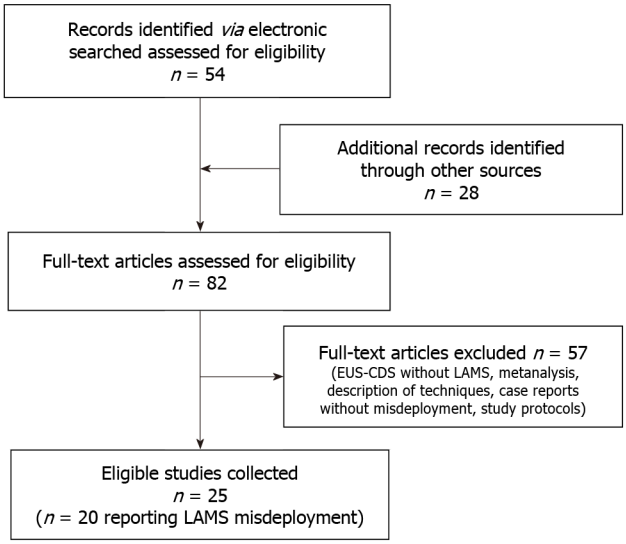
Adler *et al*[70], 2018	Retrospective multicenter	80	WON	98.7	1	Cold Axios	-	-	-	-
Curieses Luengo *et al* [88], 2019	Case report	1	WON	-	1	Hot Axios	10 mm × 10 mm	-	Distal	LAMS in LAMS technique

WON: Walled of necrosis; PFC: Pancreatic fluid collections; FCSEMS: Fully covered self-expanding metal stent; SEMS: Self-expanding metal stent; LAMS: Lumen-apposing metal stent.

causes in only seven cases (0.6%). In up to 86.8% of cases, the endoscopist managed LAMS misdeployment with an endoscopic rescue strategy during the index procedure.

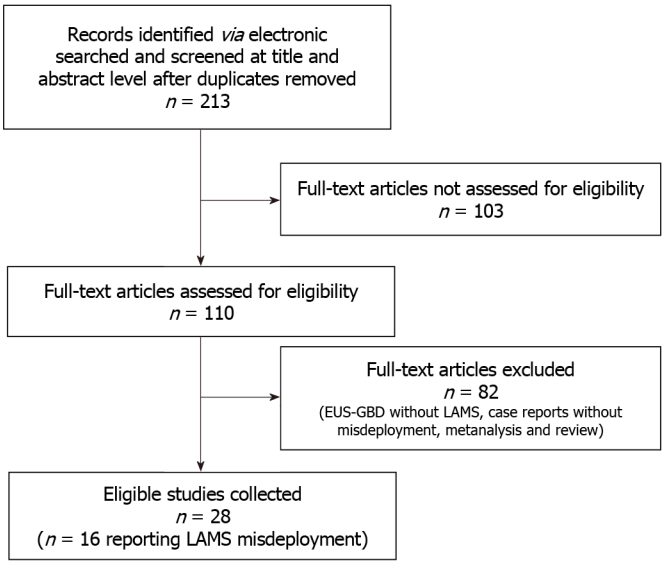
The most common endoscopic rescue technique involved misdeployed LAMS removal and over-the-wire deployment of a new stent through the same fistula tract (44.1%). SEMS were employed in most cases (76.7%). Other rescue strategies used include stent-in-stent (23.5%), EUS-guided/endoscopic rendezvous with transpapillary placement of a biliary SEMS (11.8%), and repeated EUS-drainage procedure (5.9%).

As reported in literature, the most common causes of LAMS misdeployment are related to difficult scope position[17,28,39] and small CBD diameter (< 15 mm)[21,27,34]. In a retrospective analysis by Jacques *et al*[23] involving 52 patients who underwent LAMS placement using various techniques, the technical and clinical success rates were 88.5% and 100%, respectively. In univariate analysis, CBD diameter > 15 mm, use of a 6-mm LAMS, and use of a one-step technique (direct puncture using the electrocautery system without needle puncture) were predictors of technical success in EUS-CDS. In another study, the authors performed EUS-CDS using a one-step technique in 97.1% (n = 68/70) of patients and achieved 98.6% of technical success[24]. The more frequent use of a pre-loaded guidewire in the group of patients with CBD < 15 mm compared to those with CBD ≥ 15 mm (33% *vs* 3.6%, P = 0.036) might have contributed to the comparable technical success, clinical success, and adverse event rates between the two groups. On the other hand, Di Mitri *et al*[21] reported seven cases of LAMS misdeployment in 31 patients with distal MBO who underwent EUS-CDS. CBD was ≤ 15 mm in six of the seven patients. In five cases, rescue therapies involved placing a fully covered self-expanding metal stent (FCSEMS) over the previously inserted guidewire, restoring the connection through the iatrogenic fistulous tract. In the remaining two cases, bile duct decompression after the puncture prevented the correct visualization of the CBD on EUS imaging and the possibility of approaching the CBD again; these cases were managed successfully by percutaneous- and laparoscopic-endoscopic rendezvous techniques. The authors assumed that the small caliber of the CBD forced the tip of the LAMS delivery catheter to be too close to the facing wall of the CBD and in an oblique direction, increasing the risk of misdeployment, even with the use of a small-size LAMS. In the largest study (256 patients enrolled) included in our review, Fugazza *et al*[26] reported that significantly higher technical success was achieved in patients with a larger CBD diameter compared to those with a smaller CBD diameter. The authors demonstrated that larger CBD size, use of a needle with a guidewire, fluoroscopy guidance, and LAMS placement in the proximal CBD were more likely in the non-expert group than in the expert group; however, technical [101 (94.4%) *vs* 138 (92.6%); P = 0.574] and clinical success [96 (95.0%) *vs* 134 (97.1%); P = 0.415] did not statistically differ between these two groups.



DOI: 10.3748/wjg.v29.i21.3341

Figure 1 Study flow-chart of endoscopic ultrasound-choledochoduodenostomy. EUS-CDS: Endoscopic ultrasound-choledochoduodenostomy; LAMS: Lumen-apposing metal stents.



DOI: 10.3748/wjg.v29.i21.3341

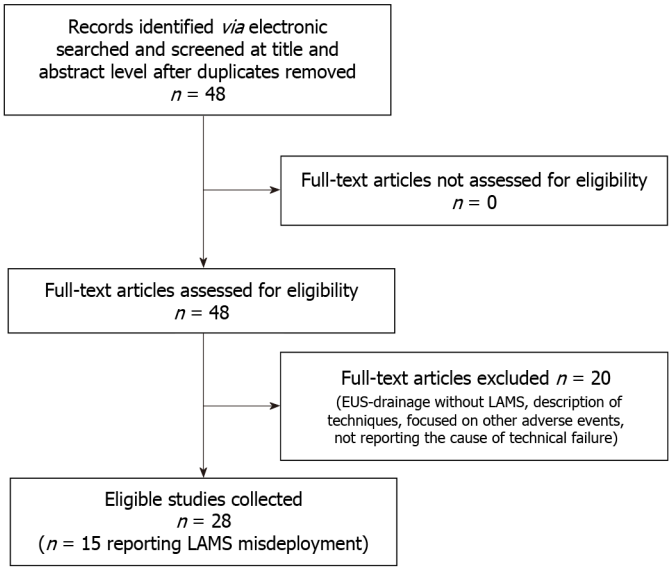
Figure 2 Study flow-chart of endoscopic ultrasound-gallbladder drainage. EUS-GBD: Endoscopic ultrasound-gallbladder drainage; LAMS: Lumen-apposing metal stents.

The use of a delivery system pre-loaded with a guidewire in complex cases (*i.e.*, endoscope instability in the duodenal bulb or smaller CBD diameter) was emphasized by Anderloni *et al*[97] because it allowed rescue using an over-the-wire stent placement in cases of LAMS misdeployment, and the single-step technique was preferred in cases of dilated CBD > 15 mm.

Wire access into the CBD could be regained in some cases, allowing endoscopic rescue maneuvers to be performed, even in cases of non-identifiable CBD on EUS imaging[28,35]. Our data show that over-the-wire deployment of a tubular stent, particularly a biliary SEMS, was the preferred rescue procedure for LAMS misdeployment during EUS-CDS (44.1%). We suppose that this technique was preferred since EUS-BD with SEMS has long been a consolidated technique for BD and that SEMS placement can be performed without EUS guidance, which may be lost in these circumstances.

EUS-GBD

Laparoscopic cholecystectomy is the standard approach for acute calculus cholecystitis. In cases of severe inflammation, adhesive disease, bleeding in the surgical area, or suspected bile duct injury, open cholecystectomy may be required to achieve safe dissection and gallbladder resection. In recent years, EUS-GBD has emerged as the preferred alternative to surgical treatment over percutaneous GBD



DOI: 10.3748/wjg.v29.i21.3341

Figure 3 Study flow-chart of endoscopic ultrasound-pancreatic fluid collections. EUS: Endoscopic ultrasound; LAMS: Lumen-apposing metal stents.

(PGBD) or endoscopic transpapillary GBD (ETP-GBD) and is included in the international guidelines for grade II cholecystitis and recommended for grade 3 cholecystitis in patients with American Society of Anesthesiologists scores ≥ 3 or Charlson Comorbidity Index ≥ 6[98,99]. It has a technical and clinical success rate of 94.65% [95% confidence interval (CI): 91.54-96.67; I^2 = 0.00] and 92.06% (95%CI: 88.65-94.51; I^2 = 0.00), respectively. The rates of adverse events associated with EUS-GBD, including perforations, misdeployment, bile leakage, stent migration into the gallbladder or peritoneum, bleeding, gastroduodenal perforation, pneumoperitoneum, and recurrent acute cholecystitis due to stent occlusion, varies between 8% and 17%[100-102]. EUS-GBD showed higher technical and clinical success rates and lower recurrence rates than those of ETP-GBD[103].

Moreover, several studies have compared EUS-GBD with PGBD, demonstrating similar technical and clinical success rates for both procedures[43,104-106]; however, EUS-GBD was associated with significantly fewer adverse events, including lower mortality, lower post-procedure pain, shorter hospital stay, and fewer readmissions and reinterventions. According to our data, LAMS misdeployment occurred in 3.4% of the patients who underwent EUS-GBD. In almost 63% of cases, LAMS misdeployment occurred when EUS-GBD was performed through the stomach.

Although studies comparing gastric and duodenal LAMS access for EUS-GBD did not find any significant differences in technical or clinical success or adverse event rates[107], the duodenum is a less mobile organ and is closer to the gallbladder than the stomach. Therefore, there is a lower risk of LAMS migration when gallbladder access is *via* the duodenum.

In 68% of cases, LAMS misdeployment involved the distal flange. This suggests that a careful choice of the position and proper advancement of the LAMS inside the GB are critical to avoiding misdeployment. The use of guidewire, reported in 68% of cases of misdeployment, helped to maintain secure access to the newly created fistula between the gastrointestinal system and GB. The most frequently performed rescue maneuver was the placement of LAMS or a longer SEMS through the lumen of the misdeployed stent (stent-in-stent strategy). The stent-in-LAMS was particularly the rescue strategy of choice in up to 60% of LAMS misdeployment cases.

Surgery or PTBD after endoscopic closure of the luminal perforation was required after the failure of endoscopic therapies[55,56,61]. LAMS removal and clip closure of digestive tract wall defect was the treatment of choice in 3 patients (one of them was treated by ETP-GBD while no further endoscopic maneuver was performed in the remaining two patients). This strategy allows a second drainage attempt during the index procedure or later in selected patients. According to our data, two patients died after LAMS misdeployment, one for surgical complications and one left to supportive care[22,56].

EUS-PFC drainage

EUS-guided transmural drainage is considered the first-line treatment option for PFC, including walled-off necrosis (WON) and pancreatic pseudocysts[108]. Transluminal drainage in the "before-LAMS age" was achieved by the placement of double-pigtail plastic stents and afterward, by biliary/esophageal FC SEMS, which were associated with risks of migration, leakage, ulceration, and bleeding[109]. The LAMS design has the advantage of supporting drainage, preventing migration, and allowing direct access inside the WON cavity for endoscopic necrosectomy because it has a larger diameter, shorter length, and stent-anchoring flanges[110].

In our study, LAMS misdeployment during EUS-guided PFC drainage occurred in 34/1684 (2.0%) patients. This result is in accordance with the high technical success rate of these procedures reported in the literature (97.6%)[111]. Distal flange misdeployment in the peritoneal cavity, external to the cystic wall, was reported in up to 36.1% of cases[77,78,80,81,85]. Over-the-wire placement of a new stent (LAMS, SEMS, or double pigtail stents/plastic) through the novel fistula tract was the rescue therapy of choice in 64.5% of misdeployments. In 50% of cases, LAMS were deployed over the guidewire to complete the procedure as initially planned. The re-insertion of the same LAMS was performed in 15% of cases[78,79], resulting in a lower cost; these cases were counted as technical successes. In a study by Khan *et al*[78] involving 208 patients who underwent EUS-PFC drainage, 5/7 cases of LAMS misdeployment were managed by repeated EUS-guided drainage during index endoscopy.

There were two cases (5.5%) of complete LAMS misdeployment inside the PFC. In such cases, Mendoza Ladd *et al*[81] decided to leave the LAMS inside the PFC, and they deployed a new one through the fistula. After LAMS dilation, direct endoscopic necrosectomy was performed. Both stents were successfully removed four weeks later. The deployment of LAMS into an adjacent organ (the splenic flexure of the colon), described as WON, was reported by Despott *et al*[83], who identified the misdeployment only at the post-procedure scan (computerized tomography). After bowel preparation through a naso-jejunal tube to bypass the gastrocolic fistula, simultaneous upper and lower gastrointestinal endoscopies were performed, and the LAMS was removed. Both the colonic and gastric perforations were closed using over-the-scope clips. Although data regarding predictive factors related to LAMS misdeployment were lacking in the studies included in our review, Curieses Luengo *et al*[88] identified excessive flexion of the echoendoscope tip due to severe inflammatory duodenal stenosis as an unfavorable condition for correct LAMS deployment.

CONCLUSION

The use of LAMS has been demonstrated to have high technical and clinical success rates in EUS-CDS, EUS-GBD and EUS-PFC drainage, however significant rates of LAMS misdeployment are reported in 5.8%, 3.4%, and 2.0% of procedures, respectively. In a relevant rate of LAMS misdeployment, endoscopic rescue management has been shown to be technically feasible and effective in completing the procedure and avoiding major clinical consequences.

Although no algorithm is available to guide the appropriate rescue strategy for each case, three endoscopic techniques have been identified: (1) Gaining wire access to the target through the newly created tract and completing the procedure; (2) "Stent-in-stent" over the wire; and (3) Repeated procedures (*ex novo* or rendezvous). When endoscopic rescue procedures are not feasible, non-endoscopic options include percutaneous drainage or surgery.

In our analysis, the preferred strategy for LAMS misdeployment in EUS-CDS was LAMS removal and over-the-wire deployment of a new stent (44.1%), frequently SEMS (76.7% of cases), followed by stent-in-stent strategy (23.5%) and endoscopic rendezvous (11.8%). In EUS-GBD, the preferred technique was the stent-in-stent strategy (60%) using a SEMS in 93.3% of cases.

In EUS-PFC, the procedure of choice was LAMS removal followed by over-the-wire deployment of a new stent (64.5%), which was a LAMS or a plastic stent, in 50% and 40% of cases respectively. In conclusion, LAMS misdeployment is a relatively common adverse event, especially in EUS-BD. Endoscopic rescue strategies are feasible, and they vary depending on type of procedure, endoscopic technique used, and experience of the operators.

ARTICLE HIGHLIGHTS

Research background

Scant data are available about rescue techniques in cases of lumen-apposing metal stents (LAMS) misdeployment which is the main cause of technical failure in endoscopic ultrasound (EUS)-guided drainage procedures. We performed a systematic review of the literature about LAMS misdeployment and rescue techniques in the biliopancreatic setting, focusing on technical aspects and success rate of endoscopic maneuvers.

Research motivation

LAMS misdeployment is a relatively common adverse event in EUS-guided drainages. There is no consensus on the best rescue approach in these cases and the choice is often made by the endoscopist relying upon the clinical scenario, anatomical characteristics, and local expertise.

Research objectives

The overall technical success rate of EUS-guided choledochoduodenostomy (EUS-CDS), gallbladder drainage (EUS-GBD) and pancreatic fluid collections drainage (EUS-PFC) was 93.7%, 96.1%, and 98.1%

respectively. Significant rates of LAMS misdeployment have been reported for EUS-CDS, EUS-GBD and EUS-PFC drainage, respectively 5.8%, 3.4%, and 2.0%. Endoscopic rescue treatment was feasible in 86.8%, 80%, and 96.8% of cases. Non endoscopic rescue strategies were required only in 10.3%, 16% and 3.2% for EUS-CDS, EUS-GBD, and EUS-PFC.

Research methods

We conducted a systematic review of the literature on PubMed searching for studies published up to October 2022 about on-label EUS-guided procedures namely EUS-CDS, EUS-GBD and EUS-PFC. The search was carried out using the exploded medical subject heading terms 'lumen apposing metal stent', 'LAMS', 'endoscopic ultrasound' and "choledochoduodenostomy" or "gallbladder" or "pancreatic fluid collections".

Research results

The overall technical success rate of EUS-CDS, EUS-GBD and EUS-PFC was 93.7%, 96.1%, and 98.1% respectively. Significant rates of LAMS misdeployment have been reported for EUS-CDS, EUS-GBD and EUS-PFC drainage, 5.8%, 3.4%, and 2.0%, respectively. Endoscopic rescue treatment was feasible in 86.8%, 80%, and 96.8% of cases. Non endoscopic rescue strategies were required only in 10.3%, 16% and 3.2% for EUS-CDS, EUS-GBD, and EUS-PFC. The endoscopic rescue techniques described were over-the-wire deployment of a new stent through the created fistula tract in 44.1%, 8% and 64.5% and stent-in-stent in 23.5%, 60%, and 12.9%, respectively for EUS-CDS, EUS-GBD, and EUS-PFC. Further therapeutic option were endoscopic rendezvous in 11.8% of EUS-CDS and repeated procedure of EUS-guided drainage in 16.1% of EUS-PFC.

Research conclusions

Stent misdeployment can be managed successfully by endoscopic rescue maneuvers to allow the completion of the procedure. In accordance with our results endoscopic rescue techniques are feasible in most cases (up to 96.8%). Three endoscopic rescue strategies have been identified: Gaining wire access to the target through the created fistula and completing the procedure; placement of a new stent through the misdeployed LAMS to the target ("stent-in-stent") and repeated drainage procedures (*ex novo* or rendezvous).

Research perspectives

LAMS misdeployment is the main cause of technical failure of EUS-drainages and it is potentially harmful to the patient. Knowledge of risk factors, classification of misdeployment and of endoscopic rescue techniques is useful to improve patient outcome and the safety of the procedure. Further prospective studies describing these issues are expected.

FOOTNOTES

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META-ANALYSIS

Association of non-alcoholic fatty liver and metabolic-associated fatty liver with COVID-19 outcomes: A systematic review and meta-analysis

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) and metabolic-associated fatty liver disease (MAFLD) are on the rise like any other liver disease, and tend to affect 25% of the United States population. The impact of NAFLD and MAFLD on patients with coronavirus disease 2019 (COVID-19) remains unclear.

AIM

To identify the association of NAFLD and MAFLD with mortality, hospitalization, hospital length of stay, and supplemental oxygen utilization in COVID-19 patients.

METHODS

A systematic review of literature on Cochrane, Embase, PubMed, ScienceDirect, and Web of Science databases was conducted from January 2019 to July 2022. Studies that evaluated NAFLD/MAFLD using laboratory methods, noninvasive imaging, or liver biopsy were included. The study protocol was registered in PROSPERO (ID CRD42022313259) and PRISMA guidelines were followed. The National Institutes of Health quality assessment tool was used to assess the quality of the studies. Pooled analysis was conducted using software Rev Man version 5.3. The stability of the results was assessed using sensitivity analysis.

RESULTS

Thirty-two studies with 43388 patients were included in the meta-analysis of whom 8538 (20%) patients were observed to have NAFLD. There were 42254 patients from 28 studies included in the mortality analysis. A total of 2008 patients died from COVID-19; 837 (10.52%) in the NAFLD group and 1171 (3.41%) in the non-NAFLD group. The odds ratio (OR) was 1.38 for mortality with a 95% confidence interval (95%CI) = 0.97-1.95 and P = 0.07. A total of 5043 patients from eight studies were included in the hospital length of stay analysis. There were 1318 patients in the NAFLD group and 3725 patients in the non-NAFLD group. A qualitative synthesis showed that the mean difference in hospital length of stay was about 2 d between the NAFLD and non-NAFLD groups with a 95%CI = 0.71-3.27 and P = 0.002. For hospitalization rates, the OR was 3.25 with a 95%CI of 1.73-6.10 and P = 0.0002. For supplemental oxygen utilization, the OR was 2.04 with a 95%CI of 1.17-3.53 and P = 0.01.

CONCLUSION

Our meta-analysis suggests that there are increased odds of hospitalization, longer hospital length of stay, and increased use of supplemental oxygen in NAFLD/MAFLD patients.

Key Words: Non-alcoholic fatty liver; Fatty liver; Coronavirus; COVID-19; Metabolic-associated fatty liver

Core Tip: Metabolic-associated fatty liver disease (MAFLD) is like non-alcoholic fatty liver disease (NAFLD) and is a hepatic presentation of metabolic syndrome. They are widely prevalent. It is estimated that 25% of the United States population have this condition. The association and effect size between fatty liver disease and coronavirus disease 2019 (COVID-19) infection is still unconfirmed. The discrepancies in the available literature may be due to study design, confounding, small study population, and heterogeneity. We performed a systematic review and meta-analysis to study the impact of NAFLD/MAFLD on mortality, hospitalization, hospital length of stay, and supplemental oxygen utilization in COVID-19 patients.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the subspecies of coronavirus that is responsible for causing coronavirus disease 2019 (COVID-19), the respiratory illness responsible for the ongoing COVID-19 pandemic. The first detected case was reported back in December of 2019 from Wuhan, China[1]. Since then, it has spread worldwide leading to a global pandemic. As of 12 December 2022, over 650 million confirmed cases have been reported with more than 6 million deaths [2]. Most COVID-19 cases are mild to moderate with usual signs and symptoms varying from general fatigue, fever, and dry cough to some very unusual ones like loss of smell and taste. In the severe form, it is associated with several comorbidities such as diabetes, hypertension, obesity, chronic obstructive pulmonary disease, and other cardiovascular diseases[3,4]. Patients with metabolic syndrome (MS) having obesity, hyperglycemia, dyslipidemia, and hypertension had worse outcomes in COVID-19[5,6]. A retrospective study from Cleveland Clinic, United States concluded that patients with MS were 77% more likely to be hospitalized, 56% more likely to be admitted to the intensive care unit, and 81% more likely to die from COVID-19[6]. A recent consensus of experts proposed redefining non-alcoholic fatty liver disease (NAFLD) as metabolic-associated fatty liver disease (MAFLD)[7-9]. It is now considered the hepatic form of MS[10] and is one of the most common etiologies of chronic liver diseases (CLDs). It has an estimated global prevalence rate of about 24%[11]. These patients may have a higher risk of hospitalization and severity of COVID-19 according to recently published reports. A cohort of Chinese patients indicated a higher risk of respiratory disease progression in MAFLD patients[12]. In another subsequent study, there was an increased risk for COVID-19 progression in younger patients with MAFLD[13]. However, there is still conflicting evidence addressing the severity of COVID-19 in MAFLD and NAFLD patients. Prior meta-analysis of a small number of studies did not detect worse outcomes in NAFLD/MAFLD patients with COVID-19[14-16].

Therefore, we conducted this systematic review and meta-analysis to assess the effects of NAFLD or MAFLD on mortality, hospitalization, length of hospital stay, and supplemental oxygen utilization among patients with COVID-19.

MATERIALS AND METHODS

This review is reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[17] as indicated in the PRISMA checklist and was registered with PROSPERO (ID CRD42022313259; www.crd.york.ac.uk/prospero).

Search and selection

A literature search was performed in six bibliographic databases PubMed, Cochrane, Embase, Science Direct, and Web of Science from January 2020 to July 2022. Using a combination of keywords and medical subject headings, we used vocabulary related to "COVID-19" OR "SARS-CoV-2" AND "NASH" OR "NAFLD" OR "non-alcoholic fatty liver disease" OR "fatty liver" OR "metabolic syndrome" (Supplementary Table 1 shows the search strategy for the study).

Six authors (Jagirdhar GSK, Qasba RK, Kashyap R, Flumignan Bucharles AC, Banga A, and Reddy ST) were involved in the study selection. After removing duplicates using Endnote reference manager software, two authors did the title and abstract screening independently using the Rayyan software (https://rayyan.ai/). Studies satisfying the inclusion criteria were retrieved and screened for full-text eligibility. Conflicts between two authors on study selection were resolved among the authors or were solved by an additional third arbiter in case a consensus could not be reached. We included observational studies that studied mortality, hospitalization, hospital length of stay, and supplemental oxygen utilization outcomes in COVID-19 patients. We included studies that assessed NAFLD/MAFLD using lab assessment (fibrosis-4 [FIB-4], aminotransferase/platelet ratio index, fibrosis score, hepatic steatosis index [HIS]), non-invasive imaging (elastography, liver ultrasound or computed tomography [CT] scan, magnetic resonance elastography, liver stiffness measurement), or liver biopsy. For studies that diagnosed NAFLD using biomarkers/lab diagnosis, we only included studies that measured NAFLD prior to index admission since acute COVID infection can increase biomarker/lab values leading to misdiagnosis as NAFLD. We excluded studies that were: (1) Systematic reviews; (2) meta-analyses; (3) literature reviews; (4) survey studies; (5) case reports; (6) animal studies; (7) single-arm studies; (8) studies that do not measure NAFLD/MAFLD/fatty liver; (9) not a retrospective or prospective study; (10) randomized controlled trials; (11) studies not related to COVID-19 patients; (12) not in English language; and (13) abstract only studies. We studied NAFLD/MAFLD patients together since recent studies are emerging that NAFLD is related to MS both mutually and bi-directionally. We included studies that compared no or mild NAFLD/MAFLD patient outcomes to moderate or severe NAFLD/MAFLD patient outcomes. We also searched systematic reviews and meta-analyses obtained from our search for eligible articles and added studies meeting our inclusion criteria. When multiple publications used overlapping study populations, we included the study with greater sample size. We grouped studies for the meta-analysis based on mortality, hospitalization, hospital length of stay, and supple-

mental oxygen utilization outcomes.

Data extraction

Three independent authors (Qasba RK, Banga A, Flumignan Bucharles AC) performed data extraction from all of the included studies into a prepiloted data extraction form in Microsoft Excel. A fourth author (Jagirdhar GSK) independently assessed the extracted data for validation. The following was extracted from each study: (1) General information: First author, title, digital object identifier, and year of publication; (2) study characteristics: Study site/country, study period, number of centers, journal, study design; (3) participant characteristics: Number of NAFLD/MAFLD and non-NAFLD/MAFLD patients and their demographic characteristics, method of assessment of NAFLD; and (4) outcomes: Number of patients with the events (mortality, hospital length of stay, hospitalization, and supplemental oxygen utilization) in NAFLD/MAFLD and non-NAFLD/MAFLD populations.

Statistical analyses

The Review Manager (RevMan) [Computer application] Version 5.4.1, the Cochrane Collaboration, 2020 was used to assess all results[18]. Using a random-effects model, crude odds ratios (ORs) for each study with corresponding 95% confidence intervals (CIs) were calculated from raw data for events and non-events from each research[19]. $P < 0.05$ was considered statistically significant for the analysis. Forest plots were generated to present the results of the meta-analyses. A previously proven technique was used to transform the median to mean to examine continuous outcomes[20]. The estimates for mean differences were then produced using the random effects model[19]. To measure study heterogeneity, the Cochrane Q and the I^2 statistics were used[19]. Low-level heterogeneity was defined as I^2 20%[19]. The stability of the results was assessed using sensitivity analysis. Egger's test and funnel plots were used to determine the likelihood of publication bias[21].

Quality assessment

The National Institutes of Health scale was used to assess case control, cohort, and randomized controlled trials for appraisal of study quality. As per the scale, studies were classified into three categories: good, fair, or poor. Five authors (Jagirdhar GSK, Qasba RK, Banga A, Rama K, Pattnaik H) independently performed the quality assessment of the included studies, and any discrepancies were resolved through discussion.

RESULTS

Search and selection

A total of 1084 records were identified from the initial search, of which 242 were excluded as duplicates and 842 articles were selected for the screening of title and abstract. Eighty-seven were chosen for full-text screening and a total of thirty-two studies met the inclusion criteria. These papers were eligible for qualitative and quantitative.

Figure 1 shows the PRISMA diagram for the study selection process. Since we included studies that compared mortality, hospitalization, hospital length of stay, and supplemental oxygen utilization in mild or no NAFLD/MAFLD to moderate to severe NAFLD/MAFLD. We excluded studies that did not present these outcomes or those that did not mention the method of NAFLD assessment[22-28].

Characteristics of included studies

We analyzed data for a total of 43388 patients from 32 studies in the meta-analysis of which 8538 (20%) patients were observed to have NAFLD and 34850 did not have NAFLD. The studies observed the outcomes of patients infected with COVID-19 with and without NAFLD/MAFLD. The outcomes recorded were the rate of hospitalization, length of stay in the hospital, supplemental oxygen requirement, and mortality of patients in both groups.

All 29 of 32 studies reported mortality data for COVID-19 infection. Ten studies reported hospital length of stay, four studies reported need for hospitalization, and four reported supplemental oxygen utilization. The main characteristics of the included studies are summarized in Table 1.

NAFLD/MAFLD and mortality outcomes in COVID-19: A total of 42254 patients from 28 studies were included in the qualitative analysis. A total of 2008 patients died from COVID-19: 837 (10.52%) in the NAFLD group and 1171 (3.41%) in the non-NAFLD group. The OR was 1.38 for mortality with a 95%CI = 0.97-1.95, I^2 = 84%, and P = 0.07. Figure 2A shows the forest plot and meta-analysis of mortality outcomes in COVID-19 patients. Figure 2B shows the sensitivity analysis of the studies. We failed to observe an association between NAFLD/MAFLD and in-hospital mortality in COVID-19 patients. Visual inspection of the standard error plots for the mortality meta-analysis (Supplementary Figure 1) suggests symmetry without an underrepresentation of studies of any precision. No publication bias was found on Egger's test, P = 0.466.

Table 1 Main characteristics of the included studies in the systematic review and meta-analysis

Serial No.	Ref.	Country	Study design	NAFLD, *n*	No NAFLD, *n*	mean ± SD	Male, *n*	Measure of NAFLD	Measure of NAFLD
1	Calapod *et al* [27]	Romania	Prospective descriptive study	47	79	66.32 ± 13.72	57.20%	Imaging evidence (ultrasound or computer tomography)	Biochemical enzymes (liver function test) within the past 12 mo
2	Campos-Varela *et al*[28]	Spain	Prospective observational (cohort) study	271	29	55.25 ± 11.69	49%	Liver steatosis by hepatic steatosis index	Transient elastography by controlled attenuation parameter
3	Chang *et al*[29]	South Korea	Retrospective cohort study	2082	1040	-	30.72%	FLI index	
4	Chen *et al*[30]	United States	Retrospective single-center cohort study	178	164	62.6 ± 15.6	53.50%	Liver steatosis	Imaging evidence of steatosis > 30 d before COVID-19 diagnosis, or hepatic steatosis index
5	Çoraplı *et al* [31]	Turkey	Retrospective cohort study	106	308	-	56.04%	Hepatic-to-splenic attenuation ratio	
6	Davidov-Derevynko *et al*[32]	Israel	Single center retrospective cohort study	47	335	58.6 ± 18.6	61%	Imaging, previous medical records, admission fibrosis-4	Prior liver enzymes
7	Demir *et al*[33]	Turkey	Retrospective cohort study	349	270	51.6 ± 9.65	58.60%	Fibrosis-4 index	
8	Ji *et al*[34]	China	Cohort	19	35	43.6 ± 14.1	58.6	Fibrosis-4 index, APRI, ultrasound	
9	Effenberger *et al*[35]	Austria	Prospective study	12	20	-	40.62%	Liver stiffness measurements and controlled attenuation parameter with a fibro scan	Liver and spleen sonography and elastography
10	Elfeki *et al*[36]	United States	Retrospective cohort study	88	285	63.3 ± 14.8	52%	Prior data lab values	
11	Forlano *et al* [14]	United Kingdom	Retrospective cohort study	61	132	-	60%	Fibrosis-4 index	Imaging (either ultrasound or computerized tomography) or past medical history
12	Hashemi *et al* [37]	United States	Retrospective cohort	55	294	63.4 ± 16.5	55.4%	Hepatic steatosis on any prior imaging studies or liver histology	
13	Huang *et al*[38]	China	Retrospective cohort study	86	194	43.6 ± 17.8	52.10%	Hepatic steatosis index	
14	Hussain *et al* [39]	Pakistan	Cross sectional study	87	63	59.73 ± 11.35	56%	Clinical parameters like hepatomegaly and lab parameters like AST, ALT	
15	Kim *et al*[40]	United States	Observational cohort study	456	411	56.9 ± 14.5	54.70%	Fibrosis by magnetic resonance elastography	Fibro scan, fibrosis-4, or biopsy
16	Madan *et al*[41]	India	Case control study	289	157	-	64.5%	Liver attenuation index	
17	Marjot *et al*[42]	United States	Cohort study	322	367	58 ± 15.6	62.40%	Reported by clinician	
18	Mushtaq *et al* [13]	Qatar	Prospective study	320	269	-	84.71%	Hepatic steatosis index	
19	Romero-Cristóbal *et al*[43]	Spain	Prospective observational (cohort) study	81	96	59.58 ± 13.79	71.96	Fibrosis-4 index	
20	Rentsch *et al* [44]	United States	Retrospective cohort study	377	139	65.8 ± 7.8	95.4	Fibrosis-4 index	
21	Shao *et al*[45]	China	Observational	37	84	60.6 ±	64.46%	Liver enzyme/GGT	

			cohort study			13.5		twice upper limit of normal	
22	Targher *et al* [46]	China	Cohort study	50	43	-	48%	Fibrosis-4	NAFLD fibrosis score
23	Tignanelli *et al* [47]	United States	Retrospective cohort study	934	25962	51 ± 23.7	56%	Elevated ALT level on 3 separate dates	
24	Trivedi *et al* [48]	United States	Case control study	45	274	65 (median)	50%	Abdominal imaging (computed tomography, magnetic resonance imaging, or ultrasound)	
25	Vázquez-Medina *et al* [49]	Mexico	Retrospective case control study	299	60	54.3 ± 14.69	22.01%	Fibrosis-4 index	
26	Moctezuma-Velázquez *et al* [50]	Mexico	Retrospective cohort study	359	111	51.6 ± 14.8	63%	Computed tomography scans	
27	Vrsaljko *et al* [51]	Republic of Croatia	Prospective observational (cohort) study	120	96	59.3 ± 12.6	63.43%	Ultrasound	Difference between liver and spleen computed tomography attenuation
28	Wang *et al*[52]	China	Retrospective cohort study	86	132	-	50.40%	Ultrasound parameters	
29	Yao *et al*[53]	China	Retrospective cohort study	38	48	43.2 ± 15.45	58.10%	Hepatic steatosis index	NAFLD fibrosis score
30	Yoo *et al*[54]	South Korea	Retrospective cohort study	629	561	-	-	Hepatic steatosis index, FLI, claims based NAFLD	
31	Younossi *et al* [55]	United States	Observational cohort study	553	2736	-	49.55%	Abdominal imaging, magnetic resonance imaging, computer tomography, ultra-sound	
32	Zhou *et al*[56]	China	Cohort study	55	55	42.1 ± 11.4	74.50%	Computed tomography	

Non-alcoholic fatty liver disease is used synonymously with metabolic-associated fatty liver disease in this table.
ALT: Alanine aminotransferase; APRI: Aminotransferase/platelet ratio index; AST: Aspartate aminotransferase; COVID-19: Coronavirus disease 2019; FLI: Fatty liver index; GGT: Gamma-glutamyl transferase; NAFLD: Non-alcoholic fatty liver disease.

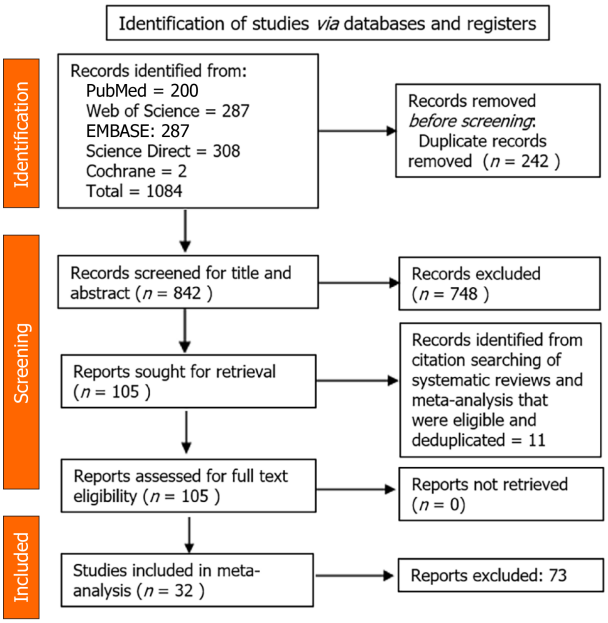

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Figure 1 PRISMA flowchart outlining the study search.

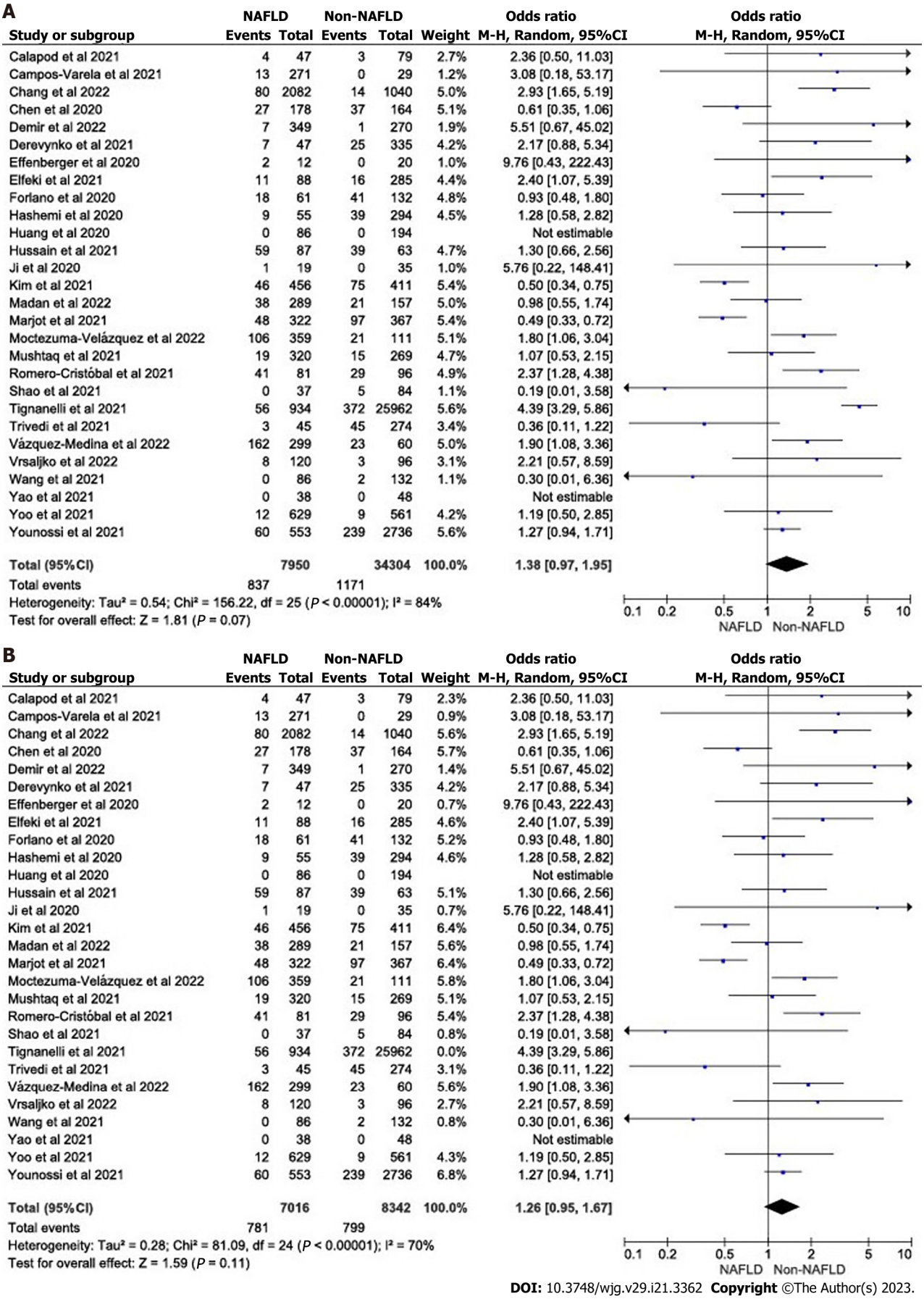

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Figure 2 Forest plot for mortality outcomes in coronavirus disease 2019 patients. A: In coronavirus disease 2019 (COVID-19) patients with and without non-alcoholic fatty liver disease/metabolic-associated fatty liver disease (NAFLD/MAFLD); B: In COVID-19 patients with and without NAFLD/MAFLD

sensitivity analysis after excluding Tignanelli *et al*[47]. CI: Confidence interval.

NAFLD/MAFLD and hospitalization in COVID-19: Four studies were taken for the quantitative analysis with 28199 patients to assess the need for hospitalization in NAFLD and non-NAFLD groups with COVID-19. A total of 4302 patients were hospitalized for COVID-19: 765 (50.83%) patients from the NAFLD group and 3537 (13.25%) patients from the non-NAFLD group. The OR was 3.25 with a 95%CI of 1.73-6.10, I^2 = 92%, and a P = 0.0002. Figure 3A shows the forest plot for hospitalization in COVID-19 patients with and without NAFLD/MAFLD. Figure 3B shows the sensitivity analysis of the studies. Visual inspection of the standard error plots for the need for hospital admission meta-analysis showed (Supplementary Figure 2) symmetry without an underrepresentation of studies of any precision. No publication bias was found (Egger's test, P = 0.254). However, as there were < 10 studies included in the analysis, publication bias cannot be completely excluded.

NAFLD/ MAFLD and hospital length of stay in COVID-19: A total of 5043 patients from 10 studies were included in the qualitative analysis. A total of 1318 patients were in the NAFLD group and 3725 patients were in the non-NAFLD group. A qualitative synthesis showed that the mean difference in hospital length of stay was 1.99 d between the NAFLD and non-NAFLD groups with a 95%CI = 0.71-3.27, I^2 = 70%, and P = 0.002. This denotes an average of about 2 d of additional hospital stays among NAFLD/MAFLD patients with COVID-19. Figure 4A shows a forest plot and meta-analysis of hospital length of stay in COVID-19 patients with and without NAFLD/MAFLD. Figure 4B shows the sensitivity analysis of the studies. Visual inspection of the standard error plots for the hospital length of stay meta-analysis (Supplementary Figure 3) suggests symmetry without an underrepresentation of studies of any precision. Publication bias was found on Egger's test, P = 0.013.

NAFLD/MAFLD and supplemental oxygen utilization in COVID-19: A total of 3609 patients from four studies were included in the qualitative analysis to assess the requirement for supplemental oxygen in COVID-19 patients during their in-hospital stay. A total of 170 (7.30%) patients in the NAFLD group and 96 (7.48 %) in the non-NAFLD group required supplemental oxygen. The OR was 2.04 with a 95%CI of 1.17-3.53, I^2 = 56%, and P = 0.01. Figure 5A shows a forest plot and meta-analysis of supplemental oxygen utilization in COVID-19 patients with and without NAFLD/MAFLD. Figure 5B shows the sensitivity analysis of the studies. Visual inspection of the standard error plots for the need for supplemental oxygen requirement meta-analysis (Supplementary Figure 4) suggests symmetry without an underrepresentation of studies of any precision. No publication bias was found on Egger's test, P = 0.500. However, as there were < 10 studies included in the analysis, publication bias cannot be completely excluded.

Quality assessment

Figures for quality assessment of case-control, cross-sectional, and cohort studies included in our study are attached with Supplementary material. For case-control studies, the quality assessment of included studies identified three as good quality, one as fair, and no poor rated studies. None of the studies were able to recruit a concurrent control or blind the outcome assessors. Only Madan *et al*[41] discussed reasons for selecting included participants, providing a sample size justification additionally, Trivedi *et al*[48] were the only ones to include a random selection of participants. For included cohorts, 19 were identified as good and 10 as fair with no poor rated studies. Only Kim *et al*[40], Yoo *et al*[54], and Zhou *et al*[56] could provide a sample size justification additionally studies scored poorly on blinding of outcome assessors with only Marjot *et al*[42] and Zhou *et al*[56] being able to do so, Furthermore Ji *et al*[34] and Huang *et al*[38] were the only ones to measure exposure more than once for each person during the study period (Supplementary Tables 2 and 3 show the Quality assessment of the studies included based on the National Institutes of Health quality appraisal tool for case control, cohort, and cross-sectional studies).

DISCUSSION

Our systematic review and meta-analysis of 32 studies and 43388 COVID-19 patients, of which 8538 (20%) patients were observed to have NAFLD/MAFLD, provided a comprehensive assessment of mortality, need for hospitalization, hospital length of stay, and need for supplemental oxygen in COVID-19-afflicted patients with fatty liver disease. In the current meta-analysis, there was no relation between pre-existing NAFLD/MAFLD and COVID-19-related mortality. The results for the unadjusted analysis showed an increased risk of mortality, but the results were not statistically significant. Similar results were seen in a prior meta-analysis[57]. Meta-analysis of seven studies by Hayat *et al*[58] on MAFLD and COVID-19 mortality found an OR of 1.45 and a non-significant 95%CI = 0.74-2.84 (P < 0.01).

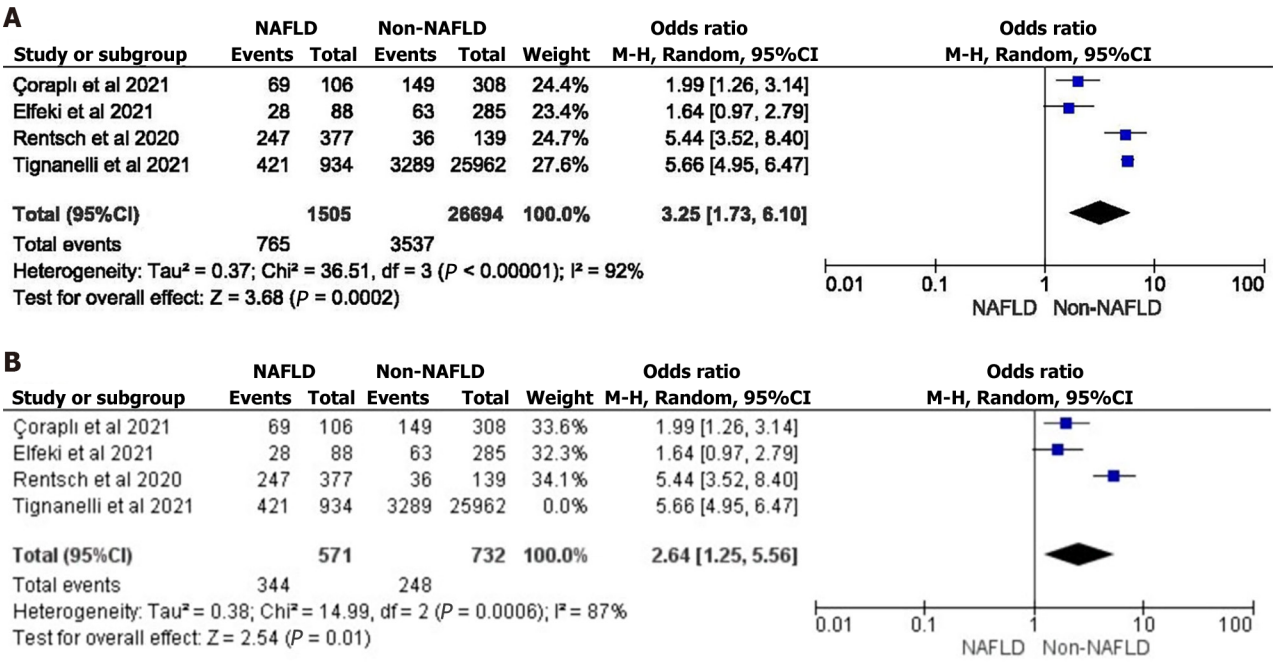

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Figure 3 Forest plot for hospitalization and need for hospitalization in coronavirus disease 2019 patients. A: Forest plot for hospitalization in coronavirus disease 2019 (COVID-19) patients with and without non-alcoholic fatty liver disease/metabolic-associated fatty liver disease (NAFLD/MAFLD); B: Forest plot for need for hospitalization in COVID-19 patients with and without NAFLD/MAFLD sensitivity analysis after excluding Tignanelli *et al*[47]. CI: Confidence interval.

As per our meta-analysis, patients with NAFLD/MAFLD had higher hospitalization rates compared to non-NAFLD/MAFLD patients (OR = 2.71, 95%CI: 1.10-6.70; P = 0.03). COVID-19 is associated with increased inflammation and thrombosis, while NAFLD/MAFLD are states of chronic inflammation. When occurring together, these may worsen disease status causing increased hospitalization rates. Contrarily, some studies have reported no increase in disease severity or need for hospitalization in NAFLD patients with COVID compared to controls. This could be due to the small study population and the retrospective nature of the study results[39,54]. Since our meta-analysis may be one of the largest to date, the results provided are expected to be closer to the true value. Our meta-analysis found that patients with NAFLD/MAFLD had a statistically significant longer hospital length of stay compared to those without. An unadjusted assessment of eight studies found that patients with pre-existing NAFLD, on average, spent an additional 2 d in the hospital compared to COVID-19 patients without NAFLD. Patients with NAFLD have ongoing inflammation processes which compound the disease process of COVID-19, thus leading to a more severe course of illness. This could explain the higher hospitalization rates and longer duration of hospital stay. Previous studies have reported similar results with longer hospital stays for NAFLD patients afflicted with COVID-19[32,58,59]. On the contrary, some studies reported no difference in the duration of hospital stay in those with or without NAFLD[52,60].

Our meta-analysis of four studies revealed that NAFLD patients had a greater need for supplemental oxygen as compared to controls. NAFLD patients may have a more severe disease course and therefore, require supplemental oxygen during their hospital stay. One previous study reported that NAFLD patients had a more frequent need for oxygen support[51].

CLD due to NAFLD has seen a rising trend over the years. Over 83 million Americans suffered from NAFLD with a prevalence rate of 26% according to data from 2015. This value is said to rise by 21% to over 100 million patients by the year 2030. The growing epidemics of obesity and diabetes mellitus II have played a major role in increasing the prevalence of NAFLD worldwide, including the progression of NAFLD to non-alcoholic steatohepatitis (NASH), cirrhosis, and even hepatocellular malignancy[61,62]. NAFLD is found in more than 50% of individuals suffering from obesity or type 2 diabetes mellitus. In their study, Estes *et al*[61] reported the median age of NAFLD patients to be about 50 years. They also found that more males suffered from NAFLD as compared to females. These results are similar to our study. The absence of an established screening method and global health policies focusing on NAFLD, and primary care interventions lead to a substantial increase in patients with NAFLD going undetected [55]. It also means that it is up to the clinician's preference and choice of the screening tool to measure NAFLD, thus creating a selection bias amongst various studies. This discrepancy could be observed in the studies included in our meta-analysis as multiple screening modalities were used in different clinical settings, including HSI, FIB-4 scoring, CT scan of the liver, liver function tests, and liver biopsy among others.

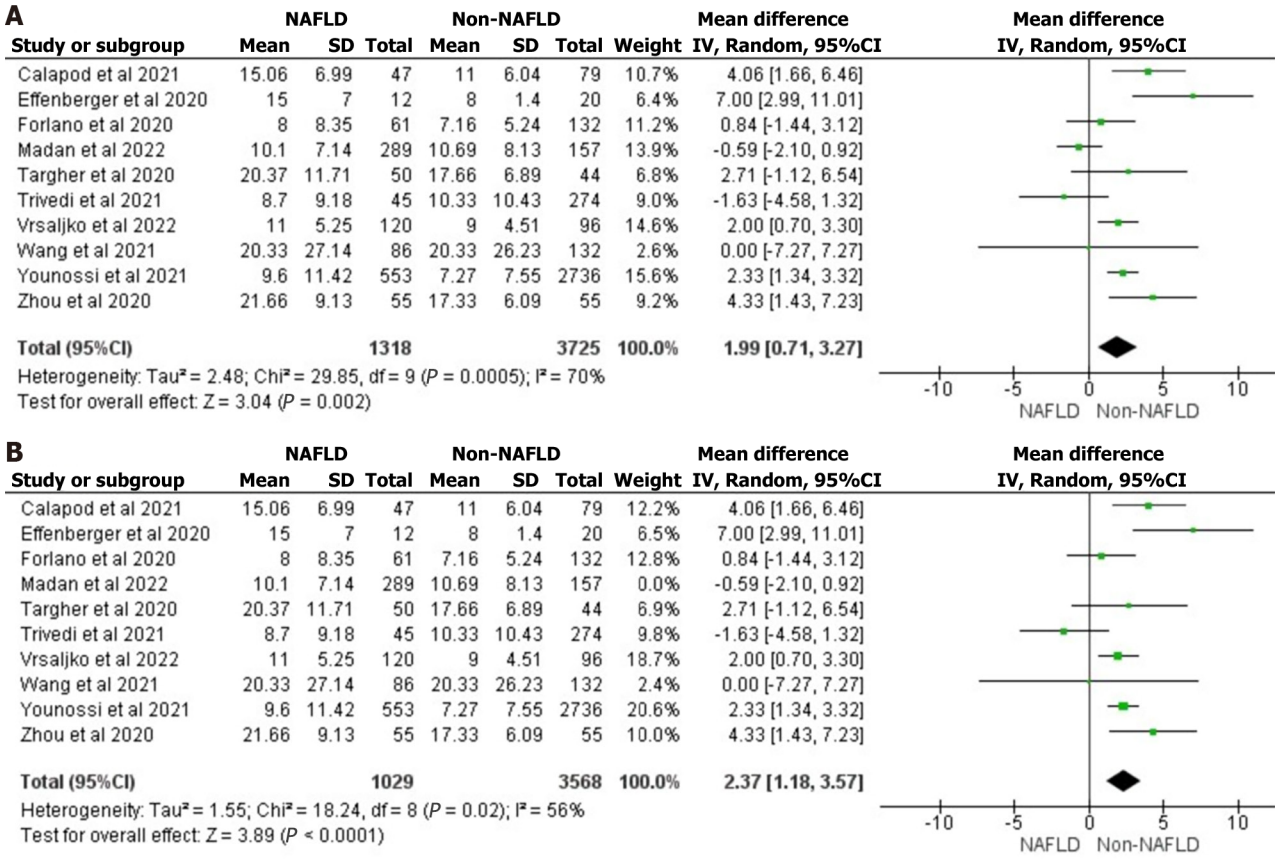

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Figure 4 Forest plot for hospital length of stay in coronavirus disease 2019 patients. A: In coronavirus disease 2019 (COVID-19) patients with and without non-alcoholic fatty liver disease/metabolic-associated fatty liver disease (NAFLD/MAFLD); B: In COVID-19 patients with and without NAFLD/MAFLD sensitivity analysis after excluding Madan *et al*[41]. CI: Confidence interval.

The presence of NAFLD confers additional susceptibility to COVID-19 infection in individuals exposed to SARS-CoV-2[63]. Pre-existing NAFLD at the time of COVID-19 diagnosis can be an indicator of increased severity of infection and utilization of health care services. As mentioned earlier, more than half of the individuals with type 2 diabetes mellitus (2nd most common comorbidity in COVID-19) have comorbid NAFLD to a certain degree[60]. Similarly, NAFLD is associated with multiple risk factors like cardiovascular diseases, obesity, and coexisting chronic lung disease which independently influences COVID-19 susceptibility and severity[61,64-68]. The impact of NAFLD on disease severity is seen through a blunted immune response to SARS-CoV-2 infection in COVID-19, leading to an increased risk of severe disease[30,13,47,55]. A retrospective observational study observed that ongoing inflammation in NAFLD puts patients with active COVID-19 infection at an increased risk of thromboembolism and associated mortality[51]. Various SARS-CoV-2 entry factors like angiotensin-converting enzyme (ACE), a disintegrin and metalloprotease 17, dipeptidyl peptidase 4, and transmembrane protease, serine 2 and NAFLD-related genes such as ACE, dipeptidyl peptidase 4, interleukin 10 (IL-10), tumor necrosis factor (TNF), and AKT1 as well as cytokine-mediated signaling, phosphoinositide 3 kinase-Akt, AMP-activated protein kinase, and mechanistic target of rapamycin signaling pathways have been identified which sheds light on the propensity for increased severity of illness in SARS-CoV-2 infected NAFLD patients. The spike protein of coronavirus has a high affinity for the receptor of ACE, which is found in the lung and the hepatobiliary cells. Furthermore, COVID-19 infection upregulates the expression of ACE receptors[69]. This leads to the hyperactivation of an immune cascade that damages the hepatocytes[70,71]. Chronic low-grade inflammatory state in individuals with NAFLD/MS creates a hypoxic environment for adipocytes leading to their dysfunction. This promotes the increased release of pro-inflammatory cytokines IL-6, IL-8, C-reactive protein, and TNF-α, and the recruitment of macrophages, B cells, and T cells. This effect is compounded by the systemic hyperactivation of the inflammatory cascade in active COVID-19 infection, which aggravates the ongoing inflammation of the hepatocytes, leading to decompensation of NAFLD and extensive hepatocyte damage[72]. Additionally, patients with NAFLD have insulin resistance, which further serves as a conducive ground for widespread inflammation[73,74].

Bramante *et al*[75] conducted a retrospective study that showed that with each additional year of having NAFLD/NASH the risk of hospitalization for COVID-19 increased. An assessment of the association between liver fibrosis scores and the clinical outcomes in patients with COVID-19 reported

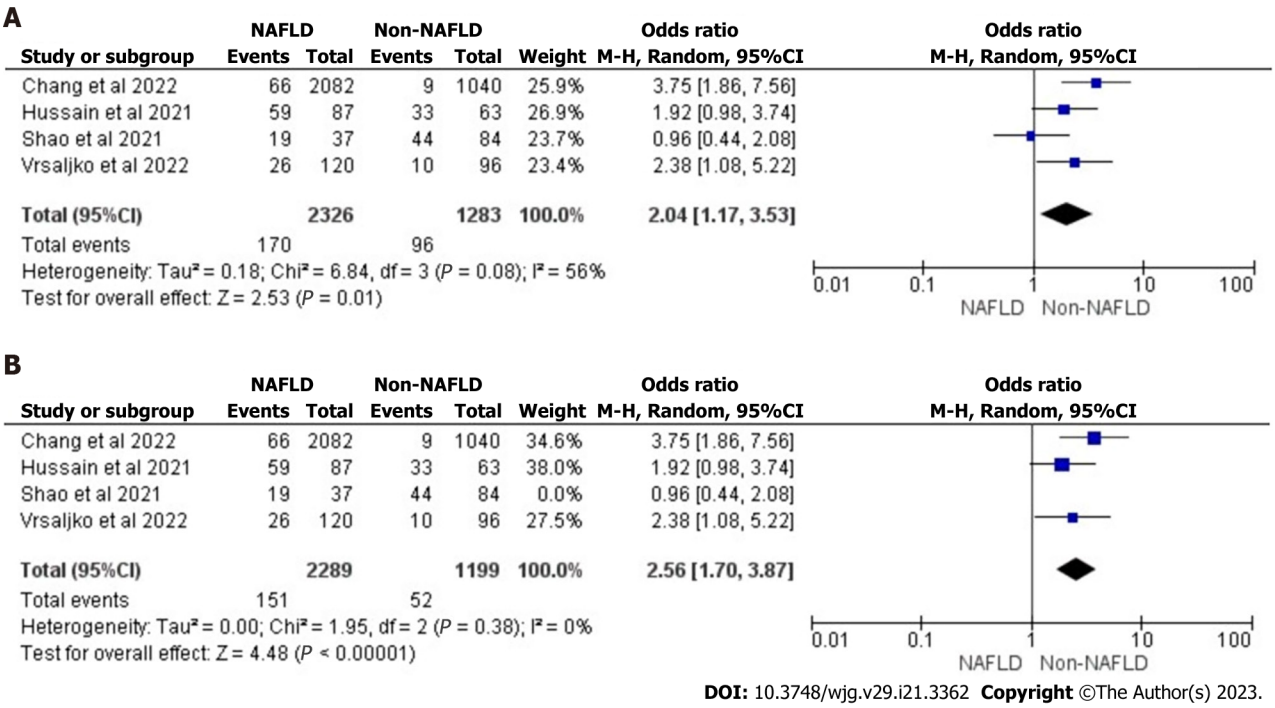

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Figure 5 Forest plot for need for supplemental oxygen utilization in coronavirus disease 2019 patients. A: In coronavirus disease 2019 (COVID-19) patients with and without non-alcoholic fatty liver disease/metabolic-associated fatty liver disease (NAFLD/MAFLD); B: In COVID-19 patients with and without NAFLD/MAFLD sensitivity analysis after excluding Shao *et al*[45]. CI: Confidence interval.

that a one-point score increase in FIB-4 was significantly associated with increased death, but not hospitalization. The authors also found that for every unit elevation in aspartate aminotransferase/alanine aminotransferase ratio, the risk of death increased by 178%[76]. Patients with higher FIB-4 > 2.67 scores had a higher risk of developing COVID-19[77]. Therefore, primary prevention is essential to control NAFLD during its development. Bramante *et al*[75] also showed that patients on prior treatments for NAFLD such as metformin and glucagon-like-peptide 1 receptor agonists had a reduced risk of hospitalization with the most significantly decreased risk from recent bariatric surgery. This highlights the importance of obesity as a cofactor to worse outcomes in COVID-19 patients and weight loss as the most significant contributing factor to improved outcomes in NAFLD[60]. Several studies found a higher incidence of obesity and NAFLD in patients hospitalized for COVID, which can also worsen disease severity[53,59,75]. This association may be confounded by the presence of obesity, which is an independent risk factor for COVID-19 severity. Previous studies reported odds ratios after adjusting for obesity and found statistically significant results in the association between NAFLD and COVID-19[77,78]. However, in a regression analysis, Li *et al*[79] observed that NAFLD independently does not affect the prognosis of severe COVID-19. Any association between NAFLD and COVID-19 is likely attributed to the confounding effect of obesity, measured in terms of body mass index, waist circumference, and hip circumference. Further studies with a larger sample size are needed to explain the varied results observed in these studies. Roca-Fernández *et al*[63] reported that higher liver fat percentages or evidence of liver fibro-inflammation, and features of NAFLD, increased the likelihood of symptomatic COVID-19. Interestingly, they found that obese patients with higher liver fat had a higher probability of having symptomatic COVID-19 compared to obese patients with normal liver fat. This is in favor of NAFLD being an independent risk factor for COVID-19 irrespective of obesity.

Strengths and limitations

We followed rigorous methodology, adhered to PRISMA guidelines, and registered our study in PROSPERO. Most of the studies were retrospective case-control and cohort studies which can be associated with the risk of bias, particularly in the absence of adjusting for confounders. NAFLD/MAFLD patients underlying medical conditions and co-morbidities that are components of MS can interfere with the outcomes studied. We excluded studies on NAFLD/MAFLD patients with other underlying causes for liver diseases including alcoholic liver disease. We understand there may be additional etiology for liver disease in patients that are underdiagnosed and may impact our study findings. The retrospective nature of most of the studies in our analysis does not imply a causal relationship between NAFLD/MAFLD and measured outcomes. We included studies that measured NAFLD/MAFLD using non-invasive procedures such as imaging and biomarker/lab diagnosis. Since they are not the gold standard for diagnosis, there may be misclassification of patients with and without NAFLD/MAFLD. The method of diagnosis of NAFLD/MAFLD varied across studies so, there may be

some misdiagnoses that can influence the results of the study. However, we included studies that compared absent or mild NAFLD/MAFLD to moderate or severe NAFLD/MAFLD to maintain homogeneity. We considered NAFLD/MAFLD patients in the same group, however, there may be important differences between these two groups that may alter the outcomes. We observed the per-study protocol in our meta-analysis. We detected significant differences in our study population for the various outcomes measured. However, there was statistical heterogeneity in our results that should be taken into consideration. We also excluded studies in a language other than English due to difficulty with translation and interpretation of results. This can introduce bias in our study results. The studies included in our meta-analysis were from 12 different countries with more studies from China and the United States, but we consider the results to be generalizable globally. However, since the studies were from different countries the level of care at each health institution may be different, which can lead to different levels of disease severity, disease progression, and death. Our study aims for hypothesis generation and further research in NAFLD/MAFLD patients to better understand the disease pathophysiology and patient risk profiles.

CONCLUSION

Our meta-analysis suggests that NAFLD/MAFLD patients appear to have higher rates of hospital admissions and longer in-hospital stays without any increase in mortality compared to non-NAFLD/MAFLD patients. Further research is needed to explore if fatty liver disease may be a risk factor that can lead to severe COVID-19 infection.

Implications for clinical practice

NAFLD/MAFLD are chronic pandemics rapidly on the rise. There is a lack of awareness and education among healthcare professionals and patients about its health impact, morbidity, and economic impact as they progress. There is a lack of systems/protocols in place to enforce the diagnosis of NAFLD globally and as a part of regular screening in primary care clinics, diabetic clinics, and routine health care visits. Propagation of information on NAFLD and involvement of international organizations, scientific societies, and the pharmaceutical industry to improve public health policies related to NAFLD. Patient education on NAFLD/MAFLD as a co-morbid risk factor for infectious diseases such as COVID-19 like other known risk factors such as hypertension, obesity, and diabetes, and measures to diagnose and treat NAFLD actively are needed.

Implication for research

Futures research on longitudinal prospective studies with larger numbers on NAFLD/MAFLD as a risk factor for COVID-19. Future studies should focus on genetics, immunology, and molecular epidemiology to better understand the mechanisms for poor outcomes in the NAFLD population.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) and its hepatic manifestation metabolic-associated fatty liver disease (MAFLD) have a rising prevalence worldwide. It is a co-morbidity like obesity, hypertension, and chronic kidney disease. NAFLD/MAFLD like obesity is considered chronic inflammatory states according to recent literature. Therefore, patients with NAFLD/MAFLD are hypothesized to have worse outcomes with coronavirus disease 2019 (COVID-19).

Research motivation

Existing literature shows conflicting information on the association of NAFLD/MAFLD in COVID-19 patients. Some studies show worse outcomes with NAFLD/MAFLD and COVID-19 infection. Some studies state it is not a risk factor for severe COVID-19. Understanding the pathophysiology and pathogenesis between fatty liver disease and COVID-19 is necessary for prevention, and management of NAFLD/MAFLD patients. Insight into this relationship will help further research and better preventative and nutritional management of these patients. It is imperative to explore the relationship of NAFLD/MAFLD with COVID-19 to improve patient care and treatment protocols for better outcomes.

Research objectives

In this meta-analysis, we investigated the association between NAFLD/MAFLD with the mortality and severity of COVID-19 infection.

Research methods

A systematic review of literature across five databases was done from January 2019 to June 2022. Observational studies were included. Studies that evaluated NAFLD/MAFLD using lab assessment/biomarker assessment, non-invasive imaging, or liver biopsy were included. We registered our study protocol in Prospero and followed the "PRISMA" guidelines (Figure 1). Meta-analysis was conducted on studies with outcomes for hospitalization, hospital length of stay, supplemental oxygen utilization, and mortality of COVID-19 infection outcomes using Rev Man version 5.3. To evaluate the validity of our studies the National Institutes of Health quality assessment tool was used. The stability of the results was assessed using sensitivity analysis.

Research results

A total of 43388 patients from thirty-two studies were included in the final analysis. There were 8538 (20%) with NAFLD/MAFLD. A total of 42475 patients from twenty-nine studies were included in the mortality analysis. There was an odds ratio of 1.36, a *P* value = 0.07 for mortality with COVID-19. A total of 5043 patients from eight studies were included in the hospital length of stay analysis. NAFLD patients spent a mean hospital stay of an additional about 2 d when compared to non-NAFLD. For hospitalization rates, the odds ratio is 3.25 and a *P* value = 0.0002. For supplemental oxygen utilization, the odds ratio was 2.04 with a *P* value = 0.01. Our meta-analysis was able to show that there is an association between NAFLD/MAFLD and COVID-19. Our study aims to increase awareness that NAFLD/MAFLD may be a potential risk factor for severe outcomes in infections. More research is needed to better explain the relationship and the pathophysiology behind it.

Research conclusions

This systematic review and meta-analysis of observational studies suggests that NAFLD/MAFLD patients had higher odds of developing severe forms of COVID-19 in comparison to non-NAFLD patients. Further research to understand the causality and strength of this relationship is needed.

Research perspectives

This review was not able to clarify why the association between NAFLD/MAFLD and COVID-19 was seen. Large size prospective studies with balanced confounding factors are necessary. Since the global burden of NAFLD/MAFLD is rapidly rising, understanding genetics and immunological mechanisms will help advance treatment and prevention strategies.

FOOTNOTES

Author contributions: Jagirdhar GSK conceived and designed the study; Jagirdhar GSK, Qasba RK, Rama K, Banga A, Reddy ST, and Flumignan Bucharles AC included studies and data extraction; Jagirdhar GSK, Qasba RK, Pattnaik H, Rama K, Banga A, Elmati PR, and Bansal V contributed to the data processing; Jagirdhar GSK, Qasba RK, Pattnaik H, Rama K, Banga A, Reddy ST, Flumignan Bucharles AC, Kashyap R, Elmati PR, Bansal V, Bains Y, and DaCosta T wrote the original manuscript; Jagirdhar GSK, Kashyap R, Bains Y, DaCosta T, and Surani S revised the paper and approved the final version.

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ACADEMIC WRITING

How to select a journal for your research

José M Ramia

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited articles; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Abstract

Publication in a peer-reviewed journal is the goal of any research project. One of the most important (and possibly the least understood) aspects of the publication process is the choice of a suitable journal that is likely to accept your work. Detailed information and tips and tricks to success are given in this editorial.

Key Words: Research; Journal; Altmetrics; Publication

Core Tip: Knowing the tips and tricks to choose the best journal for publishing your research is crucial. A narrative check list including all these tricks has been performed.

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INTRODUCTION

Publication in a peer-reviewed journal is the goal of any research project. It is through publication that your research reaches your colleagues in the field, advancing knowledge and encouraging communication between groups[1]. Although peer review can be a time-consuming and often tiring process, the publication of your manuscript validates your work and can help to advance your career, attract students and experienced staff, and obtain funding for future studies[1]. One of the most important (and possibly the least understood) aspects of the publication process is the choice of a suitable journal that is likely to accept your work[2,3]. Submitting your paper to the wrong journal may result in a series of rejections, stalling both your

research and your career[1,2,4].

Realistic assessment of your work is crucial. Both novice and well-known researchers can make the error of submitting their work to an inappropriate journal[1]. First-time authors or those who are branching out into broader areas of research may be unfamiliar with the journals in the field. In these cases, seeking out mentorship could be crucial[5]. For their part, experienced authors may be tempted to publish in the same journals as always, and thus ignore the new publication opportunities that are constantly arising. Even rigorous, high-impact work may be rejected when the topic of the research does not align with the scope of the journal and making this mistake wastes time and money and affects motivation[1] (Table 1).

EVALUATE YOUR RESEARCH

The three key features of your work to consider are its novelty, relevance, and appeal[2]. "Novelty" refers to how new your findings are compared with those previously published in your field. If your findings only offer a small step forward in what is already known, your paper is suitable for a journal with a low-to-medium impact factor (IF). However, if you feel your findings might change the way researchers in your field think about a specific topic, you should aim at a high impact factor journal[2]. "Relevance" refers to the applicability of your findings. Relevance may be geographical[2]: That is to say, if your findings with only a regional focus, choose a regional journal, but if they have worldwide implications, choose an international journal. Or relevance might be disciplinary: If your findings are highly specific for your field, choose a specialized journal, but if they have implications for researchers in other fields, choose a more general or interdisciplinary journal[2,5]. Finally, "appeal" refers to the topical interest of your study: journal editors are keen on manuscripts on current hot topics or with real-world applications[2].

You should also narrow your focus with regard to your expected readership[2,6]. The main questions to answer are the following. Is your study clinical or basic? Are the findings relevant to a broad cross-section of the scientific community, or to researchers in a specialist field? Are the findings preliminary or definitive? And finally, if you collect more data, could you try a journal with a higher IF[2]?

IDENTIFYING THE RIGHT JOURNAL

It is best to choose your journal before you start writing[2,3]. The reasons are that journals have specific criteria related to the manuscript structure, word limits, and reference style; they have specific focuses, or scopes; and editors will often look at your references to see whether you have cited articles published in their journal[2]. So, once you have chosen a journal, identify articles published there that have a bearing on your manuscript and include them among your references.

A tip for how choosing the journal is to perform a search with the keywords (or title) of your manuscript in literature databases such as Medline and PubMed. This search can identify similar or related studies and the journals where they were published[6]. Try to find between three and five manuscripts published in the last five years and decide whether they resemble yours in terms of quality and scope[7]. Identifying previously published papers in your specific subject area is an excellent way to be sure that your research topic is of interest to the readership of a particular journal, and this will obviously increase your chances of review.

There are also a variety of online resources that suggest target journals based on keywords, abstract content and so on: Journal/Author Name Estimator (Jane)®, Manuscript Matcher®, and JournalGuide® (Clarivate), *etc*. But remember that journals that have not previously published in the same area of research might also be interested in your findings.

FACTORS TO CONSIDER IN SELECTING THE BEST JOURNAL

Impact factor

IF is still the default method to assess the quality and reputation of a journal[7]. It displays the total citations made of the journal's articles[7]. IF is the most important factor that researchers consider when they submit a paper, and it is often used as a measure of a researcher's success in his/her field[2,6]. Funding agencies, Institutions, employers, and university committees also consider IF the best method to evaluate a paper[2,6,7]. Journals with higher IF often have a higher profile, and this obviously will increase your article's visibility[4,6].

Nonetheless, the validity of the Journal IF as a metric for journal quality is controversial due to the many factors that can influence the rating achieved, and to the fact that not all these factors are directly related to the quality of the publications within the journal[3]. Although it is tempting to submit a manuscript to the journal with the highest IF factor, it is important to evaluate your research objectively

Table 1 Tips and tricks for selecting best journal

Item	Description
Evaluate your research	Novelty, relevance, and appeal
Identifying the right journal	
Factors to consider in selecting the best journal	Impact factor
	Aims and scope
	Publication types
	Publication mode and rights: Type of publication; supplementary materials/media and relevant links; type of subscription; open access; and copyright
	Publication charges
	Publication frequency
	Time from acceptance to publication
	Rejection rates
	Readership
	Indexing
	Editor's preferences
	Peer-review
	Transfer cascades
	Journal reputation

and decide whether it is truly suitable for a high IF journal. Otherwise, you will risk wasting valuable time and effort as you submit your manuscript time and again to multiple journals[1].

Other scores are: Scimago Journal Rank, Eigenfactor, SNIP, Cited Halflife, Altmetric Attention Score, and Cited Score, *etc*[3,5,8].

Aims and scope

Even remarkable research may be rejected if the topic is not in line with the scope of the journal[2,6,7]. This information is usually readily available on the journal's homepage[3,6,7]. Check to see whether the journal is publishing research like yours[5,7,9]. The topics, the focus and the novelty and potential impact are all important factors[7].

Publication types

Find out which article types (*e.g.*, originals, reviews, systematic reviews, metanalysis, case reports, images, and so on) are accepted and which are not; limits on length (word count) or number of illustrations or references; whether supplementary files are allowed, or the prior inclusion of a preprint (unsubmitted draft) in a preprint server such as arXiv or bioRxiv[1-3,7,9]. Submission to a journal that does not accept the type of article you have written is a sure way of guaranteeing immediate rejection. Please read the guide to authors carefully and follow the instructions closely[1,7,9]. The abstract and cover letter are your way of presenting yourself to the Editor: Take your time over them.

To avoid initial rejection, the registration of research in international databases (ClinicalTrials, Research Registry, PROSPERO, *etc*) is often mandatory. The inclusion of a reporting Checklist, depending on the type of manuscript (*e.g.*, STROBE, PRISMA, and so on) is normally required. You should check this before sending your manuscript.

Publication mode and rights

You should be familiar with the journal's characteristics: Type of publication: print only, print plus online version (PDF), which may be a longer version, with or without early view ("early online", "online first", or "ahead of print") version, or only online version, which is becoming the most usual type[9].

Supplementary materials/media and relevant links: Many journals have links to supplementary materials in an online repository[2].

Type of subscription: Membership-based, pay per view, site license, subscription, or open access.

Open access

Open access means that the entire content of the paper is freely available to all readers, with no need to subscribe to a journal or pay to access the paper[6]. Many researchers prefer this publication model because it can help disseminate their research to a wider audience[2,6]. There is no doubt that increasing the accessibility of your article will ensure that your target audience will have access to your article worldwide, but expenses are high. There are three formats:

Green open access: which means free access to preprint or accepted manuscript (final draft) on a personal website, institutional website, or nonprofit repository such as Pubmed®, with or without a time delay before uploading; in most cases the copyright will be retained by the publisher[6].

Gold open access: free access to the final published version (typically on the publisher's website), and authors retain copyright[6]. Gold open access gives you ownership of your work after publication and ensures that the most accurate, final form of the paper is available to all readers[6].

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Publication charges

Publication charges are the fees that you will be charged to publish in a journal[6]. It is essential to read the section about charges very carefully[2]. If you are on a tight Article budget, you may need to rule out open access journals or journals with high publication charges. There are several types of charge: Submission fee, production fee and charges for color figures (usually black and white images do not incur charges)[2]. Find out whether there are free batches of reprints or online copies, and free online access for authors to published articles. Some editorials waive article processing charges to authors from developing countries for open access publication.

Find out whether the following services are provided free or included in the publication charge: Editing/illustration service; news release service; marketing; social media promotion; post-publication commenting and altmetrics (article-level metrics) tracking.

Publication frequency

Check the journal's table of contents for the number of monthly/weekly articles, articles per issue and issues per year, and how often articles appear in the journal's Online First section[2,7].

Time from acceptance to publication

There are several publication times: From submission to first/final decision, first online publication, final (online) publication. If you need rapid publication, you should specifically look for journals that offer fast response times and short periods from acceptance to publication[2].

The normal time taken for a good manuscript to be published in a reputed journal is around 9 mo to 12 mo. The optimum time limit for rejection is 4-6 wk[7]. The best time is to wait for the special editions from reputed journals and then submit a relevant paper: due to the smaller number of research papers submitted at that time, your research stands a better chance of being published[7].

Rejection rates

It is important to know the selectivity (% acceptance rate) of the journal[2]. The acceptance rate in poor journals is over 90%, while predatory journals publish almost everything, they can lay their hands on. In good journals the acceptance rate is 10%-20%[7,9].

Readership

Identify the interests of the readers[2,3] Be sure that the target audience you are trying to reach is part of the readership of the journal. Your goal is not just to be published, but also to be widely read in your field. Review the "Most Downloaded" or "Most Cited" lists from your potential journals. If you find that your manuscript is similar in scope to articles in this list from one of your potential journals, this suggests that if you publish in this journal your article will be widely read as well.

Indexing

To increase the online visibility of your article, be sure that the journal is indexed in the online databases that your target audience will use to find articles. PubMed® and Scopus® are the main indexing services used by researchers in the biomedical sciences[2,6].

Editor's preferences

It is also very useful to identify the interests of the journal's editor. Just because your manuscript may be similar in scope to the journal, this does not mean that the Editor is currently interested in your topic. Check when similar articles were published in the journal. If similar articles have been published within the last two or three years, this suggests that the Editor is probably interested in your topic. Look at recently published Editorials, Review Articles, and Special Issues, because these usually focus on topics the Editor feels are currently important for the field. If you find that your manuscript is similar in scope to others published by one of your potential journals, this suggests that the Editor may regard your research topic as important.

Peer-review

Articles published in peer-reviewed journals enjoy high esteem among the academic and scientific community[6,7,9]. The quality of the reviewers is also a crucial factor[9]. There are several types of peer-review: The most common is a closed (single- or double-blind) review, but open peer-review is also performed. Other types of review are collaborative (in which reviewers may discuss issues with each other, or reviewers/editors may discuss specific points with the authors) and transparent (in which reviews are published with or without reviewers' names). As for the speed of peer review, you should find out whether fast-track review and pre-submission inquiries are allowed. Journals with very short review times (1-2 wk) are often poor quality (though bear in mind that there are also some top journals with very short review times).

Transfer cascades

One feature of scientific publishing that many researchers are unaware of is the existence of "families" or portfolios of journals inside the same publishing house. Many portfolios with prestigious journals that receive a high volume of submissions also contain less competitive journals that publish papers on the same or similar topics. In many cases, if a publisher with this type of portfolio rejects your paper from a high IF journal, they may offer to transfer it to a lower IF journal inside the same "family". This can help accelerate the overall publication process by avoiding the need to identify a new journal, reformat the paper and prepare a new submission[6]. Bear in mind that some transfer cascades direct you to journals with publication charges.

Journal reputation

Remember that publishing in poor quality journals does not count as scientific contribution to knowledge. Avoid sending work to predatory journals, as this is a waste of time and effort[3,7]. There are many points to check in order to ensure the quality of the journal before sending your manuscript [2]: Are the journal/publisher, editor and editorial board well known? Is the journal affiliated to a professional society? Is the journal recommended by your library/society? Do your colleagues read the journal, or have they been cited there, or have they published there? Is the journal known for ethics, quality content, language, and production? Is the journal included in respected general or specific indexes, with a long history and a permanent online archive[7]? What are the journal's bibliometric scores (IF)? How old is the journal (those aged 10 years or more are often good)?

CONCLUSION

After all the hard work that goes into performing successful research, the final crucial step is choosing the right journal in which to publish. With almost 10000 journals in the Directory of Open Access Journals alone, choosing the best journal can be a daunting prospect even for experienced researchers, and making the wrong decision can cost valuable time, money, and effort. Key factors are being aware of the aims and scope of the journal; identifying papers that are similar in quality and scope; assessing the journal's restrictions; and considering the impact factor and potential reach. Despite the existence of support platforms, the development of artificial intelligence platforms may help us to decide the best journal for our research. Items like specific area, number of patients included, clinical relevance and innovation may well be able to identify the most interesting journal.

FOOTNOTES

Author contributions: Ramia JM wrote the manuscript.

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