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EDITORIAL

Relationship between mast cell, angiogenesis and pancreatic cancer: Our experience

Francesca Vescio, Michele Ammendola, Giuseppe Currò, Silvia Curcio

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Abstract

In this editorial, we focus specifically on the mechanisms by which pancreatic inflammation affects pancreatic cancer. Cancer of the pancreas remains one of the deadliest cancer types. The highest incidence and mortality rates of pancreatic cancer are found in developed countries. Trends of pancreatic cancer incidence and mortality vary considerably worldwide. A better understanding of the etiology and identification of the risk factors is essential for the primary prevention of this disease. Pancreatic tumors are characterized by a complex microenvironment that orchestrates metabolic alterations and supports a milieu of interactions among various cell types within this niche. In this editorial, we highlight the foundational studies that have driven our understanding of these processes. In our experimental center, we have carefully studied the mechanisms of that link pancreatic inflammation and pancreatic cancer. We focused on the role of mast cells (MCs). MCs contain pro-angiogenic factors, including tryptase, that are associated with increased angiogenesis in various tumors. In this editorial, we address the role of MCs in angiogenesis in both pancreatic ductal adenocarcinoma tissue and adjacent normal tissue. The assessment includes the density of c-Kit receptor-positive MCs, the density of tryptase-positive MCs, the area of tryptase-positive MCs, and angiogenesis in terms of microvascularization density.

Key Words: Mast cells; C-Kit receptor; Tryptase; Angiogenesis; Microvascular density; Endothelial area; Pancreatic tumor tissue; Adjacent normal tissue

Core Tip: This editorial focuses on the mechanisms that link pancreatic inflammation to pancreatic cancer. Pancreatic cancer remains one of the most aggressive pathologies. A better understanding of its etiology and the identification of risk factors is essential for primary prevention. Mast cells (MCs) contain pro-angiogenic factors, particularly tryptase, that are associated with increased angiogenesis. We evaluated the role of MCs in angiogenesis in both pancreatic ductal adenocarcinoma tissue and adjacent normal tissue by assessing the density of c-Kit receptor-positive MCs, the density of tryptase-positive MCs, the area of tryptase-positive MCs, and microvascularization density.

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INTRODUCTION

The pancreas is an organ belonging to the digestive system located in the retroperitoneum. Its location makes it difficult to access both in instrumental diagnostics and in the surgical approach. Pancreatic acinar cells secrete digestive enzymes including amylase, which digests carbohydrates; lipase, which breaks down fats; and trypsin and chymotrypsin, which digest proteins. The endocrine component is made up of islet cells that release insulin and glucagon to maintain glycemic balance[1]. The pancreas has a good reserve capacity, and the loss of its functionality is recognized only when the majority of the gland has been destroyed. The exocrine portion of the gland can suffer from three main diseases, acute pancreatitis (AP), chronic pancreatitis (CP), and pancreatic ductal adenocarcinoma (PDAC)[2]. In this editorial we will review the mechanisms linking CP and pancreatic cancer, outlining the possible causes involved in the transformation from benign to malignant pancreatic disease to achieve an early diagnosis.

PDAC, has a poor prognosis due to late diagnosis, early metastases, and resistance to therapy. Although there have been improvements in both diagnosis and treatment in recent years, the outcomes remain poor, with a 5-year overall survival of only 10.8% Surgery remains the only potential cure for resectable PDAC. Pancreatitis is a fibro-inflammatory disorder of the pancreas that involves the activation of digestive enzymes in the pancreas prior to their release into the small intestine, resulting in parenchymal injury, inflammation, and abdominal pain. AP or CP may be related to autoimmunity or hyperlipidemia. A controlled diet and reduction of alcoholic beverages and cigarette smoking are useful in limiting the progression of pancreatitis from acute to chronic[4]. Repeated episodes of AP lead to CP, in which irregular secretion and premature activation of enzymes result in increased damage to the residual pancreas, resulting in severe maldigestion and diabetes.

Histopathological features of CP include chronic inflammation, acinar atrophy, adipose tissue replacement, fibrosis, and abnormal ducts[5,6]. Pancreatitis has been shown to be a risk factor for pancreatic cancer[7-9]. In the pathophysiology of AP and CP, oxidative stress and the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) lead to necrosis and fibrosis of acinar cells. ROS and RNS cause DNA fragmentation, membrane disintegration, and protein misfolding. They also activate the immune system. Immune cells and other stromal components produce inflammatory cytokines and chemokines which, together with ROS and RNS, cause epithelial cell damage and increased proliferation[10]. Cytokines operate in cell signaling and are the primary operators in defining the inflammation state of the tumor microenvironment[11,12]. In a recent study, Lanki *et al*[13] analyzed 231 patients, 186 with stage I-III PDAC and 45 with CP with a serum panel including 48 inflammatory cytokines, carbohydrate antigen 19-9 (CA19-9), and C-reactive protein (CRP) to identify differences the inflammatory cytokines present in the two pathologies. They concluded that the inflammatory cytokines CTACK, GRO-α, and β-NGF together with CA19-9 and CRP may help distinguish PDAC from CP. Other inflammatory mediators, such as cyclooxygenase-2, NF-B, and STAT3, were involved in inflammatory infiltration and damage of acinar cells[14]. Numerous studies have highlighted how inflammatory stimuli in animals carrying an oncogenic Kras mutation activate a positive feedback mechanism that amplifies Ras activity to pathological levels and triggers chronic inflammation and preneoplastic lesions[15]. Ling *et al*[16] demonstrated that the Kras oncogene induces the constitutive activation of signals necessary for the establishment of PDAC. Finally, another study demonstrated that in the presence of a Kras mutant, TNF-α-induced activation of the NF-B pathway maintained transformed cells in a constant inflammatory state[17]. The immune system has great potential for reducing tumors, but its dysregulation can lead to tumor spread and reduced survival of individuals.

THE PRESENCE OF MAST CELLS IS COMMON IN THE INFLAMMATORY ENVIRONMENT OF PANCREATITIS AS WELL AS PANCREATIC CANCER

At our research center, we have studied the mechanisms that link pancreatitis and pancreatic cancer in detail. We have focused on the role of mast cells (MCs), which are bone marrow-derived cells found in many human organs and tissues and contain many pre-existing and newly formed secretory granules with specific pleiotropic functions[18].

The function of MC is especially regulated by their membrane receptor tyrosine kinase, the c-Kit receptor (c-Kit-R), which naturally binds stem cell factor. After activation by various stimuli[19], MCs release their secretory granules into the microenvironment. Recently, various research groups have shown that MCs contain several pro-angiogenic factors and synthesize and secrete a potent pro-angiogenic factor called tryptase. Tryptase is the most abundant factor stored in the secretory granules of MCs it can stimulate microvessel formation. Our studies have used immunohistochemistry and image analysis to determine the concentration of MCs positive for c-Kit-R, the number of MCs positive for tryptase, the area of MC-positive tryptase, microvascular density (MVD), and endothelial area in a series of pancreatic cancer patients undergoing radical surgery. The correlation between the parameters studied and the main clinical and pathological characteristics was also investigated[20-22].

CONCLUSION

Conclusions from these preliminary data suggest that MC granules contain many protease enzymes that, by different mechanisms, induce the formation of new microvessels that supply the tumor load. Numerous studies suggest that MC density growth is associated with MVD growth in several malignancies. A study of survival of patients with resected pancreatic cancer demonstrated that high expression of MVD was closely associated with a worse prognosis[23]. Preliminary *in vivo*/*in vitro* results have been obtained by other researchers. Their data suggest that therapeutic targeting of MC degranulation factors could be a novel strategy to inhibit tumor growth and neo-angiogenesis.

FOOTNOTES

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EDITORIAL

Understanding the molecular crossroads in acute liver failure: A pathway to new therapies

Chun-Yao Cheng, Wen-Rui Hao, Tzu-Hurng Cheng

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Abstract

In this editorial we comment on the article published in a recent issue of the *World Journal of Gastroenterology*. Acute liver failure (ALF) is a critical condition characterized by rapid hepatocellular injury and organ dysfunction, and it often necessitates liver transplant to ensure patient survival. Recent research has eluci-dated the involvement of distinct cell death pathways, namely ferroptosis and pyroptosis, in the pathogenesis of ALF. Ferroptosis is driven by iron-dependent lipid peroxidation, whereas pyroptosis is an inflammatory form of cell death; both pathways contribute to hepatocyte death and exacerbate tissue damage. This comprehensive review explores the interplay between ferroptosis and pyroptosis in ALF, highlighting the role of key regulators such as silent information regulator sirtuin 1. Insights from clinical and preclinical studies provide valuable perspectives on the dysregulation of cell death pathways in ALF and the therapeutic potential of targeting these pathways. Collaboration across multiple disciplines is essential for translating the experimental insights into effective treatments for this life-threatening condition.

Key Words: Silent information regulator sirtuin 1; Ferroptosis; Pyroptosis; P53/glutathione peroxidase 4/gasdermin D; Acute liver failure

Core Tip: Understanding the interplay between ferroptosis and pyroptosis is crucial for delineating the complex pathophysiology of acute liver failure (ALF). Targeting key regulators of these cell death pathways, particularly silent information regulator sirtuin 1, holds promise for the development of novel therapeutic strategies for mitigating hepatocyte injury and improving clinical outcomes in patients with ALF. Multidisciplinary collaborations that integrate basic science, translational research, and clinical trials should be conducted to accelerate the translation of the experimental findings into effective treatments for this life-threatening condition.

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INTRODUCTION

Acute liver failure (ALF) poses a major clinical challenge because of its rapid onset and high mortality rate. Characterized by extensive hepatocellular injury leading to organ dysfunction, ALF has various etiologies, including drug toxicity, viral hepatitis, and metabolic disorders. Despite advancements in medical care, liver transplant remains the primary therapeutic option, which indicates the urgent need to further clarify the underlying pathophysiology of ALF and develop novel treatment strategies. In a study by Zhou *et al*[1] that was published in the *World Journal of Gastroenterology*, the intricate molecular mechanisms underlying ALF progression were elucidated, shedding light on potential novel therapeutic targets. The study focused on the interplay between two distinct modes of cell death, namely ferroptosis and pyroptosis, and on their upstream regulatory pathways, particularly those involving silent information regulator sirtuin 1 (SIRT1)[1].

HALLMARK OF ALF

ALF is a severe manifestation of liver injury, often resulting from factors such as drug toxicity, viral infections, or metabolic disorders. The hallmark of ALF is the rapid and heavy loss of hepatocytes, leading to impaired liver function and systemic complications. For example, Chen *et al*[2] demonstrated the mitigative effects of boswellic acid on acetaminophen-induced hepatic injury, suggesting the potential of natural compounds in ameliorating liver damage[2]. Despite advancements in medical care, the mortality rate associated with ALF remains unacceptably high[3]. As mentioned, the study by Zhou *et al*[1] explored the molecular intricacies of ALF and focused on two pathways of cell death, namely ferroptosis and pyroptosis. Ferroptosis is characterized by iron-dependent lipid peroxidation and mitochondrial dysfunction, whereas pyroptosis is a proinflammatory form of cell death; both pathways have gained attention for their roles in various pathological conditions, including liver diseases[4,5].

SIRT1 IN ALF

Zhou *et al*[1] investigated the involvement of ferroptosis and pyroptosis in ALF by using clinical samples and animal models. They observed the dysregulation of key proteins involved in these pathways — such as GPX4, SLC7A11, p53, and GSDMD — in liver tissues from patients with ALF. Furthermore, in mouse models where ALF was induced by lipopolysaccharide and D-galactosamine, they demonstrated that inhibiting ferroptosis and pyroptosis attenuated liver injury and improved the survival rate. Central to their findings was the role of SIRT1, a protein deacetylase that is involved in multiple cellular processes, including metabolism, stress response, and inflammation. Zhou *et al*[1] demonstrated that SIRT1 activation protected against ALF by inhibiting the p53/GPX4/GSDMD signaling pathway, thereby suppressing both ferroptosis and pyroptosis. Conversely, inhibition of SIRT1 exacerbated liver injury, highlighting the therapeutic potential of SIRT1 in ALF. These findings have significant clinical implications, suggesting that the targeting of ferroptosis and pyroptosis pathways through methods such as SIRT1 modulation might lead to new therapeutic strategies for ALF. However, several questions must be addressed, such as the precise mechanisms linking SIRT1 to ferroptosis and pyroptosis and the potential side effects of pharmacological interventions targeting the related pathway.

CONCLUSION

In summary, the intricate interplay between ferroptosis and pyroptosis underscores the multifaceted nature of cell death pathways in ALF. Targeting the key regulators of these pathways, particularly SIRT1, holds promise for the development of novel therapeutic strategies for mitigating hepatocyte injury and improving clinical outcomes in patients with ALF. Further research is warranted to clarify the mechanistic complexities of cell death pathways and confirm their therapeutic potential in clinical settings. Collaboration across multiple disciplines is essential for translating experimental insights into effective treatments for this life-threatening condition.

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EDITORIAL

From macroautophagy to mitophagy: Unveiling the hidden role of mitophagy in gastrointestinal disorders

Duo-Lun Gao, Meng-Ran Lin, Nan Ge, Jin-Tao Guo, Fan Yang, Si-Yu Sun

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Abstract

In this editorial, we comment on an article titled "Morphological and biochemical characteristics associated with autophagy in gastrointestinal diseases", which was published in a recent issue of the *World Journal of Gastroenterology*. We focused on the statement that "autophagy is closely related to the digestion, secretion, and regeneration of gastrointestinal cells". With advancing research, autophagy, and particularly the pivotal role of the macroautophagy in maintaining cellular equilibrium and stress response in the gastrointestinal system, has garnered extensive study. However, the significance of mitophagy, a unique selective autophagy pathway with ubiquitin-dependent and independent variants, should not be overlooked. In recent decades, mitophagy has been shown to be closely related to the occurrence and development of gastrointestinal diseases, especially inflammatory bowel disease, gastric cancer, and colorectal cancer. The interplay between mitophagy and mitochondrial quality control is crucial for elucidating disease mechanisms, as well as for the development of novel treatment strategies. Exploring the pathogenesis behind gastrointestinal diseases and providing individualized and efficient treatment for patients are subjects we have been exploring. This article reviews the potential mechanism of mitophagy in gastrointestinal diseases with the hope of providing new ideas for diagnosis and treatment.

Key Words: Mitophagy; Gastrointestinal diseases; Parkin; Autophagic receptor; Colorectal cancer; Gastric cancer; Inflammatory bowel disease

Core Tip: Mitochondria are not only the energy factories of eukaryotic cells but are also closely related to apoptosis, and their dysfunction plays an important role in various gastrointestinal diseases. Mitophagy, an important mechanism to remove damaged mitochondria *in vivo*, has been found to alleviate the severity of inflammatory bowel diseases and plays a dual role in promoting and inhibiting the occurrence and development of gastrointestinal cancer. A complete understanding of the mitophagy pathway in gastrointestinal diseases will be helpful for developing new treatment strategies. Therefore, we investigated the mechanisms underlying mitophagy and its contribution to gastrointestinal diseases.

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INTRODUCTION

Cells are continually exposed to various threats, including pathogens[1,2], genetic mutations[3], and oxidative stress[4,5]. These challenges can lead to organelle dysfunction, subsequently inducing autophagy. There are three different types of autophagy in cells: Macroautophagy, micro-autophagy, and chaperone-mediated autophagy. Macroautophagy is usually called autophagy. Autophagy, as the second type of programmed cell death mechanism, helps cells to remove damaged organelles, pathogens or aggregates and then participates in cell growth, development and differentiation[6]. In the last 10 years, the research on the mechanism of autophagy in infection[7], cancer[8], neurodegenerative diseases[9] and other diseases has made breakthrough progress.

Mitophagy is a selective autophagy pathway, an important branch of autophagy, and has a unique mechanism. Mitophagy was named when Lemasters *et al*[10] discovered that damaged mitochondria are engulfed by autophagic vesicles and enveloped in microtubule-associated proteins light chain 3 (LC3) in serum. Mitochondria play an important role in aerobic respiration and adenosine triphosphate (ATP) production *via* oxidative phosphorylation in all eukaryotic cells, and their abnormal functions are closely related to the occurrence and progression of many diseases. Importantly, the mitochondrial quality control system can eliminate damaged mitochondrial proteins or parts of the mitochondrial network and update their components through mitophagy, maintaining a steady state of the mitochondria[11]. In the last 10 years, significant progress has been made in understanding the molecular mechanism and pathophysiological role of mitophagy in human diseases. Several key mitophagy signaling pathways have been identified, including the ubiquitin-dependent pathway mediated by the PINK1-Parkin pathway or other E3 ubiquitin ligases in the mitochondria and the receptor-mediated ubiquitin-independent pathway. With the gradual clarification of the mitophagy pathway, the pathophysiological role of mitophagy in cardiovascular, lung, liver, gastrointestinal, and other organ-related diseases has been explained, providing a new direction for the treatment of diseases[12].

Gastrointestinal diseases include gastrointestinal peristalsis, infectious inflammation (such as *Helicobacter pylori* infection, cholera, and intestinal parasites), noninfectious inflammation (such as chronic gastroenteritis and Crohn's disease), and gastrointestinal cancer[13]. Chang *et al*[14] summarized the morphological and biochemical characteristics of autophagy in gastrointestinal diseases. However, the integration of the unique mechanism of mitophagy and its role in gastrointestinal diseases is still lacking. Recent studies have indicated that mitophagy is closely related to gastrointestinal diseases (Table 1)[15-24]. In this review, we elaborate on the molecular mechanism of mitophagy, summarize its role in the occurrence and progression of inflammatory bowel diseases (IBD), gastric cancer, and colorectal cancer (CRC), and suggest that mitophagy-related pathways may be important targets for clinical treatment.

MITOPHAGY MECHANISMS

According to a known mechanism, mitophagy can be divided into ubiquitin-dependent and-independent mitophagy. Ubiquitin-dependent mitophagy is mainly coordinated by the PINK1 protein kinase and Parkin Ubiquitin E3 ligase[25]. Moreover, research has demonstrated other E3 ubiquitin ligases in the mitochondria, such as Ariadne RBR E3 Ub protein ligase 1 (ARIH1)[26], mitochondrial E3 ubiquitin ligase 1 (MUL1)[27], and Gp78[28], that can mediate ubiquitin-dependent mitophagy without relying on Parkin. Notably, ubiquitin-independent mitophagy is mainly mediated by a direct interaction between LC3 and autophagy receptor proteins (Figure 1).

PINK1-Parkin-mediated ubiquitin-dependent pathway plays an important role in mitophagy

The activation of PINK1 is one of the most upstream events in mitophagy[29]. Under normal physiological conditions, PINK1 is maintained at an extremely low level of PINK1 in the mitochondria and is almost undetectable through a series of input and degradation cycle mechanisms[30]. When affected by pathological factors, such as mitochondrial damage[31] and increased mitochondrial reactive oxygen species[32], it leads to abnormal mitochondrial membrane potential and depolarization. When mitochondria are depolarized, PINK1 is stabilized on the outer membrane of mitochondria (OMM), where it catalyzes the phosphorylation of S65 in the Ub and Ub-like domains of Parkin, thus activating the E3 ubiquitin

Table 1 Research progress of mitophagy in gastrointestinal diseases

Year of publication	Diseases of concern	Problem solved	Ref.
2017	SRMD	SRMD leads to intestinal mucosal injury: Defective mitochondria with excess O_2^- production inhibit mitophagy, ultimately triggering Bax-dependent apoptosis and NF-κB-intervened proinflammatory mucosal injury	[15]
2020	*H. pylori* associated gastritis	There was a link between *H. pylori* infection-promoted mitophagy and inflammation	[16]
2022-2024	Functional dyspepsia	Traditional Chinese medicine can improve gastrointestinal motility disorders, and the mechanism may be related to the inhibition of mitophagy and mitochondria fission	[17-19]
2023	I/R injury	Increased NET formation induces inhibition of mitophagy and lipid peroxidation in IECs, leading to ferroptosis of endothelial cells and microvascular dysfunction	[20]
2023	Malnutrition enteropathy	Dysregulation of SIRT1 and mTORC1 pathways leads to disruption of autophagy, mitochondrial homeostasis, which triggers intestinal barrier dysfunction and nutrient malabsorption	[21]
2023	IBD	Bergapten treatment alleviated NLRP3 inflammasome activation and pyroptosis by promoting mitophagy, suggesting BeG as a potential anti-inflammatory drug for the treatment of inflammatory diseases	[93]
2021-2023	IBD	Polystyrene nanoplastic induced Crohn's ileitis-like features are related to mitophagy, while Biogenic selenium nanoparticles can alleviate intestinal epithelial barrier damage by regulating mitophagy, which provides new insights for further evaluating the safety of nanoparticles	[22-24]
2023	IBD	NSAIDs induce mitochondrial stress and mitophagy in IECs, which are related to the pathophysiology of Crohn's disease	[89]
2021	CRC	Mitophagy suppresses CRC growth: PINK1 inhibits CRC growth by reducing acetyl-CoA production and activating P53	[70]
2023	CRC	Mitophagy promotes CRC growth: GPR176 activates cAMP/PKA signaling pathway and regulate mitophagy to promote the tumorigenesis and progression of CRC	[74]
2018	Gastric cancer	Mitophagy promotes gastric cancer growth: Hippo-Yap promotes tumor progression by activating SIRT1/Mfn2/ mitophagy	[63]
2023	Gastric cancer	Mitophagy suppresses gastric cancer growth: 8-paradol promoted PINK1/Parkin-associated mitophagy, mediating cell apoptosis	[67]

SRMD: Stress-related mucosal disease; *H. pylori*: *Helicobacter pylori*; I/R injury: Ischemia-reperfusion injury; NET: Neutrophil extracellular traps; IECs: Intestinal epithelial cells; SIRT1: Sirtuin 1; mTORC1: Mechanistic target of rapamycin complex 1; IBD: Inflammatory bowel diseases; NLRP3 inflammasome: NOD-like receptor thermal protein domain associated protein 3 inflammasome; CRC: Colorectal cancer; GPR176: G protein-coupled receptors 176; Mfn2: Mitofusin 2.

ligase activity of Parkin[33]. PS65-Ub can further recruit Parkin from the cytoplasm to the OMM, such that the abundance of pS65-Ub gradually increases, eventually leading to the assembly of Ub chains of about 4400 times, establishing a feed-forward loop and finally wrapping the damaged mitochondria with pS65-Ub chains[33]. Additionally, the pS65-Ub chain further recruits autophagy receptors to the damaged OMM[34], and common autophagic receptors that aggregate in the OMM include OPTN[35], NDP52[36], and P62[37]. The activation of the PINK1-Parkin system immediately activates a fraction of TANK-binding kinase 1 (TBK1), which then binds to and phosphorylates the autophagy receptor upon its binding to the Ub chain. This, in turn, enhances the affinity of the autophagy receptor for the Ub chain, extends the duration of autophagy receptors on the OMM, and facilitates mitophagy[38]. These autophagy receptors aggregated on the OMM bind to ATG8 family proteins through the LC3 interaction region (LIR) motif, and with the help of ATG8 family proteins, ubiquitinated OMM attaches to the autophagy membrane[35,37,39]. The autophagy receptors mentioned above are concentrated in the OMM. Notably, a recent study found that the autophagy receptor PHB2, located on the inner membrane of mitochondria, promotes mitophagy mediated by PINK1-Parkin by stabilizing PINK 1 and increasing mitochondrial recruitment of Parkin[40]. PHB2 is ubiquitinated by Parkin, facilitating its interaction with LC3 and accelerating autophagy clearance in damaged mitochondria (Figure 1)[41].

An in-depth study found that many factors, such as TBK1, Phosphatase and Tensin Homolog (PTEN-L), and DJ-1, regulate ubiquitin-dependent mitophagy. In addition to the function of the phosphorylated autophagy receptors mentioned above, TBK1 can promote the downstream steps of mitophagy by phosphorylating S72 in RAB7A through the Ub chain on the OMM[38]. RAB7AS72 is located in the "switch II" domain, which participates in the exchange of guanosine diphosphate/guanosine triphosphate and its interaction with other proteins, thus regulating mitophagy[38]. PTEN-L, a negative regulatory factor of mitophagy located in the OMM, effectively prevents Parkin mitochondrial translocation, reduces Parkin phosphorylation, inhibits its E3 Ligase activity, decreases the level of pSer65-Ub, blocks the feed-forward mechanism of mitophagy, and ultimately inhibits mitophagy[42]. DJ-1 is a 19.9 kda protein encoded by the PARK7 gene. Its deletion does not interfere with the activation of PINK1 or Parkin after mitochondrial depolarization but blocks downstream mitophagy by inhibiting the recruitment of the selective autophagy receptor OPTN to mitochondrial

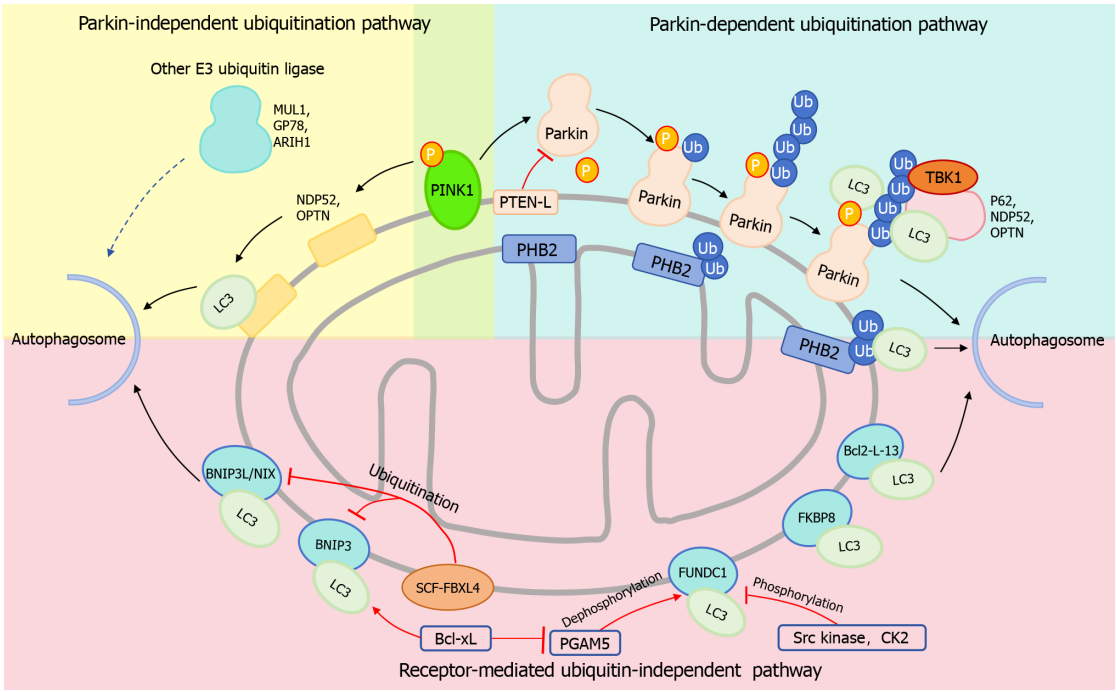

Figure 1 Major Signaling pathways of mitophagy. Parkin-dependent Ubiquitinated Mitophagy Pathway: Following the depolarization of the mitochondrial membrane, PINK1 recruits and activates the E3 ubiquitin ligase activity of Parkin, leading to the formation of ubiquitin chains. These chains then attract a series of autophagy receptors including P62, NDP52, and OPTN. Subsequently, these receptors bind to light chain 3 (LC3), facilitating the connection of the polyubiquitinated mitochondrial outer membrane to the autophagosome membrane, thereby mediating mitophagy. In this process, TANK-binding kinase 1 kinase enhances the affinity of the autophagy receptor for the Ub chain by phosphorylating the receptor. PTEN-L can reduce Parkin phosphorylation and inhibit its E3 ligase activity, thereby inhibiting mitophagy. Parkin-Independent Ubiquitinated Mitophagy Pathway: E3 ubiquitin ligases such as ARIH1, MUL1, and Gp78 may serve as compensatory pathways for Parkin-mediated mitophagy, although the precise mechanisms are yet to be elucidated. Furthermore, PINK1 has the ability to recruit NDP52 and OPTN to mitochondria, thereby directly initiating mitophagy in a Parkin-independent manner. Receptor-Mediated Ubiquitination-Independent Mitophagy Pathway: Proteins including FK506 binding protein 8, BCL2-interacting protein 3 like/NIP3-like protein X (BNIP3L/NIX), BNIP3, FUN14 domain containing 1 (FUNDC1), and Bcl2-L-13 directly bind to LC3, enabling the mitochondrial membrane to connect to the autophagosome membrane and mediate mitophagy. SCF-FBXL4 mediates the ubiquitination and degradation of BNIP3L/NIX and BNIP3, thereby inhibiting mitophagy. Under hypoxia conditions, phosphoglycerate mutase 5 (PGAM5) promotes the dephosphorylation of FUNDC1, enhancing FUNDC1-mediated mitophagy. Conversely, Src kinase and Casein kinase 2 phosphorylate FUNDC1, inhibiting its mitophagy-promoting activity. Although Bcl-xL positively regulates the binding of BNIP3 to LC3, it inhibits FUNDC1-mediated mitophagy by suppressing PGAM5. LC3: Light chain 3; BNIP3L/NIX: BCL2-interacting protein 3 like/NIP3-like protein X; FUNDC1: FUN14 domain containing 1; TBK1: TANK-binding kinase 1.

depolarization[43].

Parkin-independent ubiquitin-dependent mitophagy

ARIH1 and Parkin belong to the same RING-in-between-RING family and are widely expressed in cancer cells. Villa *et al* [26] found that they can ubiquitinate damaged mitochondria in a PINK1-dependent manner, leading to their elimination *via* autophagy. In addition, Yun *et al*[27] found that increasing the protein level of MUL1 in *Drosophila* can counteract the harmful effects caused by the deletion of PINK1 or Parkin, whereas removing MUL1 from PINK1 or Parkin mutants aggravates symptoms, suggesting that MUL1 may participate in the compensatory pathway of the PINK1/Parkin pathway. Furthermore, GP78 is a key E3 ubiquitin ligase involved in endoplasmic reticulum-mediated degradation. In HEK293 cells subjected to Parkin knockdown *via* siRNA, mitophagy triggered by GP78 remained unaffected, indicating that it operates in Parkin-induced mitophagy independently[28]. In addition, the ubiquitin-binding protein Vps13D has been found in *D. melanogaster*. Studies have shown that this protein plays a role downstream of PINK1, which is parallel to Parkin in mitophagy, and regulates the localization of ubiquitin and ATG8 around the mitochondria[44]. However, the specific working mechanisms of these proteins are not clear, and further research is needed. Another study showed that PINK1 could recruit the autophagy receptors NDP52 and OPTN into the mitochondria and directly activate mitophagy without relying on Parkin[45].

Receptor-mediated ubiquitin-independent pathway mediates mitophagy

The non-ubiquitin-dependent mitophagy pathway is mediated by the direct interactions between LC3 and mitophagy receptor proteins. These mitophagy receptor proteins include NIP3-like protein X/BCL2-interacting protein 3 like (NIX/BNIP3L), BCL2-interacting protein 3 (BNIP3), FUN14 domain containing 1 (FUNDC1), Bcl2-like protein 13 (Bcl2-L-13), and FK506 binding protein 8 (FKBP8)[46]. They directly bind to LC3 *via* the LIR region, skip ubiquitination, and directly initiate mitophagy. BNIP3 and BNIP3L/NIX were similar to some extent, indicating that they all contained an atypical BH3 domain. Under the condition of moderate hypoxia (apparent 1%-3% oxygen), hypoxia-inducible factor-1a activates the up-regulation of both transcription factors[47]. Posttranslational modifications regulate mitophagy mediated by

BNIP3L/NIX and BNIP3. SCF-FBXL 4 (SkP1/Cul1/F-box protein ubiquitin ligase complex), located in the OMM, mediates the ubiquitination and degradation of BNIP3L/NIX and BNIP3, thus inhibiting mitophagy[48]. However, phosphorylation of serine residues in BNIP3 LIR and BNIP3L LIR promoted mitophagy[49,50]. In addition, the homodimeric form of BNIP3L recruits autophagosomes more robustly than the monomeric form does[51]. Endogenous FUNDC1 is located only in mitochondria. Under hypoxia, phosphoglycerate mutase 5 (PGAM5) promotes FUNDC1 dephosphorylation and FUNDC1-mediated mitophagy, while Src kinase and Casein kinase 2 phosphorylate FUNDC1 and inhibit FUNDC1-mediated mitophagy[52,53]. Notably, Bcl-xL plays different roles in the regulation of mitophagy mediated by BNIP3 and FUNDC1; moreover, it positively regulates the binding between BNIP3 and LC3[49] but inhibits FUNDC1-mediated mitophagy by inhibiting PGAM5[54]. Moreover, iron deficiency can trigger mitophagy mediated by FUNDC1, which is, in turn, mediated by the activation of PGAM5[55]. Bcl2-L-13, a mammalian functional homolog of ATG32, mediates mitophagy by binding to LC3B through the WXXI motif of LIR[56], and the Unc-51-like Kinase (ULK1) complex is necessary for this process[57]. FKBP8 is a significant anti-apoptotic protein featuring a characteristic LIR motif at its N-terminus, which promotes non-ubiquitination of mitophagy *via* interaction with LC3A. During this process, FKBP8 can exit the mitochondria to evade degradation[46]. In addition to the LIR sequence, FKBP8 contains an LIR motif-like sequence that binds to optical atrophy1 to mediate mitochondrial fragmentation, thus inducing mitophagy[58].

THE ROLE OF MITOPHAGY IN GASTROINTESTINAL DISORDERS

Mitophagy plays an important role in gastric cancer, CRC and IBD. Numerous studies have supported the double-edged sword effect of autophagy in cancer. Specifically, mitophagy plays an inhibitory role in the initial stage of tumorigenesis or cancerous transformation; in contrast, mitophagy provides survival advantages for established and metastatic tumors and can prevent cell death induced by chemotherapy drugs[40,59]. In addition, the anti-inflammatory role of mitophagy in IBD has been extensively studied. The subsequent section will focus on the role of mitophagy in gastric cancer, CRC, and IBD and its potential as a therapeutic target (Tables 2 and 3).

Gastric cancer and mitophagy

Gastric cancer is the fifth most common cancer worldwide, with a high mortality rate[60]. The use of traditional endoscopy and ultrasonic endoscopy (EUS) facilitates the diagnosis of gastric cancer in the early stages and the evaluation of invasion depth, which is beneficial for improving, to some extent, its outcomes[61]. The inclusion of therapeutic EUS in the treatment of complex hepatobiliary, pancreatic, and gastrointestinal diseases has significantly enhanced the quality of life for tumor patients[62]. Defining the survival, migration, treatment, and drug resistance mechanisms of gastric cancer has always been a topic of great concern (Figure 2). In view of the survival and migration of gastric cancer cells, experiments have demonstrated that Sirtuin 1 (SIRT1), a Yes-associated protein (Yap) signal, activates mitophagy and promotes mitochondrial homeostasis[63]. The Yap-STRT1 mitophagy pathway blocks the caspase-9-related apoptosis axis, enhances cell migration based on F-actin, and participates in the migration and survival of gastric cancer cells[63]. Furthermore, an additional study demonstrated that gamma-glutamyltransferase 7 (GGT7) was significantly downregulated in gastric cancer cells, markedly inhibiting their growth, G1-S transition, and migration ability. This inhibition may be associated with the occurrence of GGT7-induced mitophagy[64].

Drug resistance in gastric cancer cells is primarily manifested through tumor necrosis factor α (TNFα) and chemotherapy drugs. As a pro-inflammatory and pro-apoptotic cytokine, TNFα is an important host defense system against the progress of gastric cancer; however, its therapeutic effect is limited by drug resistance[65]. Experiments indicate that TNFα treatment initiates Parkin-dependent mitophagy, and excessive mitophagy prevents mitochondrial apoptosis, mitigating the toxic effect of TNFα on cancer cells[65]. Furthermore, the suppression of mitophagy to enhance the responsiveness of gastric cancer cells to TNFα might present a novel approach to treating gastric cancer. Cisplatin remains the principal medication for managing gastric cancer; however, it demonstrates significant drug resistance, posing a crucial challenge that necessitates immediate attention in clinical settings. Moreover, research has indicated that metformin, an antidiabetic medication, may reduce the sensitivity of cancer cells to cisplatin[66]. Metformin stimulates the phosphorylation of AMPK (Thr172) and increases the expression of mitophagy markers, including Parkin and PINK1, in an AMPK signal-dependent manner, significantly increasing the mitophagy of cancer cells, reducing ATP production, and protecting gastric cancer cells from the therapeutic toxicity of cisplatin[66]. To explore the mechanism behind metformin reducing the sensitivity of gastric cancer cells to cisplatin and provide new possibilities for solving the cisplatin resistance of gastric cancer patients.

Some studies have suggested potential drugs and strategies for treating gastric cancer associated with mitophagy: 8-paradol, a phenolic compound derived from ginger, can induce cell apoptosis by enhancing mitophagy *via* the PINK1-Parkin pathway. Furthermore, the suppression of mitophagy using chloroquine ameliorates mitochondrial dysfunction and apoptosis triggered by 8-paradol. This observation underscores the pivotal role of mitophagy in the anticancer activity elicited by 8-paradol[67]. The Newcastle disease virus, a paramyxovirus, is utilized in cancer treatment. It can induce mitochondrial damage, elevate mitochondrial reactive oxygen species, and disrupt electron transport chain function. Consequently, this leads to the activation of the PINK1-Parkin pathway and the formation of a ubiquitin chain with Mitofusin 2. Furthermore, the molecular receptor p62 recognizes damaged mitochondria, mediates mitophagy, and regulates cancer cells[68].

CRC and mitophagy

According to the 2020 global cancer data, CRC is now the second leading cause of cancer-related mortality globally[60].

Table 2 Pathways regulating mitophagy in gastric cancer, colorectal cancer, and inflammatory bowel diseases

Diseases	Molecules	Effects/mechanisms	Significance	Ref.
Gastric cancer	Yap	Activates the SIRT1/Mfn2/mitophagy axis. Knockdown of Yap impairs the expression of adhesive proteins, reduces F-actin expression, and inhibits lamellipodium formation	Tumor-promoting effects: It contributes to the migration and survival of gastric cancer cells	[63]
	GGT7	Binds with the mitophagy regulator RAB7 to induce mitophagy. GGT7 inhibits ROS production and MAPK cascades	Tumor-suppressing effect: It inhibits the growth, G1-S phase transition and migration of gastric cancer cells	[64]
CRC	piR823	Promotes ubiquitination and proteasome-dependent degradation of PINK1, thereby inhibiting mitophagy	Tumor-promoting effects: It is involved in CRC tumorigenesis	[71]
	MST1	Inhibits mitophagy through the JNK/p53/BNIP3 pathway, leading to oxidative stress and initiating mitochondria-mediated apoptosis	Tumor-suppressing effect: It inhibits tumor proliferation	[73]
	GPR176	Inhibits mitophagy through the cAMP/PKA/BNIP3L axis	Tumor-promoting effects: It promotes the development of CRC	[74]
IBD	NR1D1	Acts as a positive regulator of BNIP3 expression, promoting mitophagy and maintaining the immune homeostasis of IECs	Inhibitory effect on colitis: It reduces the severity and progression of colitis	[88]

CRC: Colorectal cancer; IBD: Inflammatory bowel diseases; Yap: Yes-associatide protein; GGT7: Gamma-glutamyltransferase 7; SIRT1: Sirtuin 1; Mfn2: Mitofusin 2; JNK: c-Jun N-terminal kinase; BNIP3: BCL2-interacting protein 3; BNIP3L: BCL2-interacting protein 3 like; IECs: Intestinal epithelial cells; MST1: Mammalian sterile 20-like kinase 1; GPR176: G protein-coupled receptors 176; PKA: Protein kinase A; NR1D1: Nuclear receptor subfamily 1 group D member 1.

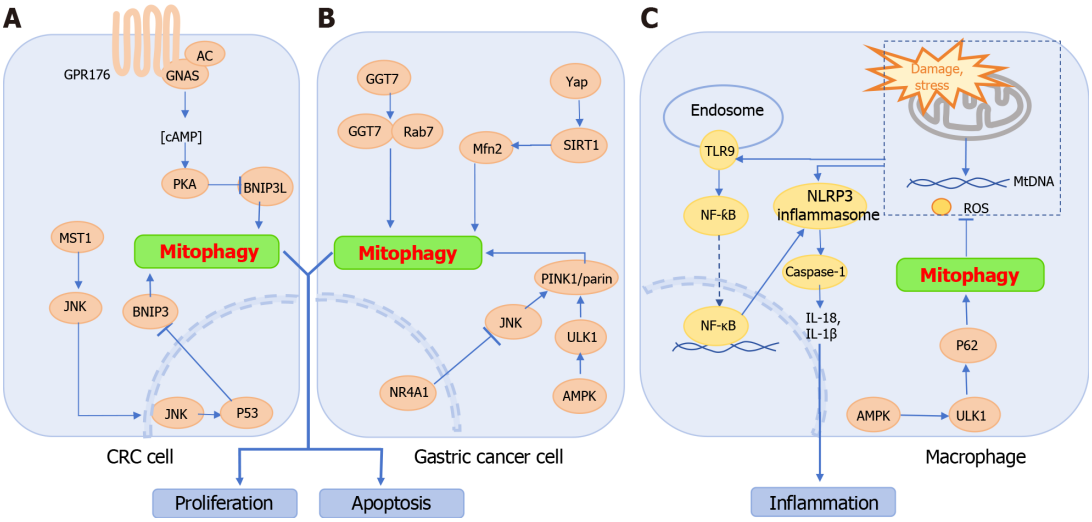

Figure 2 Pathways regulating mitophagy in colorectal cancer, gastric cancer, and inflammatory bowel disease. A: Colorectal cancer cells: GPR176 recruits GNAS to inhibit BCL2-interacting protein 3 like through the AC/cAMP/PKA pathway, thereby suppressing mitophagy. Additionally, MST1 activates the c-Jun N-terminal kinase (JNK) pathway, up-regulating P53 expression, which in turn inhibits BNIP3 transcription and activity, leading to mitophagy arrest; B: Gastric cancer cells: Interactions between GGT7 and Rab7 promote mitophagy. Yap activates sirtuin 1, enhancing Mfn2 expression and sustaining mitophagy. JNK upregulates Parkin to activate mitophagy; however, overexpression of NR4A1 inhibits mitophagy by suppressing JNK. Furthermore, the AMPK/ULK1/Parkin axis also supports mitophagy; C: Macrophages in inflammatory bowel disease: Damaged or stressed mitochondria in macrophages release mtDNA and reactive oxygen species, which directly contribute to NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome formation and activate NF-κB *via* the TLR9 pathway, triggering NLRP3 inflammasome activation and subsequent Caspase-1 activity. This results in the production of IL-1β and IL-18. Enhancing the AMPK-ULK1-P62 axis-driven mitophagy efficiently removes damaged mitochondria, inhibiting NLRP3 inflammasome activation and exerting anti-inflammatory effects. CRC: Colorectal cancer cell; NLRP3: NOD-like receptor thermal protein domain associated protein 3; SIRT1: Sirtuin 1; JNK: c-Jun N-terminal kinase.

While surgical intervention and adjuvant chemotherapy can effectively treat early-stage CRC, a significant proportion (25%-50%) of patients progress to metastasis, resulting in a dismal 5-year survival rate of approximately 14%[69]. Hence, delving deeper into the molecular mechanisms underlying CRC tumorigenesis and progression is imperative to develop new therapeutic strategies. Several studies have underscored the critical role of mitophagy in these processes (Figure 2). Thus, this subsection aims to comprehensively review how the mitophagy pathway contributes to CRC development, drug resistance, and treatment.

Table 3 Drugs affecting mitophagy in gastric cancer, colorectal cancer, and inflammatory bowel diseases

Diseases	Drugs	Effects/mechanisms	Significance	Ref.
Gastric cancer	TNFα	Activates Parkin-dependent mitophagy, and excessive mitophagy blocks mitochondrial apoptosis	Relates to the resistance of gastric cancer cells to TNFα	[65]
	Metformin	Activates AMPK signaling pathway and up-regulates the expression of mitophagy-related proteins PINK1, Parkin, and LC3B	Promotes the resistance of gastric cancer cells to cisplatin	[66]
CRC	Mito-CP, mito-metformin	Induces the release of ULK1, which promotes mitophagy	Tumor-suppressing effect: It inhibits tumor proliferation	[78]
	Aloe gel glucomannan	Activates PINK1/Parkin pathway to promote mitophagy; it activates the transcription factor EB to induce mitochondrial damage and ROS generation	Tumor-suppressing effect: It Inhibits tumor proliferation	[79]
	δ-valbetaine	Activates mitophagy through the PINK1/Parkin pathway	Tumor-suppressing effect: Inducing apoptosis of CRC cells	[80]
	Oxymatrine	Induces mitophagy and reduces NLRP3 inflammasome activation in CRC cells	Tumor-suppressing effect: Inhibit the growth and migration of CRC cells	[81]
	Small molecule andrographolide	Inactivates the NLRP3 inflammasome induced by mitophagy in macrophages	Alleviates colitis progression and reduces the risk of colitis-related cancers	[82]
IBD	Sodium butyrate	Activates Pink1/Parkin expression to promote mitophagy; it inhibits phosphorylation of NF-κB and activation of the NLRP3 inflammasome	Has an inhibitory effect on ulcerative colitis	[92]
	NSAIDs	Induces mitochondrial stress which leads to impaired mitophagy	Proinflammatory effects	[89]
	Bergapten	Promotes mitophagy and maintains mitochondrial homeostasis to inhibit NLRP3 inflammasome activation and pyroptosis	Anti-inflammatory activity	[93]
	Ginsenoside Rd	Activates AMPK/ULK1/p62 signaling pathway to trigger mitophagy, thereby inhibiting NLRP3 inflammasome	Anti-inflammatory activity	[94]

CRC: Colorectal cancer; IBD: Inflammatory bowel disease; LC3B: Microtubule-associated proteins light chain 3B; ULK1: UNC-51-like Kinase 1; ROS: Reactive oxygen species; NLRP3: NOD-like receptor thermal protein domain associated protein 3; NSAIDs: Non-steroidal anti-inflammatory drugs.

The role of PINK1 in inhibiting tumor growth within CRC has been elucidated. Yin *et al*[70] explored mouse colon cancer cells and found that PINK1 overexpression not only promoted mitophagy and decreased glycolysis through the activation of the p53 signaling pathway but also inhibited acetyl-CoA production within tumor cells, thus impeding tumor growth. The non-coding RNA piR823 interacts with PINK1, promoting its ubiquitination and proteasome-dependent degradation, thereby hindering mitophagy[71]. However, evidence suggests that PINK1 promotes survival in CRC. Chen *et al*[72] demonstrated that disruption of the mitophagy pathway due to PINK1 KD leads to a cytosolic iron imbalance, which can be rescued by ferritophagy activation through nuclear receptor coactivator 4 overexpression. These findings suggest that PINK1 regulates intracellular iron availability in conjunction with mitophagy and ferroautophagy, maintaining intracellular iron homeostasis, which is vital for supporting CRC cell survival and growth.

The receptor-mediated mitophagy pathway assumes a dual role in CRC development. Mammalian Ste20-like kinase 1, found to be down-regulated in CRC, inhibits mitophagy *via* the c-Jun N-terminal kinase (JNK)/p53/BNIP3 pathway, thereby inducing oxidative stress and initiating mitochondrial-mediated apoptosis, which contributes to the inhibition of tumor growth[73]. In contrast, the GPR176/GNAS complex inhibits mitophagy *via* the cAMP/PKA/BNIP3L axis, thereby promoting CRC development[74]. In addition to promoting or inhibiting tumor cells, Ziegler *et al*[75] found that elevated levels of mitophagy in intestinal epithelial cells induced adaptive immune responses in CD8+ T cells, providing a therapeutic target for tumor immunity.

Further, mitophagy has been implicated in drug resistance and radioresistance of CRC. Yan *et al*[76] observed that the level of mitophagy and the expression of BNIP3L were significantly increased in cancer stem cells (CSCs) after treatment with doxorubicin (DXR); however, silencing BNIP3L significantly inhibited mitophagy and enhanced the sensitivity of CSCs to DXR, suggesting that mitophagy is involved in DXR resistance in CSCs. Wei *et al*[77] proposed a mechanism by which mitophagy contributes to CRC radioresistance. Notably, excessive activation of mitophagy leads to decreased RING1b expression, which culminates in the deubiquitination of histone H2A at K119, thereby facilitating enhanced repair of radiation-induced DNA damage.

Given the significant role of mitophagy in both tumorigenesis and the progression of CRC, researchers have identified mitophagy as a promising therapeutic target for CRC. Specifically, in KRAS-mutant CRC, Mito-CP and Mito-metformin induce the release of ULK1, which promotes mitophagy and serves an anti-proliferative function[78]. Aloe gel glucomannan was found to induce mitochondrial damage and reactive oxygen species (ROS) generation, thereby inducing cytotoxic mitophagy in colon cancer cells through the PINK1/Parkin pathway and activation of the transcription factor EB[79]. Similarly, δ-valbetaine induces apoptosis in colon cancer cells by activating mitophagy through the PINK1/Parkin pathway[80]. Additionally, traditional Chinese medicine extracts have been shown to play an important role in the treatment of CRC. Oxymatrine treatment induces mitophagy in CRC cells and reduces NOD-like receptor

thermal protein domain associated protein 3 (NLRP3) inflammasome activation, inhibiting the growth and migration of CRC cells *in vitro* and *in vivo*[81]. However, the small molecule andrographolide (Andro) was shown to inactivate the NLRP3 inflammasome induced by mitophagy in macrophages, helping to mitigate colitis progression and tumor burden, thereby reducing the risk of colitis-associated cancer[82].

IBD and mitophagy

IBD is a group of autoimmune diseases characterized by gastrointestinal inflammation, primarily ulcerative colitis (UC) and Crohn's disease. In recent years, the incidence and prevalence of IBD in Asian populations have gradually increased [83]. Several studies have reported that mitochondria are related to the inflammatory response (Figure 2). When mitochondria are damaged, mtDNA and mtROS are released. mtDNA can not only activate NLRP3 inflammasome[84] but also trigger the toll-like receptor 9 pathway to induce an NF-κB and MAPK inflammatory cascade[85]. Moreover, ROS affect the secretion of inflammatory cytokines[86]. Finally, mitophagy removes damaged mitochondria, suggesting that it may be a protective factor for IBD.

NIX is an important receptor protein that mediates mitophagy. Vincent *et al*[87] found that NIX expression was upregulated in the intestinal epithelial cells (IECs) of patients with UC, and compared to wild-type mice, NIX-/- mice exhibited stronger inflammatory characteristics and loss of mucosal integrity when experimental colitis occurred. Moreover, research has demonstrated that the expression level of the circadian clock gene NR1D1 is reduced in patients with UC, and NR1D1 knockout results in a disruption of IECs immune homeostasis and a diminished mitophagy. Subsequent studies have identified that NR1D1 positively influences the expression of the autophagy receptor BNIP3, thereby enhancing mitophagy[88]. The use of non-steroidal anti-inflammatory drugs (NSAIDs) has been identified as a risk factor for IBD. This condition induces mitochondrial stress in IECs, resulting in impaired mitophagy. Such impairment leads to the release of mitochondrial damage-associated molecular patterns with pro-inflammatory potential. These mitochondrial components then act as pro-inflammatory molecules[89].

The maintenance of mitochondrial homeostasis mediated by mitophagy limits the excessive activation of the NLRP3 inflammasome[90], which plays a key role in colitis[91].

Several substances have been demonstrated to harness this process and improve outcomes in IBD. For example, sodium butyrate is effective in suppressing UC because it inhibits the phosphorylation of NF-κB and activates the NLRP3 inflammasome. Moreover, it also enhances mitophagy through the activation of PINK1/Parkin expression[92]. Similarly, Bergapten, a plant-derived hormone with anti-inflammatory properties, has been shown to inhibit NLRP3 inflammasome activation and pyroptosis in a mouse model of intestinal inflammation, further supporting mitochondrial health by facilitating mitophagy[93]. In addition, Ginsenoside Rd initiates mitophagy by activating the AMPK/ULK1/p62 signaling pathway, which in turn inhibits the NLRP3 inflammasome[94]. Furthermore, probiotics have been demonstrated to ameliorate UC. Specifically, *Lacidophilus acidophilus* enhances the levels of short-chain fatty acids, thereby stimulating the mitophagy/NLRP3 inflammasome pathway. This activation helps to maintain inflammatory homeostasis both *in vivo* and *in vitro* and contributes to the improvement of intestinal barrier function[95]. However, mitophagy-related pathway proteins may also play a role in IBD. Parkin, an E3 ubiquitin ligase, has been identified by Ma *et al*[96] as playing a significant role in the context of IBD, with the vitamin D receptor (VDR) acting as a crucial inhibitory regulator. Specifically, Parkin escalates the incidence of colitis and severe inflammation by facilitating VDR degradation *via* the p62-related autophagy-lysosomal pathway.

CONCLUSION

Mitochondria and mitophagy

Along with acting as the energy factories of cells, mitochondria play crucial roles in cell signal transduction, calcium regulation, reactive oxygen species production, cellular protein homeostasis, anti-inflammatory responses, apoptosis, and intercellular mitochondrial transfer[97-100]. The diverse and important functions of mitochondria are the basis for maintaining cell homeostasis. Under the supervision of quality control system, mitochondria will undergo continuous fission and fusion cycles in cells to maintain their shape, network and inheritance[101]. When mitochondrial function is irreparably damaged or under specific stress conditions such as hypoxia or nutritional deprivation, mitophagy is activated. This process selectively promotes the degradation of mitochondria *via* the autophagy-lysosome pathway[102]. Mitophagy, as a mechanism to maintain the quality and quantity of mitochondria, is involved in the pathophysiological processes such as cell growth, cell differentiation, cell aging and apoptosis.

Advances in mitophagy

Autophagy is an energy-intensive process. Excessive regulation of autophagy can result in cellular homeostasis imbalance, leading to unnecessary degradation and damage of organelles[103]. Therefore, it appears to be a more sensible strategy to develop selective autophagy modulators for the treatment of gastrointestinal diseases. Mitophagy regulators developed over the past decade have shown a certain efficacy in gastrointestinal disease models. In the context of gastric cancer and CRC, mitophagy serves dual functions: It not only maintains mitochondrial homeostasis to prevent cancer but also confers survival advantages and enhances drug resistance in cancer cells, influenced by the complex tumor microenvironment. Regulating mitophagy can be an effective strategy to prevent cancer, halt its progression, and enhance treatment efficacy. In IBD, mitochondrial damage can prompt the release of a series of inflammatory factors, which in turn exacerbate intestinal tissue damage. Moreover, mitophagy plays a crucial role in inhibiting the progression of IBD by removing damaged mitochondria. Thus, developing mitophagy inducers may represent a novel therapeutic approach for

IBD.

Problems to be solved

However, the research to date has demonstrated a correlation between mitophagy and several conditions, such as gastric cancer, intestinal cancer, and IBD, thus offering a new and viable direction for treatment. Nevertheless, numerous challenges remain. The signaling mechanisms of mitophagy are intricate and vary across different tissues, developmental stages, and states of stress or metabolism. Therefore, what are the temporal and spatial regulations of mitophagy under various pathophysiological conditions? Numerous experiments have demonstrated the dual role of mitophagy in cancer; however, the critical question remains: What is the threshold between its inhibitory and promotive effects? Currently, many mitophagy inducers are mitochondrial decoupling agents or mitochondrial toxins developed from *in vitro* experiments. Are these agents clinically effective? Is the pharmacological activity of these known regulators solely attributed to mitophagy regulation? Thus, identifying biomarkers and developing detection methods that can reliably and specifically measure mitophagy flux are essential. This will ensure precise regulation of mitophagy and facilitate the practical evaluation of therapeutic effects. Furthermore, the mechanism of mitophagy in additional gastrointestinal diseases, including gastrointestinal peristalsis, infectious inflammation, and chronic inflammation of the gastrointestinal tract, remains largely unexplored and warrants further investigation.

Future application fields

In summary, further analysis of the molecular mechanisms of mitophagy and its role in regulating the onset and progression of gastrointestinal diseases is warranted. Developing small-molecule drugs that target mitophagy for the treatment of gastrointestinal diseases represents a novel approach. With the appearance of an *in vivo* mitophagy imaging system[12] and a mitophagy modulator characterization system[103], it has gradually become possible to study and verify mitophagy modulators in disease animal models and to characterize drugs. Although there are many problems that need to be solved urgently in mitophagy, it is undeniable that targeted mitophagy is a promising treatment for gastrointestinal diseases.

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FOOTNOTES

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EDITORIAL

Gastroesophageal reflux after per-oral endoscopic myotomy: Management literature

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Abstract

In this editorial, we respond to a review article by Nabi *et al*, in which the authors discussed gastroesophageal reflux (GER) following peroral endoscopic myotomy (POEM). POEM is presently the primary therapeutic option for achalasia, which is both safe and effective. A few adverse effects were documented after POEM, including GER. The diagnostic criteria were not clear enough because approximately 60% of patients have a long acid exposure time, while only 10% experience reflux symptoms. Multiple predictors of high disease incidence have been identified, including old age, female sex, obesity, and a baseline lower esophageal sphincter pressure of less than 45 mmHg. Some technical steps during the procedure, such as a lengthy or full-thickness myotomy, may further enhance the risk. Proton pump inhibitors are currently the first line of treatment. Emerging voices are increasingly advocating for the routine combining of POEM with an endoscopic fundoplication method, such as peroral endoscopic fundoplication or transoral incisionless fundoplication. However, more research is necessary to determine the safety and effectiveness of these procedures in the long term for patients who have undergone them.

Key Words: Achalasia; Per-oral endoscopic myotomy; Gastroesophageal reflux disease; Transoral incisionless fundoplication; Peroral endoscopic fundoplication

Core Tip: In this editorial, we discuss the current objective measures for diagnosing gastroesophageal reflux (GER) after peroral endoscopic myotomy (POEM). We also review the factors that contribute to this adverse event, including patient and technique-related characteristics. Furthermore, we provide a list of all published studies on the various treatment options available for post-POEM GER, such as proton pump inhibitors, peroral endoscopic fundoplication, and transoral incisionless fundoplication.

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INTRODUCTION

Achalasia is a disorder of esophageal motility. Its defining characteristics are the lower esophageal sphincter's (LES) ineffective relaxation and the absence of esophageal peristalsis[1]. Peroral endoscopic myotomy (POEM) is considered the gold standard for managing achalasia[2]. After being introduced 16 years ago, we gained a better understanding of the procedure, its long-term sequence, and its outcomes[3]. Despite the high safety profile of POEM procedures done by third-space endoscopy experts, adverse events (AEs) are still reported. One of the notable AEs after POEM is gastroesophageal reflux (GER)[4]. In a meta-analysis, Repici *et al*[5] found that the incidence of GER was significantly higher following POEM than laparoscopic Heller myotomy. In this editorial, we briefly discuss the predictors of post-POEM GER and the different diagnostic and therapeutic strategies.

THE TRUTH ABOUT GERD

The Lyon consensus in 2018 determined that clinical history, questionnaire data, and response to antisecretory medication are inadequate on their own to definitively diagnose GER disease (GERD)[6]. A definitive diagnosis could be made based on the findings of objective tests. Endoscopic findings include grade C and D erosive esophagitis according to Los Angeles classification (LA), a long segment of Barret's esophagus, or strictures. At the same time, an acid exposure time (AET) of > 6% is considered diagnostic along with the subjective methods. The consensus did not include recommendations for diagnosing post-POEM GER, despite multiple studies indicating a high prevalence of GER after POEM, with rates as high as 60% in some instances[1,7,8]. Further clarification is needed regarding the term GER when describing the sequelae in patients who have undergone POEM. Several post-POEM investigations characterize GER as having a DeMeester score over 14.7 or an esophageal pH below 4 for over 5% of the observation period, similar to diagnosing GER unrelated to POEM[1,9]. According to objective testing using 24-h pH monitoring, almost 50% of those individuals have a high AET[10]. Despite the high incidence rate, only 10% of patients are symptomatic[9]. In those patients, a high AET can be attributed to either real GER, characterized by an acute decrease in pH below 3 with sluggish clearance during pH monitoring, or to fermentation of residual food due to long-standing achalasia, resulting in a gradual reduction in pH usually above 3.7[11]. Diagnosis of GER using pH monitoring should be postponed for more than 1 mo following POEM to prevent inaccurate results due to mucosal edema and damage[10].

PRECARIOUS PREDICTORS

Predicting GER after POEM has been challenging due to the lack of a standardized diagnostic approach, making it difficult to rely on previous data. In 2021, a meta-analysis was conducted by Mota *et al*[12] on the published studies in the literature discussing the risk factors for predicting the occurrence of GER after POEM. The study found that full-thickness myotomy, using a posterior myotomy approach, endoscopic findings, pH monitoring, and symptoms were more commonly associated with GER. The authors recommended using circular and anterior myotomy to minimize the risk of post-POEM GER. A study conducted by Wang *et al*[13] investigated the incidence of GERD in individuals who had undergone POEM. The diagnosis of GERD was made based on abnormal acid exposure along with symptoms and/or esophagitis. The study reported that patients who received a full-thickness myotomy had a higher likelihood of developing GERD after POEM (37.5%) in comparison to those who underwent a selective myotomy (12.5%). However, in another retrospective comparison study of 234 patients who underwent POEM, there was no significant difference in the incidence of GER between the full-thickness and circular myotomy groups[14]. Other reported risk factors for post-POEM GER include baseline LES pressure below 45 mmHg, obesity, female sex, and age over 65 years[1,7,15].

IS PREVENTION BETTER THAN CURE?

The risk of post-POEM GER could be minimized during the procedure by some measures, including performing a short esophageal myotomy[10]. In surgical myotomy procedures, myotomies shorter than 1 cm can reduce the occurrence of GER, while myotomies longer than 2 cm have been shown to be more effective in relieving the symptoms of achalasia [16]. A recent meta-analysis found that for patients who underwent POEM, the safety and effectiveness of short esophageal myotomy (ranging from 2.76 cm to 5 cm) was comparable to that of standard esophageal myotomy. Additionally, the incidence rates of GER were similar in both groups; however, patients who received short myotomy treatment had a lower risk of developing erosive esophagitis[17]. One method to determine the least effective length for endoscopic myotomy is the double-scope technique, first introduced for POEM in 2016[18]. However, Grimes *et al*[19] conducted a randomized controlled trial involving 100 patients, divided into two groups: Those who underwent a (2.6 cm) myotomy with a single scope and those who underwent a longer myotomy (3.2 cm) with double scopes. The double-scope group exhibited a greater incidence of moderate esophagitis LA grade B. Another reported measure was preserving gastric sling muscle fibers during the procedure, as Shiwaku *et al*[20] demonstrated that it could be a safe way to reduce the incidence of post-POEM GER with a 90% success rate. The two-vessel penetrating sign was initially proposed in 2018 by Tanaka *et al*[21], it could serve as a useful indicator for identifying the myotomy's endpoint.

Multiple studies have discussed the treatment strategies for post-POEM GER (Table 1). In a consensus, Inoue *et al*[22] reported that proton pump inhibitors (PPIs) are the first line for treating post-POEM GER. The role of PPIs in patients who underwent POEM is a bit controversial since most cases with high AET are asymptomatic[22]. According to studies, the majority of patients who experienced symptoms of GER after POEM were effectively treated with PPIs, and the response was confirmed using objective tests[23,24]. Although numerous algorithms have been suggested for treating post-POEM GERD, Maydeo and Patil[10] presented the most comprehensive algorithm (Figure 1).

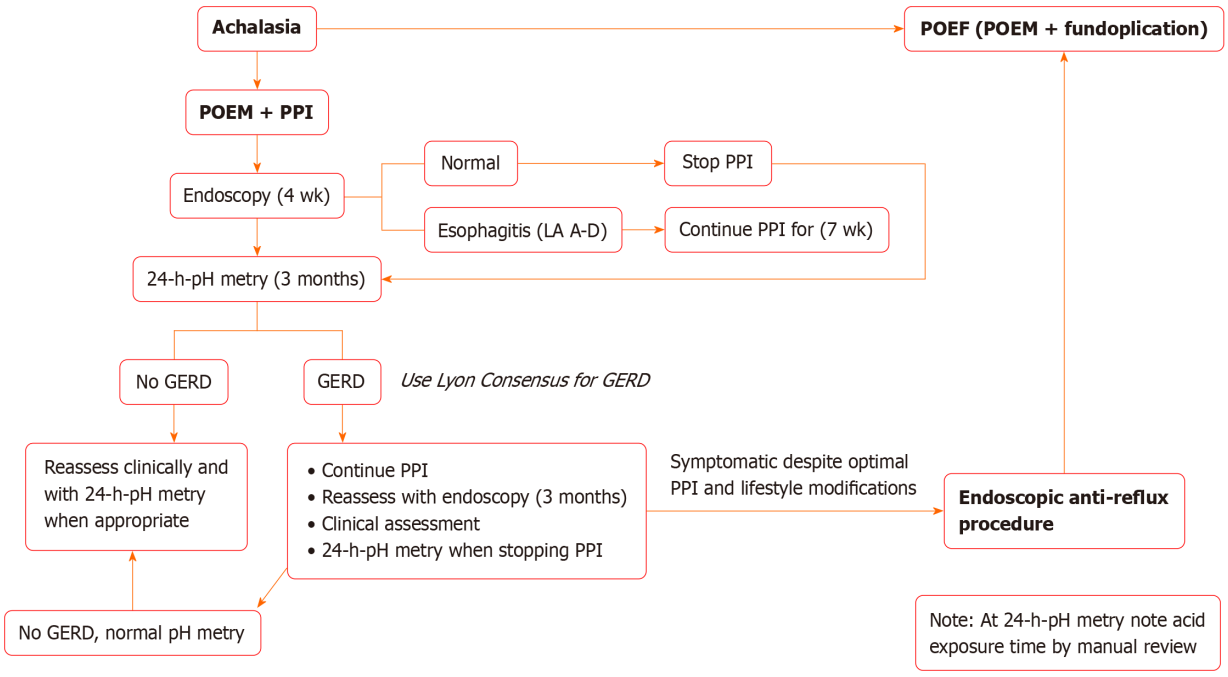

Figure 1 Algorithm of management of post-peroral endoscopic myotomy gastroesophageal reflux. The algorithm originally presented by Maydeo and Patil[10] to manage post-peroral endoscopic myotomy (POEM) gastroesophageal reflux. However, we added the option of initial fundoplication with POEM to the algorithm. EGD: Esophagogastroduodenoscopy; GERD: Gastroesophageal reflux disease; LA: Los Angeles; POEF: Peroral endoscopic fundoplication; PPI: Proton pump inhibitor.

There is a debate surrounding the incorporation of endoscopic fundoplication as a standard procedure alongside POEM. Multiple fundoplication approaches are being examined, either separate from POEM, such as transoral incisionless fundoplication (TIF)[25], or in combination with POEM, such as POEM with fundoplication (POEM + F)[26]. TIF is a therapeutic endoscopic approach used to treat chronic GERD patients[27]. Since its introduction in 2006[28], several studies have confirmed the viability of performing TIF following POEM[25,29-31]. Although most of these studies involved small groups, they demonstrated a safe and effective procedure that led to patients discontinuing the use of PPIs and resolving esophagitis.

The alternative fundoplication option is POEM + F. Inoue *et al*[26] introduced a novel endoscopic fundoplication to reduce post-POEM GER. The authors documented a reduction in the incidence of reflux symptoms with an intact wrap at 1-mofollow-up after the procedure. In a single-center study, 25 patients underwent POEM + F, in which 23 patients (92%) had a technically successful procedure. Follow-up endoscopy showed that 19 patients (82.6%) had an intact wrap, whereas only 3 patients (12%) experienced delayed complications due to endoloop or endoclip erosion of the mucosa,

Table 1 Studies reporting different treatment modalities for post-peroral endoscopic myotomy gastroesophageal reflux

Ref.	*n*	Treatment	Follow-up GER assessment method	Results and conclusion
Inoue *et al*[26], 2019	21	POEM + F	Not assessed	Technical success: 100%. Maintaining wrap at 2 mo: 95%. AE: 0%
Shrigiriwar *et al* [33], 2023	6	POEM + F + PPI	GERD-HRQL; RSI	Technical success: 100%. AE: 0%. GERD-HRQL score: 2.3 ± 3.7. RSI Score: 2.2 ± 2.5
Patil *et al*[35], 2021	20	POEM + F + PPI	24 h pHmetry; endoscopy	Technical success: 85%. Subcutaneous emphysema: 47%. Capnothorax: 17%. At 1 mo follow-up grade B esophagitis: 23.5%. At 3 mo pHmetry: High AET in those with loosening of wrap 100%. At 3 mo pHmetry: Normal AET in those who maintained wrap 100%. Maintaining wrap at 3 mo: 58.8%. Patient off PPI after 3 mo: 58.8%
Toshimori *et al* [36], 2020	1	POEF for refractory GERD with erosive esophagitis after POEM	Endoscopy	Technical success without notable AE with maintaining the wrap at a 10-mo follow-up endoscopy. Improved symptoms. No erosive esophagitis
Maydeo *et al*[37], 2023	30	EFTP	GERDQ; endoscopy. 24 pHmetry	Maintaining flap at 3 mo: 89.6%. AE: 13.8% "mild symptoms". Symptoms resolution and PPI stoppage after 6 mo: 72.4%. Improvement (> 50% from baseline) in AET: 96.6%. GERDQ improvement by > 50% at 6 mo: 55.2%
Bapaye *et al*[32], 2021	25	POEM + F	GERDQ; endoscopy; 24 pHmetry	Technical success: 92%. Maintaining wrap at 12 mo: 82.6%. AE: 12%. Abnormal AET at 2 mo: 11.1%. Erosive esophagitis at 2 mo: 18.2%
Ayoub *et al*[38], 2024	4	TIF + PPI	GERD-HRQL	75% of patients achieved either dose reduction or discontinuation of PPI. Pre-TIF GERD-HRQL: 20 ± 18.5. Post-TIF GERD-HRQL: 3.75 ± 6.2
Hoerter *et al*[39], 2022	1	TIF	Endoscopy	Technical success without notable AE. Absence of esophagitis at a 9-mo follow-up endoscopy
Kumta *et al*[40], 2015	1	TIF	Not assessed	Technical success without notable AE
DeWitt *et al*[41], 2024	17	TIF, cTIF	GERD-HRQL; endoscopy; 24 pHmetry	At 9 mo follow-up: Stopped PPI: 80%. Pre-TIF esophagitis: 88%. Post-TIF esophagitis: 50%. Pre-TIF total time reflux episode: 90.5 ± 46.9. Post-TIF total time reflux episode: 49.3 ± 32.3
Tyberg *et al*[25], 2018	5	PPI + TIF	Endoscopy	Technical success: 100%. Complete resolution of symptoms: 100%
Shiwaku *et al*[15], 2022	1886	PPI	Endoscopy	Complete resolution of symptoms: 100% at 5-yr follow up
Nabi *et al*[42], 2020	167	PPI	Endoscopy	Complete resolution of esophagitis: 81.4%
Brewer Gutierrez *et al*[43], 2020	67	PPI	Endoscopy; pHmetry	At 48 mo follow-up erosive esophagitis: 16%. 47.5 % had AET despite being on PPI

AE: Adverse event; AET: Acid exposure time; cTIF: Combined laparoscopic hernia repair and transoral incisionless fundoplication; EFTP: Endoscopic full-thickness plication GER: Gastroesophageal reflux; GERD: Gastroesophageal reflux disease; GERD-HRQL: Gastroesophageal reflux disease Health-Related Quality of Life; GERDQ: Gastroesophageal reflux disease questionnaire; POEF: Peroral endoscopic fundoplication; POEM: Peroral endoscopic myotomy; PPI: Proton pump inhibitor; RSI: Reflux symptom index; TIF: Transoral incisionless fundoplication.

which resolved spontaneously. Only 2 patients (11%) in this group developed GER after POEM[32]. In the United States, Shrigiriwar *et al*[33] conducted the first United States study with 6 patients and achieved a technical success rate of 100%. However, they did encounter some technical difficulties that need to be addressed in future research. These included the off-label use of endoscopic accessories in POEM + F and the need for surgical anatomy awareness before performing such a procedure.

In the Nabi *et al*[34] review article titled "Prediction, prevention, and management of gastroesophageal reflux after per-oral endoscopic myotomy: An update" and published in the *World Journal of Gastroenterology*, the authors provided a well-organized, comprehensive review of post-POEM GER in terms of risk factors, diagnosis, prevention, and management. They provided an algorithm for the evaluation and management of post-POEM GER. Also, they summarized the conclusions of the published papers with a simple and clear figure of the current understanding of post-POEM GER.

CONCLUSION

In our opinion, the diagnosis of GER after POEM should be determined using both objective and subjective approaches. Questionnaires and other subjective approaches for diagnosing GER can be used in conjunction with objective procedures

or to evaluate the quality of life of individuals suspected of having post-POEM GER. It has been reported that nearly 60% of patients undergoing POEM may experience AET. Therefore, performing pHmetry, especially in symptomatic patients, can prove to be helpful in assessing the need for an endoscopic anti-reflux procedure. However, delaying this testing for at least 1 mo after the POEM procedure is important to avoid inaccurate results due to mucosal edema and damage. Existing data on myotomy techniques indicate certain techniques that decrease the risk of developing GER. However, these data were inconclusive. Therefore, when performing the POEM procedure, the choice of myotomy technique should not be influenced by concerns about the development of post-POEM GER. Instead, the decision should be based on the specific circumstances of the procedure, such as the difficulty level, the complexity of using the double-scope technique, and the experience and preference of the endoscopist. However, a trial should be conducted to minimize the length of the myotomy and lower the risk of prolonged post-POEM erosive esophagitis. The first line of management for patients at risk of developing GER should always be PPIs, which are effective in treating esophagitis in most patients. POEM + F is promising yet in the early stages of development. However, this procedure needs the endoscopist to have a surgical background or be an expert in POEM and third-space endoscopic procedures, with a proficient surgical team available as a backup. Long-term studies are necessary to validate the substantial risk associated with the procedure and the long-term efficacy. In addition, accessories manufacturing companies should collaborate with endoscopists to design necessary accessories to prevent off-label use of items such as endoloop, which may lead to various risks such as tool change delays and losing position during the procedure.

FOOTNOTES

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EDITORIAL

Advancing hepatic recompensation: Baveno VII criteria and therapeutic innovations in liver cirrhosis management

Lorenzo Ridola, Sara Del Cioppo

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Abstract

The Baveno VII criteria redefine the management of decompensated liver cirrhosis, introducing the concept of hepatic recompensation marking a significant departure from the conventional view of irreversible decline. Central to this concept is addressing the underlying cause of cirrhosis through tailored therapies, including antivirals and lifestyle modifications. Studies on alcohol, hepatitis C virus, and hepatitis B virus-related cirrhosis demonstrate the efficacy of these interventions in improving liver function and patient outcomes. Transjugular intrahepatic portosystemic shunt (TIPS) emerges as a promising intervention, effectively resolving complications of portal hypertension and facilitating recompensation. However, optimal timing and patient selection for TIPS remain unresolved. Despite challenges, TIPS offers renewed hope for hepatic recompensation, marking a significant advancement in cirrhosis management. Further research is needed to refine its implementation and maximize its benefits. In conclusion, TIPS stands as a promising avenue for improving hepatic function and patient outcomes in decompensated liver cirrhosis within the framework of the Baveno VII criteria.

Key Words: Hepatic recompensation; Baveno VII; Transjugular intrahepatic portosystemic shunt; Portal hypertension; Cirrhosis; Decompensation

Core Tip: The manuscript explores the concept of hepatic recompensation outlined in the Baveno VII criteria, challenging the traditional view of decompensated liver cirrhosis as irreversible. It emphasizes the importance of addressing the underlying cause of cirrhosis, tailoring therapy accordingly, and achieving specific criteria for recompensation. Studies on alcohol, hepatitis C virus and hepatitis B virus-related cirrhosis demonstrate how targeted interventions, including antiviral therapy and Transjugular intrahepatic portosystemic shunt (TIPS) procedures, promote hepatic compensation. While promising, optimal timing and therapy selection for TIPS remain unresolved. Nevertheless, TIPS emerges as a promising avenue for hepatic recompensation, offering renewed hope for patients previously deemed untreatable.

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INTRODUCTION

The concept of hepatic recompensation, as outlined by the Baveno VII criteria, represents a significant advancement in the management of decompensated liver cirrhosis. Traditionally, decompensation has been viewed as a point of no return in the progression of liver disease. However, the Baveno VII guidelines propose a new paradigm by delineating specific criteria for hepatic recompensation, offering renewed hope for patients previously deemed ineligible for therapeutic intervention.

HEPATIC RECOMPENSATION

At the core of the Baveno VII concept of hepatic recompensation is the idea of addressing the underlying cause of cirrhosis. Whether through removal, suppression, or cure, targeting the primary etiology is essential to initiate the process of recompensation. Therapeutic modalities vary and are tailored to the specific etiology, including antiviral therapy for viral hepatitis, lifestyle modifications for alcohol-related liver disease or metabolic-associated fatty liver disease, or other targeted interventions as appropriate.

According to Baveno VII, liver recompensation is defined as the removal of the etiological factor of liver cirrhosis, resolution of ascites, hepatic encephalopathy, and absence of recurrent variceal hemorrhage for at least 12 months, along with stable improvement in liver function[1].

ALCOHOLIC ETIOLOGY

Regarding the alcoholic etiology, several studies have been conducted, such as that of Hofer *et al*[2], a retrospective study on 204 patients with alcohol-related liver cirrhosis, which analyzed the effect of alcohol abstinence on hepatic compensation. This research, based on the concept of hepatic compensation according to Baveno VII and through the measurement of hepatic venous pressure gradient, revealed that 18.1% of patients achieved compensation with a mean follow-up of 24 months. However, this compensation does not alter the risk of developing hepatocellular carcinoma, making adherence to screening programs still necessary. The study by Pose *et al*[3], a multicenter retrospective study conducted on 1001 liver transplant-listed patients for alcohol-related liver cirrhosis, hepatitis C virus (HCV)-related, and non-alcoholic steatohepatitis-related cirrhosis, indicated that 8.7% were removed from the list due to improvement after a median follow-up of 29 months. The difference in results between the two aforementioned studies depends on both the different reference patient cohorts, which in the case of the Pose *et al*[3] study is more selected, and on the diversity of the criteria chosen to define hepatic compensation, which only in the Hofer *et al*[2] study faithfully follows the Baveno VII criteria.

HCV-RELATED CIRRHOSIS

Regarding HCV-related cirrhosis, the study by El-Sherif *et al*[4] is a retrospective analysis of four clinical trials on the effects of direct-acting antiviral (DAA) therapy in patients with decompensated HCV-related liver cirrhosis. The primary outcome was improvement in liver function, defined as transition to Child-Pugh class A. Overall, 31.6% of Child-Pugh B patients and 12.3% of Child-Pugh C patients who achieved sustained virological response (VR) after DAA therapy reverted to Child-Pugh class A. The study by Gentile *et al*[5] is a multicenter prospective study involving 89 patients with Child-Pugh class B liver cirrhosis who received DAA therapy. Sustained VR (SVR) was achieved in 95.5% of patients; 61.8% of patients transitioned to Child-Pugh class A, 33.7% remained with Child-Pugh class B, and 4.5% worsened to

Child-Pugh class C. This study demonstrated that DAA therapy is safe and has a high rate of SVR and hepatic compensation. However, further research is needed to fully understand the long-term effects and impact on patient survival.

HEPATITIS B VIRUS-RELATED LIVER CIRRHOSIS

Regarding hepatitis B virus (HBV)-related liver cirrhosis, in cases of previous decompensation, therapy is based on nucleoside/nucleotide analogs that reduce circulating levels of HBV-DNA to undetectable levels in 80% of patients within a year[6-8]. The study by Jang *et al*[9] is a prospective, multicenter study involving 707 patients who experienced their first episode of decompensation. The primary endpoint of the study was 5-year liver transplantation-free survival, while the secondary endpoint was VR, serological response, and improvement in liver function. Patients treated with antivirals showed significantly better survival without transplantation compared to untreated patients. The study by Yao *et al*[10] recruited 23 patients with decompensated liver cirrhosis Child-Pugh B > 10 and treated them with Lamivudine. 60.9% of treated patients had a significant response, defined as a reduction in the Child-Pugh score of at least 3 points. Wang *et al*[11] were the first to validate the Baveno VII criteria for liver compensation in patients with HBV-related liver cirrhosis treated with entecavir. In this multicenter study, 320 patients were enrolled, treated with Entecavir for 120 weeks, and followed up for 6 months. 92.2% of the patients achieved a virological response with HBV DNA levels < 20 IU/mL, and 56.2% of the patients achieved liver compensation according to the Baveno VII criteria. These studies demonstrate how antiviral therapy significantly modifies the natural history of decompensated cirrhosis, improving liver function and increasing survival.

TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT

All the studies mentioned so far indicate the importance of treating the underlying cause of cirrhosis to achieve liver compensation according to the criteria established by Baveno VII. In a specific subgroup of patients, an interventional radiology procedure can be added to improve the clinical outcome, which is transjugular intrahepatic portosystemic shunt (TIPS).

Traditionally, TIPS has been primarily indicated for specific complications of portal hypertension, such as acute variceal bleeding or refractory ascites. The rationale behind the use of TIPS lies in its ability to ameliorate the hemodynamic derangements characteristic of portal hypertension. By creating a shunt between the portal and systemic circulations, TIPS reduces portal pressure, thereby alleviating complications associated with portal hypertension (such as variceal bleeding and ascites), resolution of which is a prerequisite for hepatic recompensation according to the Baveno VII criteria. As a consequence, it can be suggested that TIPS may play a broader role in promoting hepatic recompensation according to the criteria outlined in Baveno VII.

The retrospective evaluation by Gao *et al*[12] highlighted the potential role of TIPS in achieving hepatic recompensation in a subset of patients with bleeding varices or refractory ascites, with approximately one-third of these patients meeting the criteria for recompensation after TIPS placement, underscoring its therapeutic efficacy in selected cases. In this retrospective study, 64 patients were enrolled, considering inclusion criteria and exclusion criteria. TIPS placement was successful in 100% of cases, and no variceal bleeding occurred. The follow-up lasted for 12 months with patient reassessment at various intervals post-procedure. Several studies support the use of TIPS as a first-line treatment.

Regarding acute variceal bleeding, Liu *et al*[13] conducted a retrospective study enrolling 50 patients with liver cirrhosis and portal pressure gradient values > 25 mmHg. They divided the patients into two groups: One (35 out of 50 patients) treated with TIPS as first-line therapy in secondary prophylaxis of esophageal variceal bleeding, and the other (15 out of 50 patients) treated with TIPS as second-line therapy. The aim was to assess differences in survival and portal hypertension-related complications between the groups. During follow-up, a statistically significant difference in survival was observed between the first line and second-line groups (94.3% *vs* 66.7%, log–rank $P = 0.01$), demonstrating the effectiveness of using TIPS as first-line therapy in improving survival.

Nicoară-Farcău *et al*[14] conducted a meta-analysis of seven studies, including three randomized controlled trials and observational studies, comprising 1327 high-risk patients with liver cirrhosis and acute variceal bleeding (Child-Pugh B with active variceal bleeding or Child-Pugh C < 14). They compared the effects of preemptive TIPS, placed within 72 h of hospital admission for acute variceal bleeding, to endoscopy plus medical therapy with non-cardioselective beta-blockers. This meta-analysis clearly demonstrated beneficial effects in terms of survival, improved control of bleeding and ascites, and a lower rate of re-bleeding in this patient population.

Regarding the treatment of refractory ascites, Bureau *et al*[15] conducted a prospective study on 62 patients with liver cirrhosis complicated by decompensated ascites, requiring at least two large-volume paracenteses within a three-week period. Patients were randomized into two groups: One treated with TIPS placement (29 patients) and the other treated with large-volume paracentesis plus albumin (33 patients). All patients followed a low-salt diet and were evaluated at 1 month, 3 months, and after 1 year through blood tests and assessment of Child-Pugh and MELD scores. Additionally, Doppler ultrasound was performed at 6 and 12 months. The primary endpoint of the study was one-year transplant-free survival. The study demonstrated that the TIPS-treated group met the primary endpoint in 93% of cases, compared to 52% in the large-volume paracentesis plus albumin group. The latter experienced portal hypertension-related bleeding in 18% of cases (18% *vs* 0%; $P = 0.01$) and hernia-related complications in 18% of cases (18% *vs* 0%; $P = 0.01$). Hospitalization duration was higher in the large-volume paracentesis plus albumin group compared to the TIPS group (35 d *vs* 17 d) ($P = 0.04$). In both groups, 65% of patients did not manifest hepatic encephalopathy in the first year. This study thus demon-

strated the superiority of TIPS over large-volume paracentesis plus albumin in this patient population in terms of transplant-free survival and complications.

TIPS, however, is a technically complex procedure, with potentially fatal complications. These complications can be divided into intraprocedural, early, and late. Among the intraprocedural complications are those at the puncture site, during catheterization of the inferior vena cava or hepatic vein, during the puncture of the portal vein, hepatic artery injury, portal vein and/or mesenteric vein dissection. Early complications include hepatic encephalopathy, heart failure, bleeding, bile duct injury, STENT occlusion or migration. Late complications include TIPS dysfunction with persistence of signs of portal hypertension and endotipsitis. To reduce the rate of these complications and improve post-intervention survival, it is necessary to collaborate with a team of experts and perform adequate patient selection prior to the procedure. In this regard, the most commonly used score to predict outcome is the MELD score; for example, a MELD > 18 at time zero is associated with an unfavorable outcome. Therefore, especially with elective procedures, a careful evaluation of the patient is necessary, taking into consideration various factors that correlate with the outcome as patient age, baseline liver function using Child-Pugh score, renal function, patient nutritional status, and cognitive status.

CONCLUSION

In conclusion, TIPS represents a promising therapeutic option for achieving hepatic recompensation in patients with decompensated liver cirrhosis, as it can address both the hemodynamic consequences of portal hypertension and the underlying etiology of cirrhosis, thereby improving hepatic function in this patient population. However, the concept of hepatic recompensation is still evolving, and several issues remain unresolved, such as the optimal timing for TIPS placement and therapy selection. Therefore, further studies are needed to maximize efficacy while minimizing risks.

FOOTNOTES

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EDITORIAL

Early colorectal cancer screening–no time to lose

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Abstract

In this editorial, we comment on the article entitled "Stage at diagnosis of colorectal cancer through diagnostic route: Who should be screened?" by Agatsuma *et al*. Colorectal cancer (CRC) is emerging as an important health issue as its incidence continues to rise globally, adversely affecting the quality of life. Although the public has become more aware of CRC prevention, most patients lack screening awareness. Some poor lifestyle practices can lead to CRC and symptoms can appear in the early stages of CRC. However, due to the lack of awareness of the disease, most of the CRC patients are diagnosed already at an advanced stage and have a poor prognosis.

Key Words: Colorectal cancer; The immunochemical fecal occult blood test; Diagnostic route; Cancer screening; Stage at diagnosis

Core Tip: Briefly summarises the factors that contribute to colorectal cancer (CRC) and the early symptoms. The use of the immunochemical fecal occult blood test in different countries is explored as well as the importance of screening at an early stage. Highlighting the critical role of early screening in CRC treatment, this editorial carefully blends social context and scientific insights to reveal the dynamic landscape of this evolving field.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, and its occurrence and progression are largely attributed to genetic instability of key mutated genes[1]. Although CRC patients undergo surgery and chemotherapy, the side effects and recurrence rate remain high. Patients with advanced CRC usually have poor outcomes. Therefore, in order to improve prognosis, early treatment is necessary, which will increase patient survival[2-5]. In order to disseminate knowledge about CRC screening, public health institutions are also trying to promote the importance of CRC screening. Studies have found that approximately 30%-50% of the eligible population do not receive early screening for CRC. In addition, more than 70% of CRC cases are detected through non-screening routes[6,7].

Early symptoms of CRC are not obvious, thus it is often found only in the late stage in the clinic. In addition, CRC is also a challenging disease, and its pathogenesis is unclear. However, compared to other cancers, CRC is an easily preventable cancer. Due to no obvious symptoms in the early stages of CRC development, it is essential to develop early detection techniques.

However, there are shortcomings in traditional screening methods, and many people who are eligible for screening have not been screened[8]. This paper describes CRC diagnosed earlier in hospitals, and the methods we recommended for early CRC screening.

EARLY-ONSET CRC RISK FACTORS AND SYMPTOMS

CRC is associated with a number of risk factors. In Western countries, there is evidence that these risk factors are associated with lifestyles such as smoking, overweight, excessive alcohol consumption, consumption of red meat and processed meat, low calcium and dietary fiber intake and inadequate consumption of fruit and vegetables[9,10]. Recent work from the Nurses' Health Study found prolonged sedentary television viewing, a surrogate for an inactive lifestyle, was associated with an increased risk of early-onset CRC, particularly rectal cancer[11].

According to surveys and statistics, rectal bleeding is the most prevalent danger sign in patients with early CRC[12]. In addition, pain, changes in bowel habits such as constipation, diarrhea, unexplained weight loss, and anemia are also observed[13-16]. Previous studies have found that patients with early CRC, compared to patients with advanced CRC and control populations, present with abdominal pain, rectal pain, changes in bowel habits, rectal bleeding and weight loss. Many of these symptoms occur in the early stage of CRC. Similarly, studies have found that patients with early-onset CRC present with abdominal pain, rectal pain, changes in bowel habits, rectal bleeding, and weight loss suggestive of an increased risk of early-onset CRC when compared to patients with late-onset CRC and control populations[17].

Although early CRC can result in symptoms, the current state of treatment is not encouraging. It has been shown that patients with early stage CRC take an average of six months from onset to diagnosis[15,18-20]. There are several reasons why it takes so long to confirm the diagnosis. Firstly, patients do not realize that they are going to develop cancer, and there is very little awareness of basic care and healthcare protection. Secondly, some patients mistake the common conditions mentioned earlier for hemorrhoids, for example when bleeding from the rectum[21,22]. Although the patient is unaware, it has been established that, at the time of diagnosis, these symptoms usually predict that colon cancer is already at an advanced stage and has a poor prognosis. Therefore, the adoption and implementation of standardized diagnostic tests is extremely important for the early diagnosis of CRC.

EARLY SCREENING OF CRC AND THE IMMUNOCHEMICAL FECAL OCCULT BLOOD TEST

CRC is a highly malignant tumor, its clinical manifestations and prognosis are closely related. Early screening and early detection can greatly improve the prognosis. As the incidence of CRC increases year by year, the United States government is also actively encouraging individuals to participate in CRC screening, but unfortunately, more than 70% of eligible people are not screened[23].

The incidence of CRC has increased significantly according to annual CRC registries, and there are a growing number of high-risk and screening groups. Early screening for CRC should not be delayed. In addition, there has been a report summarising current CRC screening guidelines and highlighting future blood-based and imaging-based screening programmes[24]. To date, although some progress has been made in CRC screening, there is still a considerable number of people who are not effectively screened for CRC, and there is a lack of effective CRC screening methods and follow-up instructions[8]. Effective and necessary recommendations for CRC screening or follow-up are also needed for patients who are not adhering to treatment. CRC screening is a labor-intensive, material-intensive process, and tailoring information to each patient is costly[23,25-28]. However, screening and treatment are essential in the early stages of the disease, and the cost is greatly reduced compared to the cost of late treatment.

A recent issue of the *World Journal of Gastroenterology* published an interesting paper entitled "Stage at diagnosis of colorectal cancer through diagnostic route: Who should be screened?"[8]. This study focused on the fact that screening reduced CRC deaths in Japan. CRC treatments rely heavily on the efficacy of early screening as well as the detection of CRC at an earlier and more appropriate time plus timely removal of the lesion. Japan also provides new technical support for the early screening of CRC and precancerous lesions, as well as a theoretical basis for the early diagnosis and treatment of CRC[29]. The research data for this article were from two local hospitals. Both hospitals are among the top hospitals in Japan for treating CRC cases and are designated as Cancer Care Hospitals. They treat many CRC cases, including many CRC patients in cancer care homes. Patients registered at both institutions were enrolled in the study. The conclusion drawn on the basis of these two hospitals is therefore informative.

This article used cancer registries from two Japanese facilities to clarify the stage at diagnosis in three groups: Cancer screening, follow-up (patients detected during follow-up for other comorbidities), and symptomatic patients with some concomitant symptoms of bowel disease[8]. Symptomatic patients who show some unusual clinical manifestations are seen in hospital and are more inclined to undergo imaging and colonoscopy. It also confirms previous research that patients with some chronic diseases can be treated earlier due to their medical history[30,31].

In addition, this article highlights the significance of the local policy and medical environment in Japan for early CRC screening[8]. Japan's well-developed healthcare and universal insurance systems enable residents to make unlimited use of health resources[32] and to make screening a priority. Therefore, when a patient arrives at the hospital, having a quick test performed improves their chances of being diagnosed quickly. In addition, the advanced local healthcare infrastructure allows the use of advanced diagnostic techniques such as colonoscopy and computed tomography (CT) to help detect CRC patients earlier.

Last but not least, the immunochemical fecal occult blood test (iFOBT) is mentioned in the article[8]. The FOBT is a fairly popular method for CRC screening, especially for patients who are reluctant to undergo invasive tests such as CT colonography and colonoscopy. The FOBT consists of two tests: Chemo (gFOBT) and iFOBT. These methods are widely used in the United States and provide patients with an excellent option for early screening of CRC[33]. The use of iFOBT reduces the false-positive rate of CRC compared to the use of gFOBT (piaq-based fecal occult blood test) for CRC screening[34]. The iFOBT is also widely used in Australia. Studies have also found that 2-yearly iFOBT screening is not only beneficial to health but also cost-effective[35]. In Japan, as a result of the use of iFOBT, CRC incidence has been reduced by 10%[36] and CRC mortality by 62%[37]. An analysis of test characteristics showed that the iFOBT had a combined sensitivity of 79% for the detection of CRC[38]. The predictive value of iFOBT is used for the development of colorectal progressive adenomas in the clinical setting. In China, CRC is more likely to occur in people with advanced age, those who smoke, have diabetes mellitus, and who previously underwent a cholecystectomy; thus, attention should be paid to these groups, and use of the iFOBT has diagnostic significance for the development of adenoma in progressive stages[39]. The iFOBT is recommended for annual CRC screening because of its superior detection properties and its convenience compared with colonoscopy[40].

CONCLUSION

With the progress of research, the incidence and mortality of CRC are decreasing year by year[41]. However, its pathogenic mechanism is still unclear. The study in Japan underscores how early screening can effectively reduce the average risk of developing the disease partly due to early diagnosis and timely treatment[42]. The article also pointed out the necessity of implementing the iFOBT strategy. The health sector advocates active participation in patient health education and offers screening advice tailored to individual patient needs. This reduces pain and related complications in patients with advanced cancer. We hope that early screening, early detection and effective intervention will reduce mortality from CRC.

FOOTNOTES

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REVIEW

Role of gut-liver axis and glucagon-like peptide-1 receptor agonists in the treatment of metabolic dysfunction-associated fatty liver disease

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Abstract

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a hepatic manifestation of the metabolic syndrome. It is one of the most common liver diseases worldwide and shows increasing prevalence rates in most countries. MAFLD is a progressive disease with the most severe cases presenting as advanced fibrosis or cirrhosis with an increased risk of hepatocellular carcinoma. Gut microbiota play a significant role in the pathogenesis and progression of MAFLD by disrupting the gut-liver axis. The mechanisms involved in maintaining gut-liver axis homeostasis are complex. One critical aspect involves preserving an appropriate intestinal barrier permeability and levels of intestinal lumen metabolites to ensure gut-liver axis functionality. An increase in intestinal barrier permeability induces metabolic endotoxemia that leads to steatohepatitis. Moreover, alterations in the absorption of various metabolites can affect liver metabolism and induce liver steatosis and fibrosis. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are a class of drugs developed for the treatment of type 2 diabetes mellitus. They are also commonly used to combat obesity and have been proven to be effective in reversing hepatic steatosis. The mechanisms reported to be involved in this effect include an improved regulation of glycemia, reduced lipid synthesis, β-oxidation of free fatty acids, and induction of autophagy in hepatic cells. Recently, multiple peptide receptor agonists have been introduced and are expected to increase the effectiveness of the treatment. A modulation of gut microbiota has also been observed with the use of these drugs that may contribute to the amelioration of MAFLD. This review presents the current understanding of the role of the gut-liver axis in the development of MAFLD and use of members of the GLP-1 RA family as pleiotropic agents in the treatment of MAFLD.

Key Words: Metabolic dysfunction-associated fatty liver disease; Metabolic dysfunction-associated steatohepatitis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; Metabolic syndrome; Obesity; Gastrointestinal microbiota; Glucagon-like peptide-1; Glucagon-like peptide-2; Bariatric surgery

Core Tip: Dysbiosis of gut microbiota leading to gut-liver axis disruption is a significant contributor to the development of metabolic dysfunction-associated fatty liver disease (MAFLD). However, the precise role of enteric bacteria in the pathogenesis of MAFLD remains unclear. Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) may modulate MAFLD progression mechanisms to alleviate dysfunction, partly by modifying gut microbiota. The association between MAFLD pathogenesis and gut microbiota dysbiosis needs to be understood, as well as how GLP-1 RAs can regulate these impaired mechanisms and improve patient outcomes.

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INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a complex metabolic disorder with hepatic manifestation that was formerly known as non-alcoholic fatty liver disease (NAFLD). In this review, we will retain the former name NAFLD for older literature data due to the gradually changing nomenclature for hepatic steatosis coexisting with the metabolic syndrome. MAFLD affects approximately 25%-30% of adult patients, making it the most commonly observed liver disease in the world[1]. There has been an increase in the prevalence of this condition in rapidly growing countries [2]. The elevated prevalence of this disease is due to a sedentary lifestyle, reduced physical activity, and an unhealthy diet with a significantly higher calorie intake compared to energy expenditure[3]. MAFLD pathogenesis is closely related to mechanisms regulating occurrence of the metabolic syndrome and obesity[4]. MAFLD features an accumulation of excess lipids in the liver, causing lipotoxicity that may progress to metabolic-associated steatohepatitis (MASH). The mechanisms involved in MAFLD development and progression to MASH interact at different levels to create a complex network of processes whose role in pathogenesis has been termed the 'multiple hit' hypothesis[5]. It relates the development of MAFLD to multiple coincident factors including genetical susceptibility, abnormal gastrointestinal hormone and adipokine secretion, insulin resistance, nutritional factors, gut dysbiosis, and inflammation[6-9]. In patients with MAFLD, insulin resistance leads to hyperglycemia, elevating reactive oxygen species (ROS) synthesis in hepatocytes that affects mitochondria and promotes hepatocyte apoptosis. It was observed that patients with type 2 diabetes mellitus (T2DM) and NAFLD show faster fibrosis progression than non-diabetic patients. Metabolic dysfunction associated with cardiovascular disease, impaired renal function, or the presence of T2DM predisposes to the development of MAFLD [10]. Up to 55.5% of people with T2DM develop NAFLD and 37.3% develop non-alcoholic steatohepatitis (NASH)[11]. Moreover, the development of hepatocellular carcinoma (HCC) is much more common in those who develop NASH[12]. Lifestyle changes, such as an appropriate low-calorie diet and increased physical activity, can effectively reverse the early stages of MAFLD. If an advanced stage of the disease is detected, bariatric surgery may be necessary as it is the most effective way to treat MAFLD[13]. Additionally, clinical and laboratory manifestations of T2DM, especially cardiovascular outcomes, resolve or improve with weight loss[14,15].

Microbiota is a complex ecological community of microorganisms, including bacteria, archaea, fungi, and viruses which live in human organism as commensals, that through their collective metabolic activities and host interactions, influence both normal physiology and disease susceptibilities[16]. The gut is the primary location of human microbiota, as well as the microbiome that refers to the collection of genomes of the resident microorganisms[17]. Healthy gut microbiomes, as assessed by 16S rRNA operon sequencing, comprise over 50 phyla, are consistently dominated by bacterial phyla, and predominantly by the bacterial genera Bacteroidetes and Firmicutes[18] that belong to the bacterial phyla *Bacteroidota* and *Bacillota*, respectively, as determined by ribosomal multilocus sequence typing[19]. Gut microbiota are represented by over 1000 species of microorganisms (defined also as species-level phylotypes *i.e.* clusters of sequences with about as much diversity in their small subunit rRNA genes as in validly named species) that are estimated at over 1×10^{14} bacterial cells per mL of faeces. Many studies have shown bidirectional crosstalk among the gut microbiota and the liver along the so-called 'gut-liver' axis. This special communication controls gastrointestinal health and disease and exploits environmental and host mediators. The reciprocal interaction between the gut and liver is facilitated by the portal vein that transports gut-derived products directly to the liver. In turn, the liver secretes bile and antibodies that travel to the intestine. The intestinal barrier is a crucial anatomical and functional structure that facilitates interactions between the gut and the liver. It limits the systemic dissemination of microbes and toxins while allowing nutrients to access the circulation and reach the liver. Maintaining homeostasis of the gut-liver axis is dependent on controlling microbial

communities. The liver plays a key role in shaping these communities through bidirectional communication[20]. Significant alterations of gut microbiota and gut-liver axis disturbances play an important role in the pathomechanism of MAFLD in humans[21-25]. Changes occurring in gut microbiota of patients with MAFLD are presented in Table 1.

The emergence of novel therapeutic approaches based on or directed at mechanisms related to gastrointestinal hormones makes them an interesting topic for the analysis of interactions with other mechanisms of NAFLD pathogenesis. In adults with MAFLD, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) show beneficial effects such as reduced liver fat content, reduction of body weight, and improved metabolic parameters in laboratory tests[26]. During treatment with GLP-1 RAs, the dysbiosis associated with MAFLD is reduced, improving the function of associated mechanisms[27]. This review summarizes the factors and mechanisms involved in the pathogenesis of MAFLD in association with related gut-microbiota-liver axis disorders. It also evaluates the role of GLP-1 RAs in regulating these changes and improving patient outcomes.

GUT-LIVER AXIS AND RELATED PATHOPHYSIOLOGY

Intestinal barrier permeability

The intestinal barrier permits the selective transportation of nutrients from the intestinal lumen while restricting the transportation of pathogens and toxic metabolites. When the intestinal barrier becomes more permeable, bacterial endotoxins and digestion products can pass through non-selectively into the portal vein lumen, ultimately reaching the liver[28]. The permeability of the intestinal barrier depends on several factors, including the protective mucosal layer, antimicrobial peptides, tight junction proteins, and immune cells[29]. The mechanisms involved in the disruption of this barrier are shown in Figure 1. In patients with NAFLD, a change in the structure of gut microbiota, together with an increase in intestinal permeability, increases hepatic exposure to bacterial products from the gut, leading to the induction of metabolic endotoxemia and changes in the functionality of the 'gut-liver axis'[30].

The role of the mucus layer is to protect intestinal cells from external agents and to facilitate nutrient absorption. It consists of heavily glycosylated proteins released by the gut goblet cells[31]. The mucus layer not only represents an important bacterial niche but also displays antimicrobial properties derived from the antimicrobial peptides secreted by Paneth cells, such as defensins and IgA. The thickness and composition of the mucus layer influence the properties of this bacterial niche, while the bacteria can also impact the properties of the mucus layer[32]. *Mucispirillum* sp. bacteria present in MAFLD patients cause disruptions of the intestinal mucosal surface[33]. Intestinal dysbiosis inhibits mucin 2 gene expression, reducing the production of the main intestinal mucin and primary component of the mucus layer[32]. As the symptoms of liver damage associated with MAFLD worsen, an increase in the level of bacterial proteins in blood can be observed that is caused by increased permeability of the intestinal barrier[34].

Tight junction proteins, normally seal the junction between intestinal endothelial cells and have a vital role in preventing translocation of harmful substances from the gut into the portal system. MAFLD syndrome is characterized by a decrease in the levels of these proteins. Dysbiosis leads to an increase in endotoxin levels in the gut lumen. Lipopolysaccharide (LPS), an endotoxin of gram-negative bacteria, plays a key role in MAFLD progression through activation of toll-like receptor 4 (TLR4) in the small intestine and liver[35]. In the intestine, TLR4 forms an aggregated receptor complex with the membrane-bound protein CD14 and myeloid differentiation protein 2, as well as other adaptor proteins. CD14 plays a specific role in this complex as it is responsible for the binding of the LPS/LPS binding protein complex[36]. In the study by Nighot *et al*[37], it was shown that stimulation of the TLR4 receptor complex in the gut by LPS leads to activation of the TLR4/myeloid differentiation primary response 88 signaling pathway that in turn activates myosin light chain kinase, resulting in enhanced permeability of intestinal tight junction proteins without altering the levels of these proteins. However, other studies have shown that at different concentrations of LPS, increased intestinal barrier permeability is associated with a redistribution and change in the number of tight junctions[38-40]. In addition, the endocannabinoid system, specifically cannabinoid receptors (CB) in the gut, link gut microbiota to gut barrier integrity and metabolic endotoxemia. Administration of CB1 agonists leads to an increase in serum concentrations of LPS and a decrease in mRNA expression of tight junction proteins (ZO-1, occludin), whereas treatment with antagonists has the opposite effect[41]. The presence of *Oscillibacter* and *Desulfovibrio* bacteria in the gut has a similar effect to CB1 agonist receptors. This finding may serve as an alternative contributor to the advancement of MAFLD[42].

Immune cells of the intestinal barrier are located in the lamina propria and Peyer's patches. These cells provide protection against harmful substances but also induce tolerance to harmless substances and commensal bacteria[43]. In people with MAFLD, the number of T lymphocytes in the lamina propria is reduced and levels of pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin 6, and interferon gamma are increased compared to healthy people. Interestingly, levels of pro-inflammatory bacteria such as *Escherichia coli* and *Streptococcus* sp. are increased in NAFLD[23]. These factors are associated with impaired immune function and the subsequent increase in intestinal permeability further mediates the pathogenesis of NAFLD *via* the gut-liver axis.

The above examples suggest that multiple factors influence gut barrier integrity in MAFLD, and that gut microbiota are critical in this process, highlighting the importance of maintaining gut homeostasis.

Changes in luminal metabolism

The pathogenesis of MAFLD is mediated by numerous metabolites, such as bile acids (BAs), choline, trimethylated N-oxide (TMAO), short-chain fatty acids (SCFAs), branched-chain amino acids (BCAAs), ammonia and endogenous ethanol [44]. The mechanisms associated with metabolite disruption in MAFLD progression are presented in Figure 2.

Table 1 Changes in the gut microbiome in patients with metabolic dysfunction-associated fatty liver disease

Increase	Decrease
Genus/species (phylum)	**Genus/species (phylum)**
Anaerobacter (Firmicutes)	*Akkermansia muciniphila (Verrucomicrobiota)*
Clostridium (Firmicutes)	*Alistipes (Bacteroidetes)*
Desulfovibrio (Thermodesulfobacteriota)	*Bifidobacterium (Actinomycetota)*
Enterobacter (Proteobacteria)	*Faecalibacterium prausnitzii (Firmicutes)*
Escherichia (Proteobacteria)	*Flavonifractor (Firmicutes)*
Fusobacterium (Fusobacteriota)	*Lactobacillus (Firmicutes)*
Mucispirillum (Deferribacterota)	*Odoribacter (Bacteroidetes)*
Peptoniphilus (Firmicutes)	*Oscillibacter (Firmicutes)*
Ruminococcus (Firmicutes)	*Prevotella (Bacteroidetes)*
Streptococcus (Firmicutes)	*Rikenellaceae (Bacteroidetes)*

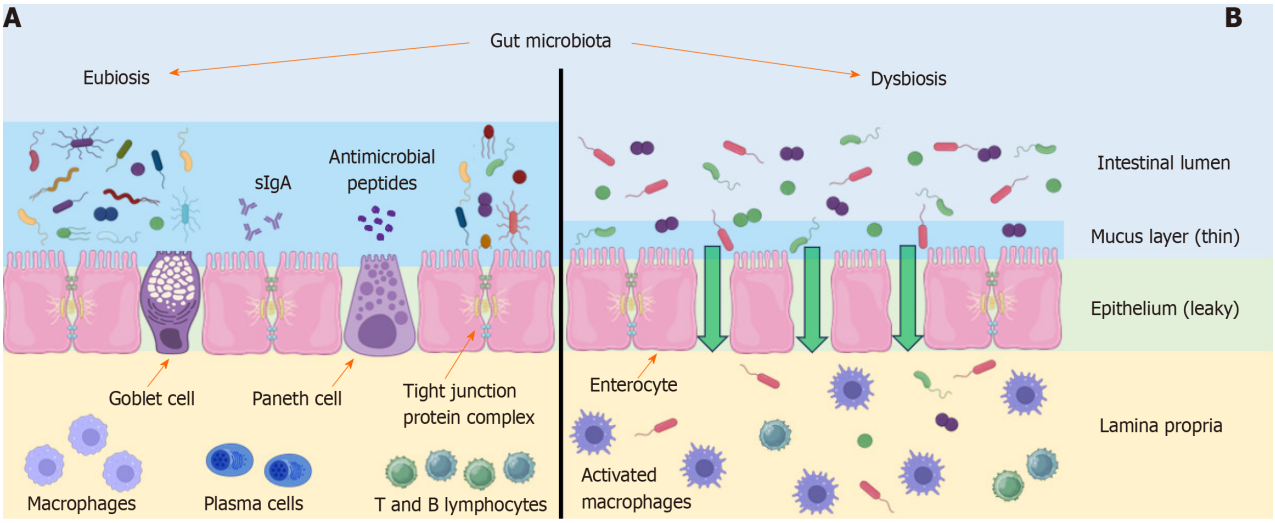

Figure 1 Intestinal barrier in healthy and metabolic dysfunction-associated fatty liver disease syndrome people. Comparison of the intestinal barrier and mechanisms involved in its disruption. A: Healthy people; B: Metabolic dysfunction-associated fatty liver disease (MAFLD) syndrome people. The composition of the gut microbiota plays a critical role in maintaining the integrity of the intestinal barrier through several mechanisms: regulation of mucus layer thickness produced by goblet cells, production of antimicrobial peptides by Paneth cells, levels of tight junction proteins responsible for epithelial cell integrity, and the activation of immune cells such as macrophages, plasma cells, and T and B lymphocytes. These mechanisms are located in different compartments of the intestinal barrier. In individuals with MAFLD syndrome, dysbiosis results in a reduction in mucous layer thickness, antimicrobial peptides production, and the levels of tight junction proteins. There is also an alteration in the number of immune cells in the lamina propria. Macrophage activation leads to the production of pro-inflammatory cytokines. These factors contribute to increased intestinal permeability and disturbance of the gut-liver axis homeostasis. The figure was created using Servier Medical Art application offered by Servier and licensed under a Creative Commons Attribution 3.0 Unported License.

BAs: BAs are synthesized in the liver through complex cholesterol metabolism. They are divided into primary BAs, such as cholic acid (CA) and chenodeoxy CA (CDCA), and secondary BAs, such as deoxy CA (DCA) and litho CA (LCA). Primary BAs are conjugated with either glycine or taurine and subsequently stored in the gallbladder until they are released into the intestine after a meal. Once in the intestine, BAs facilitate the absorption of fats, cholesterol, and fat-soluble vitamins. Primary BAs undergo deconjugation, dehydroxylation, oxidation, and desulfation by gut microbiota, leading to the formation of more hydrophobic secondary BAs. The secondary BAs are reabsorbed in the distal ileum and transported back to the liver *via* the portal vein[45]. The deconjugation process involves bacteria representing the following phyla: *Firmicutes*, *Proteobacteria,* and *Bacteroidetes*, and the species representing the following genera: *Bacillus*, *Staphylococcus*, *Bacteroides*, *Lactobacillus*, *Clostridium*, and *Enterococcus*. They produce bile salt hydrolases that deconjugate the taurine and glycine groups in the primary BAs produced in the liver[46]. *Firmicutes* bacteria, particularly strains belonging to the genus *Clostridium*, play an important role in the dehydroxylation process. They produce BA 7α-dehydroxylase that converts primary BAs (CA and CDCA) into secondary BAs (DCA, LCA)[47,48]. The main bacteria involved in BA oxidation are from the genera: *Bacteroides*, *Clostridium*, *Eubacterium*, *Escherichia*, *Eggerthella*, *Peptostreptococcus*, and *Ruminococcus*. They produce BA hydroxysteroid dehydrogenase that converts toxic BAs into urodeoxycholic

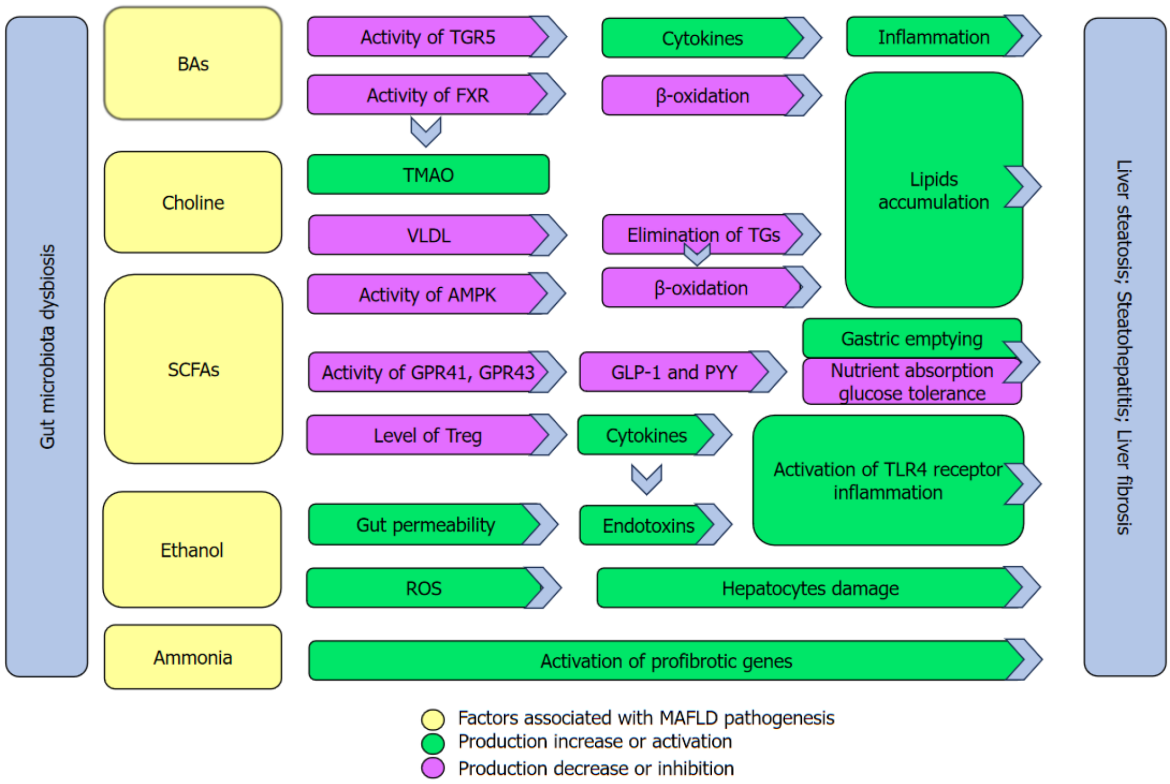

Figure 2 Key mechanisms associated with metabolite disruption in metabolic dysfunction-associated fatty liver disease progression. Gut microbiota dysbiosis in patients with metabolic dysfunction-associated fatty liver disease causes alterations of important intestinal lumen metabolites. Changes in bile acid composition lead to a decrease in the activity of Takeda G protein-coupled receptor 5 (TGR5) and Farnesoid X receptor (FXR) in the liver. TGR5 is responsible for suppressing pro-inflammatory cytokines and its reduction promotes inflammatory processes in the liver. This contributes markedly to liver fibrosis. Decreased FXR activity leads to a decline in β-oxidation processes that promotes the accumulation of lipids in the liver. Trimethylamino-N-oxide (TMAO) is a product of choline metabolism that is modified by gut microbiota and liver enzymes and is involved in the reduction of FXR activity. Increased TMAO levels reduce the bioavailability of choline in the liver that is involved in the synthesises of very-low-density lipoprotein (VLDL). Lowered VLDL levels reduce the elimination of triglycerides that promotes liver steatosis. A reduction in short-chain fatty acid levels is responsible for: (1) A decrease in the activity of liver AMP-activated protein kinase that causes a drop in the capacity of β-oxidation processes; (2) a decrease in activity of G-protein receptors (GPR41, GPR43) in intestinal enteroendocrine L cells that leads to reduced production of glucagon-like peptide-1 and peptide YY , leading to a disruption in energy homeostasis; and (3) decreased levels of T-regulatory lymphocytes that play an important role in inhibiting pro-inflammatory cytokines. Ethanol may provoke damage to the intestinal barrier that is associated with increased endotoxins levels in the portal system, ultimately reaching the liver. Activation of toll-like receptor 4 and the inflammasome by endotoxins leads to an increase in the production of pro-inflammatory cytokines that causes hepatic inflammation. Furthermore, ethanol increases the production of reactive oxygen species that results in oxidative damage to hepatocytes. Ammonia is involved in hepatic fibrosis by activating the profibrotic genes in hepatic stellate cells. BA: Bile acid; TGR5: Takeda G protein-coupled receptor 5; FXR: Farnesoid X receptor; TMAO: Trimethylamino-N-oxide; VLDL: Very-low-density lipoprotein; SCFA: Short-chain fatty acid; ROS: Reactive oxygen species; TG: Triglyceride; PYY: Peptide YY; GLP-1: Glucagon-like peptide-1; TLR4: Toll-like receptor 4.

acid that is less toxic to human cells and more water-soluble[49]. Finally, several intestinal bacteria, such as *Clostridium* sp. *strain S2*, produce sulfatases that can increase the desulfation of BAs. Desulfation of BAs by intestinal bacteria helps with the reabsorption of BAs and is crucial for maintaining homeostasis in the BAs pool[48,50]. Disruption of BA metabolism occurs during the development of NAFLD, leading to a characteristic pattern of BAs in patients with NASH. Patients with MAFLD, and particularly with MASH, exhibit increased hepatic primary and secondary BAs production, resulting in higher levels of total BAs in the blood. The results of a recent meta-analysis show that different geographic locations or disease severity influence the diversity in BA profiles. In particular, the elevated levels of taurocholic acid (TCA), taurodeoxycholic acid, taurolithocholic acid, and glycolithocholic acids were observed in patients with MASH[51]. The increase in the concentration of glycocholic acid and TCA in serum was observed to be a relevant factor of severe liver fibrosis (> F2)[52]. As signaling molecules that regulate glucose, lipids, and the immune system, BAs contribute to host cell metabolism mainly through the farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5)[53]. CDCA is the most potent FXR agonist of all of the major BAs, followed by CA > LCA > DCA[54]. The most potent BA ligands for TGR5 are ranked as follows: LCA > DCA > CDCA > CA[55]. Activation of the FXR leads to improved glucose uptake by adipocytes through the induction of fibroblast growth factor 19. However, FXR also prevents the expression of sterol regulatory element-binding protein 1c and stimulates peroxisome proliferator-activated receptor alpha that inhibits lipid accumulation in the liver and increases the efficiency of β-oxidation[56,57]. Activation of TGR5 increases glucagon-like peptide-1 (GLP-1) secretion, leading to increased glucose-dependent insulin secretion and reduced appetite, by inhibition of neuropeptide Y (NPY) and agouti-related neuropeptide neurons in the arcuate area[58,59]. Furthermore, BA-induced activation of the TGR5 receptor on Kupffer cells has been shown to inhibit LPS-induced cytokine expression, suggesting its potential role in the immune response[60]. Both receptors play a role in reducing BA secretion through a

negative feedback loop by inhibiting the expression of CYP7A1[61]. Patients with NAFLD have a reduced expression of FXR and increased levels of triglycerides (TGs) in serum[62]. In addition, the level of DCA, is elevated compared to CDCA[63]. The observed alterations in BA levels and compositions may result in a reduction in the capacity of the FXR and TGR5 receptors that in turn may contribute to the development of insulin resistance and an increase in lipid accumulation[52].

Choline: Choline is a crucial nutrient serving as the primary donor of methyl groups. It is obtained from diet, although choline synthesis can occur *de novo* in the liver[64]. In the liver, choline plays an important role in the synthesis of very low-density lipoprotein that facilitates the elimination of excess TGs. Therefore, dietary choline deficiency is an important factor in the development of MAFLD[65]. Choline is partly metabolized by intestinal microbiota in the gut, forming trimethylamine (TMA), which then travels to the liver through the portal circulation. In the liver, TMA is oxidized, leading to the formation of TMAO. The bacteria primarily engaged in choline metabolism in the gut belong to the phylum *Proteobacteria* (specifically the species *Proteus penneri* and *Providencia rettgeri*) and *Firmicutes* (*Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, and *Clostridium sporogenes*)[66]. A choline-poor diet and the presence of intestinal dysbiosis leads to increased TMAO accumulation in the liver that may result in the development of MAFLD due to decreased choline bioavailability[67]. Furthermore, TMAO may aggravate liver steatosis by suppressing BA-mediated hepatic FXR signaling[68]. TMAO levels show a robust correlation with hypertension and cardiovascular disease, suggesting its potential use as a cardiovascular biomarker in individuals with MAFLD[69].

SCFAs: SCFAs consist of molecules with 1 to 8 carbon (C) atoms and are produced primarily by the fermentation of dietary fiber in the gut. Acetate (C2), propionate (C3), and butyrate (C4) constitute the largest proportion of SCFAs in the human body[70]. *Bacteroides* spp., *Anaerostipes* spp., and other gut bacteria are primarily responsible for the production of SCFAs[71]. People with MAFLD have reduced levels of total SCFAs, particularly acetate, propionate, and butyrate, compared to healthy people[72]. SCFAs, especially butyrate, are involved in the nourishment of intestinal epithelial cells, and a decrease in SCFA levels is associated with an increase in intestinal barrier permeability. In the liver, propionate (C3) acts as a substrate for gluconeogenesis and inhibits cholesterol synthesis. Meanwhile, acetate is used as a substrate for the synthesis of long-chain fatty acids, glutamine, glutamate, and beta-hydroxybutyric acid. Butyrate is oxidized directly by hepatocytes, thereby preventing potentially harmful systemic concentrations[70]. Three G-protein receptors (GPRs) are known to interact with SCFAs: GPR41, GPR43, and GPR109A. Butyrate interacts predominantly with GPR41 and GPR109A receptors, whereas acetate and propionate have an affinity for GPR43[73]. It is noteworthy that GPR41 and GPR43 are expressed in human white adipose tissue, skeletal muscle, and liver, suggesting that SCFAs may have a direct effect on substrate metabolism in peripheral tissues[74]. Interaction with these receptors determines several physiological functions, such as the generation of ROS, promotion of neutrophil chemotaxis, and regulation of T-regulatory cells. Experimental studies have demonstrated the role of butyrate in regulating the transcription of forkhead box protein 3 factor that acts as a mediator in Treg cell development and activity whilst also suppressing the release of pro-inflammatory cytokines[75]. Acetate and butyrate may also inhibit LPS-induced TNF-α release and nuclear factor-kappa B (NF-κB) activation, leading to reduced inflammation in the liver[76] A reduction in SCFAs levels is the cause of disruption of these processes that leads to liver steatohepatitis and defective gut integrity[77]. SCFAs inhibit the growth of microorganisms by lowering the pH in the intestinal lumen. It has been observed that acetate generated by *Bifidobacterium* spp. can restrain the growth of enteropathogenic bacteria[78]. Moreover, both *in vitro* and *in vivo* studies have indicated that elevated concentrations of butyrate are linked to an augmented production of mucin and a decrease in bacterial attachment to the intestinal epithelium. These results suggest that butyrate can enhance epithelial integrity[79]. SCFAs may stimulate the production of hormones responsible for creating satiety and modulating appetite regulation, thereby preventing the development of obesity. A possible mechanism supporting the link between SCFAs and food consumption is their ability to enhance the secretion of GLP-1 and peptide YY (PYY) by activation of GPR41 and GPR43 receptors on the enteroendocrine L cells of the intestine[80]. GLP-1 and PYY reduce the secretion of NPY and stimulate proopiomelanocortin generation in the neurons of the arcuate nucleus of the hypothalamus and reduce gastric emptying[81]. Additionally, SCFAs can potentially reduce hepatic steatosis by enhancing catabolic processes. Specifically, propionate and butyrate induce the activation of AMP-activated protein kinase that promotes hepatic autophagy: A catabolic process that results in TG hydrolysis and the release of fatty acids available for β-oxidation within the mitochondria[82]. Excessive levels of SCFAs may promote the development of HCC: Therefore, any supplementation of these should be done with caution[83].

BCAAs: BCAAs, leucine, isoleucine, and valine, form a group of proteinogenic essential amino acids possessing a branched aliphatic side chain structure. They act as signaling molecules regulating the metabolism of glucose, lipids, protein synthesis, intestinal health, and immunity *via* a special signaling network, especially PI3K/AKT/mTOR signal pathway[84]. Previous reports have shown that the expression and activity of BCAA catabolic enzymes are altered in metabolic disorders. In patients with T2DM, these enzymes are downregulated[85]. Clinical studies have shown that elevated plasma BCAA levels correlate with insulin resistance and increased risk of T2DM[86]. The mechanism for this is not well understood. One potential cause of insulin resistance is the persistent activation of mTOR that may uncouple the insulin receptor from the insulin signalling mediator, insulin receptor substrate 1. Another potential mechanism is the abnormal metabolism of BCAAs in obesity that results in the accumulation of toxic BCAA metabolites. These metabolites can trigger mitochondrial dysfunction and stress signaling that are associated with insulin resistance and T2DM[87]. Interestingly, bariatric surgery was observed to cause a decrease in BCAA levels in obese individuals[88]. The bacteria *Prevotella copri* and *Bacteroides vulgatus* that correlate strongly with the amount of BCAAs, appear to be closely involved in the development of insulin resistance[89]. However, oral supplementation of BCAAs resulted in increased levels of

beneficial gut microbiota, including *Ruminococcus flavefaciens* and *Bifidobacterium* sp. and induced a reduction in hepatic fat accumulation[90]. Additionally, the elevated level of *Bifidobacterium* sp. resulted in an increase in GLP-1 secretion through the increase in SCFAs, particularly acetate[91]. The contradictory role of BCAAs may be due to the varied composition of gut microbiota. Further research is necessary to better understand the mechanism between gut microbiota composition and the role of BCAAs in MAFLD pathogenesis.

Ammonia: *In vitro* and *in vivo* studies have shown that hepatic steatosis results in a reduction in the efficiency of urea cycle enzymes. In the study by De Chiara *et al*[92] reduced levels of ornithine transcarbamylase and carbamoyl phosphate synthetase, both urea cycle proteins, were observed in patients with NAFLD. This leads to an accumulation of ammonia in the liver that activates the profibrotic genes in hematopoietic stem cells (HSCs)[93]. Fibrosis is the major cause of the increased mortality observed in patients with NAFLD, and the prevention and reduction of fibrosis should be the main aims of treatment[94]. Ammonia is produced from amino acids by gut microbiota. Therefore, the composition of gut microbiota plays an essential role in the levels of circulating ammonia. *In vivo* studies have shown that absolute anaerobic gram-positive bacteria (members of the genus *Clostridium*) and gram-negative bacilli of the family *Enterobacteriaceae* are the major contributors to ammonia production[95]. Furthermore, elevated levels of *Escherichia coli* belonging to *Enterobacteriaceae* have been consistently linked to the occurrence of NAFLD[96]. Ammonia is a neurotoxic compound that travels easily across the blood-brain barrier. Inflammation that increases as MAFLD progresses also contributes significantly to the development of hepatic encephalopathy *via* the gut-liver-brain axis and appropriate modulation of gut microbiota dysbiosis appears to be critically important in therapeutic strategies[97,98].

Endogenous ethanol: Endogenous ethanol synthesis by gut microbiota is another contributor to the development of MAFLD. Blood alcohol levels are increased after intake of alcohol-free food. However, these endogenous alcohol levels are much higher in people with NAFLD than in healthy individuals[99]. Ethanol produced in the gut stimulates NF-κB signaling molecules that can provoke tissue damage. This damage can increase intestinal permeability, leading to higher levels of LPS in the hepatic portal system. The increased LPS levels can then cause inflammation in the liver by activating TLR4 and the inflammasome[100]. In addition, alcohol dehydrogenase (ADH) levels are significantly lower in people with NAFLD than in healthy people[101]. This impaired detoxification process can lead to an increase in the production of ROS, resulting in oxidative damage to hepatocytes[102]. Bacteria from class *Gammaproteobacteria* (particularly *Escherichia coli*, *Klebsiella pneumoniae,* and other members of the *Enterobacteriaceae* family) are involved in the process of endogenous alcohol synthesis[100]. Impairments in insulin signaling may alter ADH activity in the liver, subsequently leading to impaired ethanol metabolism and elevated blood ethanol levels in patients with NAFLD[103]. Therefore, appropriate modification of gut microbiota with prevention of insulin resistance may be key to preventing the progression of MAFLD.

Glucagon-like peptides and other incretins

GLP-1 is derived from the proglucagon gene that is also the genetic origin of glucagon-like peptide-2 (GLP-2) and glucagon. In alpha cells of the pancreas, the proglucagon gene is processed to produce glucagon whose expression is stimulated by fasting and inhibited by insulin. In the enteroendocrine L-cells of the intestine, proglucagon gene expression decreases in a fasting state and increases during food consumption. Proglucagon is processed to produce GLP-1, GLP-2, and glicentin[104,105].

GLP-1 is secreted into the portal circulation from intestinal L cells that are located with increasing abundance from the duodenum to the colon. The L-cells are stimulated by nutrients, neural, and endocrine mechanisms. Different types of nutrients, such as sugars, fatty acids, amino acids, and dietary fiber, stimulate GLP-1 secretion. Secretion of GLP-1 is also stimulated by gut microbiota and their metabolites, such as SCFAs[106].

GLP-1 is an incretin, and this implies that it enhances the secretion of insulin in a glucose-dependent manner leading to a decrease in glycemia. It has also been shown to inhibit glucagon secretion when glycemia increases above fasting levels. The other peptide belonging to the incretin family is the gastric inhibitory polypeptide (GIP) secreted from K cells present in the upper intestine that also promotes postprandial insulin secretion. Besides its insulinotropic effects, GLP-1 has also been associated with various regulatory and protective effects. Unlike GIP, the action of GLP-1 is preserved in patients with T2DM[107]. GLP-1 inhibits gastric emptying and reduces acid secretion and motility, and this promotes satiety. Food intake is also reduced as a result of GLP-1 action in the hypothalamus and brainstem. GLP-1 exerts protective and regulatory effects in different tissues, including the liver, brain, heart, adipose tissue, muscles, bones, kidneys, and lungs [108].

A reduction in body weight and insulin resistance are the main mechanisms induced by GLP-1 that contribute to a decrease in hepatic steatosis, inflammation, and fibrosis observed in NASH. The anti-inflammatory effects of GLP-1 may also be mediated by modulating Kupffer cell activity in the liver and *via* anti-fibrotic effects involving inhibition of HSC activation[109].

There is limited evidence supporting the expression and function of GLP-1 receptors in hepatic tissue and their potential to reduce steatosis[110]. An RNA sequencing study by Boland *et al*[111] did not find GLP-1 receptor transcripts in bulk human liver and isolated non-parenchymal cells and this is a strong argument against GLP-1 receptor expression in human liver tissue. Therefore, currently, the beneficial effects of GLP-1 are attributed to indirect mechanisms.

GLP-2 is derived from the same gene as GLP-1 and is co-secreted, but its role is to repair the intestinal lining. It improves nutrient absorption, reduces gut permeability, and stimulates cell proliferation in the gut[112]. In a murine model, GLP-2 also showed a beneficial hepatic influence. Fuchs *et al*[113] have reported that GLP-2 treatment attenuated the activation of HSCs. GLP-2 promoted intestinal FXR-Fgf15/19 signaling resulting in reduced CYP7A1 and increased CYP2C70 expression in the liver, contributing to hepatoprotective and antifibrotic effects of GLP-2 in the Mdr2-/-mouse

model.

TREATMENT

GLP-1 RAs in the treatment of obesity and MAFLD

Endogenous GLP-1 undergoes rapid degradation by the enzyme dipeptidyl peptidase-4 (DPP-4). Two different strategies were employed to increase the effects of GLP-1: Inhibition of degradation or exogenous administration of GLP-1 RAs resistant to degradation. Endogenous GLP-1 is degraded by DPP-4 at various sites including the intestine (75%) and the liver (further 50%) that leaves only 10%-15 % of intact GLP-1 in circulation[114]. DPP-4 inhibitors have been developed to increase GLP-1 activity and are commonly used in the treatment of T2DM. Their action leads to an increase in incretin hormones, GLP-1 and GIP, and a decrease in glucose and glucagon levels. Circulating GLP-1 increases by about 2-4 fold [115]. GLP-1 RAs lead to a 10-fold increase in GLP-1 and their effect is persistent. This difference in the resulting GLP-1 activity translates into almost no weight loss with DPP-4 inhibitors.

GLP-1 RAs in obesity: GLP-1 RAs originally introduced as pharmacotherapy for T2DM improved glycemic control and also promoted weight loss that was uncommon among hypoglycemic agents. GLP-1 RAs became the preferred treatment in patients with T2DM and obesity and then obesity alone. Among GLP-1 RAs, liraglutide 3.0 mg once daily was the first medication approved by the United States Food and Drug Administration and European Medicines Agency for the treatment of obesity. The results of the SCALE trial including more than 3700 patients with obesity [body mass index (BMI) > 30] or being overweight (BMI > 27) with comorbidities confirmed its effectiveness in weight reduction. However, the benefit of 5.4% body weight loss compared to placebo observed during treatment gradually decreased after treatment cessation[116]. The STEP 5 trial assessed the efficacy and safety of a once-weekly subcutaneous dose of 2.4 mg semaglutide *vs* placebo (both plus behavioral intervention) for long-term treatment of adults with obesity or being overweight with at least one weight-related comorbidity, without T2DM. The mean change in body weight from baseline to week 104 was -15.2% in the semaglutide group *vs* -2.6% with placebo[117]. In the STEP 8 trial, liraglutide was compared to semaglutide treatment. Among overweight or obese adults without T2DM, once-weekly subcutaneous semaglutide administration compared with once-daily subcutaneous liraglutide treatment, added to counseling for diet and physical activity, resulted in significantly greater weight loss at 68 weeks. The mean weight change from baseline was -15.8% with semaglutide *vs* -6.4% with liraglutide while weight change with placebo was -1.9%[118]. In a follow-up study of the STEP 1 trial, one year after withdrawal of a once-weekly subcutaneous dose of 2.4 mg semaglutide and lifestyle intervention, participants regained two-thirds of their prior weight loss[119]. These findings indicate that continuous, possibly lifelong treatment is necessary to achieve sustained weight loss with GLP-1 RAs.

GLP-1 RAs in MAFLD: The American Association for the Study of Liver Diseases guidelines published in 2018 and joint guidelines of the European Association for the Study of the Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity published in 2016 do not consider GLP-1 RAs for the treatment of liver disease in patients with NAFLD or NASH[120,121]. Similarly, a recent review of published guidelines presents current management of NAFLD that is based on lifestyle changes that promote an energy deficit leading to weight loss. In patients with obesity and NAFLD, weight loss medications may be considered, particularly GLP-1 RAs and bariatric surgery for the morbidly obese. In patients with T2DM and NAFLD, diabetes medications could be used, such as pioglitazone and GLP-1 RAs[122]. Pharmacological therapies designed specifically for NAFLD are lacking[123]. Lifestyle modification based on a low-calorie diet and physical activity remain the mainstay of the therapy for patients with fatty liver[124]. Weight loss of 7%-10% is an effective treatment for NAFLD that results in improvement of histological features of steatosis and fibrosis[121]. The lifestyle interventions and drugs suggested in NAFLD therapy are frequently commonly used in the treatment of T2DM, metabolic syndrome, or obesity. These pharmacological treatments include pioglitazone, metformin, SGLT2 inhibitors, and GLP-1 RAs. Minimally invasive approaches, including metabolic surgery and endoscopic bariatric procedures, are also effective in patients with metabolic syndrome, fatty liver, and morbid obesity[125,126].

GLP-1 RAs are effective in T2DM and promote weight loss, ameliorate liver enzyme level perturbations and liver steatosis in patients with NAFLD/NASH[127]. In a phase 2 trial of semaglutide, 40% of NASH resolution was reported in patients with or without T2DM[128].

As GLP-1 RAs can reduce body weight, several studies have investigated the potential for GLP-1 RAs in the treatment of NAFLD and NASH. A meta-analysis published in 2016 that included 136 patients with NAFLD and T2DM treated with either GLP-1 RAs (exenatide twice daily or liraglutide) or DPP4 inhibitors, concluded that incretin-based treatment was effective in reducing biochemical biomarkers of NAFLD and significantly reduced signs of inflammation, steatosis and fibrosis in biopsy samples and observed *via* imaging[129]. In the LEAN study that included 52 patients with NASH, a dose of 1.8 mg liraglutide once daily resulted in biopsy-confirmed resolution of NASH in 39% of patients, compared with 9% in the placebo group[130]. Furthermore, in a study comparing 26 weeks of treatment with 3.0 mg liraglutide once daily to lifestyle intervention (the currently recommended treatment), patients in both groups achieved similar reductions in levels of alanine aminotransferase, liver fat fraction, liver stiffness, and body weight[131]. In a murine study of streptozotocin- and high-fat diet (HFD)-induced T2DM and NASH, liraglutide significantly ameliorated steatosis, inflammation, hepatocyte ballooning, and suppressed hepatocarcinogenesis[132].

Semaglutide treatment resulted in contradictory results. In a recent study by Loomba *et al*[133] in patients with NASH and compensated cirrhosis, semaglutide did not significantly improve fibrosis or achieve resolution of NASH *vs* placebo. In a study by Newsome *et al*[128], the percentage of patients in whom NASH resolution was achieved with no worsening

of fibrosis was dose-dependent and reached 59% in the 0.4-mg group *vs* 17% in the placebo group ($P < 0.001$). Scavo *et al* [134] described a mechanism by which GLP-1 RAs may improve NASH. In patients responding to therapy with semaglutide, the authors suggested a mechanism for reducing activation of HSCs and downregulation of extracellular matrix components such as vimentin, collagen, and fibronectin. Further, recent large retrospective cohort studies with GLP-1 RAs application revealed its potential beneficial impact in patients with chronic liver diseases and T2DM to decrease the risk of major adverse liver outcomes, including hepatic decompensation, portal hypertension, HCC, and liver transplantation. These findings suggest that treatment with GLP-1 RAs may be a promising option for reducing the risk of chronic liver disease progression[135,136].

Gut microbiota and GLP-1

GLP-1 RAs are known to affect the intestinal environment and, changes in the gut microbiota have been linked to GLP-1 RAs[137]. In general, GLP-1 is thought to have two effects, one exerted *via* the central nervous system and the other exerted *via* local receptors in the periphery. In a pioneer study investigating the mechanisms of GLP-1 RA-related changes in gut microbiota, Kato *et al*[138] reported a release of norepinephrine into the intestinal lumen *in vitro* and an activation of the sympathetic nervous system by acute administration of GLP-1 RAs with a concomitant rapid increase in *E. coli in vivo*. At the phylum level, liraglutide administration significantly decreased *Bacteroidetes* and tended to increase *Actinobacteria*. However, *Firmicutes* and *Proteobacteria* were not changed. At the genus level, liraglutide administration significantly reduced *Ruminococcus* spp. and did not increase *Akkermansia* spp. The gene expression levels of bacterial proteins that could affect the host metabolism were analysed. Expression of formate-tetrahydrofolate ligase that is related to acetic acid synthesis, was significantly increased and, conversely, the expression of butyryl-CoA: Acetate CoA-transferase that is related to butyrate synthesis, was significantly decreased. The increase in *E. coli* may help to promote bacterial translocation by attenuating intestinal tight junction gene levels under stress conditions such as colitis.

Chaudhari *et al*[139] described a GLP-1 activation pathway involving TGR5 agonist-CA-7-sulfate (CA7S). A microbial metabolite, LCA, is increased in murine portal veins after sleeve gastrectomy (SG) and by activating the vitamin D receptor, induces hepatic SULT2A enzyme expression to drive CA7S production. An SG-induced shift in the microbiome increases gut expression of the BA transporters Asbt and Ostα that in turn facilitate selective transport of LCA across the gut epithelium. Activation of this gut-liver pathway leads to CA7S synthesis and GLP-1 secretion, causally connecting a microbial metabolite with glucoregulatory benefits of SG in human patients.

Multiple peptide receptor agonists

Several novel agents acting as dual and triple incretin agonists have been developed.

GLP-1R/GcgR (glucagon receptor): The anti-obesity effects of dual GLP-1R/glucagon receptor agonists were first demonstrated in diet-induced obese mice, where dual agonism reduced body weight, hyperglycemia, and hepatic lipid compared with that resulting from GLP-1 RA treatment alone[140]. Ambery *et al*[141] studied a dual-agonist Cotadutide (MEDI0382) in overweight patients with T2DM and observed a significant improvement in post-prandial glucose excursions and reductions in body weight and liver fat. Others have shown that a related dual agonist can reduce liver fat and slow the development of hepatic fibrosis in HFD and HFD/carbon tetrachloride mouse models[142]. Furthermore, in methionine- and choline-deficient diet-fed mice with partial hepatectomy, treatment with a similar dual agonist reduced inflammation, cell death, and improved hepatic regeneration[143]. These data are suggestive of GLP-1/Gcg RAs as potential therapeutics. However, the studies were prophylactic, rather than interventional, and not conducted in a pathophysiologically relevant model of NASH[144]. Cotadutide has been engineered to balance GLP-1 and Gcg RAs (with an approximately 5:1 bias towards GLP-1 receptor affinity) to optimize beneficial outcomes for metabolic disease.

GLP-1/GLP-2: Interesting effects are expected from a combined intervention targeting both GLP-1 and GLP-2 receptors that will impact glucose homeostasis and intestine integrity. In a rodent study, GLP-1/GLP-2 co-agonists revealed effects on gut morphometry, showing a marked increase in intestinal volume and mucosal surface area. Furthermore, effects on glucose tolerance and long-term glycemic control were evident as well as a decrease in body weight and delayed gastric emptying[145]. Madsen *et al*[146] reported similar gut bacterial compositional changes following liraglutide and dual GLP-1/GLP-2 RA treatment, characterized by discrete shifts in low-abundance species and related bacterial metabolic pathways. Both compounds suppressed caloric intake, promoted a marked weight loss, improved glucose tolerance, and reduced plasma cholesterol levels. These microbiome alterations may be associated with the converging biological actions of GLP-1 and GLP-2 receptor signaling on caloric intake, glucose metabolism, and lipid handling. In another pre-clinical study, a GLP-1/GLP-2 receptor dual agonist improved BMI, glucose homeostasis, liver TGs, liver fibrosis, and intestinal barrier permeability in a murine model of NASH. SCFA-producing *Bifidobacterium* spp. increased, as well as bacteria associated with a healthy phenotype such as *Prevotella* spp., *Lactobacillus* spp., and *Akkermansia* spp. *Firmicutes,* associated with obesity, were decreased[147].

Multiple agonist effects after bariatric surgery

The metabolic effects of bariatric surgery may be attributed to a positive influence on the secretion of multiple gut hormones that results in an early improvement in glycemic control in T2DM patients[148]. These include insulin, GLP-1, GLP-2, GIP, PYY, CCK, OXY, and ghrelin. The profile of gastrointestinal hormone secretion after bariatric surgery depends on the surgical technique applied. The model technique in metabolic surgery is Roux-en-Y Gastric Bypass (RYGB). After RYGB, postprandial GLP-1 levels increase, while fasting levels remain unchanged. In a less malabsorptive version with a shorter Roux limb length, the increase in postprandial GLP-1 is more pronounced[149]. Other common procedures, such as SG and mini-gastric bypass, are based on different anatomical principles but also lead to an

improvement in the hormone profile[150].

The idea to pharmacologically replicate the effects of RYGB with multiple peptide receptor agonists is particularly attractive. In a study by Behary *et al*[151], obese patients with prediabetes or diabetes were randomized to receive an infusion of GLP-1, oxyntomodulin, and PYY (GOP) that replicates post-surgical post-prandial gut hormone levels *vs* saline for 4 weeks. The two groups of RYGB patients and very-low-calorie diet (VLCD) patients served as comparators. The glucose tolerance and variability were better with GOP, but weight loss was inferior to both RYGB and VLCD.

Both RYGB and SG lead to improvement in liver disease in patients with obesity and NAFLD or NASH. RYGB results in a significant reduction of steatohepatitis and fibrosis while SG improves steatohepatitis but does not reverse fibrosis. Both procedures significantly improve the NAFLD Activity Score and biochemical indices[152].

After bariatric surgery, gut microbiota have been considered as a factor associated with metabolic improvements and weight loss. The observed alterations in microbiota vary between procedures and individual patients. In a recent systematic review of 18 trials, Coimbra *et al*[153] reported greater amount of *Bacteroidetes*, *Proteobacteria*, and diversity after BS. *Firmicutes*, *Bacteroidetes*, and the *Firmicutes* to *Bacteroidetes* ratio was inconsistent, increasing or decreasing after RYGB and SG. There was a reduction in the relative proportion of *Firmicutes*. Moreover, a higher proportion of *Actinobacteria* was observed after RYGB that were not observed after SG.

Changes in GLP-1, GLP-2, and the gut microbiome were studied after RYGB. After surgery, zonulin decreased and an increase in area under the curve (AUC) after the meal tolerance test was observed for GLP-1 and GLP-2. Species belonging to *Streptococcaceae, Akkermansiaceae, Rickenellaceae, Sutterellaceae, Enterobacteriaceae, Oscillospiraceae, Veillonellaceae,* and *Fusobacteriaceae* families increased after intervention and correlated positively with AUC of GLP-1 and GLP-2, and negatively with glucose, hemoglobin A1c, TGs, and adiposity markers. *Clostridium perfringens and Roseburia* sp. *40_7* behaved similarly. In contrast, some species belonging to *Lachnospiraceae, Erysipelotrichaceae,* and *Oscillospiraceae* families decreased and showed opposite correlations[154]. Table 2 presents the changes in gut microbiota following various types of treatment.

Table 2 The influence of the type of treatment on the comparison of gut microbiota

Bacteria levels	GLP-1 RAs	GLP-1/GLP-2 RA	Roux-en-Y gastric bypass surgery
Increase	*Bifidobacteriaceae*	*Bifidobacteriaceae*	*Bacteroidetes*
	Actinobacteria	*Prevotellaceae*	*Actinobacteria*
	Akkermansiaceae	*Lactobacillaceae*	*Proteobacteria*
		Akkermansiaceae	*Akkermansiaceae*
Decrease	*Firmicutes*	*Firmicutes*	*Firmicutes*
	Oscillospiraceae		*Oscillospiraceae*
	Bacteroidetes		
Microbiota diversity	Increase	Increase	Increase

GLP-1 RAs: Glucagon-like peptide-1 receptor agonists; GLP-1/GLP-2 RA: Dual glucagon-like peptide-1 and glucagon-like peptide-2 receptor agonist.

CONCLUSION

Gut microbiota are strongly associated with the progression of MAFLD. The mechanisms involved in these effects are complex and depend on the functionality of the gut-liver axis with any alteration of gut permeability leading to bacterial translocation. There is also a significant role played by metabolites such as BAs, SCFAs, BCAAs, choline, ammonia, and ethanol. The composition of gut microbiota is altered in patients with obesity, metabolic syndrome, and MAFLD. It is affected by the above-mentioned pathogenic mechanisms but also influences all components of the gut-liver axis that results in a constant crosstalk. As the disease progresses from simple steatosis and early stages of MAFLD to advanced fibrosis and cirrhosis there is a loss of bacterial diversity and depletion of beneficial bacterial content. However, it is challenging to form any conclusive claims about gut microbiota profiles in MAFLD patients due to the variation in gut microbiota composition between MAFLD and non-MAFLD patients and also among different stages of MAFLD. Further insights into mechanisms and new therapeutic strategies might be offered by studies of the gut metabolome in patients with MAFLD. Gastrointestinal hormones such as insulin, glucagon, GLP-1, GLP-2, and others, are involved in the pathogenesis of MAFLD but also play an important role in maintaining gut-liver axis functionality through a complex network of reciprocal communication with gut microbiota. Novel therapeutics from a family of GLP-1 RAs effective in the treatment of T2DM and obesity have been proposed as a promising solution for patients with MAFLD. Not surprisingly, these agents affect many other physiological mechanisms and pathways including the gut-liver axis and the gut microbiome. Some insights into potential mechanisms of this interaction may be drawn from previous experience with bariatric surgery patients. Metabolic surgical procedures such as RYGB, SG, or one-anastomosis gastric bypass represent models of alterations in the gut microbiome with concomitant changes in the secretion of multiple gastrointestinal hormones in patients with MAFLD. Most of the mechanisms are not fully elucidated and further well-designed studies are needed to support therapeutic decision making as more complex therapies are being developed that involve multiple

peptide agonists.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Fifty-five cases of hepatic alveolar echinococcosis combined with lymph node metastasis: A retrospective study

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Abstract

BACKGROUND

Lymph node metastasis is a specific type of metastasis in hepatic alveolar echinococcosis (AE). Currently, there is a scarcity of describing the clinical characteristics and lymph node metastasis rules of patients with hepatic AE combined with lymph node metastasis and its mechanism and management are still controversial. Radical hepatectomy combined with regional lymph node dissection is a better treatment.

AIM

To analyse the clinical features of hepatic AE combined with lymph node metastasis to explore its treatment and efficacy.

METHODS

A total of 623 patients with hepatic AE admitted to the First Affiliated Hospital of Xinjiang Medical University from 1 January 2012 to 1 January 2022 were retrospectively analysed. Fifty-five patients with combined lymph node metastasis

were analysed for their clinical data, diagnosis and treatment methods, follow-up efficacy, and characteristics of lymph node metastasis. Finally, we comparatively analysed the lymph node metastasis rates at different sites. Categorical variables are expressed as frequencies and percentages, and the analysis of difference was performed using the χ^2 test. The Bonferroni method was used for pairwise comparisons when statistical differences existed between multiple categorical variables.

RESULTS

A lymph node metastasis rate of 8.8% (55/623) was reported in patients with hepatic AE, with a female predilection (69.1%) and a statistically significant sex difference (χ^2 = 8.018, P = 0.005). Of the 55 patients with lymph node metastasis, 72.7% had a parasite lesion, neighbouring organ invasion, and metastasis stage of P3N1M0 and above, of which 67.3%, 78.2%, and 34.5% of hepatic AE lesions invaded the bile ducts, blood vessels, and distant metastases, respectively. Detection rates of lymph node metastasis of 16.4%, 21.7%, and 34.2% were reported for a preoperative abdominal ultrasound, magnetic resonance imaging, and computed tomography examinations. All patients were intraoperatively suspected with enlarged lymph nodes and underwent radical hepatectomy combined with regional lymph node dissection. After surgery, a routine pathological examination was conducted on the resected lymph nodes. A total of 106 positive lymph nodes were detected in six groups at various sites, including 51 single-group metastasis cases and four multi-group metastasis cases. When the metastasis rates at different sites were statistically analysed, we observed that the metastasis rate in the para-hepatoduodenal ligament lymph nodes was significantly higher than that of the other sites (χ^2 = 128.089, P = 0.000 < 0.05). No statistical difference was observed in the metastasis rate between the five other groups. Clavien-Dindo grade IIIa complication occurred in 14 cases, which improved after administering symptomatic treatment. Additionally, lymph node dissection-related complications were not observed. Recurrence after 2 years was observed in one patient.

CONCLUSION

Lymph node metastasis is a rare form of metastasis in hepatic AE, which is more frequent in women. Para-hepatoduodenal ligament lymph nodes are commonly observed. Radical hepatectomy combined with regional lymph node dissection is a safe, effective, and feasible treatment for liver AE combined with lymph node metastasis.

Key Words: Echinococcosis; Radical hepatectomy; Lymph node metastasis; Lymph node dissection; Alveolar echinococcosis; Hepatic

Core Tip: We retrospectively summarized and analyzed the clinical data, diagnosis and treatment, follow-up efficacy and characteristics of lymph node metastasis in 55 patients combined with lymph node metastasis among 623 patients with hepatic alveolar echinococcosis (AE) admitted to our hospital from January 2012 to January 2022. This study is the first and largest retrospective study specifically describing the management of hepatic AE combined with lymph node metastases. We present a radical hepatectomy combined with regional lymph node dissection strategy for patients with hepatic AE combined with lymph node metastasis based on the outcomes of this study and our experience.

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INTRODUCTION

Echinococcosis multilocularis infection causes hepatic alveolar echinococcosis (AE). It is known as a "malignant parasitic disease" owing to its chronic, progressive, and infiltrative growth[1-3]. Distant metastases to the lungs, brain, kidneys, and surrounding tissues *via* the infiltrative, haematogenous, and lymphatic routes occur in hepatic AE. Reports and related studies on lymph node metastasis are scarce, and conclusive evidence supporting the regional lymphatic metastasis pathway is lacking[4,5]. Lymph node might metastases occur as a result of AE draining *via* the deep and superficial multidirectional lymphatic reflux pathways in the liver to regional lymph nodes[6]. Despite the reports on multiple related cases, more comprehensive literature reports proposing diagnostic and treatment norms for lymph node metastases do not exist[7-10]. Metastatic lymph node management is controversial. Additionally, the World Health Organization (WHO) treatment guidelines do not specify whether regional lymph node dissection should be performed as part of the radical treatment protocol for hepatic AE. Therefore, analysing the clinical characteristics and treating

patients with hepatic AE combined with lymph node metastasis is crucial. Herein, we analysed the clinical characteristics of 55 patients with hepatic AE combined with lymph node metastasis at our centre and summarised its diagnosis and treatment experience for the first time.

MATERIALS AND METHODS

Clinical information

Study subject: A total of 623 patients (295 females and 328 males) with hepatic AE were admitted to our hospital from 1 January 2012 to 1 January 2022. Fifty-five patients (17 males and 38 females) (8.8%), aged 35.5 ± 11.55 years (range: 15-66 years), who were diagnosed with combined lymph node metastasis, were selected. The liver lesions were in the right lobe in 25 cases, the left lobe in eight cases, and both the right and left lobes of the liver in 22 cases. The mean maximum diameter of the lesions was 12.93 cm, with blood vessel invasion in 43 cases (78.2%) and bile duct invasion in 37 cases (67.3%).

Symptoms: Twenty-five patients did not have any obvious symptoms. Initial symptoms of abdominal pain and bloating occurred in 21 patients. Seven patients had yellow discolouration of the skin and the sclera. One patient had a headache, dizziness, and convulsions. One other patient had chest distress, cough, and sputum production.

Medical history: Previous palliative partial hepatectomy, percutaneous transhepatic cholangial drainage, portal vein embolisation, and perforated drainage for echinococcosis-caused liquefaction and necrosis within the cavity of liver peritoneal worms were performed in ten, six, one, and two patients, respectively. History of exposure to infected areas and canines was recorded in all patients. A preoperative diagnosis was established in 14 patients, and they received irregular intermittent oral albendazole.

Staging characteristics: According to the parasite lesion, neighbouring organ invasion, and metastasis (PNM) staging suggested by the WHO, there were 12 cases of P2NIM0, three cases of P2N1M1, eight cases of P3N1M0, six cases of P3N1M1, 16 cases of P4N1M0, and 10 cases of P4N1M1. All patients belonged to the intermediate and advanced clinical stages.

Comorbidity: Extrahepatic metastases occurred in 17 cases, including 10 cases of pulmonary metastases, one case of brain metastasis, one case of pancreatic metastasis, two cases of pulmonary-cerebral metastases, and three cases of metastases to three or more organs (lung, brain, kidney, *etc*.). In addition, one patient had combined portal vein cavernous degeneration.

Adjunctive examinations: Preoperative routine blood examination, liver function tests, abdominal ultrasonography (USG), and abdominal computed tomography (CT) were performed on 55 patients. Among them, abdominal magnetic resonance imaging (MRI) was performed on 33 patients. The CT manifestations of lymph node metastasis are presented in Figure 1.

Surgical treatment

Indications: The indications for surgical treatment were as follows: (1) Patients without serious cardiopulmonary disease who could tolerate surgery; (2) Patients with Child-Pugh class A or B liver function; (3) Patients who were willing to undergo radical surgery; (4) Patients with imaging findings suggestive of lymph node metastasis or suspicious enlarged lymph nodes detected intraoperatively; (5) Patients with lung metastasis who could tolerate extended radical surgery; and (6) Patients with brain metastasis and stable brain lesions.

Contraindications: The contraindications were as follows: (1) Patients with a poor cardiopulmonary function who could not tolerate radical treatment; (2) Patients with Child-Pugh class C liver function; and (3) Patients who could not undergo radical surgical treatment due to other reasons.

Hepatic surgery: Radical hepatectomy was performed considering the location, severity, and individualised treatment of the lesion[11]. In addition, right trisegmentectomy, right hemihepatectomy, left trisegmentectomy, left hemihepatectomy, left lateral hepatic lobectomy, and *ex vivo* liver resection with autologous liver transplantation were performed in nine, fourteen, six, three, one, and twenty-two patients, respectively.

Treatment of the lymph nodes: Regional lymph nodes, including the para-cystic duct, para-common bile duct, porta hepatis, para-portal vein, para-pancreatic, celiac trunk, and superior mesenteric lymph nodes, were routinely explored intraoperatively. The detected lymph nodes that were enlarged or suspected of metastases were dissected based on the skeletal criteria (Figure 2). The hepatoduodenal ligament and the portacaval space were intraoperatively explored to avoid leakage. In addition, we prophylactically dissected the remaining suspicious lymph nodes in the lymphatic reflux pathway. The supradiaphragmatic and subdiaphragmatic lymph nodes were explored in patients who required intraoperative resection and reconstruction of the diaphragm. A routine pathological examination was performed on the dissected lymph nodes.

Treatment of intrahepatic and extrahepatic invaded tissues: Liver resection combined with organ and tissue resection should be performed according to the principles of radical tumour-free treatment when the lesion invades multiple

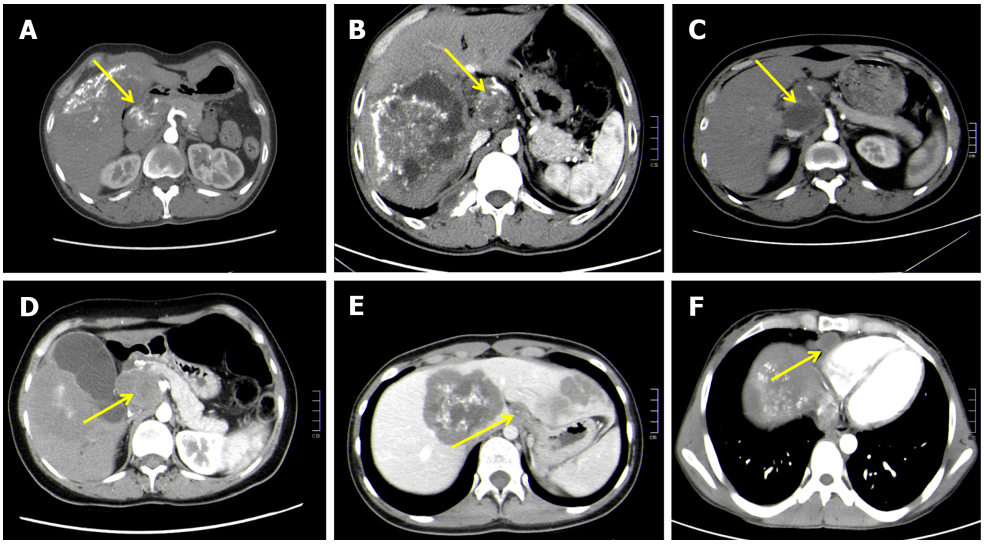

Figure 1 Computed tomography findings of lymph node metastasis in hepatic alveolar echinococcosis. A: An enlarged mixed-density lymph node with multiple nodular, eggshell calcifications next to the portal vein in the first hepatic portal area and an unenhanced liquefied necrotic area in the lesion on an enhanced scan; B: A roundish mixed-density mass next to the common hepatic artery, with multiple sand-like, nodular calcifications within the lesion, with mild to moderate enhancement of the lesion on an enhanced scan and a hypoenhanced liquefied necrotic area within the lesion; C: Enlarged lymph nodes were observed at the bifurcation level of the splenic artery and common hepatic artery on the right side of the celiac trunk, with uniform density within them, and patchy but unenhanced areas of liquefied necrosis were seen within the lesion on enhancement scans; D: A mixed-density mass in the interstitial space of the abdominal aorta posterior to the pancreatic head with a speckled high-density shadow and a patchy slightly low-density shadow, heterogeneous enhancement on an enhanced scan, and no significant enhancement in the slightly low-density liquefied necrotic area within the lesion; E: An enlarged heterogeneously dense lymph node with nodular foci next to the abdominal aorta, with a mild to moderate enhanced lesion edge on an enhanced scan and no enhancement within the lesion; F: A roundish, slightly hypointense shadow in the right diaphragmatic angle with circular enhancement at the lesion edge on an enhanced scan and no significant.

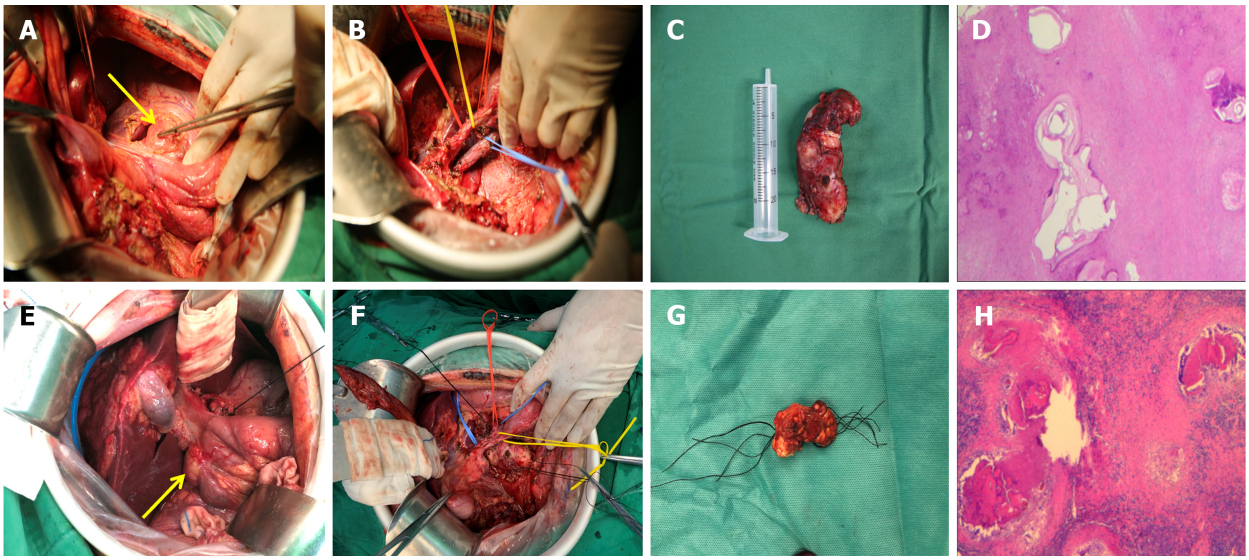

Figure 2 A 19-year-old male patient presented with intermittent epigastric pain and discomfort for 3 months and was diagnosed with hepatic alveolar echinococcosis combined with porta hepatis lymph node metastasis based on clinical examination and imaging studies. A: Para-hepatoduodenal ligament lymph node enlargement was observed intraoperatively; B: Dissection of the hepatoduodenal ligament to skeletonization; C and D: Postoperative lymph node specimens and pathological sections; E-H: A 50-year-old female patient presented with right upper abdominal distension for over 4 years and was diagnosed with hepatic alveolar echinococcosis (AE) with posterior pancreatic head lymph node metastasis based on clinical examination and imaging findings. Posterior pancreatic head lymph node enlargement was observed intraoperatively (E). The regional lymph nodes were dissected to skeletonisation (F). Postoperative lymph node specimens and pathological sections (G and H).

organs or surrounding tissues. Combined resection, repair, and reconstruction should be performed to achieve radical treatment when the intrahepatic and extrahepatic vessel and biliary system invasion are severe. Herein, hepatectomy combined with total left nephrectomy, pulmonary wedge resection, pancreaticoduodenectomy, diaphragmatic repair, portal or hepatic vein reconstruction, portal vein thrombosis removal, posthepatic inferior vena cava thrombosis removal, portal or inferior vena cava vascular grafting (six cases with autologous vessels and one case with artificial vessels), bile

duct repair and formation, and bilioenteric anastomosis were performed in one, two, one, six, seven, one, one, seven, fifteen, and seven patients, respectively.

Postoperative management and follow-up

Postoperatively, the patients were dynamically monitored for changes in vital signs and liver function. Postoperative complications were graded using the Clavien-Dindo classification[12]. After discharge, all patients were instructed to wait until normalisation of the liver function to receive regular oral albendazole (10 mg/kg) according to the guidelines. They also underwent abdominal USG or CT to evaluate regular liver and kidney function. The patients were followed up at the outpatient clinic and telephonically to check their postoperative medication status. In addition, routine blood, liver function, abdominal USG, and CT re-examinations were performed to understand recurrence and recovery.

Statistical analysis

Descriptive and correlation analyses of the data were performed using SPSS 26.0. Categorical variables are expressed as frequencies and percentages, and the analysis of difference was performed using the χ^2 test. The Bonferroni method was used for pairwise comparisons when statistical differences existed between multiple categorical variables. Statistical significance was set at $P < 0.05$.

Medical ethical approval

This study was approved by the Ethics Committee of First Affiliated Hospital of Xinjiang Medical University and conducted in accordance with the Helsinki Declaration. Written and signed informed consent was obtained from all patients or their legal custodians.

RESULTS

A total of 623 patients, comprising 295 (47.4%) females and 328 (52.6%) males, with hepatic AE, were included in this study. No statistically significant difference by sex was observed ($\chi^2 = 1.748$, $P = 0.186$). Of the 55 patients with combined lymph node metastasis, 38 (69.1%) were females, and 17 (30.9%) were males. The incidence of lymph node metastasis had a female predilection ($\chi^2 = 8.018$, $P = 0.005$).

USG, CT, and MRI are accurate, non-invasive, and effective methods for diagnosing hepatic AE. Herein, all these methods had a 100% diagnostic rate for hepatic AE; however, a lower diagnostic rate was observed for lymph node metastasis (Table 1). CT had better diagnostic significance for metastatic lymph nodes with typical morphology and large diameter. On CT, the lymph nodes had a round or ovoid appearance with mixed density or hypodense foci, which were surrounded by sand-like, ring-like, and sheet-like calcifications.

The surgery was performed successfully in all patients, and no serious intraoperative complications occurred. All patients had a transient elevation in their liver function postoperatively, which gradually decreased 3-5 d after surgery and returned to normal before discharge. Clavien-Dindo I complications (hypoproteinaemia in nine cases, bile leakage in two cases, and pancreatic fistula in one case) occurred in 10 cases during postoperative hospitalisation. These patients recovered well after symptomatic treatment with electrolyte supplementation, albumin supplementation, and adequate irrigation and drainage. Clavien-Dindo class IIIa complications (pleural effusion in ten cases, seroperitoneum in seven cases, and pneumothorax in one case) occurred in 14 patients, which improved after symptomatic treatment with puncture tube placement and drainage and closed chest drainage.

Pathological examination revealed 209 lymph nodes, 106 multilocular echinococcus cyst protoscolexes and 103 inflammatory enlarged lymph nodes. Six groups of lymph node metastases at different sites existed, including 39 cases of para-hepatoduodenal ligament lymph nodes, nine cases of posterior pancreatic head lymph nodes, four cases of para-common hepatic artery lymph nodes, five cases of para-aorta abdominalis lymph nodes, one case of celiac trunk lymph nodes, and two cases of para-diaphragmatic lymph nodes. Single-group metastasis occurred in 51 cases, and multi-group metastasis (including two cases of para-hepatoduodenal ligament + para-aorta abdominalis, one case of para-hepatoduodenal ligament + posterior pancreatic head, and one case of para-hepatoduodenal ligament + para-diaphragmatic + para-common hepatic artery metastases) occurred in four cases. The incidence of metastasis at different lymph node sites was subjected to differential analysis using the χ^2 test, yielding $\chi^2 = 128.089$ and $P = 0.000 < 0.05$. Therefore, a significant difference was observed in the rate of metastasis among the different lymph node sites. The Bonferroni method was used to perform a further pairwise comparison. The rate of para-hepatoduodenal ligament metastasis was significantly higher than the five other groups ($P = 0 < 0.01$), while no statistical difference was observed between the five other groups (Table 2).

DISCUSSION

AE is the third most dreadful foodborne parasitic disease worldwide[13]. It is projected that 91% of new cases of AE worldwide occur in China, mainly in agricultural and pastoral areas, such as Xinjiang and Tibet, seriously endangering the people's physical and mental health[14-16]. AE almost originates in the liver and is often diagnosed in the intermediate or advanced stage due to early insidious clinical symptoms. The growth characteristics of hepatic AE are aggressive and infiltrative, similar to malignant tumours, and can metastasise to surrounding tissues or distant organs *via*

Table 1 Comparison of the diagnostic compliance rate of 55 cases of hepatic alveolar echinococcosis with lymph node metastasis diagnosed by different imaging methods

Diagnosis method	Number of examined cases (*n*)	Diagnosis of hepatic AE		Diagnosis of lymph node metastasis	
		n	%	*n*	%
USG	55	55	100	9	16.4
CT	55	55	100	17	30.9
MRI	33	33	100	6	18.2

AE: Alveolar echinococcosis; USG: Ultrasonography; CT: Computed tomography; MRI: Magnetic resonance imaging.

Table 2 Comparative analysis of lymph node metastasis sites in 55 cases

Sites	Para-hepatoduodenal ligament lymph nodes	Posterior pancreatic head lymph nodes	Para-common hepatic artery lymph nodes	Para-aorta abdominalis lymph nodes	Para-diaphragmatic lymph nodes	Celiac trunk lymph nodes
Number of metastasis cases	39[a]	9[b]	4[b]	5[b]	2[b]	1[b]
Number of non-metastasis cases	16[a]	46[b]	51[b]	50[b]	53[b]	54[b]
Metastasis rate (%)	70.9	16.4	7.3	9.1	3.6	1.8
χ^2	128.089[a]					
P value	0					

[a]$P < 0.05$, para-hepatoduodenal ligament lymph nodes *vs* other five groups (posterior pancreatic head lymph nodes, para-common hepatic artery lymph nodes, para-aorta abdominalis lymph nodes, para-diaphragmatic lymph nodes, and celiac trunk lymph nodes) respectively.
[b]$P > 0.05$, compared with posterior pancreatic head lymph nodes, para-common hepatic artery lymph nodes, para-aorta abdominalis lymph nodes, para-diaphragmatic lymph nodes, and celiac trunk lymph nodes.

haematological and lymphatic pathways[17-19]. The incidence of lymph node metastasis is low (8.8% in this study), and its mechanism is unclear. The predominant assumption is that AE drains to regional lymph nodes *via* the lymphatic fluid from the intrahepatic lymphatics[4]. Radical hepatectomy is the preferred treatment for hepatic AE. Patients treated with liver transplantation for end-stage hepatic AE experience re-infection of the graft several years later[20]. This finding supports the previous hypothesis that lymph node metastasis might be a potential risk for persistent infection. In addition, serious complications, such as obstructive jaundice, portal hypertension, acute pancreatitis, and Budd-Chiari syndrome, could occur through infiltration and compression as a result of lymph node metastases in different locations. Therefore, radical treatment of the primary hepatic AE site, along with the dissection of regional lymph nodes, is crucial. However, no distinct specification exists on the scope and indications of lymph node dissection. There is a close association between the mode of lymph node metastasis and the lymphatic fluid drainage pathway. Therefore, understanding the lymphatic return pathways in the liver is essential.

The lymphatic system of the liver can be divided into deep and superficial lymphatic systems. The deep lymphatic vessels are distributed along the portal vein and the hepatic veins. The superficial lymphatic vessels are on the liver surface, comprising the visceral and diaphragmatic surfaces. A total of 80% or more of the hepatic lymph flows along the deep lymphatic system around the portal vein into the para-hepatoduodenal ligament lymph nodes and posterior pancreatic head lymph nodes[21]. Herein, six groups of different lymph node metastases sites existed. The most common site of lymph node metastasis was para-hepatoduodenal ligament (70.9%), which significantly differed from each group ($P < 0.01$). It might be the first station of lymphatic fluid flow from AE to posterior pancreatic head and para-common hepatic artery or more distant lymph nodes. In this study, patients with lymph node metastasis had more primary foci concentrated in the left interior lobe of the liver (segment IV) or the right anterior lobe of the liver (segments V and VIII), and their first hepatic invasion of the porta hepatis was more severe (69.7%) with larger lesion diameters. In addition, a high metastatic rate was observed with respect to the posterior pancreatic head lymph nodes (nine cases) and para-common hepatic artery lymph nodes (four cases), indicating that these three sites are important regional nodes. Para-aorta abdominalis lymph nodes, a distant lymph node pathway, mainly comprise three pathways converging from the porta hepatis, posterior pancreatic head, and deep lymphatic system around the hepatic vein. Herein, we observed five cases of para-aorta abdominalis lymph node metastases. In addition, we observed two cases of para-diaphragmatic

lymph node metastases attributed to the superficial lymphatic system on the liver's diaphragmatic surface that was distributed along the bilateral coronary ligaments, bilateral deltoid ligaments, and sickle ligaments. Hepatic AE lesions superior to the liver, breaching the hepatic tegument, can pass through the hepatic lymph directly through the diaphragm into the distal lymphatic system of the thoracic cavity, including the pericardial, diaphragmatic, and paraoesophageal lymphatic systems. In addition, AE lesions might also get dislodged and colonise the parietal, anterior, and posterior mediastinal lymph nodes with the thoracic lymphatic reflux when hepatic lesions invade the diaphragm and protrude into the thoracic cavity. Moreover, the posterior pancreatic head, para-common hepatic artery, para-celiac trunk, and para-aorta abdominalis lymph node metastases occurred separately in six, three, one, and three patients, respectively, indicating that AE can metastasize directly to the next-station or distant lymph nodes without passing through the first-station lymph nodes. This is because AE was not effectively terminated and eliminated at the first station lymph node, following which it spread and metastasised to the next station lymph node *via* the lymphatic flow. Multiple groups of lymph node metastasis were observed in four cases. The AE drained both ways through the deep and superficial hepatic lymphatic return system to different sites causing lymph node metastasis when there were large-diameter AE lesions that spanned the left and right lobes of the liver, from the top of the liver down to the porta hepatis and caudate lobes. Single-group, multiple-group or even jumping lymph node metastasis from deep and superficial lymphatic reflux pathways of the liver can occur, depending on the location, size, and vascular invasion of the lesion.

Lymph node metastases from hepatic AE resemble a special type of extrahepatic metastases. Intrahepatic lesions invading the right and left lobes of the liver are observed more frequently in patients with extrahepatic metastases. When the primary lesions are larger in diameter, they invade the intrahepatic and extrahepatic vessels, bile ducts, or other adjacent tissues and organs more aggressively[22]. Herein, 55 patients with a PNM stage of P3N1M0 or higher accounted for 72.7% of the cases, with most patients belonging to the intermediate or advanced stages of the disease. The mean maximum lesion diameter was 12.93 cm, and severe intrahepatic and extrahepatic vascular and biliary invasions were observed. No statistical difference was observed in terms of the sex of the 623 patients with hepatic AE in this study (χ^2 = 1.748, P = 0.186). The incidence of hepatic AE combined with lymph node metastases among the 55 patients was significantly higher among women (69.1%) than in men (χ^2 = 8.018, P = 0.005). Oestrogen receptors are present in the nucleus and envelope of some hormone-sensitive malignancies, and oestrogen binding to this receptor can directly participate in the gene transcriptional regulation of tumour cell survival and proliferation[23-24], stimulating cell proliferation, thereby enhancing their lymph node metastasis[25]. An association might exist between the high morbidity of female patients who developed lymph node metastasis in this study and the high oestrogen expression. Further clinical studies are warranted to confirm this conjecture.

Diagnosing lymph node metastases in hepatic AE based on preoperative imaging is challenging despite advances in imaging evaluation. Only large-diameter lymph node metastases and typical morphologies can be identified. Therefore, intraoperative exploration and postoperative histopathological examination are often required[7,26]. Kantarci *et al*[27] demonstrated that the USG, CT, and MRI assist in the radiological diagnosis of liver AE. Herein, a diagnostic rate of 100% for diagnosing hepatic AE using preoperative abdominal USG, MRI, and CT was reported, while the detection rate of lymph node metastasis was low (16.4%, 18.2%, and 30.9%, respectively). This might be attributed to the deeper location of the lesion, smaller diameter, or the lack of distinct boundaries between multiple lymph nodes and surrounding tissues after fusion. CT has a better diagnostic significance for lymph node metastasis compared with USG and MRI, as it can more accurately and qualitatively demonstrate lymph node metastasis. A round or ovoid shape, mixed density or hypointense foci, non-enhancement on an enhanced scan, and necrosis, nodules, and calcification in lymph nodes are the characteristic features of lymph nodes on a CT scan. Our centre believes that preoperative imaging focusing on the first porta hepatis region (regions surrounding the neck of gallbladder, para-common bile duct, and periportal vein), the second porta hepatis region (para-inferior vena cava), and retroperitoneal lymph nodes (para-aorta abdominalis) has diagnostic significance and can avoid missed diagnoses. Therefore, this step should be mandatory for preoperative evaluation.

Histopathological examination is the gold standard for diagnosing lymph node metastasis in hepatic AE. Identification of structures, such as multilocular echinococcus cyst, protoscolexes, or head hooks in lymph node histopathology sections or identifying the nucleotide sequence of Echinococcus multilocularis by polymerase chain reaction help in establishing a positive diagnosis[28]. Herein, 209 enlarged lymph nodes, 106 positive lymph nodes, and 103 inflammatory lymph nodes were identified postoperatively. Not all lymph nodes demonstrated positive results and inflamed enlarged lymph nodes were also present. This particular metastatic pattern might be associated with the body's strong and weak immune response stimulated when AE spreads *via* the lymphatic drainage to the lymph nodes in a region[29], resulting in local fibrosis and chronic inflammation. Chronic inflammation can weaken lymphatic vessel contraction, impede lymphatic flow to the subsequent station lymph nodes, and cause local lymphoedema. In addition, increased infiltration of macrophages, multinucleated giant cells, and lymphocytes can result in typical granulomatous changes[30,31]. Herein, the postoperative pathogenic diagnosis of patients highly suspected of intraoperative lymph node metastasis resulted in inflammatory enlargement. False-negative results could not be ruled out due to the diagnostic positivity, sensitivity, and specificity of unconfirmed conventional haematoxylin and eosin (H&E) staining. Therefore, immunohistochemistry is a crucial adjunctive diagnostic tool to support conventional histopathological examination when AE invasion is highly suspected in a biopsy tissue sample and when the H&E staining result is negative. Monoclonal antibody (mAb) Em^{2}G11 and mAb EmG3 are the two main antibodies that exist against multilocular echinococcus antigens[26,32]. Grimm *et al*[33] reported the presence of small particles of Echinococcus multilocularis (SPEMS) in the lymph node germ layer of patients with hepatic AE, and their immunohistochemical staining was strongly positive, along with the specificity of mAb. In addition, Hillenbrand *et al*[20] also detected SPEMS by immunohistochemical methods with mAb Em^{2}G11 in patients with negative H&E staining on histopathological examination. All histopathological examinations of the lymph node specimens in this group were performed under conventional H&E staining at high magnification. Some limitations

existed in the pathogenic diagnosis owing to the single examination modality. Therefore, histopathological examination methods need to be selected reasonably according to the actual situation to improve the lymph node metastasis diagnostic rate in patients with hepatic AE when intraoperative lymph node metastasis is highly suspected.

Radical liver resection combined with systemic regional lymph node dissection was performed on all 55 patients. The surgical feasibility of the combined procedure depends largely on the precise preoperative evaluation. The optimal surgical plan should be selected based on the size and location of the primary focus, the vascular and biliary invasion characteristics, the resectability of distant metastases, the location and number of lymph node metastases, their adjacency to important vasculature, and the patient's tolerance to the procedure. A better understanding of the multidirectional lymphatic reflux pathways in the liver is needed for targeted or prophylactic complete dissection of suspected lymph nodes when combining any surgical approach with systemic or regional lymph node dissection. Since positive and inflammatory enlarged lymph nodes cannot be accurately distinguished by intraoperative visual examination, pathologically evaluating the suspected lymph nodes together is essential. Lymph node dissection is performed by scraping and suction dissection, along with alternating electrocoagulation and pushing and peeling, to complete the skeletal dissection quickly and easily, with less bleeding and clear boundaries. In particular, the vascular sheath should be opened first and dissected close to the vessels to dissect the lymph nodes from the root and protect the vessels when the porta hepatis lymph nodes are in close proximity to blood vessels. In addition, all sections and/or margins should be treated with electrocoagulation to eliminate any residual AE, and the vessel wall is susceptible to localised effusion or secondary infection after debulking due to the loss of protection by the surrounding connective tissue, increasing the risk of late postoperative bleeding. Therefore, the porta hepatis should be routinely drained after surgery. End-stage hepatic AE changes occurred in 22 patients, making conventional surgery incurable[34]. Therefore, we performed *ex vivo* liver resection with autologous liver transplantation combined with regional lymph node dissection. Mastering organ transplantation procedures will also be a must as the foundation for combined surgical treatment when patients with lymph node metastases from hepatic AE have complex lesions that make conventional surgery inapplicable.

Serious intraoperative complications did not occur in any of the patients in this study. Postoperatively, the liver and other organs recovered well without any technique-related complications. After recovery, all patients were discharged. Clavien-Dindo grade IIIa complication occurred in 14 patients owing to high surgical trauma, preoperative jaundice, abnormal function of multiple organs, and intraoperative combined organ resection. The operation time was relatively prolonged due to lymph node dissection, while there was no association between postoperative complications and lymphatic dissection, consistent with the findings of previous studies[20]. In addition, lymph node metastasis generally occurs in para-hepatoduodenal ligament, and it can further metastasise to the posterior pancreatic head, para-common hepatic artery, or the celiac trunk, and subsequently to the para-inferior vena cava and para-aorta abdominalis lymph nodes. Pancreatic head invasion and biliary obstruction occur as a result of rapid lymph node growth that metastasises to the posterior pancreatic head[17], thereby increasing the surgical difficulty and the risk of bleeding, biliary leakage, and pancreatic fistula. Therefore, the lymph nodes are cautiously stripped by the surgeon while clearing them in strict accordance with the anatomical approach. In addition, large-diameter metastatic lymph nodes in the first hepatic porta hepatis that cause fusion can severely invade the porta hepatic structures and cause serious complications, namely portal vein spongiform degeneration and portal hypertension. Wang *et al*[35] proposed such patients can be treated safely and effectively by radical hepatectomy combined with revascularisation. Multidisciplinary collaboration can be employed to perform combined multiorgan radical resection while diagnosing and treating patients with multiorgan complex hepatic AE combined with lymph node metastasis[36]. Suturing or repair techniques should be strictly controlled to prevent postoperative stenosis or thrombosis in patients with severe vascular and biliary tract invasion requiring repair or reconstruction. Herein, portal or hepatic vein reconstruction, portal or inferior vena cava vascular grafting, bile duct repair, and formation (27.3%), and bilioenteric anastomosis (12.7%) were performed in seven (12.7%), seven (12.7%) (six cases with autologous vessels and one case with artificial vessels), 15 (27.3%), and seven (12.7%) patients, respectively. Applying the enhanced recovery after surgery protocol for postoperative patient management can also lower the occurrence of complications, accelerate liver functional recovery, and achieve rapid recovery[37].

The recurrence rate in this study was 1.8% (recurrence in one patient). *In situ* liver recurrence occurred without reinfection at the site of lymph node dissection or in the extrahepatic organs. The "infiltrative zone" where the lesion was actively proliferating was not eliminated by radical hepatectomy, resulting in recurrence at the hepatic margin. Clinical cure without disease recurrence was achieved in the remaining patients with combined regular oral albendazole. Patients who underwent revascularisation or replacement took long-term oral anticoagulants postoperatively, and complications such as thrombosis did not occur. Herein, one patient reported a 9-year history of recurrence 8 years after the previous partial hepatectomy with severe complications, such as lymph node metastasis and portal vein spongiform degeneration, and a clinical cure was achieved after this treatment. Therefore, radical hepatectomy combined with regional lymph node dissection effectively lowers the risk of disease recurrence, progression, and adjacent organ invasion.

CONCLUSION

In summary, lymph node metastasis is a specific type of metastasis in hepatic AE, which has a female predilection and has a higher incidence in intermediate to advanced hepatic AE. AE can involve single or multiple groups of lymph nodes through the unique lymphatic reflux of the liver and has a jumping metastatic characteristic. Metastases to para-hepatoduodenal ligament lymph nodes occur frequently. Therefore, intraoperative clearance of these lymph nodes should be routinely performed. Postoperative histopathological examination is the gold standard for diagnosis. Basic principles of individualised treatment should be followed while treating hepatic AE with lymph node metastasis. A

comprehensive preoperative evaluation should be performed. In addition, the most appropriate surgical approach should be selected based on rigorous planning and multidisciplinary collaboration. Radical hepatectomy combined with regional lymph node dissection is safe, feasible, and effective for treating hepatic AE combined with lymph node metastasis and is worthy of widespread clinical application.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Establishing and clinically validating a machine learning model for predicting unplanned reoperation risk in colorectal cancer

Li-Qun Cai, Da-Qing Yang, Rong-Jian Wang, He Huang, Yi-Xiong Shi

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Abstract

BACKGROUND

Colorectal cancer significantly impacts global health, with unplanned reoperations post-surgery being key determinants of patient outcomes. Existing predictive models for these reoperations lack precision in integrating complex clinical data.

AIM

To develop and validate a machine learning model for predicting unplanned reoperation risk in colorectal cancer patients.

METHODS

Data of patients treated for colorectal cancer (n = 2044) at the First Affiliated Hospital of Wenzhou Medical University and Wenzhou Central Hospital from March 2020 to March 2022 were retrospectively collected. Patients were divided into an experimental group (n = 60) and a control group (n = 1984) according to unplanned reoperation occurrence. Patients were also divided into a training group and a validation group (7:3 ratio). We used three different machine learning methods to screen characteristic variables. A nomogram was created based on multifactor logistic regression, and the model performance was assessed using receiver operating characteristic curve, calibration curve, Hosmer-Lemeshow test, and decision curve analysis. The risk scores of the two groups were calculated and compared to validate the model.

RESULTS

More patients in the experimental group were ≥ 60 years old, male, and had a history of hypertension, laparotomy, and hypoproteinemia, compared to the

control group. Multiple logistic regression analysis confirmed the following as independent risk factors for unplanned reoperation ($P < 0.05$): Prognostic Nutritional Index value, history of laparotomy, hypertension, or stroke, hypoproteinemia, age, tumor-node-metastasis staging, surgical time, gender, and American Society of Anesthesiologists classification. Receiver operating characteristic curve analysis showed that the model had good discrimination and clinical utility.

CONCLUSION

This study used a machine learning approach to build a model that accurately predicts the risk of postoperative unplanned reoperation in patients with colorectal cancer, which can improve treatment decisions and prognosis.

Key Words: Colorectal cancer; Postoperative unplanned reoperation; Unplanned reoperation; Clinical validation; Nomogram; Machine learning models

Core Tip: This study developed a machine learning model to predict unplanned reoperations in colorectal cancer patients, using data from two hospitals over two years. It employed support vector machine, least absolute shrinkage and selection operator, and extreme gradient boosting for feature selection and logistic regression to identify key risk factors. The model showed good predictive accuracy, validated by receiver operating characteristic curves, calibration curves, and decision curve analysis. Key predictors included age, gender, prior surgeries, and nutritional status. This predictive tool aims to enhance clinical decision-making, reduce reoperation rates, and improve patient outcomes in colorectal cancer care.

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INTRODUCTION

According to the World Health Organization, colorectal cancer is one of the most common malignant tumors of the digestive tract[1]. In 2018, there were more than 1.8 million cases of colorectal cancer globally, with a total of 881000 deaths — an average of 1 death out of every 10 cases[2]. Colorectal cancer is one of the top three cancer contributors to morbidity and mortality rates in the world[3]. Colorectal cancer poses a significant threat to the physical and mental health of the Chinese population. Early diagnosis of colorectal cancer in China is generally poor, and the majority of patients are in the middle-to-late stage of disease at the time of diagnosis[4]. Postoperative recurrence and metastasis of colorectal cancer are influenced by multiple factors such as lymph node metastasis, tumor type, growth location, and degree of infiltration. These factors are also key in determining the prognosis of patients with colorectal cancer[5].

Colorectal cancer is a serious malignant tumor and its treatment can include surgery, radiation therapy, chemotherapy, molecular-targeted therapy, immunotherapy, endocrinotherapy, and traditional Chinese medicine[6]. Currently, a combination approach based on surgery is the preferred strategy for the treatment of colorectal cancer[7]. Common surgical methods include radical surgery. However, in recent years, laparoscopy has been widely adopted due to its rapid recovery time, minimal trauma, and significant short-term efficacy[8].

Postoperative reoperation, particularly the rate of unplanned reoperation within 30 d, is an important indicator of surgical quality and has been adopted by the United States Centers for Medicare and Medicaid Services in its Physician Quality Reporting System[9]. Due to the high morbidity and mortality of colorectal cancer, patients undergoing surgery are at risk of later reoperation. The percentage of postoperative unplanned reoperation in patients with colorectal cancer ranges from 3% to 11%[10,11]. The causes of reoperation include complications such as anastomotic leakage, bowel obstruction, and postoperative bleeding. Understanding the causes of reoperation helps improve patient prognosis. Despite improvements in surgical techniques and perioperative management, postoperative unplanned reoperation is still closely associated with complications[12]. These complications not only affect the short-term prognosis of the patient but may also apply surgical stress on the immune system, affecting postoperative outcomes. Unplanned reoperation is an independent predictor of a patient's mortality within one year of surgery[13].

Machine learning has great potential for disease risk prediction and diagnosis. In colorectal cancer, machine learning models can accurately predict the risk of undesired postoperative return to surgery by comprehensively analyzing multidimensional data on surgical approaches, and a patient's clinical characteristics and comorbidities[14]. The ability of such techniques to learn and adapt to new data means that their predictive accuracy continues to improve over time and data accumulation, reducing unnecessary reoperations, optimizing patient prognosis, and improving quality of life.

The purpose of this study is to establish and validate a model of colorectal cancer postoperative unplanned re-operation. This model combines multidimensional data including patient clinical characteristics; surgical modalities, and comorbidities, to improve prediction accuracy. This model will help physicians to more accurately assess patient

postoperative risk, optimize treatment decisions, and reduce unplanned reoperation. This will ultimately improve patient prognosis and quality of life while reducing the economic burden of colorectal cancer on the healthcare system.

MATERIALS AND METHODS

Sample collection

Clinical data of patients with colorectal cancer admitted to the First Hospital of Wenzhou Medical University and Wenzhou Central Hospital from March 2020 to March 2022 were retrospectively collected. This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University and the Medical Ethics Committee of Wenzhou Municipal Central Hospital, No. KY2024-R016.

Inclusion and exclusion criteria

Inclusion criteria: (1) Preoperative pathological findings confirmed the diagnosis of colorectal cancer[15]; (2) laparoscopic radical resection of the primary lesion; and (3) combined with distant metastases only radical resection of the primary lesion.

Exclusion criteria: (1) Open surgery and intermediate open surgery; (2) intraoperative exploration found extensive metastases that could not be resected and only palliative surgery was performed; (3) resection of multiple bowel segments of both primary tumors or total or subtotal colectomy; (4) combined distant metastases were performed with simultaneous resection of the lesions; and (5) missing or incomplete clinical data.

Sample screening

We collected a total of 2948 patient records treated at the First Affiliated Hospital of Wenzhou Medical University and Wenzhou Central Hospital. According to the inclusion criteria, a total of 2484 samples met the requirements, and we excluded 440 samples. A total of 2044 samples were included. The patients with unplanned reoperation presenting at 30 d were assigned to the experimental group (n = 60). Among the 60 patients, 34 patients had anastomotic leakage, 21 patients had bowel obstruction, and 5 patients had abdominal cavity infection. The remaining patients were placed into the control group (n = 1984). To validate our model, we divided the patients into a training group (n = 1429) and a validation group (n = 615) based on a ratio of 7:3. Figure 1 depicts a flow chart of the process.

Clinical data collection

From the electronic medical records, we collected the clinical data of all patients, including age, gender, body mass index, history of hypertension, history of diabetes, history of stroke, history of laparotomy, preoperative hypoproteinemia, tumor site, history of preoperative radiotherapy, history of preoperative chemotherapy, tumor-node-metastasis (TNM) stage, American Society of Anesthesiologists (ASA) classification, surgical time, intraoperative bleeding, and preoperative prognostic and preoperative prognostic nutritional index (PNI).

Machine learning models

To efficiently screen feature variables associated with colorectal cancer postoperative unplanned reoperation, we used three different machine learning methods: support vector machine (SVM)[16] least absolute shrinkage and selection operator (LASSO) regression[17], and extreme gradient boosting (XGBoost)[18].

The SVM method effectively distinguishes between two classes of data points (*i.e.*, patients with or without unplanned reoperation) by finding an optimal hyperplane in a high-dimensional space. SVM is particularly effective when dealing with large datasets because it can work with high-dimensional feature spaces and nonlinear classification problems.

LASSO regression is particularly useful for feature selection as it reduces the coefficients of unimportant features to zero. This method limits the complexity of the model by adding a regularization term to avoid overfitting, while still identifying the most relevant features.

XGBoost is an integrated learning method based on decision trees, which improves prediction accuracy by constructing multiple models and combining them. It is an effective feature selection method as it optimizes the performance of the model through a gradient-boosting framework.

Model evaluation tools

To fully evaluate our unplanned reoperation, we used the following key statistical tools. The receiver operating characteristic curve (ROC) was used to assess the model's ability to discriminate between two types of outcomes (*e.g.*, occurrence and non-occurrence of unplanned reoperation). The more diagnostic the model is, the closer the area under the curve (AUC) is to 1. We also used calibration curves to test the accuracy of the model's predicted outcomes. Ideally, the calibration curve should be close to 45 degrees, showing a high degree of agreement between predicted and actual values. The Hosmer-Lemeshow test (H-L test) was used to assess the fit of the model. A high P value implies a good agreement between model predictions and actual observations. Decision curve analysis (DCA) was used to assess the utility of the model in clinical decision-making, as it identifies the thresholds at which the use of the model best improves patient care.

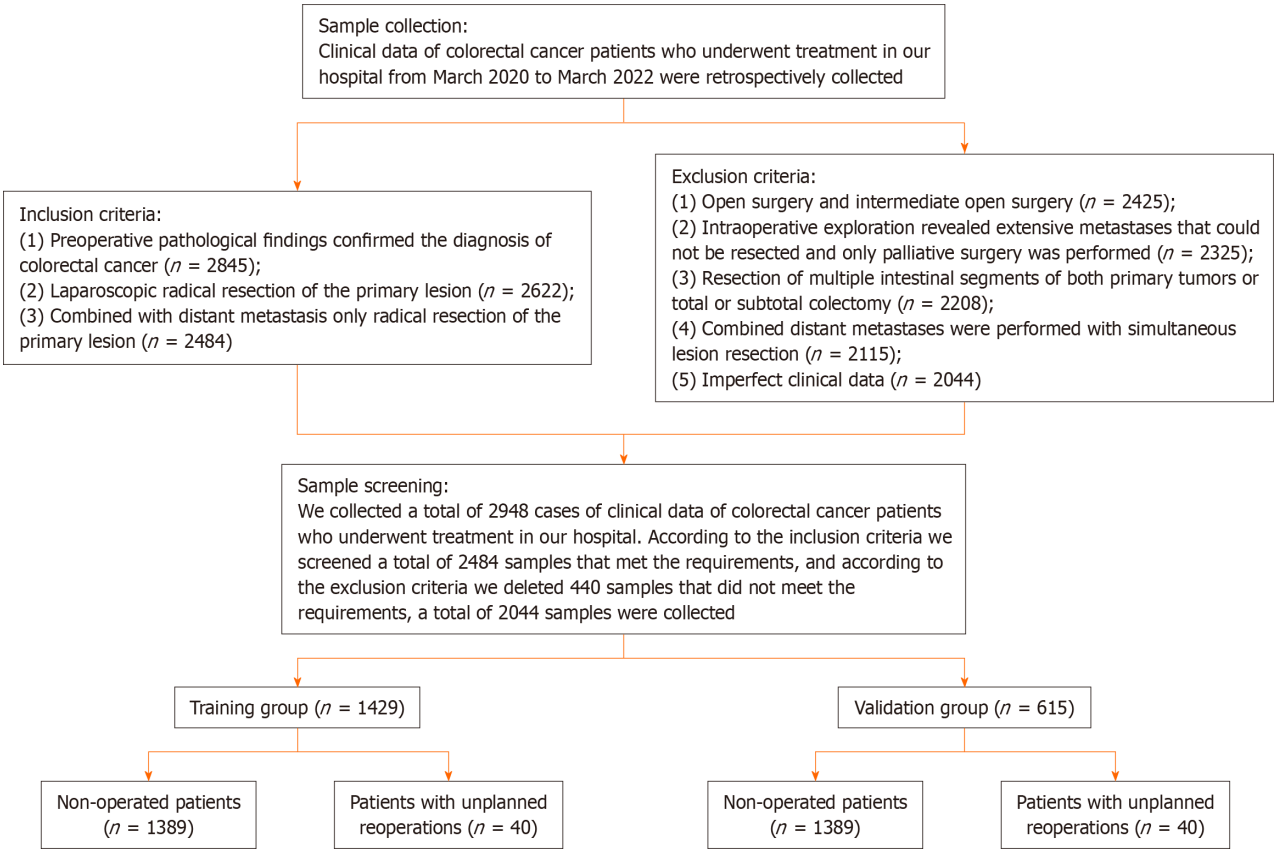

Figure 1 Sample screening flow chart.

Measurement of results

Measurement of results: (1) The differences in clinical data between the control and experimental groups were compared; (2) SVM, LASSO, and XGBoost were used to screen for unplanned reoperation feature variables, and a Venn diagram was used to identify common feature variables; (3) Independent risk factors for postoperative unplanned reoperation were screened using logistic regression; (4) A nomogram was created based on the multifactorial logistic regression; (5) ROC curve, calibration curve, H-L test, and DCA were used to evaluate the differentiation, calibration, and clinical utility of the nomogram; and (6) Based on the risk coefficients, the risk scores of patients in the training and the validation groups were calculated. The differences in the risk scores of the patients were compared, and the predictive effect of the model was verified using the ROC.

Statistical analysis

Statistical analysis was carried out using SPSS 26.0 software. For normally distributed continuous data, used mean ± SD. Comparisons between groups were made using *t*-tests. The χ^2 test was used for count data. We screened all variables using SVM, LASSO, and XGBoost, and the common variables were screened using a Venn diagram. Multiple logistic regression analysis of the common variables was used to identify the independent risk factors. Then, we constructed a nomogram prediction model based on the selected independent risk factors using R software and the rms package. We obtained the calibration curve using Bootstrap and calculated the C-index. We also plotted the independent risk factors using ROC and calculated the AUC to validate the performance of the nomogram prediction model.

RESULTS

Comparison of clinical data

Comparison of the clinical data of the two groups showed that the number of patients in the experimental group aged ≥ 60 years, male, with a history of hypertension, a history of laparotomy and hypoproteinemia, and surgical time ≥ 240 mins was significantly higher than that of patients in the control group. The PNI of patients in the experimental group was also significantly higher than that of patients in the control group ($P < 0.05$; Table 1). The remaining variables were not statistically different ($P > 0.05$).

Machine learning models screening unplanned reoperation feature variables

We screened the unplanned reoperation feature variables using XGBoost, SVM, and Lasso methods (Figure 2). We found

Table 1 Comparison of the clinical data of the two groups of patients

	Control group (*n* = 1984)	Experiment group (*n* = 60)	χ^2/t	*P* value
Age (yr)				
≥ 60	1151	48	11.609	< 0.001
< 60	833	12		
Gender				
Male	1131	43	5.120	0.024
Female	853	17		
BMI (kg/m^2)				
≥ 24	1250	36	0.225	0.635
< 24	734	24		
History of hypertension				
Yes	615	27	5.300	0.021
No	1369	33		
History of diabetes				
Yes	238	6	0.221	0.639
No	1746	54		
History of stroke				
Yes	238	9	0.495	0.482
No	1746	51		
History of laparotomy				
Yes	337	20	10.797	0.001
No	1647	40		
Hypoproteinemia				
Yes	181	12	8.058	0.005
No	1803	48		
Location of the tumor				
Colon	1250	36	0.225	0.635
Rectum	734	24		
History of preoperative radiation therapy				
Present	198	7	0.184	0.668
No present	1786	53		
History of preoperative chemotherapy				
Present	238	8	0.098	0.753
No present	1746	52		
TNM staging				
I + II	1171	32	0.778	0.378
III + IV	813	28		
ASA classification				
I + II	1786	51	1.613	0.204
III + IV	198	9		
Surgical time (min)				
≥ 240	675	30	6.580	0.010

< 240	1309	30		
Intraoperative bleeding (mL)				
≥ 120	218	4	1.123	0.289
< 120	1766	56		
PNI	46.21 ± 5.15	38.97 ± 4.75	10.749	< 0.001

TNM: Tumor-node-metastasis; BMI: Body mass index; ASA: American Society of Anesthesiologists; PNI: Prognostic nutritional index.

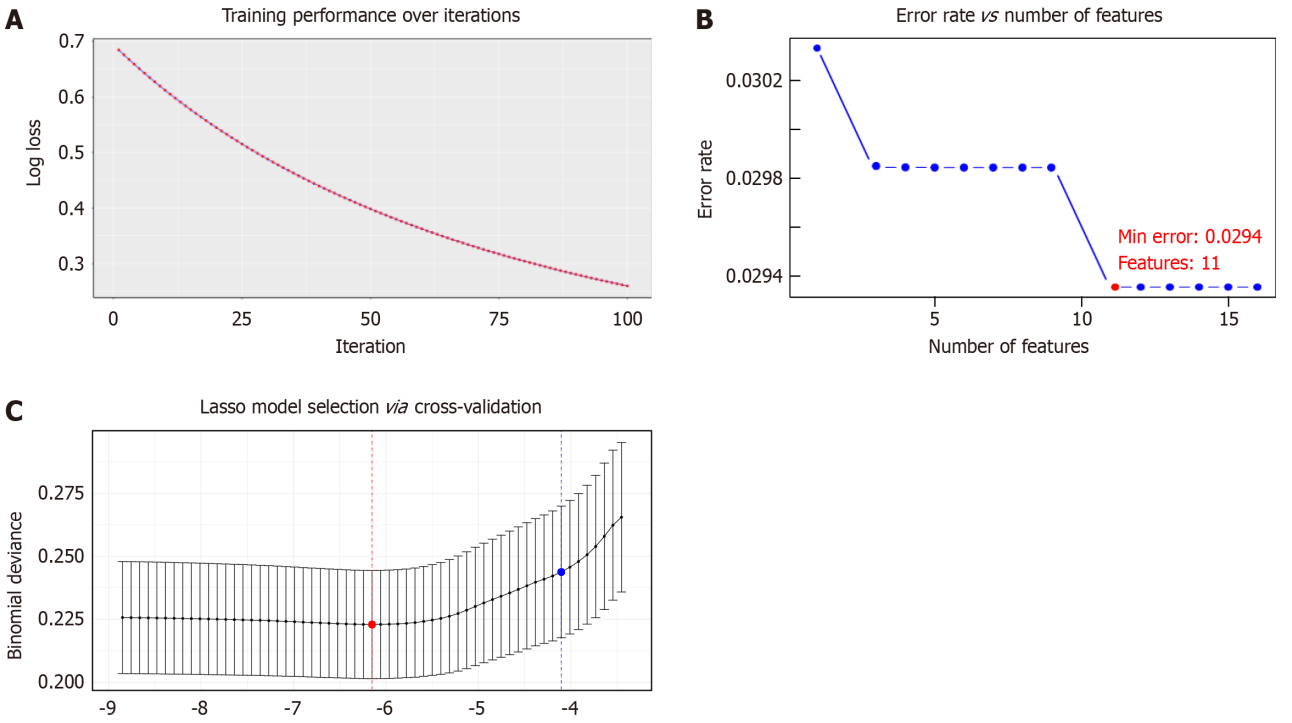

Figure 2 Comparative analysis of model performance and complexity across feature selection and regularization techniques. A: Training performance *vs* model complexity; B: Error rate *vs* number of features; C: Lasso model selection *via* cross-validation.

that XGBoost identified a total of 13 feature variables (Figure 3A), SVM identified 16 feature variables (Figure 3B), and Lasso identified 11 feature variables (Figure 3C). Using a Venn diagram (Figure 3D), we found that the 3 methods screened 10 common characteristic variables: PNI, history of laparotomy, hypoproteinemia, age, TNM staging, history of hypertension, surgical time, gender, history of stroke, and ASA classification.

Logistic regression screening for independent risk factors for unplanned reoperation

We analyzed the 10 identified signature variables using multifactor logistic regression. The 10 signature variables were first assigned values (Supplementary Table 1). The resulting analysis revealed that age, gender, history of hypertension, history of laparotomy, hypoproteinemia, and PNI were independent risk factors impacting the likelihood of unplanned reoperation ($P < 0.05$; Table 2).

Establishment of a nomogram

A nomogram prediction model was created based on the 10 predictors (age, gender, history of hypertension, history of laparotomy, hypoproteinemia, and PNI). The final prediction model equation was: Logit (P) = -6.8730575 + Age × 1.108872309 + Gender × 0.737188569 + History_of_hypertension × 0.619231168 + History_of_ History_of_hypertension × 0.619231168 + History_of_hypertension × 0.917723145 + Hypoproteinemia × 0.983183577 + PNI × 2.48620524.

The total score was obtained by summing the scores of each variable and finding the corresponding value on the "Total Score Axis". The value of the "Total Score Axis" was compared with the probability prediction line at the bottom of the nomogram to find the risk of postoperative unplanned reoperation (Figure 4).

Evaluation of nomogram

The differentiation, calibration, and clinical utility of the model were evaluated by four methods: ROC, calibration curve, H-L test, and DCA. The ROC analysis revealed that the AUC of the nomogram was 0.842, with 80.59% specificity, 76.67% sensitivity, and 57.26% Youden index (Figure 5A). This indicates that the model has a good degree of discrimination and

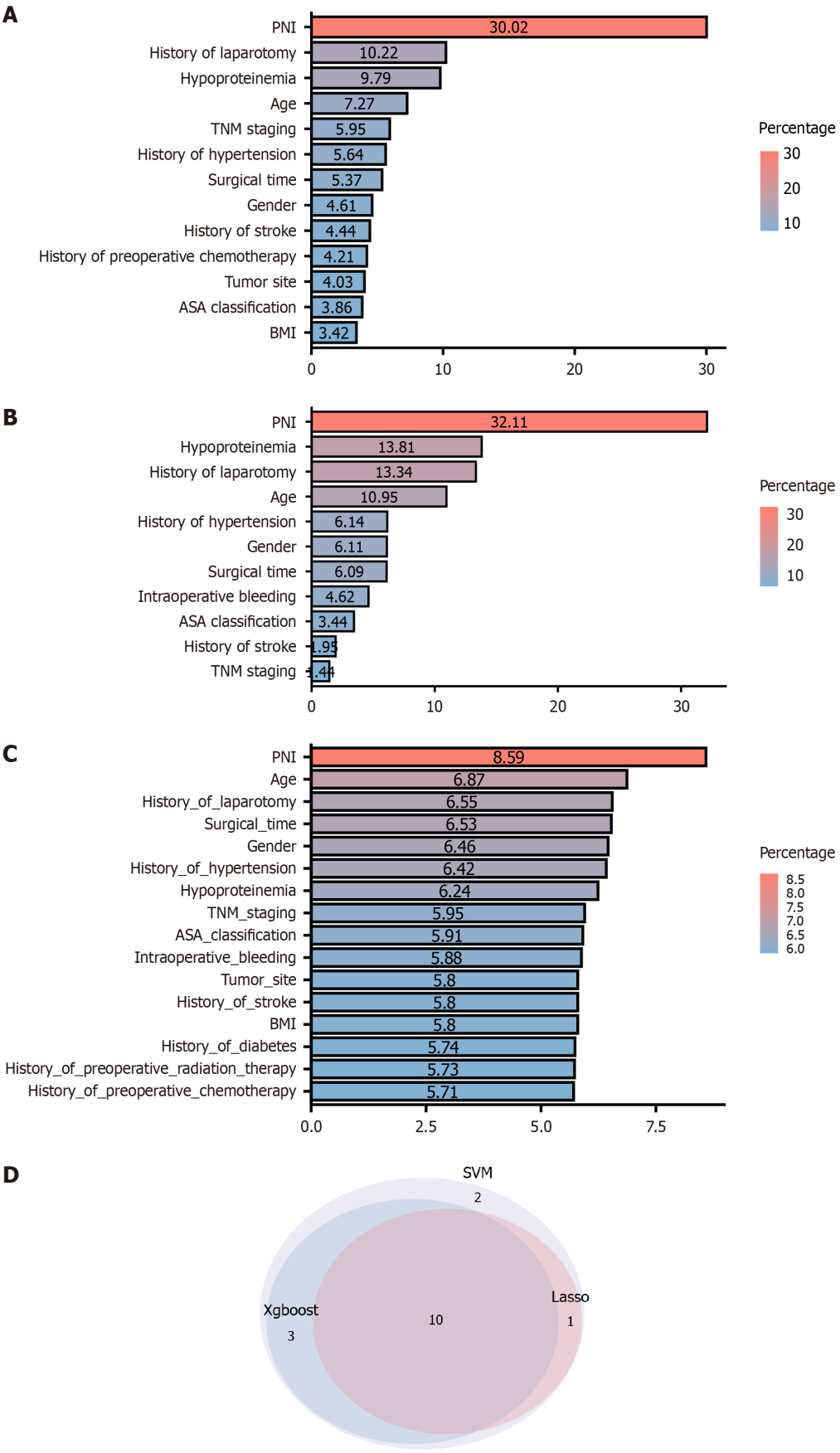

Figure 3 Signature variables of unplanned reoperation screened by machine learning. A: Extreme gradient boosting filtered unplanned reoperation feature variable; B: Supported vector machine filtered unplanned reoperation feature variable; C: Lasso filtered unplanned reoperation feature variable; D: Feature variables common to all three learning models Venn plot. TNM: Tumor-node-metastasis; BMI: Body mass index; ASA: American Society of Anesthesiologists; PNI: Prognostic nutritional index; SVM: Supported vector machine.

Table 2 Multiple logistic regression analysis

	β	SE	χ^2	*P* value	OR	95%CI	
						Lower	Upper
Age	1.072	0.335	10.268	0.001	2.922	1.516	5.63
Gender	-0.717	0.301	5.674	0.017	0.488	0.27	0.881
History of hypertension	0.592	0.278	4.545	0.033	1.807	1.049	3.115
History of stroke	0.306	0.392	0.61	0.435	1.358	0.63	2.926
History of laparotomy	0.939	0.297	9.986	0.002	2.557	1.428	4.576
Hypoproteinemia	0.918	0.357	6.59	0.010	2.504	1.242	5.045
TNM staging	-0.286	0.275	1.081	0.299	0.751	0.438	1.288
ASA classification	-0.308	0.396	0.605	0.437	0.735	0.339	1.597
Surgical time	0.509	0.276	3.387	0.066	1.663	0.967	2.859
PNI	2.476	0.369	45.073	< 0.001	11.894	5.773	24.505

TNM: Tumor-node-metastasis; ASA: American Society of Anesthesiologists; PNI: Prognostic nutritional index.

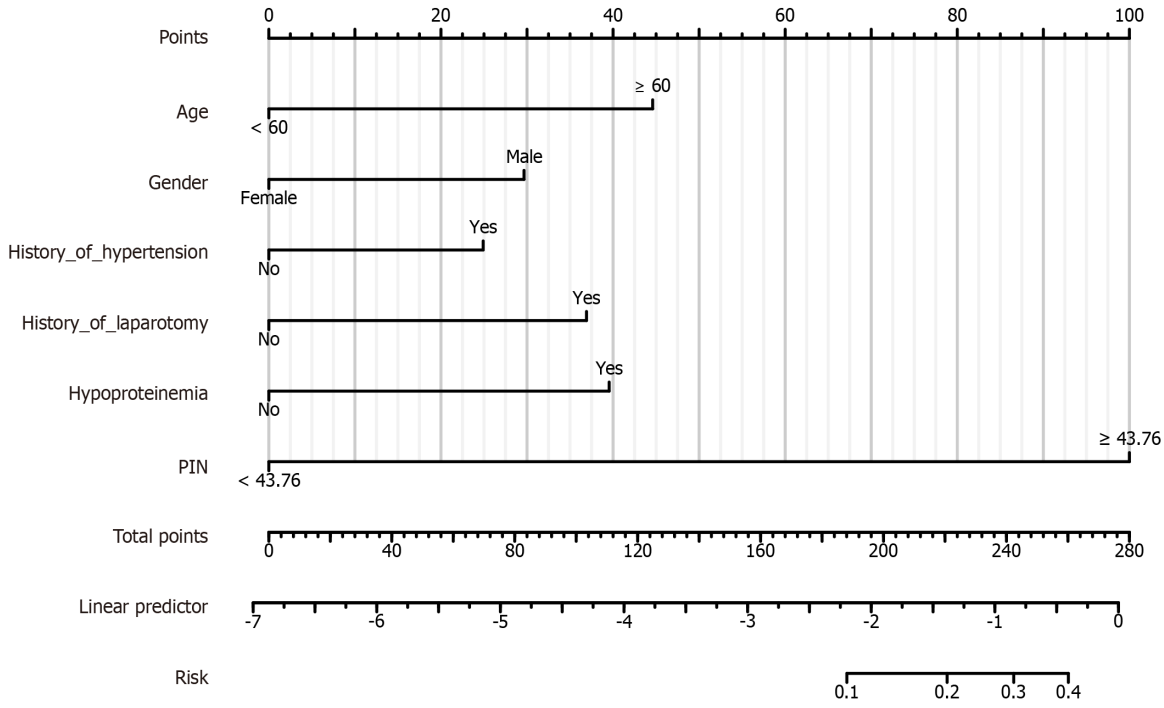

Figure 4 Nomogram of postoperative unplanned reoperation in colorectal cancer. PNI: Prognostic nutritional index.

can correctly distinguish the ending event from the non-ending event. Calibration curve analysis found that the nomogram's calibration curve had a slightly poorer overlap, but generally went in the same direction (Figure 5B). The H-L test value was 8.588 (P = 0.378). The DCA curve indicated that the unplanned reoperation net benefit rate was higher than other, *i.e.*, the blue line corresponding to the threshold probability was located to the upper right of the All line (red line), indicating that the model had some clinical utility (Figure 5C).

Validation of nomogram

We divided the data into a training group and a validation group. The risk scores were calculated separately for both groups and then validated using ROC, calibration curve, H-L test, and DCA. As before, we compared the baseline information of patients in the training group with those in the validation group. The results showed that there was no statistically significant difference between the baseline characteristics of patients in the training group and the validation group (P > 0.05; Table 3). We then calculated the risk scores of the two groups, and the results showed that the risk scores of the patients who underwent unplanned reoperation were higher than those of patients in the non-reoperation group,

Table 3 Comparison of baseline data between training group and validation group

	Training group (*n* = 1429)	Validation group (*n* = 615)	χ^2	*P* value
Reoperation				
Yes	40	20	0.309	0.578
No	1389	595		
Age (yr)				
≥ 60	853	346	2.088	0.148
< 60	576	269		
Gender				
Male	817	357	0.135	0.713
Female	612	258		
BMI (kg/m^2)				
≥ 24	891	395	0.649	0.421
< 24	538	220		
History of hypertension				
Yes	451	191	0.051	0.822
No	978	424		
History of diabetes				
Yes	164	80	0.959	0.327
No	1265	535		
History of stroke				
Yes	174	73	0.038	0.845
No	1255	542		
History of laparotomy				
Yes	248	109	0.041	0.840
No	1181	506		
Hypoproteinemia				
Yes	144	49	2.238	0.135
No	1285	566		
Location of the tumor				
Colon	894	392	0.256	0.613
Rectum	535	223		
History of preoperative radiation therapy				
Present	144	61	0.012	0.913
No present	1285	554		
History of preoperative chemotherapy				
Present	173	73	0.023	0.880
No present	1256	542		
TNM staging				
I + II	825	378	2.471	0.116
III + IV	604	237		
ASA classification				
I + II	1281	556	0.275	0.600

III + IV	148	59		
Surgical time (min)				
≥ 240	476	229	2.933	0.087
< 240	953	386		
Intraoperative bleeding (mL)				
≥ 120	145	77	2.502	0.114
< 120	1284	538		
PNI				
≥ 43.76	487	207	0.034	0.854
< 43.76	942	408		

TNM: Tumor-node-metastasis; BMI: Body mass index; ASA: American Society of Anesthesiologists; PNI: Prognostic nutritional index.

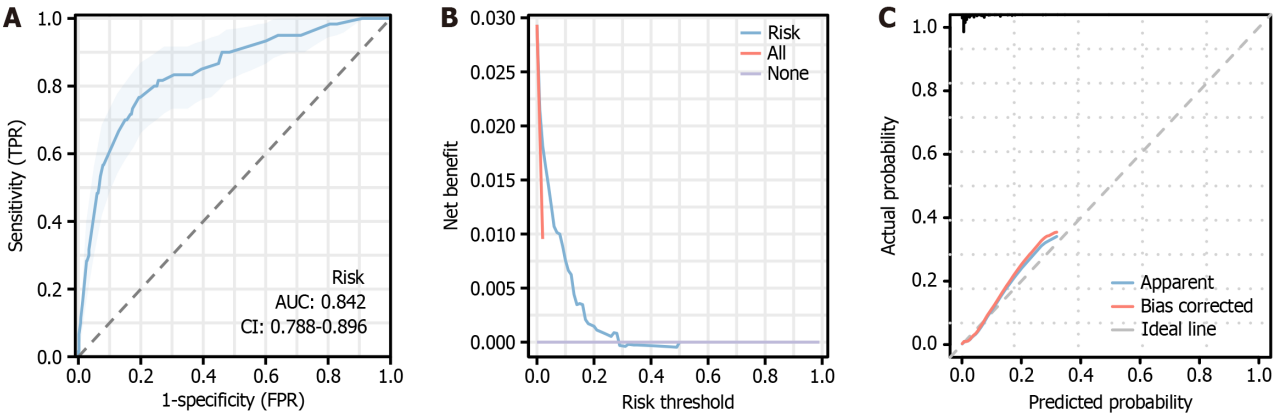

Figure 5 Evaluation and validation of nomogram. A: Receiver operating characteristic curve of postoperative unplanned reoperation in colorectal cancer; B: Calibration curve of postoperative unplanned reoperation in colorectal cancer; C: Decision curve analysis curve of postoperative unplanned reoperation in colorectal cancer.

both in the training and validation group ($P < 0.001$; Figure 6). Finally, we found that the AUC of patients in the training group and the validation group were 0.798 and 0.846, respectively (Figure 7). This suggests that the model can correctly differentiate between the outcome and non-outcome events.

Clinical validation of predictive modeling

To validate our model, we randomized the clinical data of 1 patient with unplanned reoperation. This patient was aged ≥ 60 years, male, had no history of hypertension, no history of laparotomy, hypoproteinemia, and his PNI was ≥ 43.76. The probability of occurrence was calculated for this patient (45 + 30 + 0 + 0 + 39 + 100 = 216). The results showed that the probability of the patient having unplanned reoperation was about 73% (Figure 8).

DISCUSSION

Treatment of colorectal cancer through laparoscopy allows comprehensive observation; clear peeling and resection of the lesion, as well as procedures such as hemostasis and lymph node dissection[19]. Laparoscopy has a low impact on the patient's abdominal cavity, reduces postoperative pain, and promotes recovery of gastrointestinal function[20]. However, despite the improved precision and safety of laparoscopy, unplanned reoperation remains a challenge for colorectal cancer outcomes[21]. Reoperation not only prolongs the hospital stay and increases the financial burden of the disease, but also it affects the subsequent treatment plan and significantly increases the perioperative morbidity and mortality rate [22]. Therefore, investigation of the causes and risk factors of postoperative reoperation in colorectal cancer has important clinical applications in reducing the rate of reoperation.

The absence of a standardized definition for unplanned reoperation has resulted in notable variations in the reported endpoint indicators for postoperative colorectal cancer across different medical centers. In a study by Feo and colleagues, covering 92 hospitals in China, the average reoperation rate for colorectal cancer surgeries was 9.7%[23]. Unplanned reoperation's discrepancies were primarily attributed to disparities in medical resources and treatment approaches,

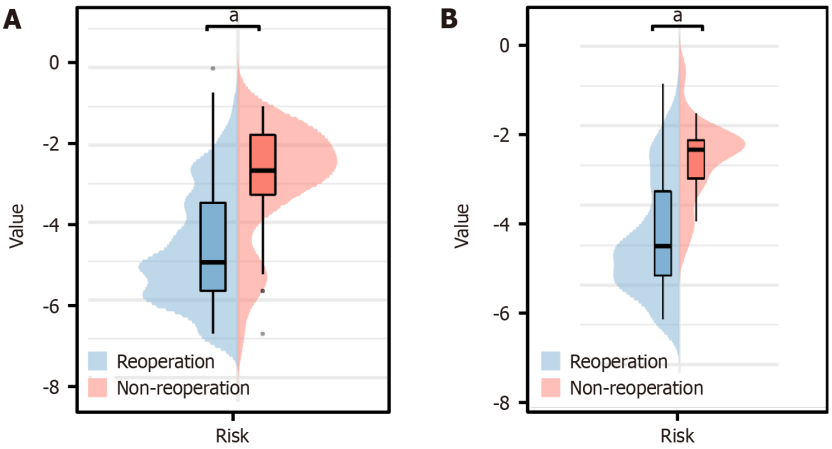

Figure 6 Calculation of risk score for training group and validation group patients. A: Calculation of patient risk score for training group; B: Calculation of patient risk score for validation group. $^{a}P < 0.001$.

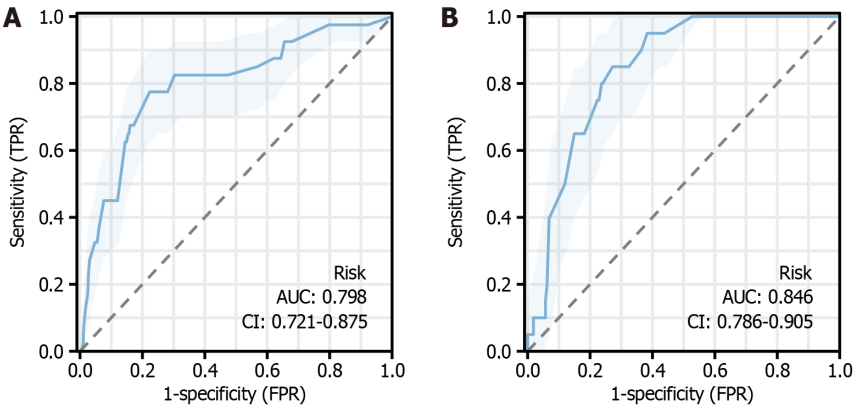

Figure 7 Receiver operating characteristic curve of training group and validation group patient risk scores in predicting patient reoperation. A and B: Receiver operating characteristic curve of risk score in predicting patient reoperation for (A) training group patients and (B) validation group patients.

which influence the risk of postoperative unplanned reoperations across various levels and regions of healthcare institutions. In contrast, the incidence of unplanned reoperations following laparoscopic surgery for patients with colorectal cancer was 2.94%. Our results generally align with the laparoscopic reoperation rate for bowel cancer (approximately 3.8%) reported by Speicher *et al*[24]. These observations reinforce the efficacy and safety of laparoscopic surgery as a preferred treatment option for colorectal surgical interventions.

Patients with colorectal cancer undergoing abdominal surgery have a higher incidence of unplanned postoperative reoperation compared to other general surgical procedures[25] due to their susceptibility to incisional and abdominal infections, venous thromboembolism, and perioperative complications[26,27]. In addition, the inherent necessity of reconstructing abdominal organs during colorectal surgery increases the likelihood of postoperative complications, thereby increasing the likelihood of subsequent reoperations.

This study aimed to develop a predictive nomogram model. To construct this predictive model, we first employed three advanced computational techniques: SVM[28], LASSO[29], and XGBoost[30]. These methods are known for their efficacy in managing high-dimensional datasets and their ability to identify critical variables in such datasets[18]. Specifically, SVM excels at handling a wide range of datasets, LASSO mitigates overfitting through a penalty-based approach, and XGBoost is particularly effective at dealing with nonlinear relationships between data points. This multifaceted methodological framework facilitates a robust assessment of the significance of variables from multiple analytical perspectives[31]. After identifying essential variables through these preliminary methods, we applied logistic regression analysis to investigate these identified variables. This analysis allowed us to identify independent risk factors that significantly impacted the probability of unplanned reoperation. Our findings suggest that age, gender, prior hypertension, history of laparotomy, hypoproteinemia, and PNI are key independent risk factors. These insights provide an understanding of the patient-specific risks associated with unplanned reoperation after colorectal cancer surgery and contribute to the clinical decision-making process.

Recent studies have identified the male gender as an independent risk factor for unplanned reoperation[32,33]. This correlation is likely attributable to the male physiology, lifestyle habits, and adherence to postoperative rehabilitation protocols. Li *et al*[34] also highlighted age as a determinant, positing that elderly patients are at an elevated risk of

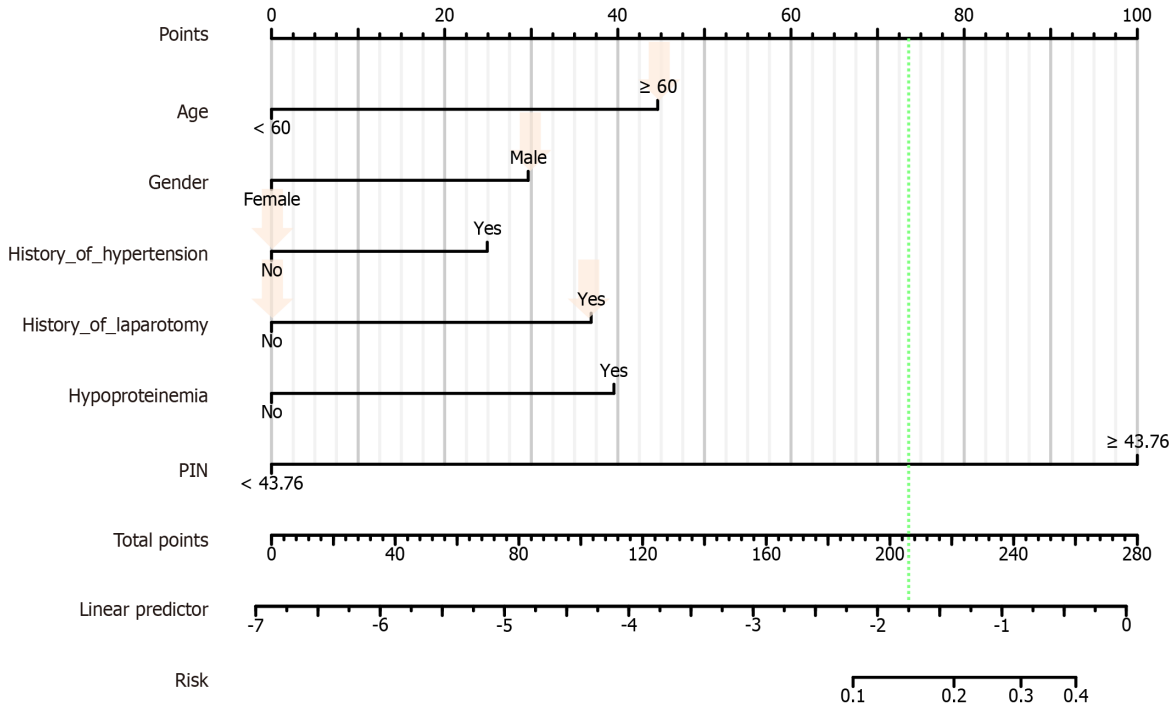

Figure 8 Clinical validation of predictive modeling. The green dashed line is the patient total score and incidence probability marker, and the light orange arrow is the patient risk factor marker. PNI: Prognostic nutritional index.

undergoing unplanned reoperations, a conclusion that aligns with our observations. While not directly causing complications, the presence of comorbidities significantly influences surgical outcomes. Therefore, a comprehensive preoperative assessment and management of comorbid conditions are imperative to mitigating the likelihood of reoperation[35].

Numerous studies have substantiated the association between preoperative hypoproteinemia and the risk of unplanned reoperation. Saadat *et al*[36] recognized preoperative hypoalbuminemia as an independent risk factor in patients with rectal cancer, a finding corroborated by Michaels *et al*[37], who linked malnutrition to increased risk of unplanned reoperation. Our study further confirms that patients with diminished preoperative albumin levels are at a heightened risk for such interventions.

The PNI is a crucial marker for evaluating a patient's preoperative nutritional and immunological status. Lower PNI values often indicate suboptimal nutritional health, which can potentially compromise wound healing through impaired collagen synthesis and fibroblast proliferation[38,39]. Improving patients' nutrition by enhancing albumin concentrations and optimizing PNI scores may significantly curtail the risk of unplanned reoperations following rectal cancer surgeries. Moreover, a history of prior abdominal surgeries is an independent risk factor for postoperative bowel obstruction following rectal resections[40]. This suggests that such historical surgical interventions may lead to extensive abdominal adhesions, thereby complicating subsequent procedures and elevating the risk of complications.

Screening patients with high reoperation risk helps clinicians target perioperative observations and interventions, thus reducing unplanned reoperation and improving patient prognosis. In this study, we successfully predicted the incidence of unplanned reoperation through a constructed nomogram. The internal validation showed that the model was highly accurate and had good predictive efficacy.

However, there are some limitations to this study. First, the retrospective design of this study may lead to information and selection bias. Second, the lack of an external independent dataset for validation limits the generalizability and reproducibility of the model. Finally, the lack of long-term follow-up data in this study prevented assessment of the long-term outcomes of surgery and patient quality of life. In the future, we hope to use a prospective design to reduce bias, conduct external validation to enhance the generalizability of the model, and include long-term follow-up to assess the long-term impact of surgery. These improvements may more accurately predict colorectal cancer postoperative risk and improve patient outcomes and quality of life.

CONCLUSION

This study successfully established and validated a postoperative unplanned reoperation risk model for colorectal cancer. Through comprehensive analysis, we accurately identified independent risk factors affecting the risk of unplanned reoperation: age; gender; history of hypertension; history of dissection; history of hypoproteinemia, and PNI. The application of the model in clinical practice can help to more accurately assess the postoperative risk of patients, thus optimizing treatment decisions, reducing the occurrence of unplanned reoperation, and improving patient prognosis and quality of life.

FOOTNOTES

Author contributions: Cai LQ designed the study; Cai LQ, Yang DQ, Wang RJ, and He H performed the study; Yang DQ, Wang RJ collected the data; Cai LQ and Shi YX analysed the data and wrote the manuscript; all authors read and approved the final manuscript.

Institutional review board statement: This study has been reviewed and approved by the Clinical Research Ethics Committee of Wenzhou Central Hospital and the First Hospital Affiliated to Wenzhou Medical University, No. KY2024-R016.

Informed consent statement: The present study was retrospective and a dispensation from informed consent has been requested.

Conflict-of-interest statement: All authors declare that there is no conflict of interest involved in this study.

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ORIGINAL ARTICLE

Prospective Study

Double contrast-enhanced ultrasonography improves diagnostic accuracy of T staging compared with multi-detector computed tomography in gastric cancer patients

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Abstract

BACKGROUND

Gastric cancer (GC) is the most common malignant tumor and ranks third for cancer-related deaths among the worldwide. The disease poses a serious public health problem in China, ranking fifth for incidence and third for mortality. Knowledge of the invasive depth of the tumor is vital to treatment decisions.

AIM

To evaluate the diagnostic performance of double contrast-enhanced ultrasonography (DCEUS) for preoperative T staging in patients with GC by comparing with multi-detector computed tomography (MDCT).

METHODS
This single prospective study enrolled patients with GC confirmed by preoperative gastroscopy from July 2021 to March 2023. Patients underwent DCEUS, including ultrasonography (US) and intravenous contrast-enhanced ultrasonography (CEUS), and MDCT examinations for the assessment of preoperative T staging. Features of GC were identified on DCEUS and criteria developed to evaluate T staging according to the 8th edition of AJCC cancer staging manual. The diagnostic performance of DCEUS was evaluated by comparing it with that of MDCT and surgical-pathological findings were considered as the gold standard.

RESULTS
A total of 229 patients with GC (80 T1, 33 T2, 59 T3 and 57 T4) were included. Overall accuracies were 86.9% for DCEUS and 61.1% for MDCT ($P < 0.001$). DCEUS was superior to MDCT for T1 (92.5% *vs* 70.0%, $P < 0.001$), T2 (72.7% *vs* 51.5%, $P = 0.041$), T3 (86.4% *vs* 45.8%, $P < 0.001$) and T4 (87.7% *vs* 70.2%, $P = 0.022$) staging of GC.

CONCLUSION
DCEUS improved the diagnostic accuracy of preoperative T staging in patients with GC compared with MDCT, and constitutes a promising imaging modality for preoperative evaluation of GC to aid individualized treatment decision-making.

Key Words: Double contrast-enhanced ultrasonography; Multi-detector computed tomography; Gastric cancer; T staging

Core Tip: This current prospective study identified double contrast-enhanced ultrasonography (DCEUS) findings in preoperative T staging, developed DCEUS criteria based on the 5-layer gastric wall structure and perfusion characteristics of CEUS, and evaluated the diagnostic performance of DCEUS in gastric cancer (GC) T staging using DCEUS criteria, a method that may overcome limitations by detailing hemodynamic changes of GCs. DCEUS showed superior performance in GC T staging to multi-detector computed tomography and constitutes a promising imaging modality for preoperative evaluation of GC to aid individualized treatment decision-making.

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INTRODUCTION

Gastric cancer (GC) is the most common malignant tumor and ranks third for cancer-related deaths among the worldwide[1]. The disease poses a serious public health problem in China, ranking fifth for incidence and third for mortality[2]. It is important to stage GC as accurately as possible due to the increased availability of minimally invasive surgery, such as endoscopic mucosal resection (EMR)/endoscopic submucosal dissection (ESD) or laparoscopic surgery [1]. Knowledge of the invasive depth of the tumor is vital to treatment decisions[1,3].

Endoscopic ultrasonography (EUS) and computed tomography (CT) are commonly used methods for baseline clinical GC staging[4-6]. However, the precision of these preoperative diagnostic tools has not kept pace with advances in GC treatment. EUS is operator dependent and lesions are difficult to identify when located in gastroesophageal junctions, subcardiac regions and lesser curvatures. The diagnostic accuracy of EUS ranges from 57% to 88% for GC T staging[7] and it differentiates T3 and T4 tumors poorly[3,7]. EUS is not routinely performed in clinical practice except for EMR/ESD indications. CT is the principal imaging modality used for staging but has limitations in terms of T staging accuracy, particularly for early GC (EGC)[1,8-12].

The limitations of available imaging methods illustrate the need for a noninvasive, reliable approach to improve GC T staging accuracy. Double contrast-enhanced ultrasonography (DCEUS) examination combines ultrasonography (US) with an ultrasonic oral contrast agent and intravenous contrast-enhanced ultrasonography (CEUS) with an intravenous contrast agent[12-14]. DCEUS has been explored in China for screening gastrointestinal diseases, including gastric and rectal tumors. Studies[12-14] have reported that DCEUS could be used for preoperative T staging based on the 5-layer structure of gastric wall, while ignoring vascularity of the gastric wall and tumors.

The current prospective study aimed to identify DCEUS findings on GC, develop DCEUS criteria based on the concept of the 5-layer gastric wall structure of US and perfusion characteristics of CEUS and evaluate preoperative GC T staging using DCEUS criteria. Diagnostic performance of DCEUS for GC T staging was compared with multi-detector CT (MDCT).

MATERIALS AND METHODS

This prospective study was approved by our hospital ethics committee and all patients provided written informed consent.

Patients

A total of 300 patients with pathological confirmation of GC were recruited from July 2021 to March 2023 and all underwent DCEUS and MDCT examinations before radical gastrectomy. Inclusion criteria were: (1) Adult; (2) no other treatment received before operation; (3) no allergy or contraindication for oral or intravenous contrast media or iodine contrast media; and (4) no contraindications for surgical resection. Exclusion criteria were: (1) The presence of other cancers; (2) no the treatment of surgery; and (3) surgical treatment accepted more than 6 wk after DCEUS and MDCT examinations. A final total of 229 patients were enrolled. The study protocol flowchart is presented in Figure 1.

DCEUS examination

Patients were fasted for > 8 h. The powdered oral contrast agent (50 g; G.F. Acoustic contrast, Yanbian, China) made from lotus root, rice, corn and soybeans was dissolved in 500 mL boiling water to form a homogenous suspension, cooled and given orally.

DCEUS examination was performed with Siemens Healthineers, equipped with a 5C1 abdominal probe and a 10 L high-frequency probe. The US examination was conducted using a 5C1 probe from the distal esophagus to the duodenal bulb in both the supine and decubitus positions. Additionally, a 10 L high-frequency probe was employed to provide a more detailed evaluation when necessary. The imaging features include the presence, location, morphology and estimated invasive depth of the lesion. CEUS examination was performed to evaluate the enhancement patterns of the lesion. The proper contrast mode, including gain, depth, acoustic window, mechanical index, and focal zone were adjusted. An injection of 1.5 mL contrast agent (SonoVue®; Bracco, Milan, Italy) was given, followed by a 5.0-mL saline flush. The timer started simultaneously when the contrast agent was being injected and the probe was kept in a stable state for 180 s to detect the gastric lesion, the surrounding normal gastric wall and the possibility of a late phase liver examination metastasis. Arterial and venous phases of the lesion were recorded for 0 s to 120 s. DCEUS images and videos were recorded in Dicom format.

Gastric wall features in DCEUS

Normal gastric wall consists of five layers, mucosa, submucosa, muscularis propria, subserosa and serosa. The five layers, including the interface echo between the gastric lumen and the mucosa, the rest of the mucosa, submucosa, muscularis propria, and serosa[15] , can be visualized by US (Figure 2). The outer serosa enhances first in the CEUS examination, followed rapidly by the submucosa and mucosa, completing the enhancement of the entire gastric wall. The layers of the mucosa and particularly the submucosa, exhibit early and intense enhancement whereas muscularis propria layer shows lesser and delayed enhancement[16] (Video 1). GCs manifest as the thickening and disruption of normal gastric wall structures on US, "hyper-enhancement" in the arterial phase and "hypo-enhancement" in the venous phase on CEUS. Therefore, the 5-layer gastric wall structure of US and perfusion characteristics of CEUS give detailed information for T staging.

The DCEUS criteria for T staging were developed according to the 8th edition of AJCC cancer staging manual, shown in Table 1. Figures 3-6 showed the typical T1a, T2, T3, T4a GCs.

The All preoperative DCEUS images and videos of 229 cancers were analyzed by two experienced radiologists before surgery who were aware that the patients had GC based on findings of preoperative endoscopy before performing the DCEUS examination. Two radiologists evaluated the T staging for DCEUS based on the DCEUS criteria. If there was disagreement, both radiologists re-evaluated DCEUS images and videos and reached a consensus.

CT examination

Patients were fasted for > 8 h and given 600-1000 mL of tap water to dilate the stomach 5 min before the CT examination. A dose of 1.5 mL/kg of a contrast agent (Ultravist, Guangzhou, China) was administered intravenously at a rate of 3.5 mL/s using an automatic power injector (MEDRAD Vistron CT, PA, United States) during the entire contrast-enhanced CT examination (Philips Brilliance 128 row 256 slice spiral CT). MDCT scans were obtained at 30 s (arterial phase) and 70 s (portal-venous phase) after administration of an intravenous contrast agent. The subject was positioned prone on the scanning table to avoid artifacts caused by air in the stomach. Two radiologists evaluated the T staging for MDCT according to the 8th edition of the AJCC cancer staging manual. They were aware of the results of preoperative endoscopy before performing the MDCT examination. In case of disagreement, a consensus was reached by re-evaluation with the two radiologists.

Pathological evaluation

Surgical resection was performed on all 229 patients and specimens subjected to histopathological evaluation as the gold standard for T staging.

Statistical analysis

All statistical analyses were performed using SPSS 25.0 software. Continuous data were expressed as means ± SD and categorical data as percentages. The χ^2 test and Fisher exact test were used to compare categorical variables. A value of $P < 0.05$ was considered statistically significant.

Table 1 Double contrast-enhanced ultrasonography criteria for T staging of gastric cancer

T stage	Pathological definition	DCEUS criteria
T1	Invasion of the mucosa or submucosa	T1a: In the arterial phase, focal thickening of the mucosa is visualized. The lesion shows slightly delayed hyper-enhancement, similar to the submucosal layer. In the venous phase, the lesion shows hypo-enhancement compared to the submucosal layer. The submucosal layer consistently shows hyper-enhancement and is continuous and intact. The muscular layer shows linear hypo-enhancement and is continuous and intact; T1b: In the arterial phase, focal thickening of the mucosa and submucosa are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer. In the venous phase, the lesion shows hypo-enhancement. The enhancing submucosal layer is continuous. The muscular layer shows linear hypo-enhancement and is continuous and intact
T2	Invasion of the muscularis propria	In the arterial phase, disruption of the mucosa, submucosa and partly muscularis propria are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer. In the venous phase, the lesion shows homogenous hypo-enhancement. The hyper-enhancement strip of submucosal layer and partly hypo-enhancement strip of the muscularis propria are disruptive
T3	Invasion of the subserosal connective tissue without invading the visceral peritoneum	In the arterial phase, disruption of the mucosa, submucosa and muscularis propria are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer. In the venous phase, the lesion shows homogenous hypo-enhancement. The hyper-enhancement strip of submucosal layer and hypo-enhancement strip of the muscularis propria are disruptive. A smooth outer margin of the serosa or a few small linear stranding within the serosa are observed. The enhancing serosa is continuous
T4	Invasion of the serosa (visceral peritoneum) or adjacent structures/organs	In the arterial phase, disruption of the mucosa, submucosa, muscularis propria and serosa are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer. In the venous phase, the lesion shows homogenous hypo-enhancement. The hyper-enhancement strip of submucosal and serosal layers and hypo-enhancement strip of the muscularis propria are disruptive; T4a: An irregular nodular margin of the serosa and densely burred or banded infiltration of the adjacent fat plane are visualized; T4b: The adjacent fat plane between the tumor and the adjacent organ is obliterated or the tumor directly infiltrates the adjacent organ

GC: Gastric cancer; DCEUS: Double contrast-enhanced ultrasonography.

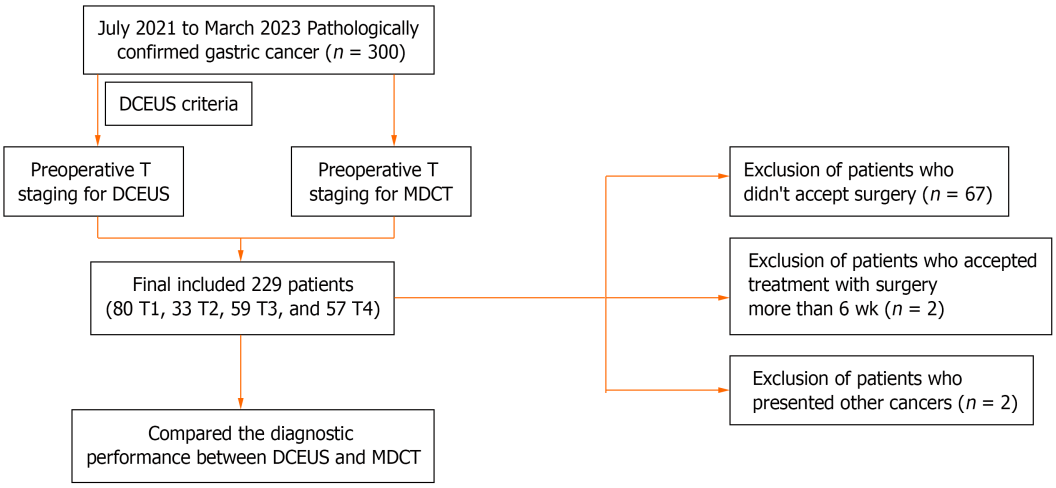

Figure 1 The flowchart of the study. DCEUS: Double contrast-enhanced ultrasonography; MDCT: Multi-detector computed tomography.

RESULTS

Clinicopathological features

Two hundred and twenty-nine patients with GC, 92 (40.2%) females and 137 (59.8%) males of mean age 54.9 ± 13.0 years, were included. Two hundred and twenty-nine lesions were classified 47 (20.5%) as T1a, 33 (14.4%) as T1b, 33 (14.4%) as T2, 59 (25.8 %) as T3, and 53 (23.1%) as T4a, and 4 (1.8%) as T4b (Table 2).

Comparison of the accuracy for T staging between DCEUS and MDCT

A comparison of the accuracy for T staging between DCEUS and MDCT is shown in Table 3. DCEUS correctly staged 199 cases (74 T1, 24 T2, 51 T3, and 50 T4), giving an overall accuracy of 86.9% with 92.5% for T1, 72.7% for T2, 86.4% for T3, and 87.7% for T4. Overestimation of T staging occurred in 16 (7.0%) cases and underestimation in 14 (6.1%). MDCT correctly staged 140 cases (56 T1, 17 T2, 27 T3, and 40 T4), giving an overall accuracy of 61.1%, 70.0% for T1, 51.5% for T2, 45.8% for T3 and 70.2% for T4. Overestimation occurred in 59 (25.8%) cases and underestimation in 30 (13.1%). DCEUS was superior to MDCT for overall accuracy (86.9% *vs* 61.1%, $P < 0.001$), T1 (92.5% *vs* 70.0%, $P < 0.001$), T2 (72.7% *vs* 51.5%, $P = 0.041$), T3 (86.4% *vs* 45.8%, $P < 0.001$), and T4 (87.7% *vs* 70.2%, $P = 0.022$) staging.

Table 2 Clinicopathological features of patients, *n* (%)

Features	Total
Sex	
Male	137 (59.8)
Female	92 (40.2)
Age (yr; mean ± SD)	54.9 ± 13.0
Pathological T staging	
T1a	47 (20.5)
T1b	33 (14.4)
T2	33 (14.4)
T3	59 (25.8)
T4a	53 (23.1)
T4b	4 (1.8)
Location	
Upper	17 (7.4)
Middle	70 (30.6)
Lower	122 (53.3)
Entire	20 (8.7)
Histopathological type	
Well differentiation	11 (4.8)
Moderately differentiation	45 (19.7)
Poorly differentiation	173 (75.5)
Bormann classification	
I	7 (4.7)
II	56 (37.6)
III	70 (47.0)
IV	16 (10.7)
Ulceration	
Yes	198 (86.5)
No	31 (13.5)
Tumor size (cm, mean ± SD)	3.5 ± 2.4

Diagnostic accuracy of DCEUS and MDCT for GC T staging based on clinicopathological features

Diagnostic accuracy of DCEUS and MDCT for GC T staging based on clinicopathological features is shown in Table 4. DCEUS showed superior accuracy in T staging for lesions located in the middle, lower and entire parts of the stomach and higher accuracy in T staging for Borrmann types II and III and histopathological types of moderately- and poorly-differentiated GC. The superiority can be seen when comparing the middle (DCEUS: 82.9% *vs* MDCT: 57.1%, $P = 0.001$), lower (DCEUS: 90.2% *vs* MDCT: 65.6%, $P < 0.001$) and entire parts of the stomach (DCEUS: 85.0% *vs* MDCT: 55.0%, $P = 0.038$). DCEUS showed higher accuracy in T staging for Borrmann types II (DCEUS: 82.1% *vs* MDCT: 48.2%, $P < 0.001$) and III (DCEUS: 82.9% *vs* MDCT: 61.4%, $P < 0.001$) and histopathological types of moderately- differentiated (DCEUS: 86.7% *vs* MDCT: 44.4%, $P < 0.001$), and poorly-differentiated (DCEUS: 86.1% *vs* MDCT: 63.6%, $P < 0.001$) GC. DCEUS accuracy in T staging was consistently superior to MDCT, stratified across lesions of varied size and the presence of ulceration.

DISCUSSION

Treatment options for GC patients depend on the tumor stage, including EMR/ESD for EGC, total/distal gastrectomy for

Table 3 Comparison of the accuracy for gastric cancer T staging between double contrast-enhanced ultrasonography and multi-detector computed tomography, *n* (%)

T staging	DCEUS (%)	MDCT (%)	*P* value
T1 (*n* = 80)	74 (92.5)	56 (70.0)	< 0.001
T2 (*n* = 33)	24 (72.7)	17 (51.5)	0.041
T3 (*n* = 59)	51 (86.4)	27 (45.8)	< 0.001
T4 (*n* = 57)	50 (87.7)	40 (70.2)	0.022
T total (*n* = 229)	199 (86.9)	140 (61.1)	< 0.001

DCEUS: Double contrast-enhanced ultrasonography; MDCT: Multi-detector computed tomography.

Table 4 Diagnostic accuracy of double contrast-enhanced ultrasonography and multi-detector computed tomography for gastric cancer T staging based on clinicopathological features, *n* (%)

Features	Total	Accuracy of DCEUS (%)	Accuracy of MDCT (%)	*P* value
Location				
Upper	17	14 (82.4)	9 (52.9)	0.067
Middle	70	58 (82.9)	40 (57.1)	0.001
Lower	122	110 (90.2)	80 (65.6)	< 0.001
Entire	20	17 (85.0)	11 (55.0)	0.038
Tumor size				
< 2.0 cm	78	67 (85.9)	48 (61.5)	< 0.001
≥ 2.0 cm	151	139 (92.1)	97 (64.2)	< 0.001
Ulceration				
Yes	198	168 (84.8)	117 (59.1)	< 0.001
No	31	31 (100)	23 (74.2)	0.005[1]
Borrmann classification				
I	7	7 (100)	5 (71.4)	0.462[1]
II	56	46 (82.1)	27 (48.2)	< 0.001
III	70	58 (82.9)	43 (61.4)	< 0.001
IV	16	14 (87.5)	9 (56.3)	0.113[1]
Histopathological type				
Well differentiation	11	11 (100)	10 (90.9)	0.306[1]
Moderately differentiation	45	39 (86.7)	20 (44.4)	< 0.001
Poorly differentiation	173	149 (86.1)	110 (63.6)	< 0.001

[1]Fisher exact test.

GC: Gastric cancer; DCEUS: Double contrast-enhanced ultrasonography; MDCT: Multi-detector computed tomography.

locally advanced GC (AGC) and chemotherapy for unresectable/metastatic AGC[1]. Therefore, correct staging is vital for selection of the optimal therapeutic regimen. The current prospective study identified DCEUS findings in preoperative T staging, developed DCEUS criteria based on the 5-layer gastric wall structure and perfusion characteristics of CEUS, and evaluated the diagnostic performance of DCEUS in GC T staging using DCEUS criteria, a method that may overcome limitations by detailing hemodynamic changes of GCs. DCEUS showed superior performance in GC T staging to MDCT.

DCEUS is an emerging modality by combining oral and intravenous contrast agents to elucidate the morphological and perfusion characteristics of gastric lesions. The oral contrast agent allows discharge the intra-gastric air and forms a homogeneous ultrasonic transmission surface to expose gastric wall layers and location, extension and morphology of gastric lesions[15] on US. It is helpful in detecting mucosal lesions for EGC and local or diffuse lesions for AGC when the distended stomach allows visualization of thickening lesions on the gastric wall. Tumors are regarded as enhancing

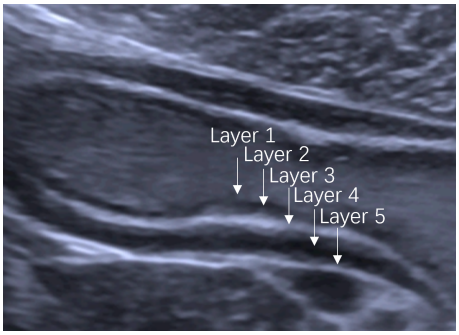

Figure 2 The 5-layer structure of the normal gastric wall on ultrasonography**.** The 5-layer structure of the normal gastric wall is numbered from the luminal side. Layer 1 is the interface echo between the gastric lumen and the mucosa, layer 2 is the rest of the mucosa, layer 3 is the submucosa, layer 4 is the muscularis propria, and layer 5 is the serosa.

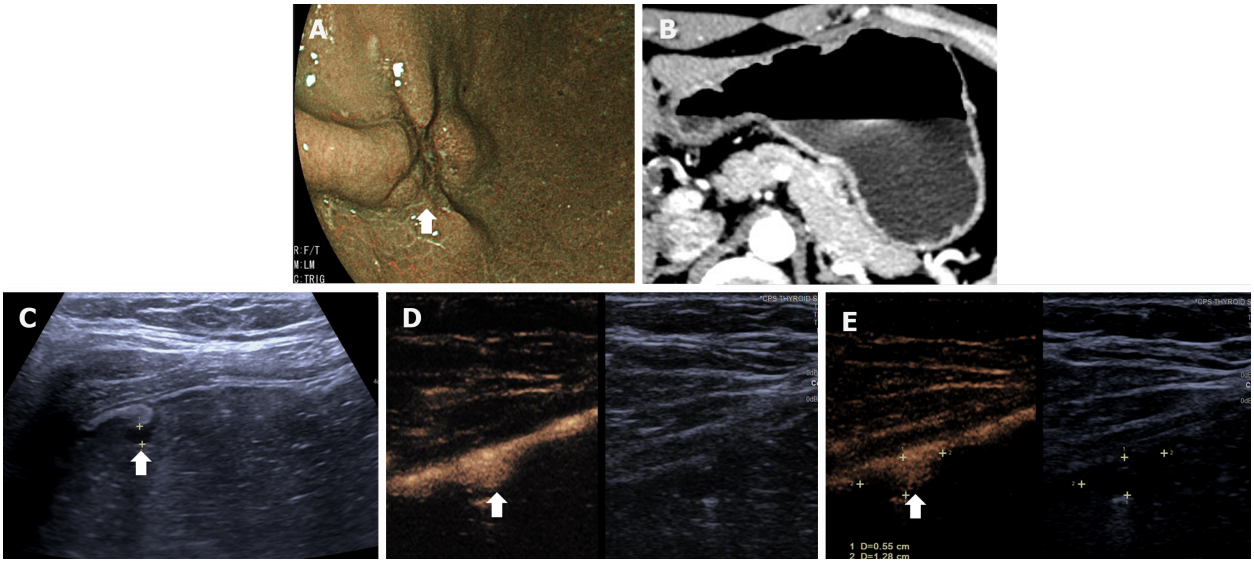

Figure 3 Images of T1a gastric cancer in a 52-year-old woman. A: Gastroscopic image shows a malignant ulcer (arrow) with converging folds and uneven margin; B: Multi-detector computed tomography image shows the gastric wall with no tumor; C: US image shows the hypoechoic lesion (arrow) with the focal thickened mucosa.; D: In the arterial phase, focal thickening of the mucosa is visualized. The lesion shows slightly delayed hyper-enhancement, similar to the submucosal layer; E: In the venous phase, the lesion shows hypo-enhancement compared to the submucosal layer. The submucosal layer consistently shows hyper-enhancement and is continuous and intact. The muscular layer shows linear hypo-enhancement, and is continuous and intact.

lesions on dynamic CEUS images due to their hypervascularity[16]. The intravenous contrast agent allows elucidation of tumor blood perfusion and enhances visualization through "hyper-enhancement" in the arterial phase and "hypo-enhancement" in the venous phase to delineate tumor location and extension[17]. The tumor contour and invasive depth may be gauged in real-time and in a dynamic manner to evaluate the invasion of adjacent structures and identify the enhancement patterns of GC for DCEUS to distinguish T1 from T2 and T3 from T4. In our study, DCEUS showed an overall accuracy of 86.9% for T staging, 92.5% for T1, 72.7% for T2, 86.4% for T3, and 87.7% for T4. Overestimation was found in 7.0% of cases and underestimation in 6.1%. Previous studies[12,14,17] have found that overall accuracies of DCEUS for T staging of GC range from 77.2% to 84.0% and accuracies from 62.5% to 90.9% for T1, 84.4% to 88.9% for T2, 78.9% to 87.9% for T3, and 82.9% to 91.3% for T4 with overestimation in 12.0% of cases and underestimation in 5.7%.

CT is the most commonly used imaging method for staging GC and allows visualization of primary tumor invasive depth, estimation of the lymph node involvement and distant metastasis. A diagnostic meta-analysis[18] comparing CT and EUS for staging GC showed that EUS was superior to CT for T1 staging, but no significant differences were found for T2-T4 lesions. Thus, it is suggested that CT might replace EUS for preoperative staging, as its accuracy in T staging is almost equivalent to that of EUS[19]. However, CT does not give clear visualization of the 5-layer gastric wall or indicate the invasive depth of the lesion. Recent studies[1,8-12] have reported relatively low accuracies of CT for T staging, ranging from 43% to 86% for overall accuracy of T staging, and 27% to 46% for T1, 53% to 56% for T2, 42% to 86% for T3, 59% to 86% for T4, respectively. MDCT had an overall accuracy of 61.1% in current work, 70.0% for T1, 51.5% for T2, 45.8% for T3, and 70.2% for T4. A CT scan is usually a diagnostic tool for preoperative staging but is not a primary screening tool for GC. The current study classified lesions that were not depicted on MDCT images as T1a[20], perhaps causing MDCT to perform better in the determination of EGC compared with previous studies. DCEUS was superior to MDCT for diagnosis of EGC in the current study, consistent with the previous studies[13,21]. MDCT gave a higher rate of

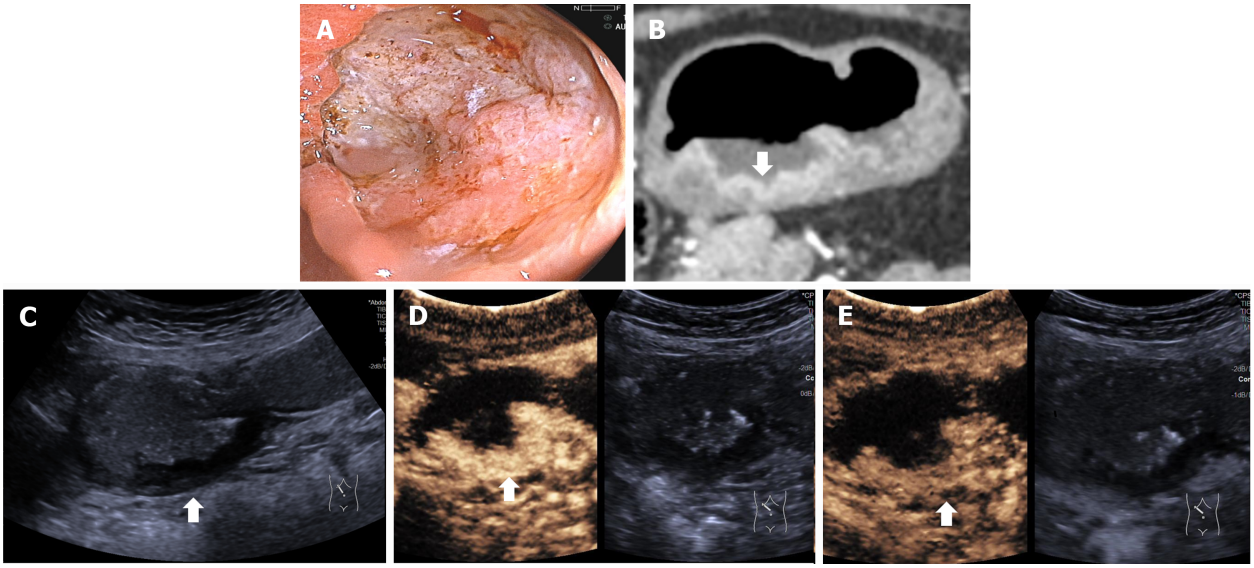

Figure 4 Images of T2 gastric cancer in a 65-year-old woman. A: Gastroscopic image shows a typical ulcerative tumor; B: Multi-detector computed tomography image shows transmural, enhancing tumor (arrow) with smooth outer border of gastric wall; C: ultrasonography image shows the hypoechoic ulceroinfiltrative tumor (arrow). The mucosa, submucosa and partly muscularis propria are disruptive; D: In the arterial phase, disruption of the mucosa, submucosa and partly muscularis propria are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer; E: In the venous phase, the lesion shows homogenous hypo-enhancement. The hyper-enhancement strip of submucosal layer and partly hypo-enhancement strip of the muscularis propria are disruptive.

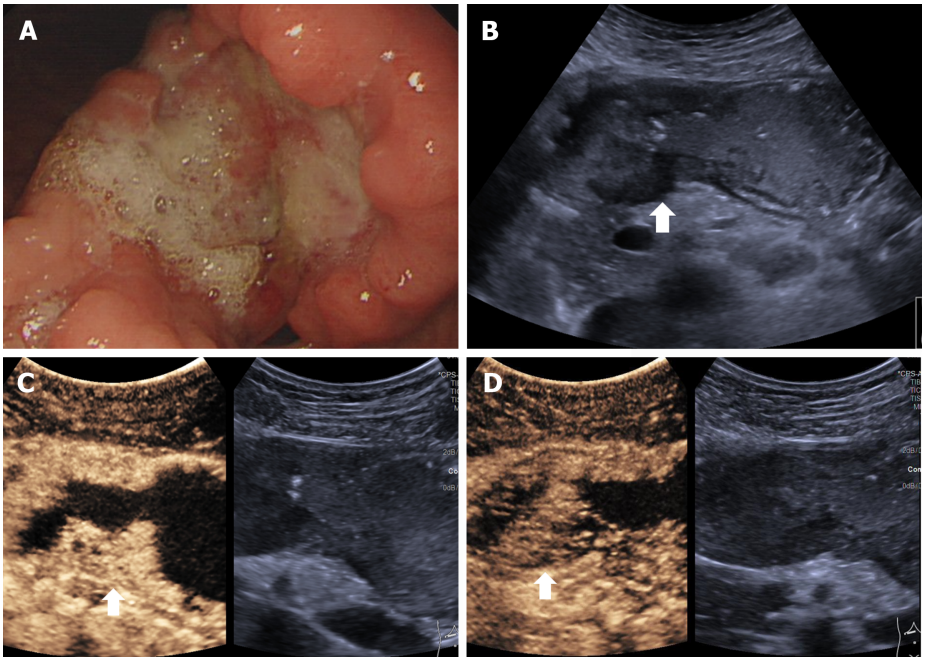

Figure 5 Images of T3 gastric cancer in a 58-year-old man. A: Gastroscopic image shows an ulcerative tumor; B: US image shows the hypoechoic tumor (arrow) with disruption of the mucosa, submucosa and muscularis propria. The outer margin of the serosa is slightly blurred; C: In the arterial phase, disruption of the mucosa, submucosa and muscularis propria are visualized. The lesion shows homogenous hyper-enhancement, similar to the normal submucosal layer; D: In the venous phase, the lesion shows homogenous hypo-enhancement. The hyper-enhancement strip of the submucosal layer and hypo-enhancement strip of the muscularis propria are disruptive. A few small linear stranding within the serosa is observed. The enhancing serosa is continuous.

overestimation and underestimation for T staging due to difficulties in observing the multilayered pattern of the gastric wall especially in thinner regions and partial volume averaging effects in areas scanned obliquely. By contrast, DCEUS showed the 5-layer structure of the gastric wall with clearer visualization of lesion invasion on CEUS. Thus, DCEUS is a more promising candidate for T staging of GC than CT.

By comparing diagnostic accuracy of T staging for GC between DCEUS and MDCT stratified across the tumor location, tumor size, status of ulceration, histopathological type and Borrmann type for AGC, our study further highlights the advantages of DCEUS in preoperative T staging. DCEUS had higher accuracy of T staging than MDCT for lesions located

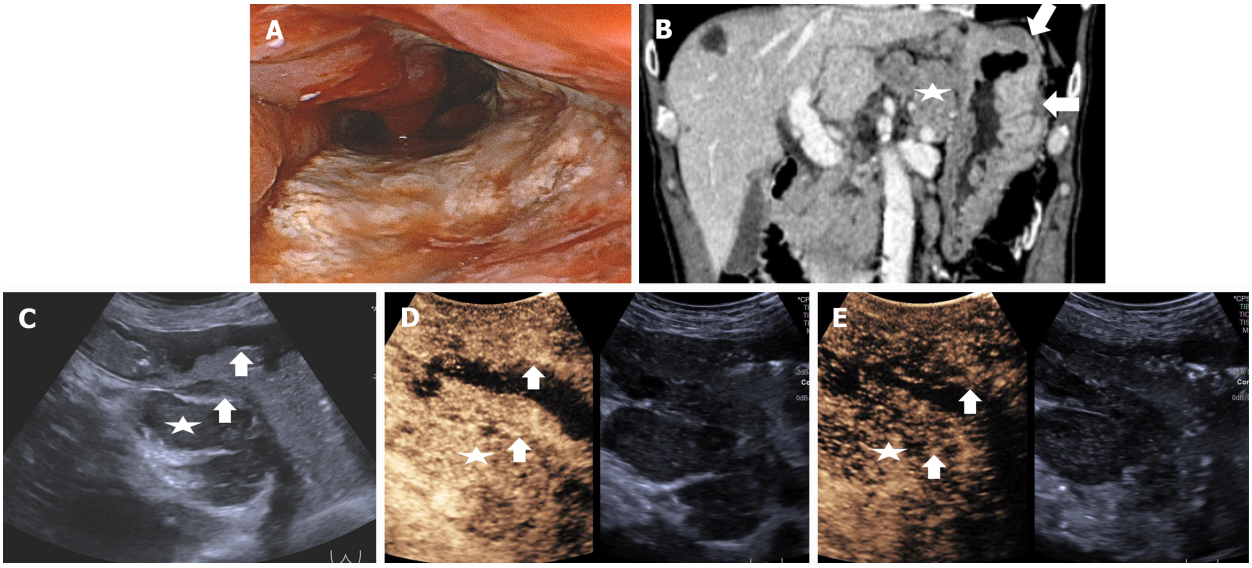

Figure 6 Images of T4a gastric cancer in a 60-year-old man. A: Gastroscopic image shows a diffuse ulcerative tumor; B: MDCT image shows the tumor (arrows) with diffuse thickening of the whole gastric wall, band-like perigastric fat infiltration, and cluster of enhancing nodes (star) around perigastric region; C: US image shows the hypoechoic diffuse tumor (arrows) with irregular margin of the serosa, banded infiltration of the adjacent fat plane, and cluster of nodes (star) in perigastric region; D and E: CEUS images show the tumor (arrows) with surrounding perigastric fat plane and cluster of nodes (star) synchronous hyper-enhancement in the arterial phase (D), and synchronous hypo-enhancement in the venous phase (E). The hyper-enhancement strip of the submucosal and serosal layers and hypo-enhancement strip of the muscularis propria are disruptive.

in the middle, lower and entire parts of the stomach. Previous reports have identified the cardia of stomach as producing the least accurate results due to inadequate filling with water[19]. However, DCEUS showed good diagnostic performance of T staging lesions located in the upper parts due to distention of the whole stomach lumen in our study. Previous studies[22,23] have reported that lesion size and ulceration affected the accuracy of T staging for CT and EUS. In our study, DCEUS was consistently superior to MDCT for T staging, regardless of lesion size and the presence of ulceration.

AGCs are classified into four Borrmann types, depending on gross appearance and are usually accompanied by different degrees of inflammation. Determination of invasive depth by MDCT is difficult, especially when diffuse inflammation is present[9,10]. Dynamic DCEUS images delineate tumors more precisely due to real-time observation of the 5-layer structure of the gastric wall and perfusion characteristics on CEUS. Tumor invasion is difficult to distinguish from inflammatory effect for Borrmann type II and III tumors with ulceration. DCEUS had better diagnostic performance than MDCT with significantly higher accuracy of T staging in Borrmann type II and III tumors. However, no significant differences were found in diagnostic accuracy of T staging for Borrmann type I and IV tumors between DCEUS and MDCT. Both DCEUS and MDCT showed good delineation for Borrmann type I due to the morphologic characteristics of nodular polypoid tumors. Borrmann type IV AGC is characterized by diffuse infiltration of the gastric wall without ulceration or distinct elevation and is difficult to recognize by endoscopy. CT has been shown superior for lesion detection and characterization of Borrmann type IV[24]. And our study additionally shows that DCEUS exhibits superior diagnostic performance to CT for T staging of Borrmann type IV AGC.

Histopathological type may affect the accuracy of T staging between DCEUS and MDCT. In our study, DCEUS showed higher accuracies of T staging for moderately- and poorly-differentiated GCs than MDCT. Compared with the differentiated type, undifferentiated types are hypovascular with diffuse infiltration[22,23]. Tumor vascularity could be evaluated by CEUS and contrast enhancement has been shown to correlate with histological vessel density in rectal cancer, gastrointestinal neuroendocrine tumors and stromal tumors[25,26]. And our study confirms that the enhancement characteristics of the lesion contributes to higher accurate diagnosis of T staging for moderately- and poorly-differentiated GC.

The study has several limitations. First, this was a single-center study which may result in potential bias. Second, radiologists who reviewed the DCEUS and MDCT data had prior knowledge of the endoscopic results which may lead to overestimation of accuracies for T staging of GC.

CONCLUSION

In conclusion, DCEUS shows superior accuracy for T staging of GC compared with MDCT and may facilitate optimal treatment decision in GC patients.

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FOOTNOTES

Author contributions: Xu YF and Ma HY contributed equally to the manuscript; Xu YF, Ma HY, Huang GL, Zhang YT, Wang XY, Wei MJ, and Pei XQ designed the research study; Xu YF, Ma HY, Huang GL, and Zhang YT performed the research; Xu YF and Ma HY analyzed the data and wrote the manuscript; and all authors have read and approved the final manuscript.

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

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