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REVIEW

Acute pancreatitis: A review of diagnosis, severity prediction and prognosis assessment from imaging technology, scoring system and artificial intelligence

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Abstract

Acute pancreatitis (AP) is a potentially life-threatening inflammatory disease of the pancreas, with clinical management determined by the severity of the disease. Diagnosis, severity prediction, and prognosis assessment of AP typically involve the use of imaging technologies, such as computed tomography, magnetic resonance imaging, and ultrasound, and scoring systems, including Ranson, Acute Physiology and Chronic Health Evaluation II, and Bedside Index for Severity in AP scores. Computed tomography is considered the gold standard imaging modality for AP due to its high sensitivity and specificity, while magnetic resonance imaging and ultrasound can provide additional information on biliary obstruction and vascular complications. Scoring systems utilize clinical and laboratory parameters to classify AP patients into mild, moderate, or severe categories, guiding treatment decisions, such as intensive care unit admission, early enteral feeding, and antibiotic use. Despite the central role of imaging technologies and scoring systems in AP management, these methods have

limitations in terms of accuracy, reproducibility, practicality and economics. Recent advancements of artificial intelligence (AI) provide new opportunities to enhance their performance by analyzing vast amounts of clinical and imaging data. AI algorithms can analyze large amounts of clinical and imaging data, identify scoring system patterns, and predict the clinical course of disease. AI-based models have shown promising results in predicting the severity and mortality of AP, but further validation and standardization are required before widespread clinical application. In addition, understanding the correlation between these three technologies will aid in developing new methods that can accurately, sensitively, and specifically be used in the diagnosis, severity prediction, and prognosis assessment of AP through complementary advantages.

Key Words: Acute pancreatitis; Imaging technology; Scoring system; Artificial intelligence; Severity prediction; Prognosis assessment

Core Tip: In this review, we comprehensively analyzed, discussed, and summarized the latest progress in the diagnosis, severity prediction, and prognosis assessment of acute pancreatitis from the aspects of imaging technologies, scoring systems, and artificial intelligence. This review provided comprehensive guidance and suggestions with clinical value for the diagnosis and treatment of acute pancreatitis.

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disorder resulting from intracellular activation and leakage of improper proteolytic enzymes, including active inflammation and pancreatic injury[1,2]. AP can result in nausea, vomiting, severe upper abdominal pain, abnormal release of pancreatic juice, or a systemic inflammatory response syndrome with fever, low blood pressure, and in some cases failure of one or more organs[2]. AP is one of the most common causes of hospitalization from gastrointestinal diseases, with a global incidence rate ranging from 13 to 45 cases per 100000 individuals annually[2,3]. Globally, the incidence of AP varies, with the North America and Western Pacific regions (as defined by the World Health Organization) experiencing the highest rates, surpassing 34 cases per 100000 individuals annually[4]. The incidence of AP has steadily increased over time in most countries of the Western world[5]. In the United States, the rate of AP-related hospitalization increased from 65.4 to 81.9 per 100000 adults from 2001 to 2014[6].

Classification of AP based on severity

AP, often worsened by comorbidities and demographic factors such as obesity, type 2 diabetes, cardiovascular and renal diseases, alcohol use disorder, and age over 45, is classified by severity into three categories: Mild AP (MAP), moderately severe AP (MSAP), and severe AP (SAP)[7]. SAP typically results in pancreatic necrosis, systematic inflammation, and multi-organ dysfunction and failure. Its mortality rate, ranging from 20%-40%, significantly surpasses those of MAP and MSAP[8]. The volume of extrapancreatic necrosis positively correlates with the complication rate of SAP, potentially serving as an indicator for predicting adverse outcomes in AP[9]. Early prediction of SAP with high mortality remains a challenge due to the limited accuracy of current predictive tools and the complex clinical features of SAP[10].

MSAP is characterized by transient organ failure, local complications, or exacerbation of comorbid disease, and SAP is defined by persistent organ failure lasting more than 48 h[11]. MSAP is linked to transient organ failure, while SAP involves persistent organ failure, often necessitating intensive care management[7]. The early identification of SAP is critical for the stratification and treatment of patients. Additionally, for SAP, it is crucial to avoid interventions that are either excessive and premature or insufficient and delayed; instead, a progressive intervention approach should be implemented at the appropriate time. The development of risk stratification tools that meet clinical needs and guide clinicians in terms of resource allocation, patient consultation and clinical audit, and the multidisciplinary approaches including evidence-based care are essential to achieve optimal clinical outcomes[12]. Therefore, early assessment of the etiology and severity of AP is essential for prompt treatment and close monitoring of severe patients.

Pathophysiology of AP

The pathophysiology of AP involves acinar cell damage, resulting in premature intrapancreatic activation of digestive proteases[13]. The pathological factors of AP includes calcium (Ca^{2+}) overload, mitochondrial dysfunction, impaired autophagy, endoplasmic reticulum stress, unfolded protein response, intraductal fluid stasis, genetic mutations (*e.g.*,

PRSS1 or *CTFR* gene), unsaturated fatty acids, and exosomes, which mainly lead to inappropriate activation of trypsinogen, infiltration of inflammatory cells, and destruction of secretory cells[14,15]. Ca^{2+} overload is a prevalent mechanism causing cell damage in the body[15]. Intracellular Ca^{2+} overload and mitochondrial dysfunction, induced by cholecystokinin, excessive alcohol consumption, and bile acids, have been identified as key steps in SAP development caused by acinar cell dysfunction[15]. Mitochondrial dysfunction hinders cell autophagy, leading to increased production of reactive oxygen species and cytokines, which exacerbates pancreatic cell damage[15]. Mitochondrial injury exacerbates endoplasmic reticulum stress and lysosomal damage, promoting the release and activation of cathepsinogen and trypsinogen, which results in cytoplasmic protein degradation and cell necrosis[15].

Uncertainty of serum amylase and lipase in diagnosing AP

Common biochemistry markers used in clinical practice include amylase and lipase in serum, but clinicians must be aware of the difference in half-life between the two[12]. In serum, amylase returns to normal limits within 3-5 d, and lipase returns to normal limits within 8-14 d[12]. Elevated serum amylase and/or lipase levels support the clinical suspicion of AP, and the measurement of amylase is more widely used[16]. However, about 40% of serum amylase is derived from the pancreas, with the rest primarily from the salivary glands[16]. Therefore, the elevation of serum total amylase is not specific for pancreatitis, and other intra-abdominal diseases should be considered[16]. For example, Gumaste *et al*[17] reported that the sensitivity of serum amylase in detecting AP was 72% and the specificity was 99%. In a prospective study including 500 patients with acute abdominal pain, the serum amylase assay had a sensitivity of 85% (with a cutoff value of 300 U/L for the upper reference limit) and a specificity of 91%[18]. Another prospective study showed that the sensitivity and specificity of total amylase in serum were 45% and 97%, respectively, at the calculated diagnostic threshold of 175 U/L[19].

In some non-pancreatic diseases, there is also a false elevation of serum amylase. For example, Hu *et al*[20] reported a case of hyperamylasemia with an average serum amylase value of 881 U/L, significantly exceeding the reference range of 10-220 U/L. In addition, elevated levels of amylase and lipase, while indicative, are not exclusive to AP and may result from conditions such as bowel obstruction, infarction, cholecystitis, or perforated ulcer[21]. However, the sensitivity of serum lipase ranges from 85%-100%; while some studies reported it was less sensitive than serum amylase, others contended it surpassed amylase in sensitivity[22].

Current clinical diagnosis of AP

The definition of severity in AP is pivotal for determining the therapeutic approach. Patients with MAP typically respond to conservative treatment, while those patients with necrotizing pancreatitis often experience organic dysfunction, necessitating intensive care and regular therapeutic interventions, with a more uncertain prognosis[1]. Currently, the clinical diagnosis of AP necessitates meeting two of the following three criteria: (1) Abdominal pain consistent with AP; (2) Serum levels of amylase or lipase exceeding three times the upper normal limit; and (3) Cross-sectional abdominal imaging findings consistent with AP[23]. It is important to note that two of these criteria alone may fail to identify one-quarter of AP patients and misdiagnose it in one-tenth of patients[23].

At present, there is still no single scoring system that can cover all the issues related to the management and evaluation of AP. AP continues to be one of the most intricate digestive disorders in terms of clinical course and outcome, and its inherent variability in each case makes it both challenging and captivating[24]. Meanwhile, to predict the severity and mortality of AP, clinicians evaluate clinical data, including assessing organ function, conducting laboratory tests and imaging, and utilizing severity-of-the-disease rating systems, such as Ranson, Acute Physiology and Chronic Health Evaluation (APACHE) II, Balthazar's computed tomography severity index (CTSI), modified Mortele's CTSI (MCTSI), Bedside Index for Severity in AP (BISAP), harmless AP score (HAPS), and the first artificial intelligence (AI) model, EASY-APP[25]. In addition to these, the latest imaging studies and clinical scoring systems for the early diagnosis, prognosis assessment, and severity prediction of AP have been extensively studied and reported. In this review, we provided a detailed discussion and analysis of the latest imaging examinations and some scoring systems applied in this field to afford more valuable guidance to more accurately diagnose, predict, and assess AP.

IMAGING TECHNOLOGY

Imaging technology still plays a fundamental role in the initial evaluation, identification of severe cases, prognosis prediction, and decision-making for the treatment and management of AP patients[1]. An accurate description of imaging findings is crucial in all diseases, particularly in diseases like AP where the appropriate therapy depends on precise diagnosis[26]. The manifestations of pancreatic diseases are variable, and imaging plays an important role in the diagnosis and treatment of pancreatic diseases[27]. Imaging evaluation is still essential to validate the clinical diagnosis, ascertain the etiology, exclude other causes of pain related to elevated levels of amylase and/or lipase, and assess the severity and extent of AP[1].

Imaging modalities for the pancreas encompass plain X-ray, ultrasonography (US), endoscopic ultrasound (EUS), endoscopic retrograde cholangiopancreaticography (ERCP), computed tomography (CT), magnetic resonance imaging (MRI), and magnetic resonance cholangiopancreatography[27]. US is usually considered to be the only appropriate modality in the early phase of AP with typical presentations and is used for the detection of gallstones. CT and MRI are suitable for patients in the early phase of AP with equivocal presentation[28]. In emergency situations, CT and US are the preferred imaging modalities due to their advantages of accessibility, speed, and lower cost[29]. Early detection of CT imaging may influence the diagnosis or treatment in up to 15% of AP patients presenting to the emergency department,

particularly in older patients with a history of pancreatitis and biliary interventions.

However, abdominal US may offer a more precise screening for biliary etiologies and provide a more informed direction for subsequent treatment[30]. Based on the fact that US often shows a regular pancreatic structure, the main role of transabdominal US in AP is to identify gallstones and/or choledocholithiasis, which is useful especially for the evaluation of biliary tract[31,32]. However, because of the presence and overlap of bowel gas, US is not possible to visualize pancreatic distal abnormality in the detection of AP[31,32].

In the late phase, typically 48-72 h post-presentation, CT and MRI serve as primary imaging modalities for AP patients, facilitating the assessment of etiology, complications, disease extent, interventions, and subsequent follow-up[28]. For example, as early as 2007, Stimac *et al*[33] reported that non-enhanced MRI was comparable to contrast-enhanced CT (CECT) in the early assessment of AP severity, with both methods demonstrating equal efficacy in predicting local and systemic complications of AP. MRI of the pancreas serves as both a problem-solving tool following CT or US evaluations and an initial imaging examination of choice. Furthermore, magnetic resonance cholangiopancreatography is valuable for detecting and evaluating pancreatic ductal anomalies, such as pancreas divisum and annular pancreas[34].

Abdominal CT

Radiological evaluation, especially by CT, plays a pivotal role in the definition of managing severe cases, particularly in characterizing local complications that impact the prognosis and dictate the therapeutic approach[1]. CT is an outstanding noninvasive diagnostic tool for discerning the origins of endocrine and exocrine pancreatic insufficiencies in most patients, and its significance has grown considerably in the diagnosis, treatment, and follow-up of AP patients[21,27,35]. CT is commonly used to assess the severity of the inflammatory process, ascertain the presence and extent of pancreatic necrosis, and identify local complications[21]. CT with high spatial resolution and rapid acquisition is the preferred for diagnosing AP and associated local complications[36]. Moreover, CT can clearly display the pancreas and adjacent tissues and is more precise than US in diagnosing and delineating the extent of pancreatic disease[36,37].

CECT plays a pivotal role in assessing the scope and progression of AP and stands as the primary imaging modality for initially pinpointing local complications. Typical cross-sectional imaging features encompass pancreatic enlargement, pancreatic edema, uneven density, peripancreatic fat stranding, and fluid collection[15,38]. For example, on CECT, SAP patients typically exhibit larger amounts of peripancreatic retroperitoneal fluid[39]. Approximately 7 d after the onset of AP, initial CECT plays a significant role in predicting infected pancreatic necrosis, which underscores the significance for clinicians to contemplate the initial imaging of the pancreas[40]. In addition, CT is regarded as the gold standard for imaging evaluation of AP due to its satisfactory effectiveness, outstanding timeliness, and widespread availability[1]. A lack of clinical response to appropriate conservative treatment within 48-72 h often indicates the necessity for a CT scan to verify the initial diagnosis, assess the severity of the onset, and identify any complications[41].

In 1990, Balthazar *et al*[42] developed CTSI by integrating observations of peripancreatic inflammation, phlegmon, and the degree of pancreatic necrosis evident in initial CT examinations. To enhance the accuracy in predicting the prognosis of AP patients, Mortele *et al*[43] simplified the assessment of fluid collections and the extent of pancreatic necrosis in CTSI and added features that reflect organ failure and extrapancreatic complications, leading to the development of MCTSI. MCTSI grading of AP was significantly associated with duration of hospitalization, requirements for intensive care unit (ICU), necessity for intervention, and organ failure[44]. CTSI is an easy-to-calculate and informative tool and is considered to be a good predictor of mortality and severity of AP[45].

A prospective study including 50 patients evaluated prognostic correlation and clinical outcome of AP using both Balthazar's CTSI and modified Mortele's CTSI[27]. In this study, Raghuwanshi *et al*[27] concluded that the scores derived from the modified Mortele's CTSI exhibited a more robust correlation for all outcome parameters in all the patients compared to Balthazar's index. They asserted that CECT served as an outstanding diagnostic tool for staging the inflammatory process, identifying pancreatic necrosis, detecting local complications, and grading the severity of AP[27]. Contrary to expectation, the 2012 Revised Atlanta Classification (RAC) demonstrated greater accuracy than the modified Mortele's and Balthazar's CTSI in assessing mortality and organ failure among AP patients[27].

In a study including 178 patients with interstitial edematous pancreatitis, Song *et al*[46] indicated that the initial CECT findings of peripancreatic fluid and heterogeneous enhancement in the pancreatic parenchyma could serve as useful predictors for the progression to necrotizing pancreatitis (NP) in patients initially diagnosed with interstitial edematous pancreatitis. However, it was disconcerting that the early CT scan might not conclusively diagnose NP[46]. Tasu *et al*[47] demonstrated that a pancreatic enhancement threshold of less than 30 UH on post-contrast CT images during the portal phase provided an accurate and consistent criterion for diagnosing NP. Badat *et al*[48] highlighted that using the 2012 RAC to categorize pancreatic and peripancreatic collections by CT yielded moderate interobserver agreement, underscoring the potential necessity to either devise a new semiology for characterizing peripancreatic collections by CT or to employ alternative imaging modalities like MRI for more precise analysis of collection contents.

However, the latest relevant clinical research also has encouraging results. A retrospective cross-sectional study enrolled 1924 patients experiencing their first episode of AP from three tertiary referral centers in three different prefecture-level cities of Sichuan Province in China and revealed a positive rate of 96.7% (1860/1924) for CT findings in AP diagnosis based on CECT[49]. Among these 1860 AP patients with affirmative CT results, MCTSI exhibited positive correlations with both the 2012 RAC and APACHE II, as evidenced by Spearman's rank correlation coefficients[49].

However, there remains a puzzling contradiction, *i.e.*, CTSI and MCTSI remain inconsistent in assessing the severity and clinical outcome of AP. Bollen *et al*[50] determined that there was no notable distinction between CTSI and MCTSI in assessing AP severity. Both CT indexes were more accurate for diagnosing AP severity and had a better correlation with the need for intervention and pancreatic infection in comparison with APACHE II. Sahu *et al*[51] concluded that both CTSI and MCTSI significantly correlated with the clinical outcome of AP and aligned well with RAC grading of severity. MCTSI demonstrated higher sensitivity albeit with lower specificity than CTSI in differentiating MAP from MSAP/SAP.

Alberti *et al*[52] determined that CT indexes surpassed APACHE II in assessing the severity in AP, with CTSI holding a slight advantage over MCTSI. Additionally, CTSI precisely predicted pancreatic infections and intervention requirements. Liao *et al*[53] indicated that both CTSI and MCTSI were significantly associated with clinical prognosis, offering higher accuracy in predicting infectious pancreatic necrosis but less precision in predicting persistent organ failure compared to APACHE II.

Another important factor affecting the effectiveness of CECT in assessing AP severity is the appropriate timing. Dachs *et al*[54] indicated that early abdominal CT did not offer benefits to afebrile patients experiencing their first episodes of AP. The evidence-based guidelines from the International Association of Pancreatology/American Pancreatic Association recommend that the optimal timing for an initial CT assessment should be between 72-96 h following the onset of symptoms[55]. However, until now, the appropriate point in time for when CECT should be performed to provide an accurate assessment for AP has not been well established in clinical practice. For example, in a retrospective study with 309 SAP patients, Huang *et al*[56] highlighted that the optimal timeframe for CECT evaluation of SAP-associated complications was between 72 h and 1 wk following the onset of SAP, particularly for SAP patients with infection. Their findings revealed that the severity of the disease and its alterations manifested as expanded areas of acute peripancreatic fluid collection (APFC) and increased exudation of pleural effusion within the first 1 wk of SAP onset[56]. However, the former showed a decrease after 4 wk or more, while the latter reduced after 2 wk or more[56].

Pocard and Soyer[57] found that a meticulous review of the current literature failed to offer compelling evidence regarding a specific interval between symptom onset and CT examination, suggesting that the pertinent matter of timely CT examination in AP patients remains inadequately addressed by the existing studies. In this regard, an important and outstanding issue is that the optimal time point for CECT to evaluate SAP patients' needs to be determined by larger multicenter clinical studies to improve accuracy of disease diagnosis, avoid unnecessary CECT tests, promote early intervention, and thus improve prognosis.

Chest CT

In AP patients, thoracic complications encompass pleural effusion, pulmonary consolidation, atelectasis, pulmonary embolism, cardiac tamponade, pericardial effusion, elevated diaphragms, mediastinal pseudocysts, and acute respiratory distress syndrome (ARDS), the first two of which are common in AP[58-65]. In AP patients, pleural effusion accounted for 50% on admission, and the emergence of pulmonary consolidation was associated with the onset of respiratory failure [66]. A retrospective study from three Chinese Acute Pancreatitis Centers showed that 232 out of 465 AP patients had positive pleural effusion, accounting for 49.9%[67]. In a study including 358 AP patients from seven European centers, more than half of the patients had pleural effusion, with the proportion of 54.4% (195/358), and pleural effusion appeared mostly bilaterally (150/195, 76.9%)[64]. It has been reported that AP patients with bilateral pleural effusion had a significantly worse 1-year survival[64]. Bilateral pleural effusion/pulmonary consolidation was suggestive of SAP to a certain extent, and it was considered that measurement of these two parameters has certain clinical value in assessing the severity and prognosis of AP[68]. Moreover, the early onset of pleural effusion highlights its clinical significance and predicts a poor prognosis in AP[63].

In a single center study with 309 AP patients, Peng *et al*[65] explored the predictive significance of semiquantitative pleural effusion and pulmonary consolidation in determining AP severity using chest CT. In AP patients without organ failure, the values of pleural effusion and pulmonary consolidation were 25.4 ± 23.5 mL and 0.8 ± 1.0 points, respectively, which were lower than the corresponding values of 137.4 ± 116.9 mL and 2.4 ± 1.2 points observed in AP patients with organ failure[65]. Simultaneously, the values of pleural effusion and pulmonary consolidation in AP patients without death were 39.0 ± 36.0 mL and 1.0 ± 1.1 points, respectively, and were lower than the corresponding values of 144.0 ± 140.3 mL and 3.0 ± 1.1 points in the patients who died[65]. In addition, in predicting SAP, the accuracy of pleural effusion volume (mean value of 41.7 ± 38.0 mL, range of 1-1079 mL) and pulmonary consolidation score (mean value of 1.0 ± 1.2 points, range of 0-5 points) was similar to that of CTSI, APACHE II, and BISAP. For predicting organ failure, both the parameters had the same accuracy with the three scores, suggesting that the two parameters could provide prediction of SAP occurrences and organ failure in the early stage[65]. More importantly, this clinical study may increase the application value of CT due to the important role of these two parameters in predicting AP severity.

In a retrospective study from three medical centers, Yan *et al*[67] reported that the mean volume of pleural effusion was 98.8 ± 113.2 mL in 465 AP patients. The volume of pleural effusion exhibited significant and robust correlations with C-reactive protein (CRP), duration of hospital stay, and scoring systems, such as Ranson, BISAP, Marshall, APACHE II, CTSI, and extrapancreatic inflammation on CT, and displayed considerable accuracy in predicting outcomes like severity, infection, mortality, procedural needs, ICU admission, and organ failure[67]. Luiken *et al*[64] categorized the volume of pleural effusion in 195 AP patients as low (48.2%, 94/195), moderate (30.3%, 59/195), and severe (21.5%, 42/195). Their findings suggest that the presence of bilateral and/or moderate to severe amounts of pleural effusion in the early phase of AP could independently predict SAP[64].

Thus, the volume of pleural effusion can serve as a dependable radiological biomarker to predict the severity and clinical outcome of AP. In addition, larger and more multi-center prospective studies need to be conducted to promote the clinical application of pleural effusion in the prediction of AP severity. So far, there is an absence of an established quantitative grading system for pleural effusion, meriting attention in forthcoming clinical research.

Practical problems in the application of CT in AP

Based on advances in predicting and diagnosing AP severity, CECT is considered the diagnostic criterion for assessing AP. However, there is a non-negligible situation where contrast CT is contraindicated in patients with renal dysfunction and in pregnant women, and it is not possible to replicate follow-up studies due to cost and radiation exposure. When uncomplicated AP is diagnosed both clinically and biochemically, CT is superfluous; minimizing its overuse will not only

curtail healthcare costs but also diminish radiation exposure to patients[69]. CT on admission to predict outcome does not appear to have an advantage compared with the simpler and more readily available clinical scoring systems. Therefore, CT on the day of admission to assess severity is not recommended[70]. Improvement measures aimed at curbing the overuse of early imaging in AP patients may diminish superfluous imaging, elevate quality of care, and curtail wastage [71].

In addition, CT possesses limitations in assessing the severity of AP, and it is difficult to distinguish between necrosis and local effusion in small nonenhanced areas of the pancreas[36]. Without pancreatic parenchymal necrosis, small organized peripancreatic fluid collections might be misconstrued as pseudocysts on CT, leading to an underestimation of extrapancreatic necrosis[72]. These disadvantages limit the use of CT in some situations, and there is a need to develop other methods that can be used for the diagnosis and prognosis evaluation of AP. Furthermore, it is recommended that future studies should incorporate reliable non-radiological and laboratory-based categorization tests to enhance the precision in determining and assessing the severity and prognosis of AP, thereby reducing morbidity and mortality associated with post-necrotic inflammation of the pancreas.

MRI

While CT remains the prevalent choice for evaluating AP, MRI has demonstrated greater sensitivity than CT in detecting AP[34]. MRI, a noninvasive technology boasting high tissue contrast and multiple acquisition sequences, effectively aids in determining the diagnosis, complications, and severity of AP[36]. When CT yields negative results but there remains a strong clinical suspicion of AP, fat-saturated turbo spin echo T2-weighted or diffusion-weighted imaging sequences can reveal nuanced pancreatic and/or peripancreatic inflammation[73]. MRI holds a pivotal role in the diagnosis of AP and is instrumental in assessing and characterizing extrapancreatic necrosis, inflammation, splenomegaly, and tissue involvement, including vascular, transverse-mesocolon, interfascial plane, and the gastrointestinal tract, in AP patients [21,74-80]. MRI can effectively capture the intra-abdominal inflammatory spread that affects mesenteric and omental fatty regions, indicative of a pathological manifestation of intra-abdominal fat edema combined with fat necrosis resulting from AP[81].

MRI is particularly beneficial for imaging of patients with iodine allergies or renal insufficiency, characterizing fluid collections and evaluating abnormalities or disconnections in the pancreatic duct[38]. As an alternate method for diagnosing AP, MRI shows great potential in clinical applications. MRI offers superior capabilities in diagnosing early extrapancreatic necrosis compared to CT, without the need for radiation, making it suitable for repeated follow-up assessments[74]. MRI more adeptly identifies the subtlest changes in AP and can delineate the constituents of mild extrapancreatic inflammatory effusions that might be missed on CT[82]. Fat-saturated T2-weighted MRI offers superior sensitivity in detecting fluid and no liquefied material in extrapancreatic collections compared to CT, while T1-weighted MRI is beneficial for identifying pancreatic or peripancreatic hemorrhage[74].

MRI in hemorrhage, tissue necrosis, and APFC

Compared to CT, MRI demonstrates superior sensitivity in visualizing hemorrhages, which appear hyperintense on T1-weighted imaging during the acute phase and maintain their signal intensity longer than on CT[36]. In necrotizing pancreatitis, MRI offers superior soft tissue contrast compared to CT and excels in visualizing hemorrhage and tissue necrosis[36]. A retrospective study including 539 AP patients demonstrated that MRI was superior in detecting hemorrhage associated with AP compared to CT, even when CT showed no signs of hemorrhage[83]. This study revealed that pancreatitis in AP patients with accompanying hemorrhage presented with greater clinical severity, increased susceptibility to organ failure, and prolonged hospital stays, suggesting that early hemorrhage detection on MRI could serve as a novel severity indicator in AP associated with poorer prognosis[83]. Additionally, due to its enhanced tissue resolution, MRI is poised as the frontline imaging technology for evaluating AP and its complications, notably the identification of hemorrhage[83].

In a retrospective analysis including 301 AP patients, MRI revealed that 24.9% exhibited at least one peripancreatic vascular abnormality related to AP, and the incidence of peripancreatic vascular involvement was notably more pronounced in necrotizing pancreatitis compared to edematous pancreatitis[76]. The common manifestations of early AP on MRI were splenic vein phlebitis and splenic artery involvement/arteritis, and 6.3% of the patients had splenic artery arteritis complicated with hemorrhage in the early phase of AP[76]. The findings highlighted the efficacy of MRI in delineating the progression of inflammatory processes and associated vascular changes during treatment, and early-stage vascular involvement detected by MRI might serve as a valuable indicator of AP severity[76].

Since the introduction of abdominal US and CT in the early 1970s, there has been a marked increase in the identification of acute fluid collections in AP, accompanied by a deeper insight into their natural progression and management [84]. APFC can complicate acute interstitial edematous pancreatitis, manifesting in approximately 30%-50% of such cases [85]. If APFC was associated with high BISAP (≥ 3) and CRP levels (≥ 150 mg/L) after 48 h from admission or with persistent clinical symptoms reflecting prolonged inflammatory responses, SAP patients with APFC were more likely to develop late complications[86]. Acute necrotic collections, observed exclusively in necrotic pancreatitis within the first 4 wk of onset, comprise varying amounts of fluid and necrosis, with the latter potentially affecting the pancreatic parenchyma or peripancreatic tissues, or both[36]. Pancreatic necrosis, characterized by focal, multifocal, or diffuse devitalized tissue within the pancreas, either superficial or deep, is deemed a critical imaging indicator of necrotizing pancreatitis[81]. A significant correlation exists between the presence of pancreatic necrosis and extrapancreatic fluid collections in relation to the clinical parameters, with an increase in extrapancreatic fluid collections aligning with the escalating severity of AP[87].

While CT has emerged as the primary noninvasive tool for identifying local complications in AP, it is difficult to distinguish between APFC and acute necrotic collection in the early phase due to its limited sensitivity in revealing the necrosis debris of peripancreatic tissue[81]. Given its exceptional resolution for soft tissues, MRI surpasses CT in delineating pancreatic/peripancreatic fluid collections, especially in quantifying solid debris and fat necrosis, serving as an alternative in cases with CT contraindications[88]. In MRI findings, hemorrhage in the pancreas and/or surrounding tissues may intermingle with necrosis of these same regions, manifesting as spotted, patchy, or extensive regions of hyperintensity on T1-weighted fat-suppressed images[81]. In a retrospective study including 70 AP patients, Zhou *et al* [74] discovered that MRI characteristics of extrapancreatic collections, particularly its extent and amount, could differentiate early extrapancreatic necrosis from peripancreatic fluid collections, suggesting the presence of extrapancreatic necrosis. Moreover, the more extensive the extrapancreatic collections and the broader the scope of extrapancreatic inflammation associated with hemorrhage in AP on MRI, the higher the likelihood of extrapancreatic necrosis[74].

In a meta-analysis encompassing a total of 566 patients, MRI demonstrated superior accuracy and sensitivity compared to CT for diagnosing AP[89]. While no study has yet shown that MRI can decrease AP mortality or enhance prognosis, MRI serves as an invaluable diagnostic tool for distinguishing individuals with suspected AP and is regarded as the premier imaging choice for the clinical diagnosis of AP[89]. Tang *et al*[82], utilizing MRI and APACHE II, devised a novel model through logistic regression for the early prediction of AP severity and ascertained that the combined model of extrapancreatic inflammation on MRI (EPIM) and APACHE II excelled in predicting AP severity, surpassing individual parameters. This retrospective analysis including 363 AP patients suggested that merging MRI and APACHE II for gauging AP severity was both viable and more accurate than other scoring mechanisms, potentially facilitating the creation of tailored treatment and management[82].

MRI severity index in AP

MRI severity index (MRSI), derived from CTSI, evaluates the severity of AP by integrating both peripancreatic inflammation and pancreatic parenchymal necrosis, achieving an effect comparable to that of CTSI in assessing AP severity[36]. In patients with pancreatitis, MRSI outperformed APACHE II in assessing local complications, while APACHE II demonstrated superiority in determining systemic complications[90]. MRSI is pivotal for the initial assessment, staging, and prognosis of AP. The clinical relevance of MRSI allows for prediction of the severity of AP based on initial MRI findings in the early phase, and it holds a significant correlation with APACHE II, incidence of systemic complication, duration of hospital stay, and overall clinical outcome[81]. In a retrospective study including 337 AP patients, Zhou *et al* [75] reported that in the early stages of AP EPIM based on MRI proved more effective in assessing the severity than extrapancreatic inflammation on CT . Moreover, the predictive accuracies of EPIM for SAP and organ failure aligned with those of APACHE II and BISAP, surpassing the accuracy of MRSI[75].

Overall, MRI serves as an excellent instrument for identifying and distinguishing prevalent local complications subsequent to AP. MRI offers diagnostic and prognostic value on par with CT, though it presents certain limitations in clinical practice. The scans necessitate greater cooperation of the patient, including prolonged immobility and apnea, and are more time-consuming and costly[1]. Additionally, MRI has the limitation of a restricted field of view, preventing it from capturing extensive regions of the chest and pelvic cavity simultaneously, as CT can[81].

US

Based on its quick, simple, repeatable, radiation-free, bedside applicability, US is the first-line imaging method in most medical centers to confirm the diagnosis of AP and exclude other causes of acute abdomen. In the early period, the advantages of US are its capability of assessing the gallbladder and biliary tract, detecting gallstones, and identifying bile duct dilatation[21]. However, US may show normal pancreas in MAP patients and is not able to differentiate the diagnosis between interstitial and necrotizing pancreatitis because of not allowing the assessment of parenchymal perfusion[21]. EUS can identify choledocholithiasis and hidden pancreatic tumors that remain elusive on CT or MRI in recurrent AP patients. EUS-guided fine needle puncture biopsy can distinguish focal pancreatitis from a pancreatic tumor, and color Doppler US can be used to assess vascular complications such as false arterial aneurysms or portal vein thrombosis[21]. Xu *et al*[91] reported that EUS outperformed CT in accurately categorizing symptomatic peripancreatic fluid collections and emerged as a preferred imaging modality for detecting solid necrotic debris. EUS-guided lumen-apposing metal stents for pancreatic fluid collections were feasible and effective with preferable technical and clinical success rates[92].

In a retrospective analysis with a cohort of 6069 patient, Froes *et al*[93] evaluated the impact of abdominal ultrasound (AUS) on the length of service (LOS) for patients hospitalized for AP who lacked radiographic evidence of AP on CT of the abdomen and pelvis (CTAP). Additionally, they further assessed how AUS affected the probability of subsequent interventions, such as ERCP or cholecystectomy[93]. In patients with AP, undergoing AUS within 48 h resulted in a reduced LOS by 1.099 d. Those who underwent AUS were 1.126 times more likely to proceed with subsequent ERCP compared to those who only had CTAP; patients receiving AUS after CTAP had a 2.711 times higher likelihood of undergoing subsequent cholecystectomy[93]. In this cohort of patients admitted for AP, conducting AUS within 48 h after negative CTAP correlated with reduced LOS. Moreover, patients undergoing AUS were not only more inclined to undergo ERCP but also exhibited a higher likelihood of undergoing cholecystectomy[93].

In a study with a total of 196 patients, Cai *et al*[94] investigated the diagnostic accuracy of US and contrast-enhanced US (CEUS) for AP. They demonstrated that CEUS outperformed US in diagnosing AP and SAP and produced excellent results in the staging of AP severity[94]. In this study, compared to results from CECT, the diagnostic rates for pancreatic swelling using US and CEUS were 121% (148/122) and 91% (111/122), respectively, while for peripancreatic fluid collection, they were 84.8% (151/178) and 96.6% (172/178), respectively[94]. The findings confirmed that CEUS surpassed US in specificity when visualizing pancreatic parenchyma edema, pancreatic border-capsula, collection fluid of

peripancreas, and peripancreatic necrosis. This discrepancy between US and CEUS might arise from the ability of CEUS to visualize vessels upon contrast agent injection[94]. The conclusion drawn was that CEUS serves as a trustworthy method for diagnosing and monitoring AP and SAP, potentially acting as an alternative to CECT[94].

Summary

The application of imaging in patients with AP is an essential aspect of modern clinical management. While there are challenges associated with their use, continuous research, technological advances, and thoughtful implementation of guidelines can optimize their role in patient management.

Imaging technologies for diagnosing and managing AP have made great strides, but inappropriate imaging tests can increase economic costs to the health system, subject patients to excess radiation, and elevate complication rates without benefiting patients. The choice of appropriate imaging modality for AP depends exactly on available time, technique, and clinical situation of the patient. Although imaging examination is widely used and carefully evaluated during the diagnosis process of AP, it remains unclear when imaging should be performed, especially given the economic costs associated with imaging and the financial burden on patients. In terms of the economic and financial implications of diagnostic imaging for AP patients, early imaging may not be advisable for those presenting with characteristic clinical symptoms and pronounced laboratory results. However, when clinical manifestations are unclear, early imaging examination is often used to identify suspected AP, discover potential etiology, diagnose complications, assess severity, implement risk stratification, and guide treatment. For AP patients, imaging technologies remain pivotal in initial diagnosis, identification of severe cases, assessment of prognosis, and decision of therapeutic management.

Radiomics is a data science technique that extracts a large number of quantitative features from medical images using advanced algorithms. These features capture subtle differences in the texture, shape, and intensity of image regions, which may be difficult for human observers to discern. By extracting these features, radiomics can transform images into high-dimensional data that can be analyzed and mined using machine learning and other data science techniques. This allows for more objective and precise diagnosis, treatment planning, and prognosis evaluation in AP. Therefore, radiomics has the potential to revolutionize medical imaging and improve patient outcomes in the 21st century.

CLINICAL SCORING SYSTEMS

Over the decades, many clinical scoring systems have been developed and applied, and their efficacy and accuracy have been compared. Clinically, an ideal scoring system should be responsive, simple, reliable, and universally applicable across diverse patient populations and geographical areas, maintaining its relevance over time. Such clinical scoring systems are imperative to predict complications, severity, mortality, and ICU admission requirements in AP patients[95]. Numerous "traditional" multifactorial clinical scoring systems, such as APACHE II, Ranson, Glasgow, Systemic Inflammatory Response Syndrome (SIRS), HAPS, Japanese Severity Score (JSS), CTSI, Sequential Organ Failure Assessment (SOFA) and BISAP, provide insights into systemic complications to some extent and possess commendable predictive capabilities for severity and mortality of disease[12,82,96-98]. Based on the 2012 RAC, these scoring systems primarily stratified the severity of AP into MAP, MSAP, and SAP[99].

Development of the original APACHE severity-of-illness classification system began in 1978, and APACHE II was derived from the results of a simplified effort based on the 12 most commonly used physiological measures included in the original APACHE system[100]. APACHE II, initially designed for intensive care applications, necessitates the aggregation of numerous parameters, some of which might not be pertinent to the prognosis in AP, while it overlooks key indicators such as pancreatic injury and significant regional complications[101,102].

Ranson was first used to assess the severity of AP in 1974 and has been used for nearly 50 years[103]. Ranson is relatively accurate in classifying the severity of AP patients; however, its limitation is the 48-h duration required for completion, thereby missing a crucial early therapeutic opportunity[102]. The main limitation of Glasgow, much like Ranson, is the need for a 48-h duration to finalize the calculation[96]. However, based on the local characteristics of CT examinations, CTSI mainly emphasizes local complications but falls short in representing the systemic inflammatory response[42]. In addition, for SAP, MCTSI demonstrates prognostic value for short-term mortality, while CTSI effectively predicts the necessity for intervention[104]. SOFA, similar to APACHE II, is a detailed scoring system that takes into account acute and chronic illness, signs, and laboratory values in patients[12].

Comparison of different scoring systems used in AP

For a more thorough understanding of the various attributes inherent in distinct scoring systems, we will embark on a comprehensive discussion and detailed analysis of the utilization of commonly employed clinical scoring systems within the context of AP in the following sections.

In two independent, prospectively enrolled cohorts [training (n = 256) and validation (n = 397)] of AP patients, Mounzer *et al*[105] compared the accuracy of the scoring systems including APACHE II, BISAP, Glasgow, HAPS, JSS, Ranson, and SIRS in predicting persistent organ failure. In this study, they discovered that these scoring systems exhibited moderate accuracy, with area under the curve (AUC) at admission ranging from 0.62-0.84 in the training cohort and 0.57-0.74 in the validation cohort. Notably, Glasgow emerged as the superior classifier at admission in both cohorts [105]. In a retrospective study including 161 patients, statistically significant cutoff values in predicting SAP were APACHE II ≥ 8, Ranson ≥ 3, BISAP ≥ 2, CTSI ≥ 3, and CRP_{24} ≥ 21.4 mg/dL. APACHE II had the highest accuracy in predicting SAP[106].

Confusingly, different studies have shown that these scoring systems vary widely in accuracy, sensitivity, and specificity for the desired purpose of prediction, as follows. In a retrospective study including 326 patients diagnosed with hyperlipidemic AP (HLAP), the predictive abilities of APACHE II, BISAP, Ranson, and MCTSI were compared for assessing MSAP and SAP, local complications, and HLAP mortality[107]. The results showed that the four scoring systems have their own advantages and characteristics. For example, Ranson lacked a distinct advantage in predicting severity and prognosis of HLAP compared to other three scoring systems. APACHE II excelled in predicting HLAP severity but fell short in predicting local complications. MCTSI demonstrated exceptional prowess in predicting local complications yet was less adept in predicting severity and mortality. BISAP offered a commendable accuracy in evaluating the severity, local complications, and mortality of HLAP, yet there remains room for refining its precision in future assessments[107].

In a prospective study including 50 AP patients, Kumar and Griwan[108] assessed the accuracy of APACHE II, BISAP, Ranson and MCTSI in predicting the severity of AP, referencing the 2012 RAC. In this study, MCTSI demonstrated the highest AUC values for predicting SAP (0.919), pancreatic necrosis (0.993), organ failure (0.893), and ICU admission (0.993); meanwhile, APACHE II ranked second in accuracy for predicting SAP (0.834) and organ failure (0.831)[108]. The findings indicated that APACHE II demonstrated a high sensitivity in predicting pancreatic necrosis (93.33%), organ failure (92.86%), and ICU admission (92.31%) while also maintaining a substantial negative predictive value (NPV) for predicting pancreatic necrosis (96.15%), organ failure (96.15%), and ICU admission (95.83%)[108].

Keskin *et al*[109] retrospectively investigated 690 patients who had been admitted due to AP by five scoring systems including HAPS, Ranson, BISAP, Glasgow, and JSS. In this study, NPV of each score was notably superior to their respective positive predictive value (PPV)[109]. Of the five scoring systems, JSS exhibited the highest value of AUC across all endpoints (0.80 for in-hospital major adverse events, 0.94 for in-hospital mortality, 0.91 for 30-d mortality); nevertheless, none of the five scoring systems effectively predicted 30-d readmission[109].

Li *et al*[110] conducted a retrospective assessment of four scoring systems (Ranson, BISAP, Glasgow, and APACHE II) to predict AP outcomes in 918 patients, categorizing them into two age groups: The elderly (≥ 60-years-old) and the younger (< 60-years-old). In this study, they drew several following conclusions: BISAP effectively predicted the severity, pancreatic necrosis, and mortality in elderly AP patients; APACHE II was more suitable for assessing severity in younger patients; both Ranson and Glasgow were generally applicable for evaluating most AP patients; and Ranson demonstrated heightened efficacy in assessing severity among younger patients[110]. In this study, the criterion of predicting SAP was different between the elderly and the younger (the elderly: Ranson ≥ 4, Glasgow ≥ 3, APACHE II ≥ 9, BISAP ≥ 3; the younger: Ranson ≥ 3, Glasgow ≥ 2, APACHE II ≥ 8, BISAP ≥ 2), suggesting that the scoring cutoffs for the elderly were consistently one point higher than those for the younger[110]. The variation in the cutoff value for predicting SAP enhanced the specificity of the four scoring systems albeit with a marginal reduction in their sensitivity to SAP[110].

In a retrospective analysis including 653 AP patients, Teng *et al*[111] investigated and compared the characteristics of six scores in predicting SAP, ICU admission, and mortality, including Ranson, Glasgow, APACHE II, BISAP, HAPS, and SOFA. In predicting SAP, SOFA exhibited the lowest sensitivity at 13.6% but boasted the highest specificity at 99.7%. Conversely, Ranson maintained the highest sensitivity at 92.6% but had one of the lowest specificities at 51.9%, with only HAPS registering a slightly lower specificity at 49.7%[111]. In predicting ICU admission, APACHE II and Ranson displayed a sensitivity at 100.0%, BISAP demonstrated the lowest sensitivity at 25.0% and a specificity at 93.4%, and SOFA demonstrated the highest specificity at 99.2%[111]. In predicting mortality, APACHE II and Ranson displayed a sensitivity at 100.0%, BISAP showcased the lowest sensitivity at 25.0%, and SOFA had the highest specificity at 98.9%, similar to ICU admission[111]. All scores had high and comparable NPVs in the prediction of SAP, ICU admission, and mortality in AP patients[111]. In this study, they concluded that SOFA and 48-h Ranson outperformed other clinical scorings (Glasgow, APACHE II, BISAP, HAPS) in predicting severity, ICU admission, and mortality[111].

In a prospective observational study including 164 patients, Venkatesh *et al*[112] reported that, based on receiver operating characteristic (ROC) curves, Ranson at admission demonstrated superior diagnostic accuracy in predicting severity, organ failure, and mortality and outperformed the other three scores (APACHE II, BISAP, and modified Glasgow) in predicting AP severity. In addition, this study revealed that while BISAP might be calculated within 24 h of admission, both APACHE II and modified Glasgow demonstrated superior diagnostic accuracy, with APACHE II exhibiting the strongest association with mortality in SAP patients[112].

Asfuroğlu Kalkan *et al*[113] retrospectively analyzed 1150 AP patients, and reported that these scoring systems including BISAP, Ranson, HAPS, APACHE II, and Glasgow were capable of predicting mortality. However, APACHE II predicted mortality with a sensitivity of 90% and specificity of 92%[113].

Drawing on the insights gleaned from the aforementioned body of literature, we have meticulously synthesized a detailed appraisal of the application of various clinical scoring systems in prognosticating severity, local complications, organ failure, and mortality rates associated with AP. These summarizations are comprehensively depicted in Table 1. Various scoring systems exhibited diverse levels of sensitivity, specificity, and accuracy in forecasting the severity, local complications, organ failure, and associated mortality. Further, it is noteworthy that numerous studies have indicated the existence of substantial differences among these scoring systems, highlighting their lack of uniform standards and, in some instances, a concerning degree of inconsistency in their projections. Given the variability in accuracy among diverse scoring systems for predicting the severity, local complications, organ failure, and mortality associated with AP, there is a plausible need for further refinement and design optimization of each scoring system to enhance the precision of these predictions. Moreover, another potential area of research could be the amalgamation of multiple existing scoring systems to boost the predictive accuracy for AP through a more comprehensive scoring approach.

To enhance a more comprehensive understanding of the clinical utility of prevalent scoring systems, such as BISAP, SOFA, and qSOFA, in predicting AP outcomes, we conducted independent discussions and analyses on the latest advancements of these tools to provide invaluable reference and guidance for their practical application in clinical

Table 1 Comparison of existing clinical scoring systems used in patients with acute pancreatitis for predicting the severity of acute pancreatitis, such as severe acute pancreatitis, mortality, organ failure, intensive care unit admission, location complications, in-hospital adverse events, and pancreatic necrosis

Prediction	Scoring system (cutoff value)	Sensitivity, %	Specificity, %	Accuracy, %	PPV, %	NPV, %	AUC	No. of patients	Ref.
MSAP and SAP[1]	BISAP (≥ 3)	54	86	-	68	-	0.795	326	[107]
	Ranson (≥ 3)	46	84	-	54	-	0.766		
	APACHE II (≥ 8)	57	89	-	67	-	0.814		
	MCTSI (≥ 4)	36	94	-	66	-	0.654		
SAP[1]	Ranson (≥ 3)	85.7	44.3	-	18.8	95.3	0.69	161	[106]
	BISAP (≥ 2)	61.9	72.1	-	25.0	92.7	0.74		
	APACHE II (≥ 8)	81.0	65.7	-	26.2	95.8	0.78		
	CTSI (≥ 3)	66.7	67.1	-	23.3	93.1	0.69		
SAP[1,2]	Ranson (≥ 4/≥ 3)	81.4/92.0	84.2/92.8	-	28.9/37.7	98.3/99.6	0.867/0.964	368/550	[110]
	BISAP (≥ 3/≥ 2)	88.9/96.0	86.5/88.0	-	34.3/27.6	99.0/99.8	0.922/0.942		
	APACHE II (≥ 9/≥ 8)	85.2/96.0	61.0/93.0	-	14.7/42.9	98.1/99.8	0.784/0.951		
	Glasgow (≥ 3/≥ 2)	85.2/80.0	84.2/88.2	-	29.9/24.4	98.6/98.9	0.913/0.881		
SAP	HAPS (≥ 1)	79.0	49.7	53.3	18.2	94.4	0.687	653	[111]
	BISAP (≥ 3)	24.7	95.3	86.5	42.6	89.9	-		
	APACHE II (≥ 8)	80.2	63.3	65.4	23.6	95.8	-		
	Ranson (≥ 3)	92.6	51.9	57.0	21.4	98.0	0.857		
	Glasgow (≥ 3)	76.5	68.5	69.5	25.6	95.4	-		
	SOFA (≥ 7)	13.6	99.7	89.0	84.6	89.1	0.966		
SAP[3]	APACHE II (≥ 6)	50	100	68.3	100	53.57	0.771	164	[112]
	BISAP (≥ 2)	25.96	100	53.1	100	43.80	0.640		
	Modified Glasgow ≥ 3)	75.96	100	84.8	100	70.59	0.649		
	Ranson (≥ 2)	32.69/58.65	100/100	57.3/73.8	100/100	46.15/58.25	0.848/0.817		
SAP[3,4]	APACHE II (≥ 6)	63.7	77.1	68.2	84.6	51.9	-	69	[112]
	BISAP (≥ 2)	31.8	85.7	50.0	81.4	38.9	-		
	Modified Glasgow (≥ 3)	79.9	31.4	63.4	69.6	44.0	-		
	Ranson (≥ 2)	44.9/63.7	91.4/51.4	60.5/59.6	91.1/72.1	45.7/41.8	-		
Mortality[1]	BISAP (≥ 3)	89	80	-	15	-	0.867	326	[107]
	Ranson (≥ 3)	78	77	-	9	-	0.842		
	APACHE II (≥ 8)	89	78	-	10	-	0.854		
	MCTSI (≥ 4)	78	86	-	14	-	0.839		
Mortality in AP	HAPS (≥ 1)	83.3	46.6	29.9	2.8	99.3	-	653	[111]

	BISAP (≥ 3)	25	93.1	91.9	6.4	98.5	-		
	APACHE II (≥ 8)	100	58.7	59.1	3.6	100	-		
	Ranson (≥ 3)	100	47.3	48.2	3.4	100	0.917		
	Glasgow ≥ 3)	75.0	63.8	64.2	4.1	99.5	-		
	SOFA (≥ 7)	50.0	98.9	98.0	46.2	99.1	0.968		
Mortality[1]	BISAP (≥ 2.5)	92.0	90.0	-	-	-	0.92	106	[113]
	HAPS (≥ 1.5)	49.0	98.0	-	-	-	0.83		
	Ranson (≥ 3.5)	75.0	71.0	-	-	-	0.78		
	JSS (≥ 3.5)	84.0	94.0	-	-	-	0.92		
	Glasgow (≥ 2.5)	89.0	86.0	-	-	-	0.91		
	APACHE II (≥ 5.5)	90.0	92.0	-	-	-	0.94		
Persistent organ failure[1,5]	APACHE II (≥ 7)	84/94	71/44	-	49/14	93/99	0.77/0.71	256/397	[105]
	BISAP (≥ 2)	61/62	84/76	-	54/20	87/96	0.72/0.69		
	Glasgow (≥ 2)	85/65	83/82	-	61/22	95/97	0.84/0.74		
	HAPS (≥ 1)	70/73	53/58	-	32/12	85/97	0.62/0.66		
	JSS (≥ 2)	59/42	92/89	-	70/23	88/95	0.76/0.66		
	Ranson (≥ 2)	66/46	78/80	-	49/16	88/95	0.72/0.63		
	SIRS (≥ 2)	70/69	71/58	-	43/11	88/96	0.70/0.64		
Organ failure[1]	Ranson (≥ 3)	88.89	96.67	-	88.89	96.67	0.757	50	[108]
	BISAP (≥ 3)	90.00	83.87	-	64.29	96.30	0.762		
	APACHE II (≥ 8)	92.86	69.44	-	54.17	96.15	0.831		
	MCTSI (> 4)	92.86	75.00	-	59.09	96.43	0.893		
Association with organ failure[3]	APACHE II (≥ 6)	48.5	36.2	40.3	27.8	28.1		164	[112]
	BISAP (≥ 2)	8.5	55	39.4	8.8	54.2	0.640		
	Modified Glasgow (≥ 3)	68.5	20.2	36.5	30.3	56	0.649		
	Ranson (≥ 2)	14.2/22.8	68.1/36.2	50/31.7	18.5/15.3	61/48	0.848/0.817		
ICU admission[1]	Ranson (≥ 3)	80.00	96.55	-	88.89	93.33	0.910	50	[108]
	BISAP (≥ 3)	90.91	86.67	-	71.43	96.30	0.877		
	APACHE II (≥ 8)	92.31	65.71	-	50.00	95.83	0.885		
	MCTSI (> 4)	92.86	75.00	-	59.09	96.43	0.993		
ICU admission	HAPS ≥ 1	90.0	47.2	29.9	5.1	99.3	-	653	[111]
	BISAP ≥ 3	25.0	93.4	91.3	10.6	97.5	-		
	APACHE II ≥ 8	100	59.6	60.5	6.6	100	-		
	Ranson ≥ 3	100	47.9	49.5	5.7	100	0.946		
	Glasgow ≥ 3	75.0	64.5	65.1	7.0	99.3	-		
	SOFA ≥ 7	40.0	99.2	97.4	61.5	98.1	0.943		
Location complications[1]	BISAP (≥ 3)	54	81	-	21	-	0.731	326	[107]
	Ranson (≥ 3)	57	79	-	20	-	0.698		

	APACHE II (≥ 8)	43	78	-	15	-	0.580		
	MCTSI (≥ 4)	68	90	-	38	-	0.791		
In-hospital adverse events[1,6]	HAPS ≥ 2	66.2	70.6		36.0	89.1	0.70	690	[109]
	Ranson ≥ 3	66.9	62.8	-	31.2	88.3	0.68		
	BISAP ≥ 2	61.9	75.9	-	39.3	88.7	0.74		
	Glasgow ≥ 2	51.8	83.7	-	44.0	87.3	0.71		
	JSS[7]	81.9	66.0	-	38.2	93.4	0.80		
Pancreatic necrosis[1]	Ranson (≥ 3)	80.00	96.55	-	88.89	93.33	0.910	50	[108]
	BISAP (≥ 3)	81.82	83.33	-	64.29	92.59	0.822		
	APACHE II (≥ 8)	93.33	71.43	-	58.33	96.15	0.855		
	MCTSI (> 4)	93.33	77.14	-	63.64	96.43	0.993		

[1]The predictive accuracy of each scoring system was measured by the area under the curve.
[2]In this study, 918 patients with were divided into two groups, namely the elderly group (368 patients who were ≥ 60-years-old) and the younger group (550 patients who were < 60-years-old). The former value corresponds to the elderly group, and the latter value corresponds to the younger group.
[3]The Ranson score in this study involved two time points: at admission and 48-h after admission. For Ranson, the former value corresponds to at admission, and the latter value corresponds to 48-h after admission.
[4]Computed tomography (CT) abdomen in 69 patients showed modified CT severity index ≥ 8 in all 69 (100%) patients.
[5]In this study, two prospective cohorts were involved, namely the training cohort and the validation cohort. The former value corresponds to the training cohort, and the latter value corresponds to the validation cohort.
[6]In-hospital adverse events included all in-hospital complications, pancreatic necrosis, and in-hospital mortality.
[7]Severe acute pancreatitis according to the Japanese Severity Score was defined if the prognostic factor was ≥ 3 or CT grade ≥ 2.
AP: Acute pancreatitis; APACHE: Acute Physiology and Chronic Health Evaluation; AUC: Area under the curve; BISAP: Bedside Index for Severity in Acute Pancreatitis; CTSI: Computed tomography severity index; HAPS: Harmless acute pancreatitis score; ICU: Intensive care unit; JSS: Japanese Severity Score; MCTSI: Modified Mortele's computed tomography severity index; MSAP: Moderately severe acute pancreatitis; NPV: Negative predictive value; PPV: Positive predictive value; SAP: Severe acute pancreatitis; SIRS: Systemic Inflammatory Response Syndrome; SOFA: Sequential Organ Failure Assessment.

settings.

BISAP

In a large population-based study, Wu *et al*[101] identified five variables for prediction of in-hospital mortality by Classification and Regression Tree analysis to derive a prognostic scoring system (BISAP) including blood urea nitrogen (> 25 mg/dL), age (> 60 years), SIRS, pleural effusion, and impaired mental status. Blood urea nitrogen emerged as the most efficient primary discriminative variable, age and SIRS further distinguished between high-risk and low-risk cases, and mental status and pleural effusion further refined the categorization of intermediate-risk patients[101]. Introduced in 2008, BISAP, with its advantages of simplicity and precision, had been employed for the early identification of AP patients with an elevated risk of in-hospital mortality[101]. BISAP is adept at identifying AP patients at heightened risk of mortality, representing the advancement of intermediate markers of severity within 24 h of onset, and its risk stratification ability could hold potential for enhancing clinical care and streamlining enrollment in clinical trials[114]. BISAP was considered to be as good as APACHE II in predicting severity, death, and especially organ failure in AP. It outperformed Ranson, CTSI, CRP, hematocrit, and body mass index, with a score of 2 being a statistically significant cutoff value[115]. BISAP is a streamlined scoring system designed to predict the severity of AP and is instrumental in early risk stratification of AP.

A prospective study of 87 patients experiencing their first episode of AP revealed that BISAP (≥ 2) demonstrated comparability to both APACHE II (≥ 8) and MCTSI (≥ 8) in metrics of accuracy, sensitivity, specificity, and NPV[116]. In a systematic review and meta-analysis, a pooled analysis from 12 prospective cohorts showcased the exemplary performance of BISAP in predicting SAP across diverse patient populations and disease severity[117]. Furthermore, the performance of BISAP was notably superior when severe pancreatitis was characterized by the persistence of organ failure for 48 h or more[117]. A European cohort study indicated that BISAP effectively predicted SAP, mortality, and ICU admission, making it invaluable for triaging patients toward ICU care[118].

Chen *et al*[119] assessed the accuracy of BISAP in predicting the severity and prognoses of AP in Chinese patients. In this study, they retrospectively analyzed clinical data from 497 AP patients comparing BISAP with APACHE II, Ranson, and CTSI regarding their predictive capacities for the severity of AP and the occurrence of mortality, pancreatic necrosis, and organ failure in SAP patients[119]. They highlighted that BISAP outperformed traditional scoring systems in terms of simplicity and speed, and maintained a performance comparable to other scoring systems in predicting both SAP and its associated prognoses[119].

Zhang *et al*[120] evaluated the efficacy of BISAP, APACHE II, and Ranson in predicting the severity, mortality, and pancreatic necrosis of AP based on the 2012 RAC at a tertiary care center in China. From their study involving 155 patients, they determined that BISAP might serve as a reliable tool for risk stratification and prognostic assessment in Chinese AP patients[120]. Gao *et al*[121] conducted a meta-analysis to systematically assess the accuracy of BISAP in predicting mortality and SAP and affirmed that BISAP served as a dependable tool for identifying AP patients at elevated risk for adverse outcomes. While BISAP demonstrated superior specificity compared to Ranson and APACHE II, it exhibited a slightly diminished sensitivity for both mortality and SAP[121].

An Indian study with 119 AP patients showed that BISAP was an accurate means of risk stratification, and patients with BISAP ≥ 4 invariably developed SAP or pancreatic necrosis and had high mortality[122]. The available studies collectively demonstrated that BISAP performs very well in predicting SAP, and the simplicity and accuracy of the calculation make BISAP a valuable tool for clinical care of AP patients. Additionally, before confidently advocating for the adoption of BISAP, its integration into clinical practice should be evaluated to determine its potential to enhance outcomes in AP.

BISAP, an easily computed clinical prediction scale, leverages data from initial assessment of patients and routine laboratory results, demonstrating excellent performance in predicting SAP. BISAP is less cumbersome to calculate and more economical, which makes it an ideal scoring system. It is considered that BISAP should be popularized at primary and secondary care institutions for severity classification and risk stratification of early AP. Therefore, SAP patients can be referred to higher-level medical centers for more reasonable clinical intervention.

However, as more and more clinical studies have been conducted, BISAP has shown inconsistent predictive power and results in predicting SAP, as reported in the next studies. A prospective study including 51 patients showed that BISAP was inferior to APACHE II in predicting the severity of AP, especially for SAP[123]. In a meta-analysis including 1972 subjects, Yang and Li evaluated the diagnostic performance of BISAP in predicting SAP[124]. They concluded that despite its high specificity BISAP was not the optimal standalone method for assessing AP severity due to its low sensitivity[124].

In a prospective study including 50 AP patients, the accuracy of BISAP in predicting SAP was 84%, surpassing that of serum procalcitonin (PCT) (≥ 3.29 ng/mL) at 76%, which was on par with APACHE II; moreover, in logistic regression analysis, BISAP demonstrated greater statistical significance than serum PCT[125]. They determined that BISAP outperformed serum PCT, APACHE II, Glasgow, and BCTSI in accurately predicting AP severity, positioning it as a promising tool for gauging the clinical progression of AP[125]. Hagjer *et al*[126] evaluated the usefulness of BISAP and PCT for AP prediction in a prospective observational study including 60 patients. Based on this study, in predicting severity, mortality, and organ failure, they finally concluded that BISAP was as effective as APACHE II and surpassed Ranson, CTSI, CRP, hematocrit, and body mass index in evaluating AP patients. PCT was a good independent prognostic marker and was comparable with BISAP and APACHE II in accuracy[126].

A multicenter validation study is essential to corroborate these findings and further elucidate the role of BISAP in AP. Meanwhile, further well-designed prospective studies are warranted to investigate the conditions under which BISAP can be used to more accurately, sensitively, and specifically assess severity and prognosis in AP.

Combination of BISAP and other diagnostic indicators

In a retrospective analysis including 114 cases, the severity and mortality of AP escalated with the increase of BISAP, and BISAP exhibited a positive correlation with CRP, D-dimer, and serum glucose and negatively correlated with serum Ca^{2+} [127]. Based on the positive correlation between CRP and APACHE-II, Ranson, BISAP, and CTSI, when CRP was included into BISAP, the AUC of predicting SAP and death were 0.873 and 0.909, respectively, showing that the combination of BISAP and CRP had better predictive value for severity and death of AP[127]. In a study including 117 SAP patients, Wu *et al*[128] reported that combining BISAP with miR-155 yielded a superior AUC compared to individual predictions, suggesting that this combination could enhance the clinical predictive accuracy for AP severity.

Early diagnosis and timely assessment of the severity are critical because early aggressive treatment reduces morbidity and mortality of AP. However, an ideal multifactor scoring system for early assessment of AP severity has not been determined. Based on an analysis of the available data and evidence, we recommend that BISAP as a multifactor scoring system is combined with characteristic biochemical markers present at 48 h, in order to achieve optimal early assessment of AP severity.

SOFA

In October 1994, the European Society of Intensive Care Medicine convened in Paris to establish the SOFA score, aiming to quantitatively and objectively describe the degree of organ dysfunction/failure over time in patient groups or even in individual cases[129]. Although SOFA is primarily designed for patients with sepsis, it was deemed necessary to expand its application beyond this specific patient group[129]. At present, SOFA is widely utilized in the ICU to evaluate, prognosticate, and assess patients; since its validation, it has been applied in diverse medical settings, including trauma, surgical, cardiac, and neurological ICUs[130].

Minne *et al*[131] conducted a systematic review on the utility of SOFA-based models for predicting the risk of mortality in ICU patients and recommended an integration of a traditional model derived from data within the initial 24 h post-ICU admission with sequential SOFA. SOFA could be easily integrated into contemporary cardiac ICU through an electronic algorithm, and the day 1 SOFA demonstrated strong predictive capability for short-term mortality among a broad spectrum of patients in the cardiac ICU[132]. Among the critical care systems, SOFA has distinct benefits, including its simplicity in computation, incorporation of therapeutic needs, and facilitation of comparisons of AP with other critical care diseases[24].

Adam *et al*[133] retrospectively evaluated the efficacy of APACHE II, SOFA, and modified Ranson in predicting mortality among 43 SAP patients as well as other factors influencing mortality in patients admitted to the ICU and concluded that SOFA was superior to Ranson and APACHE II in determining prognosis. In this study, SOFA had a significant correlation with mortality, and all patients with SOFA ≥ 11 at any point during the ICU stay exhibited a heightened mortality risk, with a sensitivity of 80% and a specificity of 79%[133].

Tee *et al*[134] retrospectively obtained serial measurements of Ranson, APACHE II, and SOFA in 159 patients with SAP, assessing the efficacy of serial measurement using these three scoring systems. In this study, besides acquiring Ranson and APACHE II on admission and at 48 h, they took serial weekly measurements of SOFA, including data from admission, 48 h, and days 7, 14, and 21[134]. The three scoring systems reliably predicted both overall and ICU mortality. However, the SOFA on day 7 exhibited the largest AUC, with any increase or lack of change in SOFA on day 7 of hospitalization correlating with elevated mortality[134]. They concluded that both APACHE II and SOFA were sensitive in predicting mortality for AP. Serial SOFA proved reliable for guiding clinical decisions, and day 7 of hospitalization was a reasonable time for SOFA reassessment to predict late mortality in SAP[134].

A retrospective study enrolling 146 AP patients demonstrated that an increase in SOFA independently heightened the likelihood of adverse outcomes during hospitalization for AP patients, and SOFA > 5 was highly predictive of in-hospital mortality compared to other scores[135]. Utilizing a straightforward tool like SOFA, validated in intensive care settings, could enhance the stratification of in-hospital mortality risk and clinical deterioration among AP patients admitted to medical wards. Teng *et al*[111] reported that both SOFA and 48-h Ranson effectively predicted the severity, ICU admission, and mortality associated with AP, with SOFA showing particularly favorable results.

qSOFA

The qSOFA includes respiratory rate (breaths per minute), systolic blood pressure (mm Hg), and Glasgow Coma Scale score[136]. In a 17-year observation study including 1059 patients, the ROC curve analysis revealed that the AUC values of APACHE II, SOFA, and qSOFA scores in predicting the prognosis of infected patients were 0.713, 0.744, and 0.662, respectively[137]. In this study, Qin *et al*[137] posited that qSOFA, due to its advantages of rapid acquisition, would serve as an efficient tool for assessing the prognosis of ICU patients with infections. Given its extraordinary simplicity, qSOFA would be an appropriate score particularly for the initial patient evaluation in the emergency department and was considered to be a rapidly available prognostic score in AP with limited prognostic validity[138]. In a cohort study including 203 patients, Rasch *et al*[138] reported that qSOFA could predict ICU admission and multiple organ dysfunction syndrome in AP.

In a retrospective cohort study involving 161 patients with the diagnosis of alcohol-induced AP, a qSOFA score of 2 or higher both upon admission and 48 h post-admission exhibited a specificity of 94% or greater and sensitivity of 33% or higher for assessing pancreatitis severity and determining the necessity for intensive care admission, intubation, or vasopressor[139]. In a 3-year cohort study from the United States, Hallac *et al*[140] evaluated the ability of qSOFA and SIRS in predicting extended hospital stays among patients presenting with AP to the emergency department and hospital ward. A qSOFA of 2 or higher was linked to a diagnosis of significant AP with a specificity of 99% and a sensitivity of 4%. In contrast, a SIRS score of 2 displayed a specificity of 61% and a sensitivity of 80% in detecting patients with significant AP[140]. Based on their findings, they inferred that relying solely on qSOFA for triaging AP patients could lead to under recognition and potential undertreatment[140].

HAPS

HAPS was calculated rapidly from the following three parameters: presence or absence of rebound tenderness or guarding; hematocrit (> 43 mg/dL for males or > 39.6 mg/dL for females); and serum creatinine (> 2 mg/dL)[105,141]. Oskarsson *et al*[142] reported that HAPS predicting nonsevere AP progression had a specificity of 96.3% and a corresponding PPV of 98.7% in 531 patients experiencing either a first-time or a recurrent attack of AP, emphasizing HAPS as a highly specific scoring algorithm predicting nonsevere AP progression[142]. In a prospective pilot study with 103 AP patients from India, the sensitivity, specificity, PPV, NPV, and AUC of HAPS as a predictor of nonsevere disease were 76.3%, 85.7%, 93.8%, 56.6%, and 0.848, respectively[143]. In a study including 703 AP patients from China, the sensitivity, specificity, PPV, NPV, and AUC of HAPS on admission for predicting MAP was 48.2%, 97.7%, 95.6%, 64.1%, and 0.749, respectively[97]. These studies validated the utility of HAPS at admission in predicting nonsevere AP in India and MAP in China, respectively. Maisonneuve *et al*[144] evaluated the PPV of HAPS by performing a meta-analysis of 20 reports covering 6374 patients. They concluded that HAPS accurately identified patients with nonsevere AP who would not require ICU care, enabling the pinpointing of patients suitable for brief general ward stays or home-based care[144].

HAPS may offer significant advantages in the triage of AP patients when compared to other scoring systems, underscoring its potential utility in optimizing patient classification and guiding treatment strategies. In a study including 60 patients with the first attack of AP, Gupta *et al*[145] reported that the sensitivity, specificity, PPV, NPV, and AUC of HAPS predicting SAP were 90.91%, 59.81%, 33.33%, 96.67%, and 0.75, respectively. The high NPV indicated that HAPS could very accurately identify within the first hour of admission patients who had a mild course of disease, did not require intensive management, and were not at risk of dying from the disease[145]. Based on this result, they argued that the patient typically tended to experience a milder course of illness if the evaluation of HAPS yielded a negative result [145]. Conversely, in instances where the score was positive, the patient's clinical progression could unfold in any direction, demonstrating the uncertainty associated with such an outcome[145]. In this same study, the sensitivity, specificity, PPV, NPV, and AUC of BISAP in the prediction of SAP were 63.64%, 100%, 100%, 92.45%, and 0.82, respectively[145]. In comparison to BISAP in this study, HAPS demonstrated a heightened sensitivity towards processes predicting mortality and severity and played a pivotal role in determining whether patients necessitated costly imaging procedures, thereby potentially enabling significant hospital cost savings[145]. In a study with 116 patients, Al-Qahtani *et*

al[146] compared HAPS with Ranson in predicting the severity of AP and concluded that HAPS was effective in rapidly identifying patients likely to experience a nonsevere course of the disease.

Of significant importance is the fact that assessment of HAPS can be accomplished within the first hour of a patient visit, offering a distinct advantage in terms of time efficiency. In contrast, while Ranson might offer superior accuracy, it necessitates a full 48 h to reach completion, highlighting a potential trade-off between speed and precision in these scoring systems. Considering that the substantial majority of individuals diagnosed with AP typically exhibit a milder form of the disease, the capacity to accurately distinguish these patients of MAP is of utmost significance. Drawing upon the aforementioned analysis and discussion, HAPS appears to be a commendable choice for assisting physicians in evaluating the severity of AP. Furthermore, HAPS could potentially be perceived as a gold standard for facilitating both the early identification and cost-effective management of this disease. In addition, due to the readily accessible parameters required for its computation, HAPS can be effectively utilized in a wide range of healthcare facilities, including those located in developing countries. This ease of implementation makes HAPS an inclusive and practical tool for global health contexts.

Other recent clinical scoring systems

Hong *et al*[147] developed a prognostic score termed SABP, encompassing systemic inflammatory response syndrome, serum albumin, blood urea nitrogen, and pleural effusion. The SABP score could serve as an instrumental tool to categorize patients at risk of developing SAP as per the latest revised Atlanta criteria. Its application on admission may enhance clinical care and refine management approaches for AP[147]. He *et al*[148] retrospectively analyzed the clinical data of 469 patients with AP, and selected seven prognostic indicators to establish an unweighted predictive score and weighted predictive score for MSAP and SAP. The early multi-indicator prediction models for MSAP and SAP demonstrated robust predictive efficacy, offering a meaningful clinical benchmark for diagnosis and treatment[148].

In a retrospective analysis encompassing a total of 1295 AP patients, Feng *et al*[149] developed an independent predictive tool, known as a nomogram, to predict the likelihood of sepsis occurrence in this patient population. In this study, the predictive performance and clinical utility of the newly established nomogram surpassed those of other scoring systems such as SIRS, BISAP, SOFA, and qSOFA[149]. The innovative risk-prediction system could precisely estimate the likelihood of sepsis in AP patients, assisting clinicians in formulating personalized treatment strategies for the patients. By doing so, it not only alleviated the disease burden of the patients but also facilitated the reasonable distribution of medical resources, which was a crucial aspect of tertiary prevention[149]. The nomogram incorporated all the independent prognostic factors, including body temperature, phosphate, Ca^{2+}, sodium, lactate, albumin, platelet count, urinary output, mean blood pressure, Glasgow Coma Scale, and Charlson Comorbidity Index[149]. These diverse elements collectively contributed to its predictive strength.

Summary

Score systems, utilizing 4-25 factors, have been developed to predict severity, yet they frequently rely on multiple parameters not measurable daily and often require over 24 h to finalize, leading to critical time loss[150]. While these scores can predict failure or severity of specific organs, their reliance on dichotomous parameters leads to information loss, limiting their practical application in clinical settings[150]. Based on the current literature, here are the identified problems and potential solutions for applying clinical scoring systems to the diagnosis, severity prediction, and prognosis assessment of AP.

Inconsistency: Different scoring systems like Ranson, Glasgow, BISAP, and CTSI may yield inconsistent results, leading to confusion in clinical decision-making.

Solution: Research to validate and compare different scoring systems can help identify the most accurate and reliable ones. Standardizing the use of a particular scoring system across healthcare settings can reduce inconsistency.

Complexity: Some scoring systems are complex and require multiple parameters, making them time-consuming to calculate. This complexity can hinder their practical application in urgent care settings.

Solution: Creating simplified and user-friendly scoring systems that maintain accuracy can make them more practical for clinicians to use, especially in urgent care settings.

Lack of sensitivity and specificity: Some scoring systems may lack sensitivity or specificity in predicting the severity and prognosis of AP, leading to inaccurate assessments.

Solution: Combining scoring systems with comprehensive clinical assessment can lead to more accurate care. This solution is more of a clinical recommendation rather than a documented research finding.

Lack of personalization: Scoring systems are often based on population-level data and may not account for characteristics of individual patient, leading to generalized predictions that may not be applicable to all patients.

Solution: Considering patient-specific factors, such as comorbidities, lifestyle, and preferences, in conjunction with scoring systems, can lead to more personalized and effective care.

Over-reliance on scoring systems: Sole reliance on scoring systems without considering clinical judgment and other patient-specific factors may lead to suboptimal care.

Solution: Providing education and training to healthcare professionals on how to effectively use scoring systems, including their limitations, can enhance their application in clinical practice.

In conclusion, while clinical scoring systems are valuable tools in managing AP, they present challenges that are recognized both in clinical practice and in the research literature. The solutions outlined above, grounded in current research and clinical wisdom, can enhance the effectiveness of these systems in providing accurate and personalized treatment for patients with AP.

AI

In the era of AI, machine learning algorithms have been devised to accurately predict the severity, complications, recurrence, mortality, and even the optimal timing of surgery for AP patients. However, the quality of research evaluating the accuracy of AI is still low and lacks studies comparing AI with these commonly used clinical scores. Therefore, more research is needed before we can routinely use AI in our daily clinical practice. Prior to this, the easy-to-calculate and applicable scoring systems seems to be the most reasonable choice.

Recently, AI applications, utilizing machine learning, have been progressively integrated into the medical field, demonstrating superior performance in predicting complications compared to logistic regression analysis[151]. AI-based machine learning is booming and creating a technological revolution, especially in the healthcare industry[152]. Machine learning, a subset of AI, employs statistical methods to train algorithms for predictions, enabling a computer system to self-learn and enhance its performance based on experience[150]. Machine learning has garnered significant attention and recognition from clinicians, driven by advancements in statistical theory and computer technology[153]. Machine learning adeptly discerns intricate relationships between diseases and variables, categorizes variables based on specific criteria, predicts outcomes from foundational features, and recognizes objects with analogous patterns[152]. Innovative machine learning technologies have been extensively employed in predictive models for a spectrum of diseases, consistently demonstrating superior performance over traditional logistic regression or Cox regression analyses[153].

In this age of technological advancement, AI stands as a pinnacle of innovation, proficiently discerning the intricate non-linear proficiently between numerous biochemical parameters and their associated disease outcomes[150]. For example, a retrospective study demonstrated that when juxtaposed with the traditional logistic regression model machine learning models [extreme gradient boosting (XGBoost) and random forest (RF)] utilizing readily accessible features upon admission exhibited superior performance in predicting acute kidney injury among AP patients[151]. Leveraging such machine learning algorithms in predictive models could enable clinicians to foresee acute kidney injury at an early stage, potentially mitigating further renal damage[151].

Based on an international cohort of 1184 patients and a validation cohort of 3543 patients, Kui *et al*[154] devised a user-friendly web application named EASY-APP, which employs multiple continuous variables accessible at admission. The EASY prediction score serves as an effective tool for pinpointing patients at elevated risk for severe AP within hours of hospitalization, and the web application was made available to clinicians, enhancing the utility and precision of the model[154].

Zhou *et al*[155] demonstrated that the XGBoost algorithm possesses the capability to precisely predict the severity of AP, offering clinicians valuable assistance in identifying severe AP at an early stage. In a prospective cohort study integrating necrosis prediction with AI, the XGBoost machine learning algorithm was employed to analyze the data from 2387 AP patients[156]. This model in the predictive capability rivals those existing clinical scoring systems, and its performance is anticipated to improve with continued use[156]. In the United States, Thapa *et al*[7] applied machine learning algorithms to predict which AP patients need SAP treatment and developed three models using logistic regression, neural networks, and XGBoost. In this study, machine learning models were trained and tested to utilize data from 61894 patients, with the XGBoost model surpassing the performance of both logistic regression and neural network-based models[7]. Furthermore, the XGBoost model achieved a superior AUROC compared to both HAPS and BISAP in identifying patients likely to be diagnosed with SAP[7]. They concluded that machine learning has the potential to refine the precision of AP risk stratification methods, facilitating prompter treatment and intervention initiation[7].

In a large retrospective study enrolling 5460 patients, Yuan *et al*[157] developed and validated a novel machine learning tool, APCU, leveraging clinical, laboratory, and radiologic data to predict ICU admission among AP patients. They showed that the APCU effectively categorized AP patients into high-risk and low-risk groups, demonstrating a superior discriminative capability compared to other risk scores like Ranson, APACHE II, SIRS, and NEWS in predicting ICU admission for AP patients and specific subgroups within 48 h of hospitalization[157]. Notably, this study marked the inaugural application of a machine learning algorithm for the predictions of ICU admission in AP patients within 48 h of hospitalization, relying on widely accessible clinical, laboratory, and radiologic data[157].

In a retrospective analysis involving 648 AP patients, Hong *et al*[158] developed RF and logistic regression models using a training sample; the RF model, notable for its interpretability, showcased the most superior discriminative performance in predicting SAP. In a retrospective study involving 631 AP patients, Luo *et al*[159] developed a machine learning model, culminating in a nomogram designed for the early identification of SAP during the progression of AP. Their findings indicated that the RF model delivered optimal predictive performance, with the nomogram offering a visual scoring model suitable for clinical application[159]. Such models have the potential to act as functional tools, enabling personalized treatment choices and enhancing clinical results by stratifying AP patients prior to treatment[159]. In a study with a total of 1012 patients, Yin *et al*[160] developed a series of effective models for early prediction of SAP based on automated machine learning (AutoML) platform, and these models outperformed the existing scoring systems, which might offer insights into AutoML applications in future medical studies. The AutoML model based on the GBM algorithm for early prediction of SAP showed evident clinical practicability[160].

In a recent retrospective study involving a cohort of 460 AP patients to predict ARDS in these patients at admission, Zhang *et al*[161] constructed and optimized four machine learning models, including support vector machine, ensembles of decision trees (EDTs), Bayesian classifier (BC), and nomogram models, based on 31 features with significant differences between the groups with and without ARDS. Among the four models, the BC algorithm exhibited superior predictive performance with the highest AUC (0.891), surpassing support vector machine (0.870), EDTs (0.813), and the nomogram (0.874) in the test set[161]. Concurrently, the EDT algorithm achieved the highest accuracy at 0.891, precision at 0.800, and F1 score at 0.615 but registered the lowest FDR at 0.200 and the second-highest NPV at 0.902[161]. In terms of predictive performance for ARDS as a complication of AP, they concluded that BC was the superior predictive model in the test set, and EDTs exhibited promising potential for predicting large samples[161].

Summary

The application of AI in the diagnosis, severity prediction, and prognosis assessment of AP represents an exciting development in the field of medicine. However, based on these current studies, we recognize several limitations and potential challenges that must be addressed to fully leverage the capabilities of AI in this context.

Data quality and availability: AI algorithms require high-quality, comprehensive, and diverse data to build robust and accurate models. In the context of AP, such data sets may not be readily available, especially for rare subtypes of the disease or patient populations with specific comorbidities. Furthermore, incomplete or inconsistent data can lead to biased or flawed results.

Interpretability: AI models, especially those employing complex algorithms like deep learning, often operate as 'black boxes,' providing outputs without clear, understandable reasons for their decisions. This can limit their acceptance in the clinical setting, as healthcare professionals typically prefer to understand the reasoning behind a diagnosis or prediction.

Standardization: AI algorithms are typically designed and validated on specific datasets. Their generalizability to other populations or healthcare settings, especially those that are vastly different from the original context, is not guaranteed. This lack of standardization can lead to inconsistent results when the models are used in different settings.

Generalizability: Models trained on a specific set of data may not perform well when applied to different datasets, especially if there are demographic or geographical differences. For example, an AI model trained on data from a high-income country might not work as well in a low-income setting due to differences in healthcare infrastructure, disease prevalence, and patient characteristics.

Regulation: The use of patient data to develop and apply AI models raises significant concerns around data privacy, consent, and security. It is crucial that these concerns are addressed to ensure ethical usage and maintain public trust. For instance, who is responsible if an AI system makes an incorrect diagnosis or prognosis? How is patient data privacy ensured?

Implementation: The successful implementation of AI in healthcare settings requires clinicians to have a certain level of understanding and trust in the technology. This can be challenging due to varying levels of digital literacy among healthcare providers and resistance to change.

Given these challenges, ongoing research is critical to improve the reliability, interpretability, and generalizability of AI tools in healthcare and to address the ethical, legal, and workflow integration issues associated with their use. It is important that as we move forward, these tools are developed and used in a manner that complements the expertise of healthcare professionals rather than seeking to replace it.

CONCLUSION

Early aggressive treatment of AP has been proven to reduce the incidence and mortality rates. Therefore early diagnosis and severity assessment of AP are extremely necessary, and there is a particular need for early technological approaches to evaluate and predict the progression of AP.

In recent years, there has been heightened interest in leveraging imaging technologies, scoring systems, and AI to improve the diagnosis, severity prediction, and prognosis evaluation of AP. Different imaging modalities, such as CT, MRI, and US, are used to assess the severity and extent of pancreatic inflammation and detect any complications that may arise. Several scoring systems have been developed to assess the severity of AP and predict the risk of complications, such as Ranson, APACHE II, BISAP, SOFA, and HAPS. These scoring systems take into account various clinical and laboratory parameters, such as age, blood pressure, serum glucose, and white blood cell count, to provide a numerical score that reflects the severity of the disease. AI is a rapidly developing field that has the potential to revolutionize the diagnosis and management of AP. AI algorithms can be trained to analyze large datasets of imaging and clinical data to predict the severity and prognosis of AP. AI algorithms have been developed to analyze CT scans of patients with AP to predict the risk of complications such as pancreatic necrosis, abscess, or pseudocyst. The algorithms can detect subtle changes in the pancreas that may be missed by human radiologists and can provide more accurate and timely predictions of the risk of complications.

The integration of imaging technologies, scoring systems, and AI in the diagnosis, severity prediction, and prognosis assessment of AP has several advantages, including: (1) More accurate diagnosis. Imaging technologies and AI algorithms can provide more accurate diagnoses, reducing the risk of misdiagnosis and unnecessary treatment; (2)

Improved risk assessment. Scoring systems and AI algorithms can provide more accurate risk assessments, which can help healthcare providers make more informed treatment decisions; (3) Personalized treatment. The combination of imaging technologies, scoring systems, and AI can provide a more personalized approach to treatment, taking into account each patient's unique circumstances; and (4) Improved patient outcomes. The earlier and more accurate diagnosis, as well as the more personalized treatment options, can lead to improved patient outcomes and reduced healthcare costs.

Despite these advantages, there are several challenges that need to be addressed when integrating imaging technologies, scoring systems, and AI in the management of AP. These challenges include the need for standardized imaging protocols and scoring systems, the need for large datasets of imaging and clinical data to train AI algorithms, and ethical and legal challenges associated with the use of AI in healthcare. In conclusion, the integration of imaging technologies, scoring systems, and AI has the potential to revolutionize the diagnosis, severity prediction, and prognosis assessment of AP.

FOOTNOTES

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REVIEW

New insights into the pathogenesis of primary biliary cholangitis asymptomatic stage

Vasiliy Ivanovich Reshetnyak, Igor Veniaminovich Maev

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Abstract

Primary biliary cholangitis (PBC) is a chronic cholestatic progressive liver disease and one of the most important progressive cholangiopathies in adults. Damage to cholangiocytes triggers the development of intrahepatic cholestasis, which progresses to cirrhosis in the terminal stage of the disease. Accumulating data indicate that damage to biliary epithelial cells [(BECs), cholangiocytes] is most likely associated with the intracellular accumulation of bile acids, which have potent detergent properties and damaging effects on cell membranes. The mechanisms underlying uncontrolled bile acid intake into BECs in PBC are associated with pH change in the bile duct lumen, which is controlled by the bicarbonate (HCO_3^-) buffer system "biliary HCO_3^- umbrella". The impaired production and entry of HCO_3^- from BECs into the bile duct lumen is due to epigenetic changes in expression of the X-linked microRNA 506. Based on the growing body of knowledge on the molecular mechanisms of cholangiocyte damage in patients with PBC, we propose a hypothesis explaining the pathogenesis of the first morphologic (ductulopenia), immunologic (antimitochondrial autoantibodies) and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage. This review focuses on the consideration of these mechanisms.

Key Words: Primary biliary cholangitis; Antimitochondrial autoantibodies; MicroRNA 506; Inositol-1,4,5-trisphosphate receptor type 3; Chloride/bicarbonate anion exchanger 2; Biliary bicarbonate umbrella; Dihydrolipoyl transacetylase (E2 subunit); Pyruvate dehydrogenase complex

Core Tip: This review considers the mechanisms contributing to the damage of the E2 subunit of the pyruvate dehydrogenase complex, formation of antimitochondrial autoantibodies (AMAs), and the development of ductulopenia in primary biliary cholangitis asymptomatic stage. A hypothesis explaining the pathogenesis of the initial morphological (ductulopenia), immunologic (AMAs), and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage is proposed.

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INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic cholestatic progressive liver disease of autoimmune etiology, characterized by the destruction, necrosis, and apoptosis of small bile duct epithelial cells, in the terminal stage of which cirrhosis develops[1-3]. PBC development is preceded by a long asymptomatic period[1,2], during which time there are no physical signs of the disease. The earliest and most common symptoms are fatigue, weakness, and malaise. Detection of serum antimitochondrial autoantibodies (AMAs) at a titer ≥ 1: 40 during this period serves as a pathognomonic marker of PBC development. AMAs are detectable months to years before PBC manifests clinically, indicating their primary immunopathogenetic role rather than a secondary phenomenon occurring as a consequence of cholestasis[4,5]. However, AMAs titer does not correlate with disease activity or duration[4-7]. Identifying the causes and mechanisms of AMAs formation may contribute to understanding the pathogenesis of the development of clinical, morphologic, biochemical, and immunologic signs of PBC.

AMAs are not strictly specific for PBC[4]. They are classified as immunoglobulin (Ig) M which reacts with multiple antigens in mitochondria, designated M1-M9[8]. The highly sensitive and most frequent (> 95%) AMAs found in PBC are anti-M2 IgM[8]. In patients with classical PBC, the antigenic components of AMAs are related to the dihydrolipoyl transacetylase (E2 subunit) of the pyruvate dehydrogenase (PDC) complex (E2 PDC), which localizes to the inner mitochondrial membrane[8]. Immunization of laboratory animals with E2 PDC recombinant polypeptide leads to AMAs formation but not cholangiocyte damage[9], indicating that AMAs do not trigger the destruction of biliary epithelial cells [(BECs), cholangiocytes].

It is critical to understand how the E2 PDC antigen of BECs, located on the inner membrane of mitochondria in small and medium-sized bile ducts, can be a target of immune effector mechanisms[4]. The theory of antigenic mimicry is discussed below.

AMAs AND THE THEORY OF ANTIGENIC MIMICRY

The PDC in prokaryotes is structurally similar to that of eukaryotes[10]. Antibodies from the serum of patients with PBC have been shown to react with yeast and bacterial proteins[11,12]. Therefore, it has been suggested that AMAs in PBC arise due to cross-reactivity to exogenous bacterial antigens (antigenic mimicry)[13,14] and that the disease may have a bacterial origin[15]. However, there is no clear evidence of an infectious agent[4]. In classic bacterial antigenic exposure, IgM is the first antibody secreted by the adaptive immune system, and after 3-4 wk, IgG is produced. IgM can persist in the blood for up to 3 mo, followed by its decline. However, in PBC, the level of IgM-related AMAs does not decline over the course of the disease, which does not align with the bacterial nature of antigens triggering the production of AMAs. With continuous exposure to thymus-independent antigens and decreased immune tolerance to them, IgM synthesis can become stable[16]. However, there is low likelihood that bacterial antigen is constantly present in patients with PBC and triggers the production of AMAs[10]. It is more logical to assume that the antigen is from the patients' own tissues, namely the epithelium of the biliary tract. The production of AMAs in patients with PBC is initiated once E2 PDC becomes an immunomodified antigen, leaves the mitochondria, exits the cholangiocyte, enters the blood, and meets immunocompetent T and B lymphocytes which recognize it as a foreign antigen. To date, the triggers and mechanisms that initiate these processes in cholangiocytes remain unknown.

In the last decade, scientific data have shown that the "protective umbrella" of bicarbonate (HCO_3^-) protects cholangiocytes from the toxic effects of bile acids. In PBC, the production of HCO_3^- decreases, which leads to increased bile acid intake into cholangiocytes (theory of defective "biliary HCO_3^- umbrella"). Based on these data, we hypothesize that the gradual accumulation of bile acids in BECs may serve as a trigger mechanism for AMAs formation, ductulopenia development, and one of the early clinical signs of PBC, namely weakness in the asymptomatic stage.

AGGRESSIVE AND DEFENSE FACTORS OF CHOLANGIOCYTES

Bile is an aggressive medium for cholangiocytes, which are epithelial cells lining the intrahepatic and extrahepatic bile ducts. The presence of bile acids in bile, which have potent detergent properties, can cause damage to the cell membranes of cholangiocytes. Hydrophobic bile acids are cytotoxic to many cell types[17]. However, BECs under physiological conditions are exposed to very high (millimolar) concentrations of hydrophobic bile acids without signs of cytotoxicity [18], indicating the presence of mechanisms protecting cholangiocytes from the toxic effects of bile acids.

The conjugation of bile acids and formation of mixed micelles with cholesterol and phospholipids are considered defense mechanisms at the levels of hepatocytes, bile capillaries, and Hering's canals[18]. Defense factors that enter the bile during its passage through bile ducts include the production and secretion of mucin and HCO_3^-[19]. Under physiological conditions, the main function of cholangiocytes is biliary secretion of HCO_3^-[20]. HCO_3^- is produced by cholangiocytes lining the biliary tree. Mucin glycoprotein is produced by peribiliary glands (PBGs)[21], which are located in the wall of large intrahepatic and extrahepatic bile ducts and are directly connected with the bile duct lumen. Experimental evidence indicates that the glycocalyx, which covers the apical surface of large cholangiocyte membranes with glycosylated mucins and other glycan-containing membrane glycoproteins, stabilizes the biliary HCO_3^- umbrella, thereby helping to protect human large cholangiocytes from bile acid toxicity[22]. In addition, the mucin produced by PBGs protects the cholangiocytes of only large bile ducts[19]. Thus, the cholangiocytes of large intrahepatic and extrahepatic bile ducts have dual protection: The mucin produced by PBGs and HCO_3^-. Intralobular, interlobular, and septal bile ducts do not contain PBGs, which is accompanied by the absence of mucin in them[21]. As a result, only HCO_3^- serves as a factor of BEC defense at the levels of intralobular, interlobular, and septal ducts. Under physiological conditions, there is a balance between aggressive factors (bile acids) and defense factors (HCO_3^- and/or mucin secretion).

CHOLANGIOCYTE DEFENSE MECHANISMS

Cholangiocytes are polarized epithelial cells that line the intrahepatic and extrahepatic bile ducts and are responsible for regulating bile volume, modifying bile, and maintaining bile pH (alkalinity)[23,24]. They play an important role in modifying the composition of primary bile by secreting water, chloride (Cl^-), and HCO_3^-[25], and by absorbing bile acid salts, amino acids, and glucose. Small and large cholangiocytes are distinguished depending on their size and location in small and large bile ducts[26]. They are differently involved in the processes of secretion and absorption[27]. The secretion of HCO_3^- with human bile accounts for 25%-40% of the total volume of secreted bile and maintains physiologic pH in the lumen of bile ducts[17,28,29].

In the process of bile formation, predominantly conjugated bile acids and a minimal amount of unconjugated bile acids enter the bile capillary. Under physiological conditions, both conjugated and unconjugated bile acids are secreted into bile by hepatocytes in anionic (deprotonated, ionized, negatively charged) form[30]. HCO_3^-, which is secreted by cholangiocytes into the lumen of the bile duct, creates a slightly alkaline pH of hepatic bile due to its buffering properties, keeping bile acids in a deprotonated state. The ionized form of bile acids are impermeable to BECs due to the presence of negatively charged HCO_3^- molecules on the apical surface of the cytoplasmic membrane of cholangiocytes[30]. Thus, the secretion of HCO_3^- ions protects cholangiocytes from uncontrolled transmembrane bile acid entrance and their cytotoxicity. This protective mechanism, which preserves cholangiocytes and normal bile flow along the biliary tree, is called the biliary HCO_3^- umbrella.

MAIN REGULATORS OF HCO_3^- PRODUCTION AND SECRETION BY CHOLANGIOCYTES

The pH fluctuation in bile ducts depends on the rate of HCO_3^- production by cholangiocytes. The signaling pathways regulating HCO_3^- secretion differ in large and small cholangiocytes[31]. In small cholangiocytes, activation of HCO_3^- secretion is due to biliary adenosine triphosphate (ATP) secreted from upstream hepatocytes of Hering's canals (Figure 1). Cholangiocytes express apical membrane proteins of the purinergic receptor (P2YR) family, which are stimulated by ATP[32]. Luminal ATP binds to P2YR, stimulating intracellular calcium (Ca^{2+}) release *via* inositol-1,4,5-trisphosphate receptor type 3 ($InsP_3R3$)[33]. In cholangiocytes, $InsP_3R3$ is the major receptor isoform localized in the apical region[31,34]; it is involved in $InsP_3R3$-mediated cell signaling and Ca^{2+} secretion[35]. $InsP_3R3$ is the only receptor that promotes the opening of intracellular Ca^{2+} channels and release of Ca^{2+} ions[34]. Ca^{2+} is one of the second messengers in cholangiocytes that modulates and regulates diverse cellular functions such as ion channel activation, secretion, cell proliferation, and apoptosis[34,36]. Ca^{2+} release from subapical stores in the endoplasmic reticulum triggers and locally activates transmembrane 16A Cl^- channels (TMEM16A) on the apical membrane of cholangiocytes (Figure 1)[36-38]. The appearing Cl^- concentration gradient on the apical membrane activates the Cl^-/HCO_3^- anion exchanger 2 (AE2), also Slc4A2, which leads to the secretion of HCO_3^- into the lumen of the bile duct.

In large cholangiocytes, with the exception of the Ca^{2+}-dependent pathway of HCO_3^- secretion, there is an additional mechanism involving the hormones secretin and somatostatin[39] (Figure 1). Secretin is produced by the S cells of the duodenal mucosa, and stimulates the production of HCO_3^- by the intestinal mucosa itself as well as by cholangiocytes and pancreatic ductular epithelial cells[39]. Secretin regulates the secretion of HCO_3^- and Cl^- into bile by large cholangiocytes through interaction with secretin receptors (SRs) located on the basolateral membrane of BECs[39-42] (Figure 1).

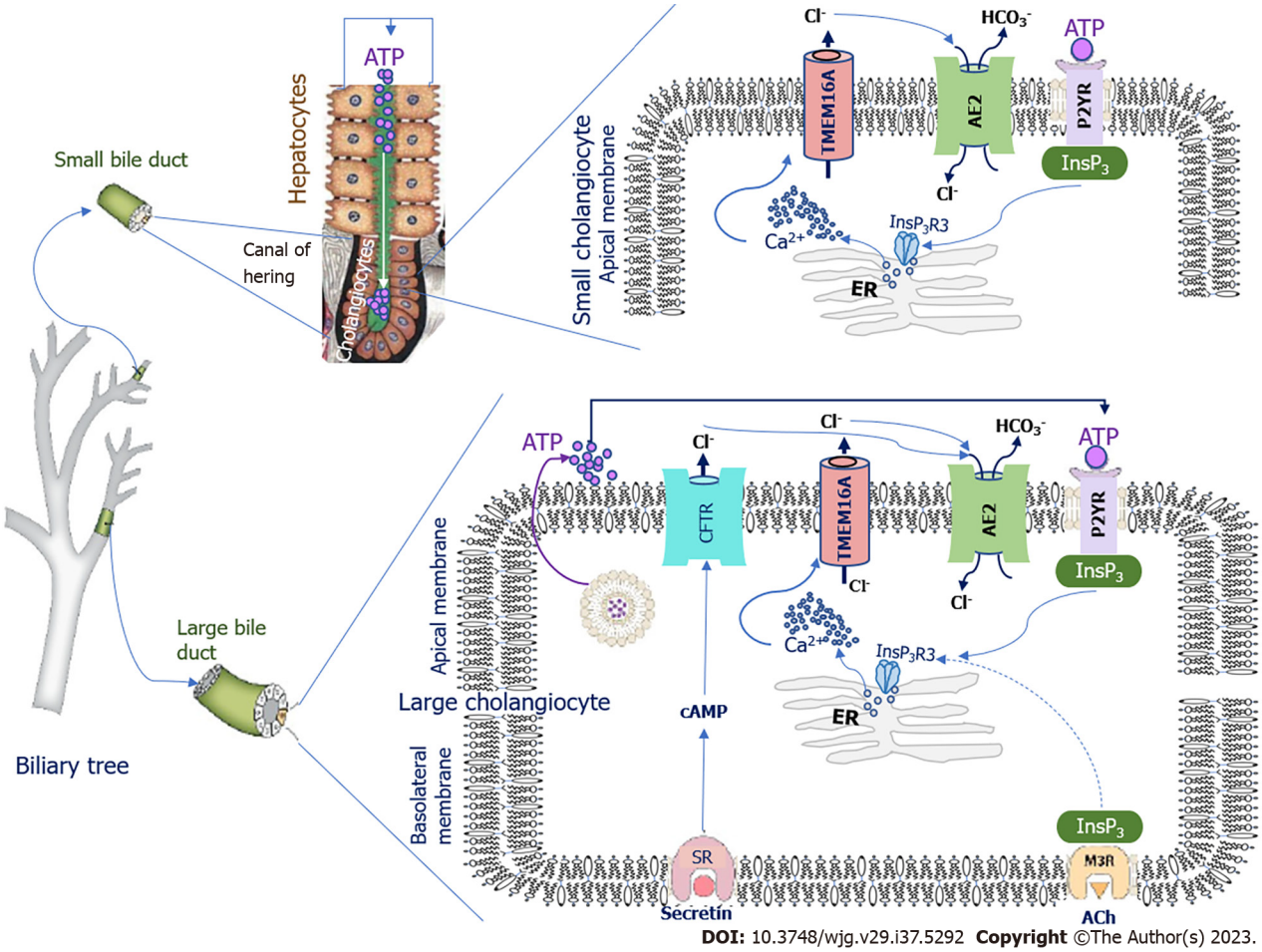

Figure 1 Schematic of bicarbonate secretion by small and large cholangiocytes. AE2: Chloride/bicarbonate anion exchanger 2; TMEM16A: Transmembrane 16A chloride channels; ATP: Adenosine triphosphate; P2YR: Purinergic receptor family; $InsP_3$: Inositol-1,4,5-trisphosphate; $InsP_3R3$: Inositol-1,4,5-trisphosphate receptor type 3; ER: Endoplasmic reticulum; SR: Secretin receptor; cAMP: Cyclic adenosine monophosphate; Ach: Acetylcholine; CFTR: Cystic fibrosis transmembrane conductance regulator; M3R: Muscarinic acetylcholine M3 receptor; HCO_3^-: Bicarbonate; Cl^-: Chloride; Ca^{2+}: Calcium.

As a result of this interaction, the formation of cyclic adenosine monophosphate (cAMP) is stimulated *via* G proteins. cAMP activates the cystic fibrosis transmembrane conductance regulator (CFTR) through adenylate cyclase, resulting in the secretion of Cl^- ions into the bile duct[28]. The appearing Cl^- concentration gradient on the apical membrane of cholangiocytes activates AE2, which leads to the secretion of HCO_3^- into the bile duct lumen in exchange for the intracellular entry of Cl^- ions into the cholangiocytes[20,24,26,43]. In parallel, ATP from cholangiocytes enters the bile duct lumen by exocytosis, which stimulates the secretion of HCO_3^- *via* a Ca^{2+}-dependent mechanism[44]. SRs and CFTR are not found in small cholangiocytes; therefore, secretin is unable to stimulate the secretion and entry of HCO_3^- and Cl^- into bile in small cholangiocytes[24]. However, a Ca^{2+}-dependent mechanism of HCO_3^- secretion is present in both small and large cholangiocytes (Figure 1)[20,24]. Somatostatin, which binds to the somatostatin receptor, counteracts the stimulating effect of secretin, inhibits fluid secretion, and slows the production and entry of HCO_3^- from cholangiocytes into the lumen of the bile duct[45].

MECHANISMS OF BILE ACID PROTONATION-DEPROTONATION AND THEIR ENTRY INTO CHOLANGIOCYTES

Uncontrolled, carrier-independent, passive diffusion of unconjugated primary bile acids into BECs is determined by their polarity and degree of protonation[18,46,47]. Protonation of bile acids is an exponential function of pH. When the pH of hepatic bile acidifies, bile acids may undergo protonation. The degree of protonation of bile acids depends on both their dissociation constant (pKa) and the pH of the bile. The pKa values for unconjugated primary bile acids are 5-6[48-50]. Conjugation of primary bile acids with amino acids reduces pKa values to 4-5 for conjugates with glycine and to 1-2 for conjugates with taurine, which improves their solubility in water and reduces their lipophilicity[48-50]. The low pKa values of the taurine conjugates of primary bile acids indicate that they are stronger acids than glycine conjugates. Therefore, taurine-conjugated bile acids are in a dissociated (deprotonated) form even at acidic bile pH values. Whereas glycine conjugates, with higher pKa values, are weak acids and will quickly change to a protonated state at the slightest

acidification of bile[50].

Ionized (deprotonated, negatively charged) bile acids are unable to overcome the biliary HCO_3^- umbrella on the outer hemi leaflet of the apical cytoplasmic membrane of cholangiocytes[18,47]. Under normal physiological conditions, a small amount of unconjugated protonated primary bile acids is taken up by cholangiocytes. The neutral intracellular pH further promotes the transport of unconjugated protonated primary bile acids into the peribiliary vascular plexus with subsequent return to hepatocytes and re-release into biliary capillaries[51]. Such a biliary-hepatic shunt aims to prevent the accumulation of toxic bile acids with strong detergent properties in cholangiocytes[18,47].

Conjugated bile acids can be transported through the apical and basolateral membranes of cholangiocytes with the aid of specific transporters[26,52-56]. Conjugates of bile acids with glycine in human hepatic bile account for three-quarters of all conjugated bile acids and have a pKa close to 4[57]. At physiologic pH (approximately 7.4), glycine conjugates of primary bile acids, as relatively weak acids, are partially protonated (nonpolar), which promotes their absorption by cholangiocytes in micromolar amounts. Small shifts in local pH to an acidic region in biliary ducts lead to an increase in protonated glycine conjugates of primary bile acids. A significant increase in the ratio of protonated:deprotonated glycine-conjugated bile acids will lead to increased absorption by cholangiocytes.

Bile acid conjugates with taurine in hepatic bile account for one-quarter of all conjugated bile acids. They are stronger acids and have a pKa of 1-2[57,58]. Therefore, changes in biliary pH have little effect on their protonation. Most of the taurine conjugates of bile acids are in anionic form and thus are not able to enter cholangiocytes. Therefore, taurine conjugates of primary bile acids are less toxic to cholangiocytes.

Active functioning of bile acid transporters in the basolateral membrane of cholangiocytes, as a rule, leads to the rapid removal of hydrophobic bile acids from the intracellular space and their delivery back into hepatocytes[59]. Therefore, the accumulation of toxic bile acids with detergent properties in cholangiocytes does not occur under normal conditions.

THEORY OF DEFECTIVE BILIARY HCO_3^- UMBRELLA IN PBC

In PBC, there is a reduction in the protective role of HCO_3^- for cholangiocytes. The theory of defective "biliary HCO_3^- umbrella" has been extensively discussed[1,17,22]. This theory is based on a number of clinical and experimental works showing insufficient HCO_3^- supply to the bile ducts in PBC, which leads to a shift in the pH of intraductal (hepatic) bile to the slightly acidic region and an increase of pH in cholangiocytes to the slightly alkaline region. The reasons for the insufficient production of HCO_3^- by cholangiocytes are unknown. The involvement of $InsP_3R3$ and AE2 in this process is discussed. The expression of $InsP_3R3$ and AE2 genes is reduced in the liver biopsy specimens and blood mononuclear cells of patients with PBC, indicating their dysfunction and involvement in the pathogenesis of this disease[60,61]. The decreased expression and activity of $InsP_3R3$ and AE2 is associated with increased microRNA 506 (miR-506) expression in cholangiocytes[62]. MiRNAs are small noncoding RNAs 22-23 nucleotides long that inhibit gene expression by full or partial pairing with initial sequences located in the 3′-untranslated region (3′-UTR) of mRNA[62].

The 3′-UTR region of the mRNA of $InsP_3R3$[62] and the 3′-UTR region of the mRNA of AE2[63] contain binding sites for miR-506. MiR-506 binding to the 3′-UTR regions of the mRNA of $InsP_3R3$ and AE2 prevents the translation of these proteins. In this way, miR-506 is a regulator of $InsP_3R3$ and AE2 expression (Figure 2). The expression of miR-506 likely undergoes epigenetic regulation and can vary by individual as a result of polymorphisms in nuclear factor kappa B[30].

An increase in the amount and activity of X-linked miR-506 has been reported in the cholangiocytes of patients with PBC[63], which leads to the decreased expression and activity of $InsP_3R3$ and AE2, potentially explaining the prevalence of this disease in women[30] (Figure 2).

Decreased expression and activity of $InsP_3R3$ in cholangiocytes in PBC[64] impairs intracellular Ca^{2+} secretion and its use as a messenger in signaling to the transmembrane Cl^- channel TMEM16A[44]. Impaired Ca^{2+} signaling in cholangiocytes in PBC is evidenced by the absence of ATP stimulation of P2YRs on the apical membrane[36]. Decreased Ca^{2+}-dependent activity of TMEM16A on the apical membrane of cholangiocytes leads to the decreased secretion of Cl^- ions into the lumen of bile ducts, which is accompanied by decreased activity of the chlorine/HCO_3^- anion exchanger and impaired secretion of biliary HCO_3^-. In models of cholangiocytes expressing miR-506, $InsP_3R3$-mediated reduction in intracellular Ca^{2+} release and decreased fluid and HCO_3^- secretion into bile ducts has been shown[36,44]. Binding of miR-506 to the 3′-UTR of AE2 mRNA also contributes to decreased Cl^-/HCO_3^- anion exchanger activity and decreased HCO_3^- secretion by cholangiocytes (Figure 2). Human cholangiocytes isolated from biopsy specimens of patients with PBC show decreased AE2 activity[65]. Thus, the homeostasis of intracellular pH (pHi) in cholangiocytes and bile duct pH in patients with PBC may undergo changes[30]. Changes in intra- and extracellular pH in PBC associated with the loss of $InsP_3R3$ and decreased activity of AE2 promote the protonation of bile acids, their entry into cholangiocytes, and the development of damage to the latter[44].

Destruction of biliary epithelium of small intrahepatic bile ducts during the early asymptomatic stage of PBC is most likely related to the imbalance between the aggressive factors (bile acids) and defense factors (biliary HCO_3^- umbrella) of cholangiocytes. Because intralobular, interlobular, and septal bile ducts, which are damaged in PBC, do not contain PBGs producing mucin glycoproteins (mucin supraepithelial layer)[21], this defense mechanism for small cholangiocytes most likely does not play a pathogenetic role in the development of PBC.

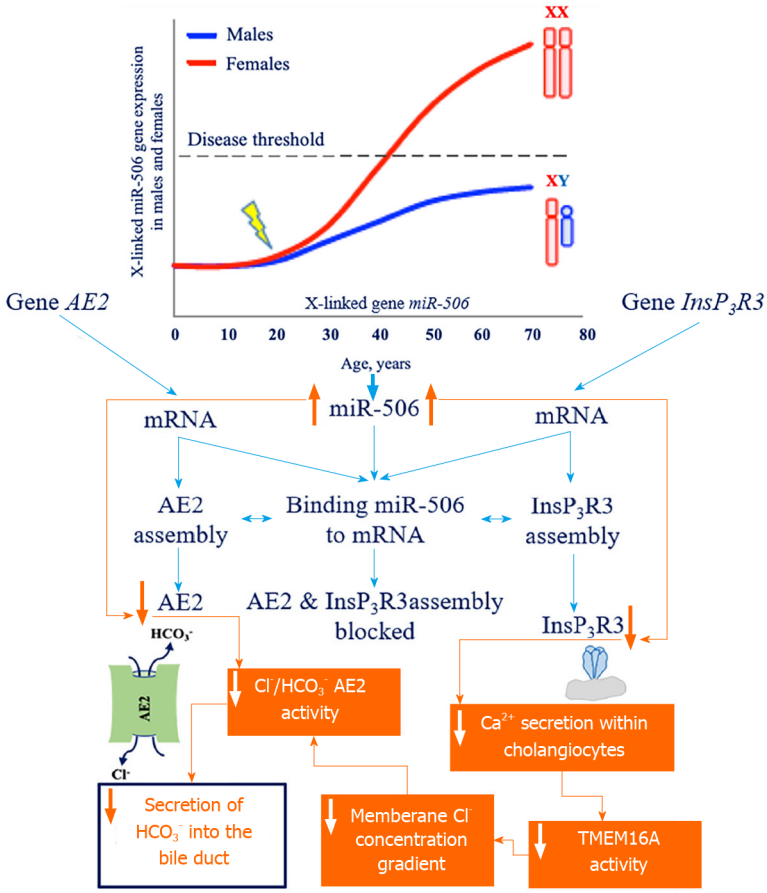

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Figure 2 Mechanism of inositol-1,4,5-trisphosphate receptor type 3 and chloride/carbonate (chloride/bicarbonate) anion exchanger 2 gene expression reduction due to the increase in the amount of micro-RNA 506 and its activity. $InsP_3R3$: Inositol-1,4,5-trisphosphate receptor type 3; AE2: Chloride/bicarbonate anion exchanger 2; miR-506: Micro-RNA 506; TMEM16A: Transmembrane 16A chloride channels; HCO_3^-: Bicarbonate; Cl^-: Chloride.

MECHANISM OF CHOLANGIOCYTE DAMAGE AND DESTABILIZATION OF BILIARY HCO_3^- UMBRELLA

Decrease of HCO_3^- supply to bile ducts, due to a decrease in $InsP_3R3$ and AE2 activity, will shift pH in the bile duct lumen to an acidic region[66]. Simultaneously, due to the retention and accumulation of HCO_3^- in the cytosol of cholangiocytes, there is gradual alkalinization of intracellular pHi in patients with PBC[30,65,67]. Complete AE2 deficiency will lead to intracellular alkalosis of cholangiocytes[61]. However, the reduced (rather than absent) expression of $InsP_3R3$ and AE2 genes has been observed in patients with PBC[61].

A shift of pH to a slightly acidic region in the lumen of bile ducts will increase the amount of protonated unconjugated and glycine-conjugated primary bile acids. This will lead to their increased entry into the small cholangiocytes (intralobular, interlobular, septal) of the bile ducts. Once in the slightly alkaline pHi within the cholangiocytes, the protonated bile acids will undergo deprotonation. Alkalinization of pHi and ionization of glycine-conjugated and unconjugated primary bile acids within cholangiocytes reduces the process of their difundation from intracellular to peribiliary space. As a result, there is a delayed and gradual accumulation of glycine-conjugated and unconjugated primary bile acids in small cholangiocytes. The theory of defective biliary HCO_3^- umbrella helps to explain the intracellular uncontrolled increased entry and accumulation of bile acids in small BECs.

The presence of the mucin-containing glycocalyx layer on the apical surface of large cholangiocytes protects them from penetration and the damaging effect of protonated conjugated and unconjugated bile acids. Intracellular accumulation of hydrophobic bile acids is a prerequisite for their cytotoxic effects[68]. As strong detergents, they are able to solubilize phospholipids and cholesterol from membrane structures of cholangiocytes, which leads to damage and destruction of cytoplasmic membrane and membranes of cell organelles (Figure 3). Thus, the entry and accumulation of unconjugated bile acids with stronger detergent properties in small cholangiocytes is more toxic for the cell than the accumulation of conjugated bile acids.

The chronic damaging effect of bile acids on membrane structures triggers accelerated senescence, necrosis, and/or apoptosis of BECs[69]. Bile acids destroy the membranes of cell organelles and nuclear membrane of cholangiocytes with the release of apoptogenic factors. The barrier function of the biliary epithelium is impaired, resulting in concomitant damage, inflammation, and oxidative stress. Cytokines, chemokines, and pro-inflammatory mediators released by cholangiocytes probably stimulate apoptotic and proliferative responses as well as activate fibrogenesis[70]. Bile acids

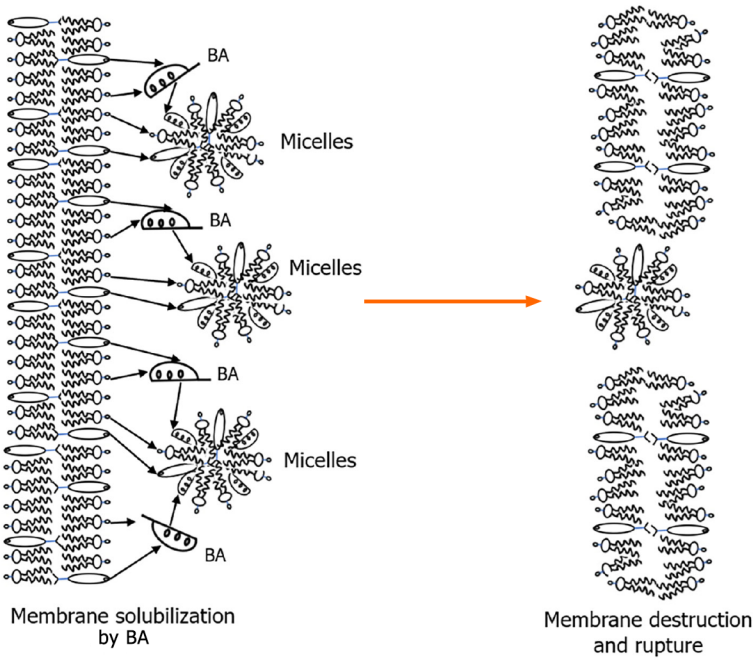

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Figure 3 Solubilization of phospholipids and cholesterol from membrane structures by bile acids. BA: Bile acids.

also mediate their toxic, apoptotic effects through specific signaling pathways at the intracellular level. The intrinsic apoptotic pathway is activated, including mitochondrial translocation of B-cell lymphoma 2-associated X protein, release of cytochrome C from mitochondria, activation of caspase 3, cleavage of poly (ADP-ribose) polymerase, and DNA fragmentation[71]. There is evidence that miR-506 activates the apoptosis pathway upon stimulation with toxic bile acids [66].

Proinflammatory cytokines additionally increase miR-506 expression[30]. A vicious circle develops that supports senescence, apoptosis, and proliferation of cholangiocytes. Ultimately, ductulopenia develops[30]. Together, this reflects the direct effects of bile acids on cholangiocytes rather than the nonspecific effects resulting from periportal inflammation [31].

From a pathophysiologic point of view, common to all cholangiopathies is the coexistence of cholangiocyte death and proliferation, as well as various degrees of portal inflammation and fibrosis[70]. Cell death induces the activation of inflammatory and profibrogenic pathways that trigger the development and progression of fibrosis, which gradually leads to small bile duct ductulopenia[72]. The conceptual mechanisms of these processes have been described in reviews [69,72].

Disruption of apoptosis is considered a trigger of PBC and already in the asymptomatic stage leads to the development of small bile duct ductulopenia, one of the early morphologic signs of the disease[73]. Apoptosis depends on mitochondrial permeabilization associated with excessive intracellular accumulation of bile acids[74-76]. In addition, bile acids and incomplete apoptosis of BECs diverted to necrosis can lead to pathogenic effects on intracellular components with subsequent generation of AMAs[77].

MECHANISM OF MITOCHONDRIAL PERMEABILIZATION AND AMAs FORMATION

Solubilization of phospholipids and cholesterol from the outer membrane of mitochondria by bile acids leads to their permeabilization[78]. There is an increase in the permeability of the mitochondrial outer membrane to ions and solutes [71,78]. There is leakage of the contents of the intermembrane space into the cytosol and loss of membrane potential. Mitochondria swell, their outer membrane swells, and the release of apoptogenic factors occurs[78]. The inner mitochondrial membrane, the main target for AMA formation, is opened. Further solubilization by bile acids of phospholipids and cholesterol from the inner membrane and destruction of mitochondria can lead to the release and degradation of PDC. The latter includes three enzymes: E1 PDC, E2 PDC, and E3 PDC[73]. Each of these enzymes, in addition to the protein part, has cofactors: E1 PDC contains thiamine pyrophosphate as a cofactor; E2 PDC contains lipoic acid and coenzyme A; and E3 PDC contains flavin adenine dinucleotide and nicotinamide adenine dinucleotide. E1 and E3 PDC are protein complexes that do not contain lipid components. Therefore, they are unlikely to be affected by bile acids accumulated in cholangiocytes, since they have an effect on lipid components. Sera from PBC patients do not show serologically detectable reactivity against the E1 and E3 components of PDC[79].

E2 PDC is a lipoprotein with two lipoic acid binding sites[8]. E2 PDCs contain an essential lysine residue in the lipoyl domain to which lipoic acid is covalently attached[8]. The lipoic-lysine bond at position 173 is highly conserved across species and is essential for antigen recognition[80]. AMAs target immunodominant epitopes containing lipoic acid.

The importance of chemical xenobiotics capable of modifying lipoic acid in E2 PDC has been previously shown for the appearance of serologic reactivity of this complex[81,82]. Alteration of the conformational structure of the lipoyl domain of E2 PDC, due to chemical modification of lipoic acid may contribute to the loss of immune tolerance[83,84]. Most likely, such chemical modifiers in PBC are bile acids accumulating in cholangiocytes upon loss of the protective properties of the biliary HCO_3^- umbrella. Bile acids can interact with the lipoic acid of the antigen-recognized E2 PDC. The result of such an interaction may be immunomodification of the E2 PDC complex with acquisition of autoantigenic properties and loss of immune tolerance[66]. This assumption is supported by a number of studies performed at the end of the last century. In these works, it was shown that the main immunogenic region on E2 PDC recognized by sera from patients with PBC is localized in the lipoyl-containing domain[85-87]. The lipoic acid content of E2 PDC is thought to play a role as a potent adjuvant[88]. The presentation of immunomodified E2 PDC complex to lymphocytes can lead to stimulation of the T-cell subpopulation and specific production of AMAs[66,88,89].

A defective biliary HCO_3^- umbrella triggers a continuous and endless process of accumulation and detergent action of bile acids on small cholangiocytes with the formation of AMAs. Since the disruption of HCO_3^- entry into the lumen of the bile duct is constant, the production of AMAs will be continuous. As a result, an elevated level of IgM (M2) will be constantly maintained in the plasma of PBC patients. The appearance of AMAs in serum is another early immunologic pathognomonic sign of PBC, which occurs in the asymptomatic stage of the disease.

DYSFUNCTION OF THE PDC COMPLEX AND THE FIRST CLINICAL SIGNS OF THE ASYMPTOMATIC STAGE OF PBC

AMAs detection in the asymptomatic stage of the disease is accompanied by the appearance of the first subjective clinical signs, namely weakness, malaise, fatigue, and decreased performance[90]. Fatigue is the most common symptom of PBC in the asymptomatic and early stage of the disease[91-93], occurring in about 40%-80% of patients[94,95]. However, there is no correlation between fatigue and the severity or duration of the disease[95-98].

The mechanism underlying fatigue development is closely related to gradually progressive energy deficiency[99]. The latter is most likely related to the involvement of the PDC in the pathologic process of PBC development. The PDC is a very important metabolic enzyme. PDC functions in every cell and is required for the conversion of pyruvate to acetyl-CoA, which is incorporated into the Krebs cycle and is essential for the body to obtain energy in the form of ATP[73]. As mitochondria in cholangiocytes permeabilize and PDC becomes involved in AMAs production, there is a gradual decrease in ATP synthesis. This leads to the development of local energy deficiency, which in turn, enhances the senescence and apoptosis processes of small BECs initiated by bile acids. A vicious cycle occurs, contributing to the progression of ductulopenia and AMAs formation. In this case, AMAs are able to react with polypeptides including E2 PDC in the mitochondria of almost any cell. It has been shown that antibodies to PBC cross-react with polypeptides in the mitochondria of beef heart, presumably related to E2 PDC[100]. ATP production decreases and energy deficiency develops throughout the organism.

The development of energy deficiency is accompanied by an increase in glycogenolysis and a decrease in glycogenogenesis. The study by Green *et al*[101] showed that in the initial stages of PBC, there was a gradual decrease in glycogen stores in the liver associated with increased glycogenolysis and decreased glycogenogenesis. The authors also demonstrated that glucokinase activity significantly dropped (down to zero) in patients with PBC, indicating a decrease in glycogen formation in the liver[101]. At the same time, hexokinase (phosphorylates hexoses), which is responsible for glycogen synthesis mainly in muscles, was significantly increased in patients with PBC during this period compared to healthy individuals[101]. As a result of the developing energy deficiency in the asymptomatic stage of the disease, the first clinical signs of expressed weakness, rapid fatigue, decreased performance, functional status, and quality of life appear in patients with PBC[90,102-104].

CONCLUSION

Based on the growing body of knowledge on the molecular mechanisms underlying the development of cholangiocyte damage in patients with PBC, we proposed a hypothesis to explain the pathogenesis of the initial morphologic (ductulopenia), immunologic (AMAs) and clinical (weakness, malaise, rapid fatigue) signs of the disease in the asymptomatic stage (Figure 4).

Evidence suggests that in susceptible individuals, an unknown initial trigger causes an X-linked epigenetic change that leads to gene reactivation and increased expression of miR-506[30]. The increased synthesis and activation of miR-506 leads to inhibition of $InsP_3R3$ and AE2 translation[105]. As a result, HCO_3^- entry into the bile duct lumen is reduced and HCO_3^- accumulation in the cytosol of cholangiocytes occurs[30]. Changes in extra- and intracellular pH alter the protonation (in the lumen of the bile duct) and deprotonation (intracholangiocyte) of bile acids, leading to an increase in the uncontrolled entry and accumulation of unconjugated and glycine-conjugated bile acids into the BECs.

The detergent properties of bile acids trigger cell membrane disruption, senescence and apoptosis of cholangiocytes, mitochondrial permeabilization, destruction and immunomodification of E2 PDC, followed by AMAs formation. Senescence, apoptosis, and proliferation of cholangiocytes lead to the gradual development of ductulopenia. The involvement of PDC in the pathological process contributes to insufficient ATP synthesis, development of energy deficiency, and occurrence of the nonspecific clinical sign of fatigue. The development of ductulopenia is accompanied by

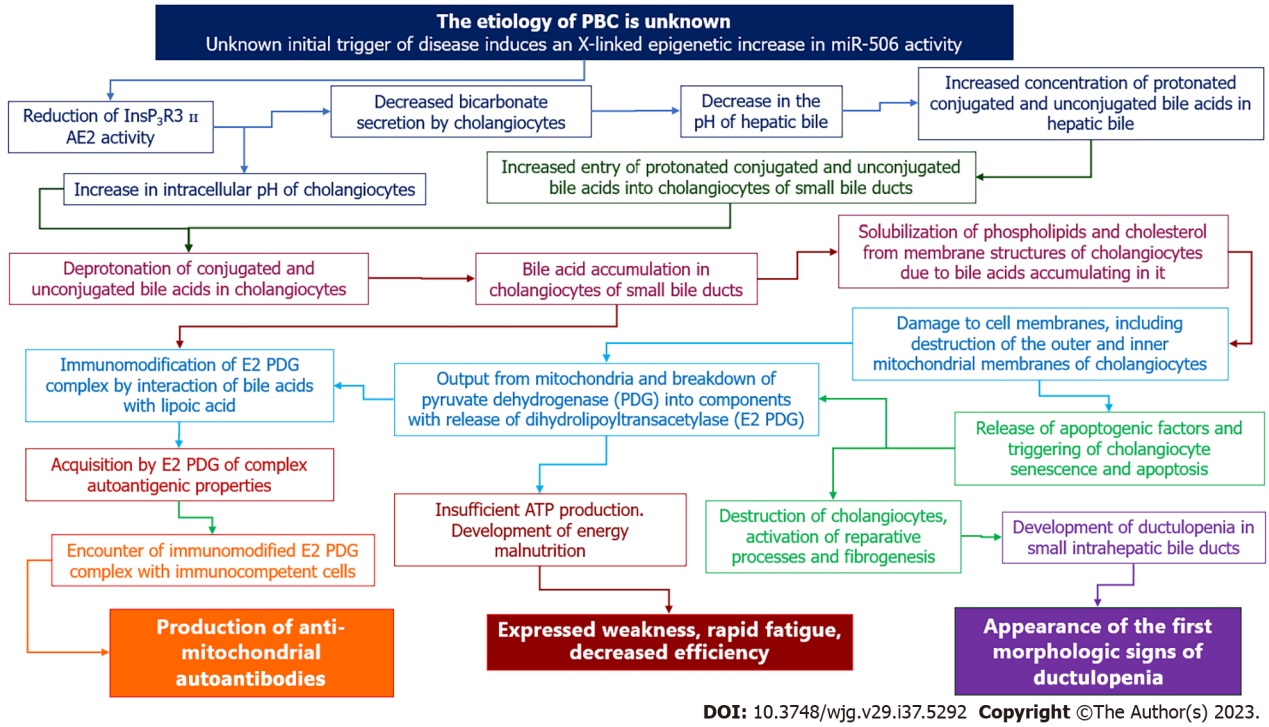

Figure 4 Mechanism of anti-mitochondrial antibody formation, development of ductulopenia, weakness, fatigue and malaise in the asymptomatic stage of primary biliary cholangitis: hypothesis. $InsP_3R3$: Inositol-1,4,5-trisphosphate receptor type 3; AE2: Chloride/bicarbonate anion exchanger 2; PDG: Pyruvate dehydrogenase; ATP: Adenosine triphosphate.

the development of intrahepatic cholestasis. Cholangiocytes are the main target in the initial stage of PBC. However, as soon as cholestasis develops, hepatocytes are also involved in the pathological process, which leads to their damage[30].

FOOTNOTES

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MINIREVIEWS

Sequence of events leading to primary biliary cholangitis

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Abstract

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease that is observed more frequently in middle-aged women. This disorder is considered an autoimmune disease, since liver injury is sustained by the presence of self-directed antimitochondrial antibodies targeting the bile duct cells. The prognosis may vary depending on an early diagnosis and response to therapy. However, nearly a third of patients can progress to liver cirrhosis, thus requiring a liver transplant. Traditional immunosuppressive therapies, commonly employed for other autoimmune diseases, have limited effects on PBC. In fact, dramatic functional changes that occur in the biliary epithelium in the course of inflammation play a major role in perpetuating the injury. In this minireview, after a background on the disease and possible predisposing factors, the sequential cooperation of cellular/molecular events leading to end-stage PBC is discussed in detail. The rise and maintenance of the autoimmune process, as well as the response of the biliary epithelia during inflammatory injury, are key factors in the progression of the disease. The so-called "ductular reaction (DR)", intended as a reactive expansion of cells with biliary phenotype, is a process frequently observed in PBC and partially understood. However, recent findings suggest a strict relationship between this pathological picture and the progression to liver fibrosis, cell senescence, and loss of biliary ducts. All these issues (onset of chronic inflammation, changes in secretive and proliferative biliary functions, DR, and its relationship with other pathological events) are discussed in this manuscript in an attempt to provide a snapshot, for clinicians and researchers, of the most relevant and sequential contributors to the progression of this human cholestatic disease. We believe that interpreting this disorder as a multistep process may help identify possible therapeutic targets to prevent evolution to severe disease.

Key Words: Primary biliary cholangitis; Cholangiocyte; Biliary secretion; Biliary proliferation; Ductular reaction; Antimitochondrial antibody; Cellular senescence; Liver fibrosis

Core Tip: Primary biliary cholangitis is a chronic cholestatic human disease. The pathological processes that favor the evolution toward end-stage liver disease during this disorder are only partially elucidated. The aim of this minireview is to summarize the sequential pathological contributors, supporting the progression of liver injury, in the course of this disease.

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INTRODUCTION

Primary biliary cholangitis (PBC), previously named primary biliary cirrhosis[1], was probably already described in the middle of the nineteenth century. In fact, important similarities with PBC were reported in a clinical case observed at Guy's Hospital in 1851[2]. Almost one century later, a description of the clinical course of the disease was reported in a group of patients, mainly achieving diagnosis after a surgical approach[3]. The etiology of PBC remains elusive today, even if the pathogenesis is related to the onset of an autoimmune response characterized by the presence of anti-mitochondrial antibodies (AMA) in the sera of affected patients[4]. The target of the immune response is represented by the intrahepatic biliary tract, thereby determining progressive chronic cholestasis, fibrosis, and a possible evolution toward liver cirrhosis[5]. Women are prevalently affected by PBC, with an age of onset that usually falls within the fourth or fifth decade[5]. The prevalence can change among different countries, accounting in general for 20-30/100.000 inhabitants in United States and Europe[5,6]. Liver biopsy is generally not required for diagnosis since AMA positivity, coupled with increased cholestasis indexes in the absence of biliary obstruction, represents a specific and characteristic presentation[7]. Ursodeoxycholic acid (UDCA), 13-15 mg/kg daily is the main and mandatory treatment to slow the progression in patients affected by PBC. The use of obeticholic acid (OCA) should help increase the response to UDCA or in subjects UDCA intolerant[8]. Despite the identification of effective medical treatments for PBC, a third of patients have a scarce response, thus evolving toward liver cirrhosis that may also require liver transplantation[9]. This problem and uncertainty about the mechanisms of onset and progression of the disease suggest that further research is needed to decipher the natural/molecular history of PBC. In this review, which also describes possible predisposing factors, we examine current knowledge on sequential molecular and cellular mechanisms in the onset of PBC. Reversion of these chain processes may possibly prevent the most severe sequelae of this chronic cholestatic disorder. The manuscript was prepared on the base of a literature search (PubMed, Scopus, Web of Science) using several key words (alone or in combination) such as: PBC, primary biliary cirrhosis, cholangiocyte, bile duct cells, secretion, proliferation, cholestasis, molecular mechanism, risk factors, pathology, antibody, immunology, and others.

Before PBC (predisposing factors)

Some generic risk factors, such as female sex and age, have been identified in patients with PBC. More interestingly, and suggesting a genetic predisposition, studies examine the prevalence of autoimmune disorders in patients with PBC and their relatives[10]. The family history of PBC seems, in fact, to be the strongest predisposing factor for the disease with an odds ratio of nearly 10 compared to controls. Furthermore, this familiar occurrence also increases the possibility of developing other autoimmune disorders such as polymyositis, systemic lupus erythematosus, and others[10]. These observations claim a possible similar genetic background between PBC and other autoimmune disorders, as also suggested by a clinical study focusing on this category of diseases[11]. However, we must emphasize that in PBC, such as other complex disorders, genetics represents only a permissive trait, which requires exposure to possible environmental/external factors for the development of injury[12]. Human leukocyte antigens (HLA) have long been investigated for their possible relationship with the onset of immune diseases[13]. Similarly, early data suggested an association between PBC and HLA DRB1*08[12]. This finding was later expanded, demonstrating an opposite protective effect of DRB1*11 and DRB1*13 and not only with regard to PBC, but also extended to other hepatic diseases such as viral B and C hepatitis[14]. The approach of genome-wide association studies has more recently allowed to identify other genetic changes in PBC, including several non-HLA variants[15]. These may affect the interleukin-12/JAK-STAT pathway, the B cell response, and other steps of inflammatory signalling. However, the trigger factors that may cause disease in the presence of a favourable genetic background are still not clearly defined. Smoking or drinking habits have been associated with the development of PBC, as well as recurrent urinary infections, hair dye, and hormonal replacement therapy in women[10]. The large diffusion of these conditions, together with the limited prevalence of PBC, allow us to foresee the scarce utility of possible preventive strategies for this disease, at present.

The beginning of injury: the antimitochondrial antibody

In parallel with what has been observed in other autoimmune disorders, the presence of self-directed antibodies (AMA) is the basis of the physio-pathological process in PBC. The main target of AMA is represented by the mitochondrial E2

subunits of the pyruvate dehydrogenase complexes (PDC-E2)[4]. Since E2 is a highly preserved portion in other species and bacteria, the possible origin of AMA by molecular mimicry (cross-reaction with self-antigen after a previous exposure to exogenous pathogens with similar moieties) has long been suggested[16]. Several agents have been proposed in the past to induce AMA formation, including microorganisms or environmental substances[17]. Among bacteria, *Escherichia coli (E. Coli)* has long been suggested as a possible important actor in eliciting the immune response in PBC[18]. The contribution of *E. Coli* may also explain the frequent association between the onset of PBC and recurrent urinary tract infection, as this microorganism is an important causative agent of this latter disorder. More recently, exposure to *Novosphingobium aromaticivorans (N. Aromaticivorans)*, a ubiquitous gram-negative bacteria capable of metabolizing xenobiotics, has been suggested as a possible trigger factor in PBC. In fact, the immune reactivity of sera from patients with PBC is 100 times stronger for *N. Aromaticivorans* compared to *E. Coli* and the ubiquity of this bacteria is demonstrated by its presence in 25% of fecal samples from both PBC patients or control[19]. Regarding the mechanism linking bacteria to PBC progression, also a role for intestinal dysbiosis has been claimed[20]. This may increase intestinal permeability and lipopolysaccharide flux toward the liver, thus resulting in an enhanced immune/inflammatory response. Interestingly and supporting this view, UDCA treatment was reported to attenuate the difference in intestinal microbioma between PBC patients and normal controls[21]. However, to underscore that studies on gut microbial composition are particularly complex, affected by individual, environmental and dietary factors as well as by sampling procedure, so that a conclusive picture on this issue is not available at present. The development of an AMA titer in blood has been considered in the clinic to play an important causal role in the following biliary damage. However, in this regard, while some authors suggest that the onset of this antibody is an important predictive factor for the development of PBC[22], others underscore the higher prevalence (1/1000) of AMA compared to PBC (0.4/1000), therefore suggesting that some subjects may be healthy for life despite displaying these autoantibodies in blood[4]. With this regard, some data show that AMA does not seem pathogenic by itself, and its complex with the corresponding antigen is needed to prompt immunity. However, why the formation of immune complexes occurs in PBC patients is a question that remains unanswered at present[23]. The AMA target within PDC-E2 is represented by the lipoyl domain, and the different degradations of this epitope seem related to the reason that the antibodies mainly target bile duct cells (*i.e.*, cholangiocytes). In fact, PDC-E2 is ubiquitously present in cells; however, it appears that after the apoptotic death of cholangiocytes, differently from other epithelial cells, the epitope is released in its intact form, thus maintaining the immune response and lymphocyte homing[17]. This pattern appears to be also followed by the salivary and lacrimal glands, explaining the frequent association between PBC and Sjögren syndrome[24], being the latter a disease that presents characteristically with dryness in the mouth and eyes and also recognizing an autoimmune origin[25]. Finally, from a clinical point of view, the AMA titer does not have a significant relationship with the prognosis or extent of liver damage in PBC[26] while some patients with this disease exhibit an antinuclear antibody (ANA) instead of AMA (AMA negative PBC)[27].

Onset of chronic inflammation

Biliary cell apoptosis is a critical event in maintaining the injury since the early stages of PBC. This process allows exposure to specific epitopes that support the autoimmune response[28]. The possible contribution of innate immunity to the inflammatory process of PBC remains an argument for debate. Destruction of biliary cells has been described after toll-like receptor 4 stimulation of natural killer cells (NK) in the presence of interferon alpha[29]. Interestingly, biliary damage is proportional to the degree of NK infiltration of the biliary tract[30]. In fact, when the ratio of NK/bile duct cells is low, no biliary damage occurs. On the contrary, high NK infiltration is associated with extensive cholangiocyte damage and epitope exposure. These are targeted by T cells, which in turn maintain chronic injury realizing the adaptive immune response[30]. In this perspective, the activity of NK cells would be the main determinant of the evolution from an innate to an adaptive immunological response, during the onset of PBC. Anti-PDC $E2_{163-176}$ specific T cells then expand and are preferentially located in the lymph nodes and liver. These cells are recovered only in patients with PBC, and their specific location in the liver supports their pathogenic role in chronic injury involving this organ[31]. Once the inflammatory process is established, changes that occur in the activities of bile duct cells further contribute to injury, as reported in the following paragraphs.

Changes in biliary physiology

Several changes occur in the function of cholangiocytes when the PBC inflammatory damage is established. These involve the secretory and proliferative processes of the biliary epithelium and other aspects. These issues are discussed in the following paragraphs, including their contribution to perpetuating and worsening the damage.

Impairment of secretive processes

Several studies in recent decades have elucidated the paramount importance of biliary epithelia in bile secretion and the physiopathological changes that occur in this system in cholestatic disorders (when the bile does not reach the intestine) such as PBC[32]. Differently from hepatocytes, in which bile secretion is constant and is driven by canalicular bile acids (BA) transfer, the cholangiocyte bile output can change significantly according to exposure to different gastro-intestinal hormones or peptides[32]. Moreover, endogenous (inflammatory) or exogenous stimuli (bacterial, toxic, drug-induced, and others) altering the ductal microenvironment can change cholangiocyte secretory response[33]. The classical molecular process that stimulates biliary bile secretion recognizes as a first step the stimulation by secretin (Sec) of a specific sec receptor (SR) expressed only in cholangiocytes within the liver[34]. Sec/SR binding activates a cascade of molecular events involving, in turn: (1) Increased intracellular cyclic adenosine 3',5'-monophosphate (cAMP); (2) phosphorylation of protein kinase A (PKA); (3) Cl^- extrusion in canalicular space by cystic fibrosis transmembrane

regulator; and (4) reabsorption of Cl^- and its replacement with bicarbonate operated by the chloride-bicarbonate exchanger (AE2)[34]. This determines the realization of a BA-independent bicarbonate-enriched choleresis that may contribute, under some conditions, to more than 50% of total bile flow. Several human and experimental evidences demonstrated impaired AE2 activity in PBC. In 1993, a decrease in AE2 mRNA was first described in the liver of PBC patients[35]. In a subsequent human study, a reduction in AE2 was also demonstrated with respect to protein synthesis, employing the immune staining technique[36]. Finally, the same group demonstrated with positron emission tomography reduced (both basal and Sec-stimulated) bicarbonate biliary secretion in patients with PBC[37]. Further evidence supporting the impairment of AE2 in PBC came from the Ae2 knockout mouse model, which resembles several features of this human disease[38]. The mechanism at the base of the down-regulation of AE2, in the course of PBC, is not clear at present. However, research demonstrated epigenetic changes in the AE2 promoter region (both in liver and peripheral blood mononuclear cells of PBC patients) characterized by enhanced methylation, thus determining reduced mRNA transcription[39]. Despite the uncertainty with respect to the chain of events that leads to decreased AE2 activity, the outcome of this occurrence is well evidenced by the impairment of bicarbonate-enriched choleresis after Sec stimulation[37]. The fall in biliary bile flow and, in particular, of bicarbonate secretion, opens the door to BA-induced cytolitic damage, according to the theory of bicarbonate umbrella[40]. In agreement with this view, given the decreased AE2 activity, the delicate bicarbonate film (bicarbonate umbrella), laying on the luminal side of the cholangiocyte, would be altered, allowing cellular damage by hydrophobic BA monomers. The main determinants of this process are shown in Figure 1. In this perspective, two pathological components would contribute to chronic biliary damage during PBC: (1) The autoimmune inflammatory process; and (2) the cytotoxic BA-related injury. The role of hydrophobic BA accumulation, in perpetuating PBC damage, is also indirectly supported by the beneficial results obtained when the BA pool is supplemented with less detergent and more hydrophilic BA such as UDCA or when their synthesis is inhibited with OCA.

Changes in proliferative processes

Bile duct proliferation is a complex process, characterized by important changes during pathological conditions. As observed with respect to the secretive process, intracellular cAMP levels also play a key role in modulating biliary growth [41]. After enhanced cAMP formation, the PKA/Sc/MEK/ERK1/2 axis is activated in order to stimulate cellular proliferation[42]. Several gastro-intestinal hormones and neuropeptides may enhance cholangiocyte proliferation using the cAMP route (such as Sec, acetylcholine, serotonin, histamine, *etc.*), while others (such as gastrin and somatostatin) may negatively regulate this pathway, obtaining the opposite effect[43]. Finally, BA are also important key molecules for biliary growth[44], interacting with specific cholangiocyte receptors. For example, the interaction of BA with the cholangiocyte Takeda G protein-coupled receptor 5 modulates proliferation, with an opposite effect in ciliated and nonciliated cells[45]. From a morphological point of view, three different proliferative pictures can be recognized in the biliary tree [46]. Type I 'typical' proliferation is observed in humans after high-grade acute biliary obstruction or in the early phase of chronic cholestatic disease, including PBC. In Type I, biliary proliferation is restricted to portal spaces and characterized by ducts with a preserved architecture and orientation. In Type 2 'atypical' proliferation, the extension of truncated, poorly organized, and possibly nonfunctional ducts, with an indefinite lumen, is observed in the liver parenchima, usually coupled with enhanced inflammatory processes. Type II proliferation is generally observed in the more advanced stage of PBC and may represent the introductive step toward the progressive loss of bile ducts (ductopenia) found in the late phase of the disease. Finally, Type III 'oval cell' proliferation is also reported. This irregular proliferation (distorted duct with architectural changes in the liver parenchima) is observed in the early phase of experimental carcinogenesis and may have a relationship with the onset of cholangiocarcinoma, as it originates from multipotent (oval) cells[44,46]. As mentioned above and according to this morphological classification, the repairing process during PBC: (1) Would stimulate biliary growth that; (2) may evolve from a typical phase to an atypical phase, and possibly; and (3) may lead to a ductopenic end stage. This complex cellular/molecular process is morphologically recapitulated in the so-called 'ductular reaction (DR)', which probably promotes the evolution towards liver fibrosis and duct loss, both characteristic features of end-stage PBC.

Mixing biliary proliferation, fibrosis, and cellular senescence(CS): The DR

The term DR was coined and is generally preferred to proliferation in liver pathology, since it implies a reactive expansion of the ductular phenotype that may or may not derive from a ductal origin[47]. This suggested definition comes from evidence reporting the presence of inflammatory cells, as well as cells with an intermediate progenitor phenotype, in the DR microenvironment. Although DR may be detected in various liver disorders, it seems particularly frequent and widespread in chronic cholestatic diseases, compared to the normal liver[48,49]. The pathogenetic role of DR in the course of chronic cholestatic diseases has not yet been fully elucidated. However, some experimental data suggest that inhibition of biliary growth in bile duct ligated mice also negatively regulates hepatic stellate cells (HSC) activation and fibrous tissue deposition, suggesting a close link between DR and the onset of liver fibrosis[50]. On the other hand, DR has also been associated with CS[51]. CS refers to a cell that shows permanent G1 phase growth arrest. This cell acquires a so-called senescence-associated secretory phenotype that has been implicated in the pathogenesis of cholangiopathies[52]. With regard to PBC, CS is largely observed within biliary cells and its occurrence (since the possible replacement of vital cells with senescent ones) has been related to the evolution toward ductopenia[53]. In agreement with this view, CS has generally been observed in the late stage of PBC. On the other hand, autophagy, likely supported by the impairment of the bicarbonate umbrella[54], is more frequent in the early phase of the disease, suggesting that this last cellular event may be a possible trigger for CS[55].

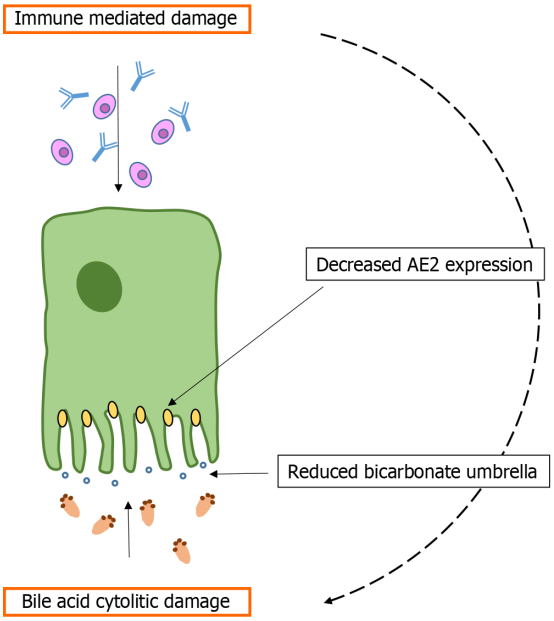

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Figure 1 From immune mediated damage to bile acids injury. In primary biliary cholangitis the inflammatory process induces a decrease in chloride-bicarbonate exchangerexpression. This in turn determines a reduction of bicarbonate film (bicarbonate umbrella) on the canalicular portion of bile duct cells, exposing them to hydrophobic bile acids injury. AE2: Chloride-bicarbonate exchanger.

As described above, it is clear how the PBC liver represents a pathology compendium in which several cellular/molecular determinants (inflammation, changes in biliary secretion, HSC activation, CS, autophagy, *etc.*) combine to support the injury. The common field in which all these events are recapitulated or prompted could possibly be represented by DR. In this perspective, we emphasize that DR began to attract the attention of pathologists more than 60 years ago, due to its characteristic features and its relationship with human liver injury[56]. In PBC, a recent clinical study again demonstrated DR as the most relevant pathological characteristic in predicting the stage of the disease, fibrosis progression, and response to UDCA therapy[57]. Unfortunately, most of the research focusing on DR and its relationship with other liver pathological features (such as fibrosis or cell senescence) is mainly descriptive. Mechanistic studies are needed to reveal the molecular pathways that trigger the sequential cascade of pathological events in PBC. In Figure 2 the relationship between DR and other pathological processes during PBC, is depicted.

CONCLUSION

Several sequential molecular/cellular events may occur to develop end-stage PBC. While the presence of the principal predisposing factors (family inheritance or gender) should not be modified, a deep knowledge of the mechanisms involved in the onset and perpetuation of the disease can reveal possible therapeutic targets. However, to decipher the progression of injury during PBC, research cannot be restricted to the study of the immune-inflammatory process, since important physiological and pathological changes in cholangiocytes also contribute to liver damage. To support this view, clinical studies approaching PBC with immunosuppressive or anti-inflammatory drugs, which are usually effective in other autoimmune disorders, gave limited results in these patients[8]. In this perspective and as observed in other liver diseases (such as alcoholic and nonalcoholic steato-hepatitis)[58], multiple hits are likely needed in PBC to develop severe liver damage. Therefore, in an attempt to design a possible pathological route leading to this disorder, a series of events linking together should be considered. As reported in Figure 1 and described in this minireview, the autoimmune process not only promotes inflammation, but also radically changes the secretive and proliferative activities of bile duct cells. Regarding biliary secretion, decreased expression of AE2 negatively regulates biliary flow and bicarbonate secretion. This in turn allows BA cytolitic damage from the impairment of the bicarbonate umbrella. Parallel to this, biliary growth is enhanced in an attempt to balance cellular loss. The chronic enhancement of proliferative activities is recapitulated in the pathological finding of the DR. This represents a microenvironment in which CS and profibrotic processes originate, leading to ductopenic/fibrotic end-stage liver injury (Figure 2). While previous reviews consist in a conventional broad description of PBC or consider a specific aspect of the disorder (genetic, immunology, diagnosis, therapy, *etc.*), in this manuscript we aimed: (1) To design with clarity the sequential steps allowing the PBC evolution from liver inflammation to end-stage fibrosis; and (2)to examine and define in detail the key consecutive molecular/cellular events supporting this path. We believe that deciphering the natural history of PBC as a step by step route, may facilitate the identification of important therapeutic targets. It is clear that several pieces are still lacking to complete the pathogenetic puzzle of this disease. In this sense, studies that examine in detail the molecular pathways that lead to different changes/responses of cholangiocytes may improve our knowledge of PBC and should be helpful in developing new pharmacological

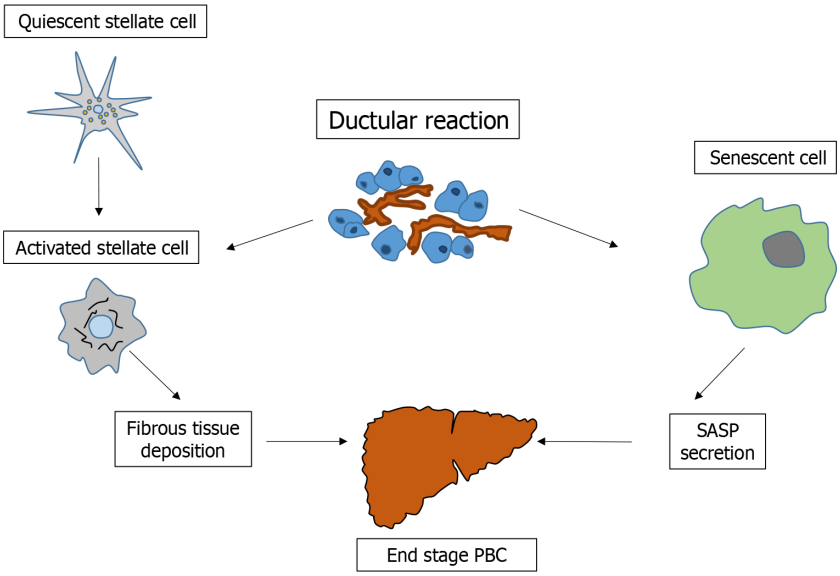

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Figure 2 Central role of ductular reaction in the progression of primary biliary cholangitis. Ductular reaction contributes to the activation of hepatic stellate cells and simultaneously favours a cell senescent phenotype. These events support the ductopenic/cirrhotic evolution of liver tissue during this cholestatic disease. PBC: Primary biliary cholangitis; SASP: Senescence-associated secretory phenotype.

treatments.

FOOTNOTES

Author contributions: Lenci I acquisition of data, analysis and interpretation, drafting of manuscript, critical revision; Carnì P, Milana M, Bicaj A, and Signorello A acquisition of data, critical revision; Baiocchi L proposal of study, study conception, correction of manuscript, critical revision.

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ORIGINAL ARTICLE

Case Control Study

Leukocyte immunoglobulin-like receptor B2 overexpression as a promising therapeutic target and noninvasive screening biomarker for colorectal cancer

Qian-Qian Wang, Lei Zhou, Geng Qin, Chang Tan, Yuan-Chen Zhou, Shu-Kun Yao

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Abstract

BACKGROUND

Colorectal cancer (CRC) has become the second most deadly malignancy in the world, and the exploration of screening markers and precise therapeutic targets is urgent. Our previous research identified leukocyte immunoglobulin-like receptor B2 (LILRB2) protein as a characteristic protein of CRC, but the association between LILRB2 expression and clinicopathological features, the internal mechanism related to CRC progression, and screening diagnostic efficacy are not clear. Therefore, we hypothesized that LILRB2 is significantly highly expressed in CRC tissues, correlated with advanced stage and a poor prognosis, and could be used as a therapeutic target and potential screening biomarker for CRC.

AIM

To explore whether LILRB2 can be used as a potential therapeutic target and noninvasive screening biomarker for CRC.

METHODS

Patients who underwent radical surgery for CRC at China-Japan Friendship Hospital between February 2021 and October 2022 were included. Cancer and paracancerous tissues were collected to verify LILRB2 expression, and the association between LILRB2 expression and clinicopathological features was analysed. Serum was collected from CRC patients, adenoma patients and healthy controls during the same period to assess the diagnostic value of LILRB2 as a

noninvasive screening biomarker, and its diagnostic value was further compared with that of the traditional markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9).

RESULTS

A total of 58 CRC patients were included, and LILRB2 protein was significantly overexpressed in cancer tissues compared with paracancerous tissues ($P < 0.001$). Angiopoietin-like protein 2 (ANGPTL2) protein, as the ligand of LILRB2, was synergistically overexpressed in CRC tissues ($P < 0.001$), and overexpression of LILRB2 and ANGPTL2 protein was significantly correlated with poor to moderate differentiation, vascular involvement, lymph node metastasis, distant metastasis, advanced tumor-node-metastasis stage and a poor prognosis ($P < 0.05$), which suggested that LILRB2 and ANGPTL2 are closely associated with CRC progression. In addition, serum LILRB2 concentrations increased stepwise in healthy individuals, adenoma patients and CRC patients with statistically significant differences. The sensitivity of serum LILRB2 for the diagnosis of CRC was 89.74%, the specificity was 88.89%, the area under the curve was 0.95, and the diagnostic efficacy was better than that of conventional CEA and CA19-9.

CONCLUSION

LILRB2 protein can be used as a potential novel therapeutic target and noninvasive screening biomarker for CRC, which is beneficial for early screening and precise treatment.

Key Words: Colorectal cancer; Leukocyte immunoglobulin-like receptor B2; Angiopoietin-like protein 2; Therapeutic target; Noninvasive screening biomarker

Core Tip: Based on prior proteomic research rather than simple data mining, this study innovatively proposed and validated that leukocyte immunoglobulin-like receptor B2 (LILRB2) and its ligand, the angiopoietin-like protein 2 protein, are significantly overexpressed in colorectal cancer (CRC) and closely associated with tumour progression and a poor prognosis. In addition, this study is the first to propose that serum LILRB2 concentration can be used as a novel screening biomarker with a sensitivity of 89.74%, a specificity of 88.89% and an accuracy rate of 89.63% for the diagnosis of CRC. The sensitivity and accuracy were significantly higher than those of carcinoembryonic antigen and carbohydrate antigen 19-9. Therefore, LILRB2 could be a promising therapeutic target and noninvasive screening biomarker for CRC.

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INTRODUCTION

The latest cancer statistics show that more than 1.9 million new cases of colorectal cancer (CRC) and 935000 deaths occurred in 2020, accounting for approximately one-tenth of all new cancer cases and deaths. CRC has become the third ranked malignancy worldwide in terms of incidence and the second ranked in terms of mortality, second only to lung cancer[1-3]. The 5-year overall survival (OS) rate for early-stage CRC can be as high as 80%-90%, while the prognosis for advanced-stage patients is poor, with a 5-year survival rate of less than 20%[4,5]. Although targeted therapy or immunotherapy has improved the OS of CRC patients, limited treatment options, moderate response rates and drug resistance remain major challenges for CRC treatment. Therefore, the exploration of novel therapeutic targets and noninvasive screening biomarkers is essential to improve the outcomes of CRC patients.

Proteomics has been widely used in cancer research[6,7]. In preliminary research, we screened leukocyte immunoglobulin-like receptor B2 (LILRB2) protein as a characteristic protein of CRC[8]. LILRB2 is a transmembrane glycoprotein with a structure similar to immune checkpoint protein programmed cell death protein-1 (PD-1)/PD-ligand 1; it has four extracellular immunoglobulin-like structural domains and intracellular tyrosine-based immune receptor inhibitory motifs (ITIMs); it is widely expressed on the surface of dendritic cells, macrophages, and other myeloid cells and can inhibit immune cell function and participate in important pathological processes such as tumour immune microenvironment (TME), immune escape, and promotion of tumour progression[9]. Angiopoietin-like proteins (ANGPTLs) are a family of seven secreted glycoproteins that play an important role in angiogenesis, the inflammatory response, and tumour progression[10]. In recent years, it has been revealed that LILRB2 is a receptor for ANGPTLs, with the strongest affinity for ANGPTL2, ending the status of ANGPTLs as "orphan ligands"[11]. The interaction of the LILRB2 receptor with the ANGPTL2 ligand has been reported to promote the progression of tumours such as acute leukaemia and non-small cell

lung cancer (NSCLC)[10,12,13]. However, such interactions have not been reported in CRC.

Therefore, this study hypothesized that LILRB2 could be a potential novel therapeutic target and screening biomarker for CRC, but validation of LILRB2 and ANGPTL2 protein expression and interaction in CRC tissues is first needed to further explore the feasibility of LILRB2 protein as a noninvasive screening biomarker.

MATERIALS AND METHODS

Study subjects

Patients who underwent radical surgery for CRC at China-Japan Friendship Hospital between February 2021 and October 2022 were recruited. Inclusion criteria were as follows: patients diagnosed with CRC by the department of pathology who intended to undergo radical resection. The exclusion criteria were as follows: (1) Patients with familial adenomatous polyposis, hereditary nonpolyposis CRC (lynch syndrome), synchronous multiple tumours, inflammatory bowel disease-related CRC; (2) CRC with neuroendocrine manifestations; (3) Patients who received radiotherapy, targeted therapy, immunotherapy, *etc.*, within 6 mo prior to radical surgery; and (4) Patients who lacked informed consent or patients with missing information.

Colorectal adenoma patients and healthy controls were recruited at the Endoscopy Center of China-Japan Friendship Hospital during the same period. Inclusion criteria for adenoma patients were as follows: Patients with pathologically confirmed colorectal adenoma who were intended to undergo elective endoscopic mucosal resection. The exclusion criteria were as follows: (1) Severe atypical hyperplasia, high-grade intraepithelial neoplasia or carcinoma in situ; (2) Familial adenomatous polyposis or inflammatory bowel disease-associated colorectal polyps; and (3) Refusal to sign the informed consent form. Patients with no abnormalities according to colonoscopy were included in the healthy control group. The study was approved by the ethics committee of China-Japan Friendship Hospital and conducted in accordance with the Declaration of Helsinki.

Sample collection

Cancer and paracancerous tissues (> 5 cm from the cancer margin) were collected from CRC patients after surgical resection, and the samples were rinsed with ice-cold saline, soaked in 10% formalin solution with a volume ratio of 1:7, fixed for 24 h, rinsed under running water, dehydrated and waxed at 55 °C and embedded in paraffin. The paraffin blocks were stored at room temperature and subsequently used for immunohistochemical staining.

Fresh whole blood samples were collected from CRC patients before (within 7 d) and after (within 24 h) surgery, adenoma patients before surgery (within 7 d) and healthy controls and centrifuged at 2-8 °C and 3000 r/min for 15 min at room temperature. The supernatant serum was collected into 2 mL freezing tubes, transferred to a -80 °C refrigerator in liquid nitrogen for freezing and storage, and subsequently used for enzyme-linked immunosorbent assay (ELISA), and the samples avoided repeated thawing before the examination.

Data collection

In the electronic medical record system, applications were made to view the medical records of the enrolled patients and collect clinical information (age, sex), tumour markers [carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9)], histopathological features [tumour location, size, pathological type, degree of differentiation, vascular involvement, nerve involvement, lymph node metastasis, and tumor-node-metastasis (TNM) stage] and molecular pathology (KRAS, NRAS, PIK3CA, BRAF, MS). The normal reference ranges were CEA < 5 ng/mL and CA19-9 ≤ 30 U/mL. TNM staging was based on the American Joint Committee on Cancer 8th edition TNM staging system.

Immunohistochemistry

Paraffin blocks were cut into 4-μm-thick sections, and the sections were sequentially placed in dewaxing solution I for 15 min, dewaxing solution II for 15 min, dewaxing solution III for 15 min, anhydrous ethanol I for 5 min, anhydrous ethanol II for 5 min, 85% alcohol for 5 min, and 75% alcohol for 5 min and then washed with distilled water. The slides were placed in a repair box filled with EDTA antigen repair buffer (pH 9.0) in a microwave oven for antigen repair, and after natural cooling, the slides were placed in phosphate buffered saline (PBS) (pH = 7.4) on a decolorization shaker and washed 3 times for 5 min each. Sections were placed in 3% hydrogen peroxide solution, incubated for 25 min at room temperature and protected from light, and the slides were placed in PBS (pH = 7.4) on a decolorizing shaker and washed three times for 5 min each time to block endogenous peroxidase. The tissue slides were covered uniformly with 3% BSA in a dropwise manner and then blocked at room temperature for 30 min; the slide was gently shaked to remove the blocking solution, and a primary antibody (anti-LILRB2, LSBio, Cat No. LS-B9762-50, 1:150; anti-ANGPTL2, Proteintech, Cat No. 12316-1-AP, 1:200) in a certain ratio of PBS was added dropwise onto the section. Sections were incubated flat in a wet box overnight at 4 °C. After washing and shaking the slides dry, HRP-labelled goat anti-rabbit secondary antibody (Servicebio, GB23303, 1:200) was added dropwise in the circle to cover the tissue and incubated at room temperature for 50 min. After washing and shaking the slides dry, freshly prepared DAB colour development solution was added dropwise, the colour development time was controlled under the microscope, and the positive colour was brownish yellow. Haematoxylin was used to restain the nuclei for approximately 3 min, and the sections were washed with tap water. Finally, microscopic examination was performed, and images were acquired for analysis. Image-Pro Plus image analysis software was used to quantify the immunohistochemical images; five fields of view were randomly selected after magnification of each section at 200 × to assess the integrated optical density and area, and the average optical density

value was calculated.

Correlation and survival analysis

The Gene Expression Profiling Interactive Analysis (GEPIA) platform can analyse differentially expressed genes or mRNAs between normal and cancer tissues online for survival and correlation analysis in a variety of tumours. In this study, we used GEPIA V2.0 (http://gepia2.cancer-pku.cn/) for survival analysis and correlation analysis of CRC at the mRNA level as a complement to proteomics and immunohistochemistry (IHC) at the protein level.

ELISA

The assay was performed according to the instructions of the Human LILRB2 ELISA Kit (Abcam, Cat No. ab269551). Prepare blank wells and wells with multiply diluted standards, add 100 μL of properly diluted serum samples to be tested in the reaction wells, seal the plate with sealing film and incubate at 37 °C for 1-2 h. Discard the liquid, add 300 μL of washing solution to each well, soak for 1-2 min, pat dry on absorbent paper and repeat 3-5 times. Then, 100 μL of diluted biotinylated antibody working solution was added to each well, the plate was sealed with sealing film and incubated at 37 °C for 1 h. The liquid was discarded, 300 μL of washing solution was added to each well, and the washing was repeated. Then, 100 μL of diluted enzyme conjugate working solution was added to each well, the plate was sealed with sealing film and incubated for 30 min at 37 °C, and the washing process was repeated. TMB substrate solution (100 μL) was added to each well and incubated for 10-30 min at 37 °C, protected from light, until a clear colour gradient appeared in the wells of the standards diluted in multiples. The reaction was terminated by adding 100 μL of 2 M sulfuric acid to each well, and the colour changed from blue to yellow. The OD value of each well, including wells with enzyme standards and blank wells, was measured at 450 nm. The blank control wells were adjusted to zero. A standard curve was made according to the concentration and OD value of the standards, and then the sample concentration was calculated according to the equation of the standard curve, and the result was multiplied by the dilution factor for the sample.

Statistical analysis

SPSS 25.0 was used for statistical analysis. Quantitative data are expressed as the mean ± SD, and an independent sample *t* test was used for comparisons between two groups. If the quantitative data did not obey a normal distribution or the variance was not uniform, the median (quartiles) was used, the Mann-Whitney *U* test was used for two-group comparisons, the Kruskal-Wallis *H* test was used for multigroup comparisons, and the Bonferroni method was required to correct the significance level for multiple two-group comparisons. Categorical data were expressed as percentages, and two or more groups were compared using the χ^2 test or Fisher's exact test. Quantitative data correlation analysis was performed using Pearson correlation or Spearman correlation analysis (nonnormally distributed). OS curves and progression-free survival (PFS) curves were constructed using the Kaplan-Meier method and analysed using the log rank test. $P < 0.05$ was considered to indicate a significant difference.

RESULTS

LILRB2 and ANGPTL2 protein expression in CRC tissues and paracancerous tissues

From February 2021 to October 2022, a total of 58 CRC patients met the criteria, and 58 pairs of tissues were successfully collected. Figure 1 demonstrates the actual expression of LILRB2 and ANGPTL2 proteins in primary CRC tissues and paracancerous tissues. Figure 1A shows that LILRB2 protein expression significantly higher in CRC tissues than in paracancerous tissues ($P < 0.001$), and ANGPTL2 protein was significantly overexpressed in CRC tissues compared with paracancerous tissues ($P < 0.001$) (Figure 1B).

Association between LILRB2 and ANGPTL2 protein expression and clinicopathological features of CRC

The associations between LILRB2 and ANGPTL2 protein expression and clinicopathological characteristics of CRC are shown in Table 1. The results showed that the overexpression of LILRB2 protein and ANGPTL2 protein was significantly correlated with poor to moderate tumour differentiation, vascular involvement, lymph node metastasis and advanced TNM stage. In addition, high LILRB2 protein expression was significantly correlated with nerve involvement ($P = 0.019$), confirming that LILRB2 and ANGPTL2 protein are closely associated with the progression of CRC.

Correlation of LILRB2 and ANGPTL2 expression at the mRNA and protein levels in CRC

Figure 2 demonstrates the correlation between *LILRB2* and *ANGPTL2* expression at the mRNA and protein levels in primary CRC tissues. The correlation between *LILRB2* and *ANGPTL2* expression at the mRNA level was first analysed using the GEPIA database, and the results showed that *LILRB2* mRNA expression was significantly and positively correlated with *ANGPTL2* mRNA expression in CRC tissues ($r = 0.63$, $P = 0$) (Figure 2A); similarly, LILRB2 protein expression was significantly and positively correlated with ANGPTL2 protein expression ($r = 0.63$, $P < 0.0001$) (Figure 2B).

Observation of IHC images showed that LILRB2 protein was expressed in the cell membrane, cytoplasm or both in CRC cells, and inflammatory cells also showed brown staining/positive expression, while ANGPTL2 protein was mainly expressed in the cytoplasm of CRC cells. LILRB2 protein was highly expressed in 67.2% of CRC tissues, and ANGPTL2 protein was highly expressed in 48.3% of CRC tissues. However, in the paracancerous tissues, LILRB2 protein and ANGPTL2 protein expression was significantly lower or not detected (Figures 2C-H).

Table 1 Associations between leukocyte immunoglobulin-like receptor B2 and angiopoietin-like protein 2 protein expression and clinicopathological features of colorectal cancer

	LILRB2		*P* value	ANGPTL2		*P* value
	Low (*n* = 19)	High (*n* = 39)		Low (*n* = 30)	High (*n* = 28)	
Clinical features						
Age (yr)			0.670			0.986
≤ 50	4 (21.1%)	5 (12.8%)		14 (46.7%)	13 (46.4%)	
> 50	15 (78.9%)	34 (87.2%)		16 (53.3%)	15 (53.6%)	
Sex			1.000			0.473
Male	10 (52.6%)	21 (53.8%)		24 (80.0%)	25 (89.3%)	
Female	9 (47.4%)	18 (46.2%)		6 (20.0%)	3 (10.7%)	
Histopathological features						
Location			0.737			0.065
Right-side colon	2 (10.5%)	7 (17.9%)		2 (6.60%)	7 (25.0%)	
Left-side colon	6 (31.6%)	9 (23.1%)		11 (36.7%)	4 (14.3%)	
Rectum	11 (57.9%)	23 (59.0%)		17 (56.7%)	17 (60.7%)	
Size (cm)			0.224			0.436
> 4.9	7 (36.8%)	21 (53.8%)		13 (43.3%)	15 (53.6%)	
≤ 4.9	12 (63.2%)	18 (46.2%)		17 (56.7%)	13 (46.4%)	
Pathological type			0.287			0.589
Ulcer	12 (63.2%)	29 (74.4%)		23 (76.7%)	18 (64.3%)	
Bulge	6 (31.6%)	5 (12.8%)		5 (16.7%)	6 (21.4%)	
Mix	1 (5.20%)	5 (12.8%)		2 (6.60%)	4 (14.3%)	
Differentiation			0.009			0.013
Poor	0 (0.00%)	10 (25.6%)		1 (3.40%)	9 (32.1%)	
Moderate	15 (78.9%)	27 (69.2%)		25 (83.3%)	17 (60.7%)	
High	4 (21.1%)	2 (5.20%)		4 (13.3%)	2 (7.20%)	
Vascular involvement			0.001			0.016
Yes	1 (5.30%)	19 (48.7%)		6 (20.0%)	14 (50.0%)	
No	18 (94.7%)	20 (51.3%)		24 (80.0%)	14 (50.0%)	
Nerve involvement			0.019			0.754
Yes	7 (36.8%)	27 (69.2%)		17 (56.7%)	17 (60.7%)	
No	12 (63.2%)	12 (30.8%)		13 (43.3%)	11 (39.3%)	
Lymph node metastasis			< 0.001			0.008
Yes	2 (10.5%)	29 (74.4%)		11 (36.7%)	20 (71.4%)	
No	17 (89.5%)	10 (25.6%)		19 (63.3%)	8 (28.6%)	
Molecular pathology						
KRAS			0.186			0.249
+	6 (31.6%)	19 (48.7%)		11 (36.7%)	14 (50.0%)	
-	13 (68.4%)	20 (51.3%)		19 (63.3%)	14 (50.0%)	
NRAS			0.594			0.613
+	2 (10.5%)	2 (5.10%)		3 (10.0%)	1 (3.60%)	
-	17 (89.5%)	37 (94.9%)		27 (90.0%)	27 (96.4%)	

PIK3CA			1.000			1.000
+	0 (0.0%)	1 (2.60%)		1 (3.30%)	0 (0.0%)	
-	19 (100%)	38 (97.4%)		29 (96.7%)	28 (100%)	
BRAF			0.548			1.000
+	0 (0.0%)	2 (5.10%)		1 (3.30%)	1 (3.60%)	
-	19 (100%)	37 (94.9%)		29 (96.7%)	27 (96.4%)	
MS			1.000			1.000
MSS	18 (94.7%)	37 (94.9%)		28 (93.3%)	27 (96.4%)	
MSI-H	1 (5.30%)	2 (5.10%)		2 (6.70%)	1 (3.60%)	
Clinical stage						
pT			0.001			0.006
T1	0 (0.0%)	0 (0.0%)		0 (0.0%)	0 (0.0%)	
T2	7 (36.8%)	1 (2.60%)		7 (23.3%)	1 (3.60%)	
T3	11 (57.9%)	27 (69.2%)		21 (70.0%)	17 (60.7%)	
T4	1 (5.30%)	11 (28.2%)		2 (6.70%)	10 (35.7%)	
pN			< 0.001			0.001
N0	19 (100%)	11 (28.2%)		22 (73.3%)	8 (28.6%)	
N1	0 (0.0%)	15 (38.5%)		6 (20.0%)	9 (32.1%)	
N2	0 (0.0%)	13 (33.3%)		2 (6.70%)	11 (39.3%)	
pM			0.044			0.002
M0	19 (100%)	31 (79.5%)		30 (100%)	20 (71.4%)	
M1	0 (0.0%)	8 (20.5%)		0 (0.0%)	8 (28.6%)	
pTNM stage			< 0.001			< 0.001
I-II	18 (94.7%)	10 (25.6%)		22 (73.3%)	6 (21.4%)	
III-IV	1 (5.30%)	29 (74.4%)		8 (26.7%)	22 (78.6%)	

LILRB2: Leukocyte immunoglobulin-like receptor B2; ANGPTL2: Angiopoietin-like protein 2; CRC: Colorectal cancer; MS: Microsatellite state; MSS: Microsatellite stability; MSI-H: Microsatellite instability-high.

Associations between LILRB2 and ANGPTL2 expression and the prognosis of CRC

ANGPTL2 mRNA was significantly negatively correlated with the OS (P = 0.0042) and PFS (P = 0.0013) of CRC patients, while no association was found at the protein level (Figures 3A and B). In contrast, *LILRB2* mRNA expression was not associated with the OS or PFS of CRC patients (P > 0.05) (Figure 3C), while LILRB2 protein overexpression was significantly associated with reduced OS (P = 0.045) (Figure 3D), suggesting a poor prognosis in CRC patients and indicating the procancer role of LILRB2 protein in CRC progression.

Screening and diagnostic efficacy of serum LILRB2 concentration

From February 2021 to October 2022, 313 serum samples were collected, including 117 preoperative and 85 postoperative serum samples from CRC patients, 93 serum samples from adenoma patients and 18 serum samples from healthy controls.

Comparison of the differences in serum LILRB2 concentrations among CRC patients, adenoma patients and healthy controls showed that serum LILRB2 concentrations were not identical in the three groups, and the differences were statistically significant (H = 75.25, P < 0.001). The Bonferroni method was used to correct for significance levels, and a two-by-two comparison showed that serum LILRB2 concentrations in patients with CRC (7074.69 pg/mL *vs* 3931.33 pg/mL, adjusted P < 0.001) and in adenoma patients (4855.88 pg/mL *vs* 3931.33 pg/mL, adjusted P = 0.036) were significantly higher than those in healthy controls (Figure 4A). The serum LILRB2 concentration in CRC was again significantly higher than that in the adenoma group (7074.69 pg/mL *vs* 4855.88 pg/mL, adjusted P < 0.001). When compared before and after CRC radical surgery, postoperative serum LILRB2 concentrations were significantly lower than preoperative concentrations (5665.30 pg/mL *vs* 7074.69 pg/mL, adjusted P < 0.001) (Figure 4B), and this result was consistent with the analysis of 50 paired serum samples (5431.44 pg/mL *vs* 6579.17 pg/mL, P = 0.002).

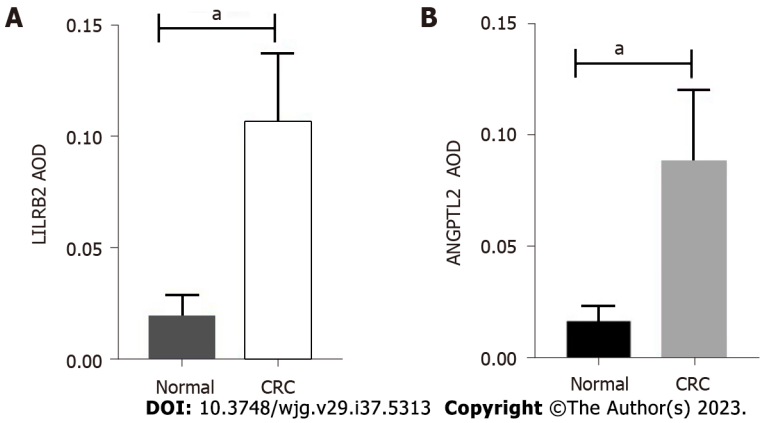

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Figure 1 Leukocyte immunoglobulin-like receptor B2 and angiopoietin-like protein 2 protein expression in colorectal cancer tissues and paracancerous tissues. A: Comparison of leukocyte immunoglobulin-like receptor B2 protein expression in colorectal cancer (CRC) and paired normal tissues; B: Comparison of angiopoietin-like protein 2 protein expression in CRC and paired normal tissues. Quantification of protein expression using immunohistochemistry and Image-Pro Plus software. LILRB2: Leukocyte immunoglobulin-like receptor B2; ANGPTL2: Angiopoietin-like protein 2; CRC: Colorectal cancer; AOD: Average option density. $^{a}P < 0.001$.

Further stratified analysis of CRC patients showed significant differences in serum LILRB2 concentrations between tumours with different infiltration depths ($H = 42.13$, $P < 0.001$). Compared with those in healthy controls, serum LILRB2 concentrations at pT2 (adjusted $P = 0.004$), pT3 (adjusted $P < 0.001$) and pT4 (adjusted $P < 0.001$) were significantly higher (Figure 4C). LILRB2 concentrations were significantly higher in pT1-T2 stage (adjusted $P = 0.002$) and pT3-T4 stage (adjusted $P < 0.001$) patients than in healthy controls (Figure 4D). There was also a significant difference between samples with different degrees of differentiation ($H = 38.525$, $P < 0.001$), with significantly higher serum LILRB2 concentrations in both the poor differentiation (adjusted $P < 0.001$) and moderate differentiation groups (adjusted $P < 0.001$) than in the healthy controls (Figure 4E).

The recommended cut-off value of serum LILRB2 concentration for screening CRC was 5256 pg/mL, with a sensitivity of 89.74% (105/117), a specificity of 88.89% (16/18), an accuracy rate of 89.63% (121/135), an area under the curve (AUC) of 0.95 (0.89, 1.00) and $P < 0.0001$ (Figure 4F). The diagnostic efficacy of LILRB2 was superior to that of the traditional serum markers CEA and CA19-9 (Figure 4G).

DISCUSSION

The high morbidity and mortality of CRC has created a more urgent clinical need for novel therapeutic targets and screening biomarkers. Preliminary high-throughput proteomics research identified LILRB2 as a signature protein for CRC. In this study, IHC was used to confirm that LILRB2 protein was significantly highly expressed in CRC and that increased LILRB2 protein expression was significantly associated with clinicopathological features such as poor to moderate degree of differentiation, lymph node metastasis, advanced TNM stage and poor prognosis. ANGPTL2, as the ligand for LILRB2, was significantly highly expressed in CRC tissues and synergistically overexpressed with LILRB2 at both the mRNA and protein levels, suggesting that their overexpression and interaction are associated with CRC progression. In addition, serum LILRB2 concentrations increased in healthy controls, adenoma patients and CRC patients sequentially with statistically significant differences; the sensitivity for the diagnosis of CRC was 89.74%, the specificity was 88.89%, and the AUC value was 0.95, and these results were superior to those of traditional CEA and CA19-9 in terms of diagnostic efficacy. Therefore, we believe that LILRB2 protein can be used as a potential therapeutic target and noninvasive screening biomarker for CRC; the biomarker could facilitate early screening and precise treatment and improve the prognosis of CRC patients.

At present, there are relatively few basic experimental or clinical studies on the relevance of LILRB2 to CRC. One study explored the expression of LILRB2 in human normal colorectal mucosal cells and various CRC cell lines, and the results showed that LILRB2 was significantly highly expressed in CRC cell lines, and the malignant biological behaviour of cancer cells was inhibited after blocking LILRB2, suggesting that LILRB2 is involved in and regulates the proliferation, migration and invasion of CRC cells and promotes cancer development[14,15]. The results of this cellular assay complement and validate the results of our clinical study. In addition to the basal cell assay, another study analysed clinical samples from CRC patients, but unlike our study using IHC, the researchers prepared cell suspensions from frozen CRC tissues and analysed the immune checkpoint protein LILRB2 by flow cytometry; the results showed that LILRB2 expression was associated with distant lymph node metastasis, advanced stage, and shorter survival and that LILRB2 overexpression may be an independent prognostic risk factor for CRC patients[15]. This study used flow cytometry analysis, and the study technique is innovative, but because flow cytometry requires fresher of tissue samples, prolonged cryopreservation may affect the accuracy of LILRB2 expression detection by flow cytometry. Our used traditional immunohistochemical staining, and the findings with the two study methods corroborated each other. Ultimately, the findings further supported that LILRB2 promotes colorectal carcinogenesis and development.

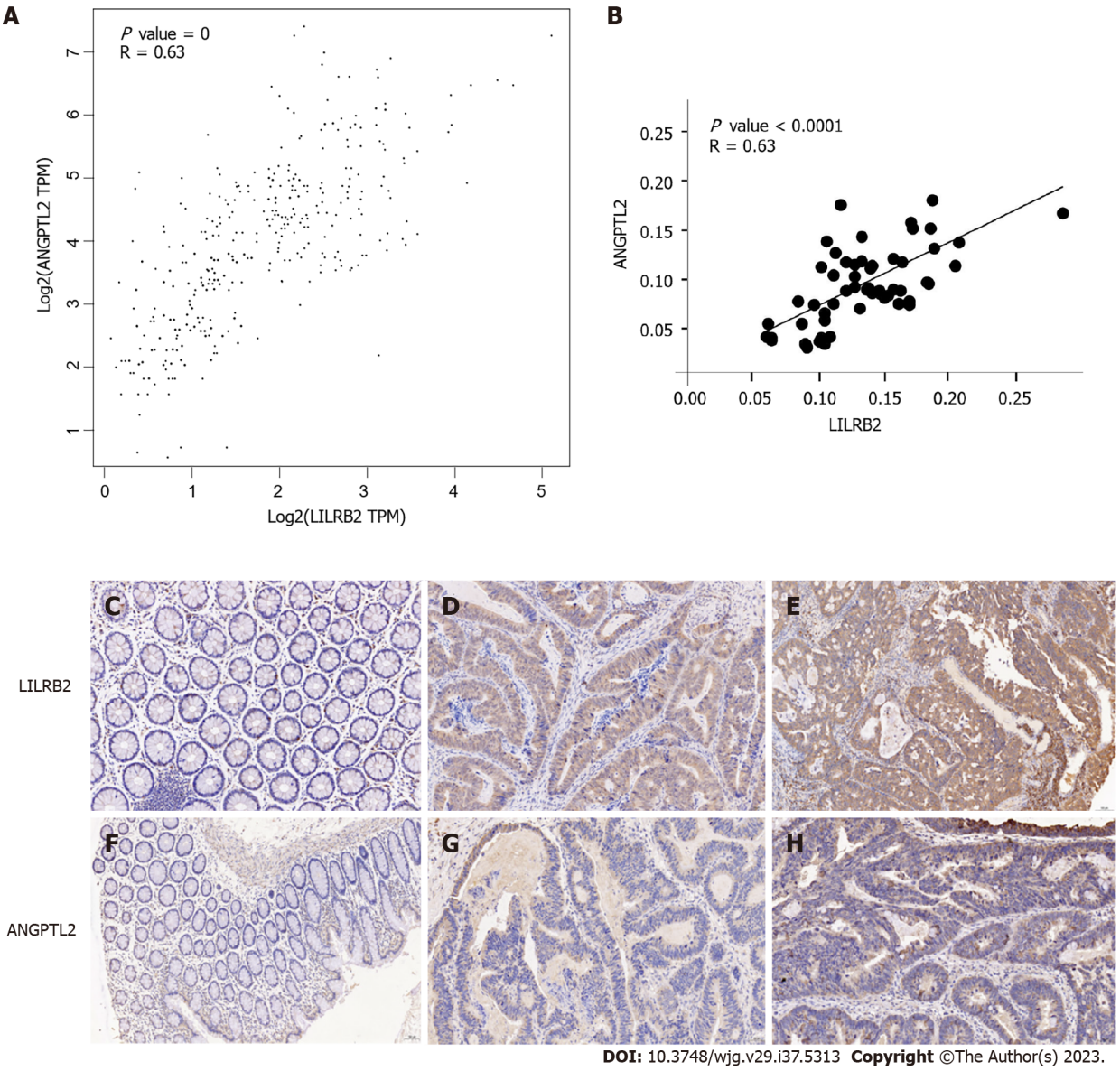

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Figure 2 Correlation of leukocyte immunoglobulin-like receptor B2 and angiopoietin-like protein 2 expression at the mRNA and protein levels in colorectal cancer. A: Correlation at the mRNA level in colorectal cancer (CRC); B: Correlation at the protein level in CRC; C: Immunohistochemical staining of leukocyte immunoglobulin-like receptor B2 (LILRB2) in paracancerous tissue; D: Low expression of LILRB2 in CRC tissue; E: High expression of LILRB2 in CRC tissue; F: Immunohistochemical staining of angiopoietin-like protein 2 (ANGPTL2) in paracancerous tissue; G: Low expression of ANGPTL2 in CRC; H: Overexpression of ANGPTL2 in CRC. LILRB2: Leukocyte immunoglobulin-like receptor B2; ANGPTL2: Angiopoietin-like protein 2; CRC: Colorectal cancer.

Since the discovery of the LILRB2 receptor, ANGPTLs have lost their designation as "orphan ligands"; the LILRB2 receptor interacts with the highest affinity with ANGPTL2 to support the development of a variety of malignancies, such as CRC, acute myeloid leukaemia (AML)[12] and NSCLC[13]. The present study confirmed the synergistic expression of LILRB2 and ANGPTL2 in CRC: There was a significant positive correlation between their expression at both the mRNA and protein levels and a significant association with clinicopathological parameters, suggesting that LILRB2 receptor and ANGPTL2 ligand interactions are closely associated with the development of CRC. A study analysing more than 9000 human leukaemia samples revealed that *LILRB2* mRNA levels could be elevated several-fold in human AML cells. In a mouse leukaemia model, defects in the mouse immunoglobulin-like receptor (PirB), a homologue of human LILRB2, led to increased differentiation of leukaemic stem cells and significant downregulation of the expression of many pro-oncogenes, suggesting that PirB promotes leukaemia development[10]. Another study further indicated that the LILRB2 receptor accelerated leukaemia development by binding to overexpressed ANGPTL2, and the binding of both induced activation of tyrosine phosphatases SHP-1/SHP-2 and calmodulin-dependent protein kinase downstream of iTIMs, stimulating leukaemia development, and suggested that targeting ANGPTL2 ligands could serve as a certain type of AML as a potential disruption strategy[12,16]. LILRB2 is significantly highly expressed in NSCLC tissues and correlates with a poor prognosis[17]. There have been corresponding advances in the study of the mechanisms by which LILRB2 promotes NSCLC development and immunotherapy tolerance, which are expected to bring new therapeutic opportunities to NSCLC patients. On the one hand, tumour cell-derived ANGPTL2 overexpression, by acting on LILRB2 receptors, promotes increased tumour angiogenesis, epithelial mesenchymal transition, reduced intercellular adhesion,

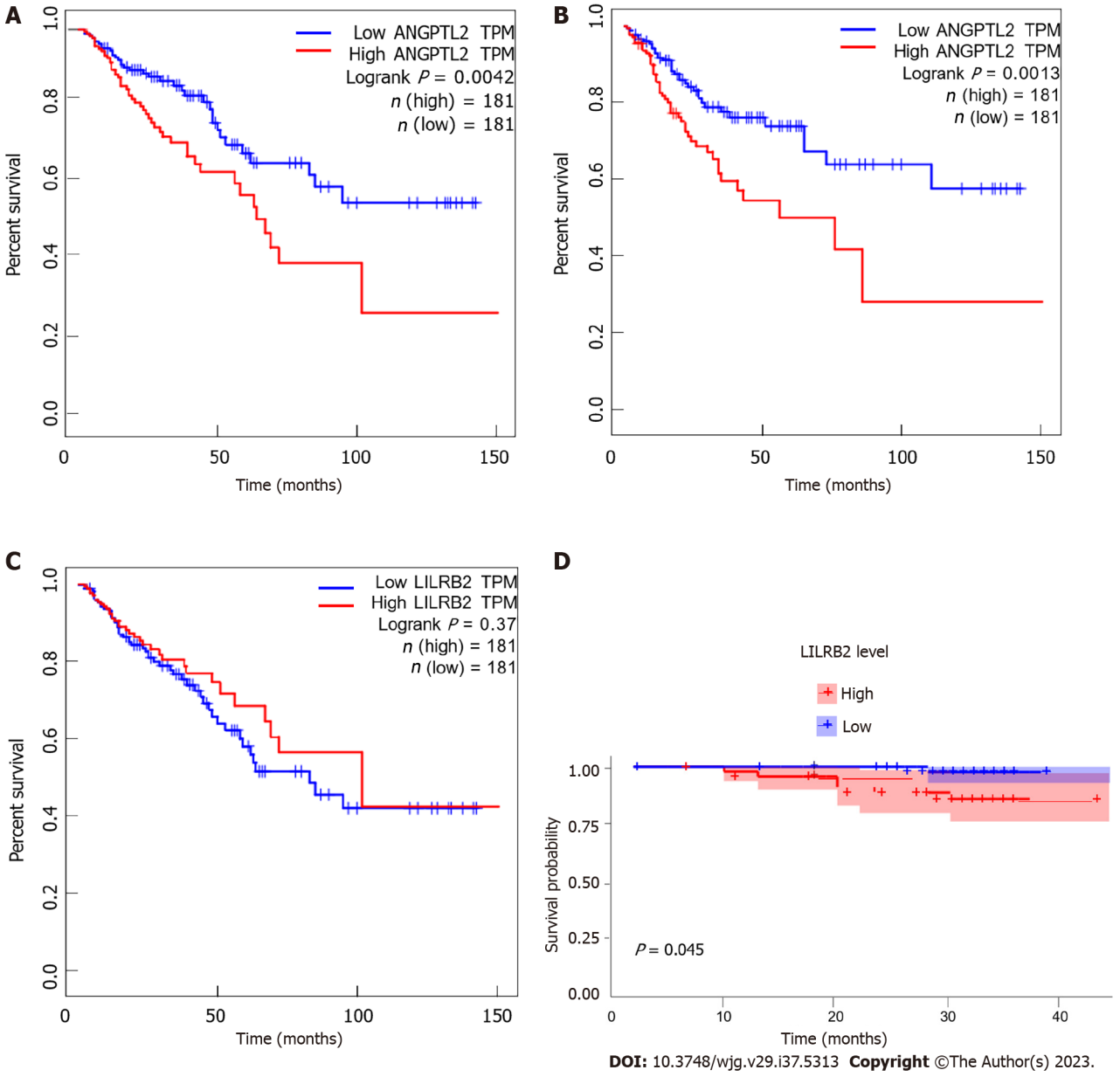

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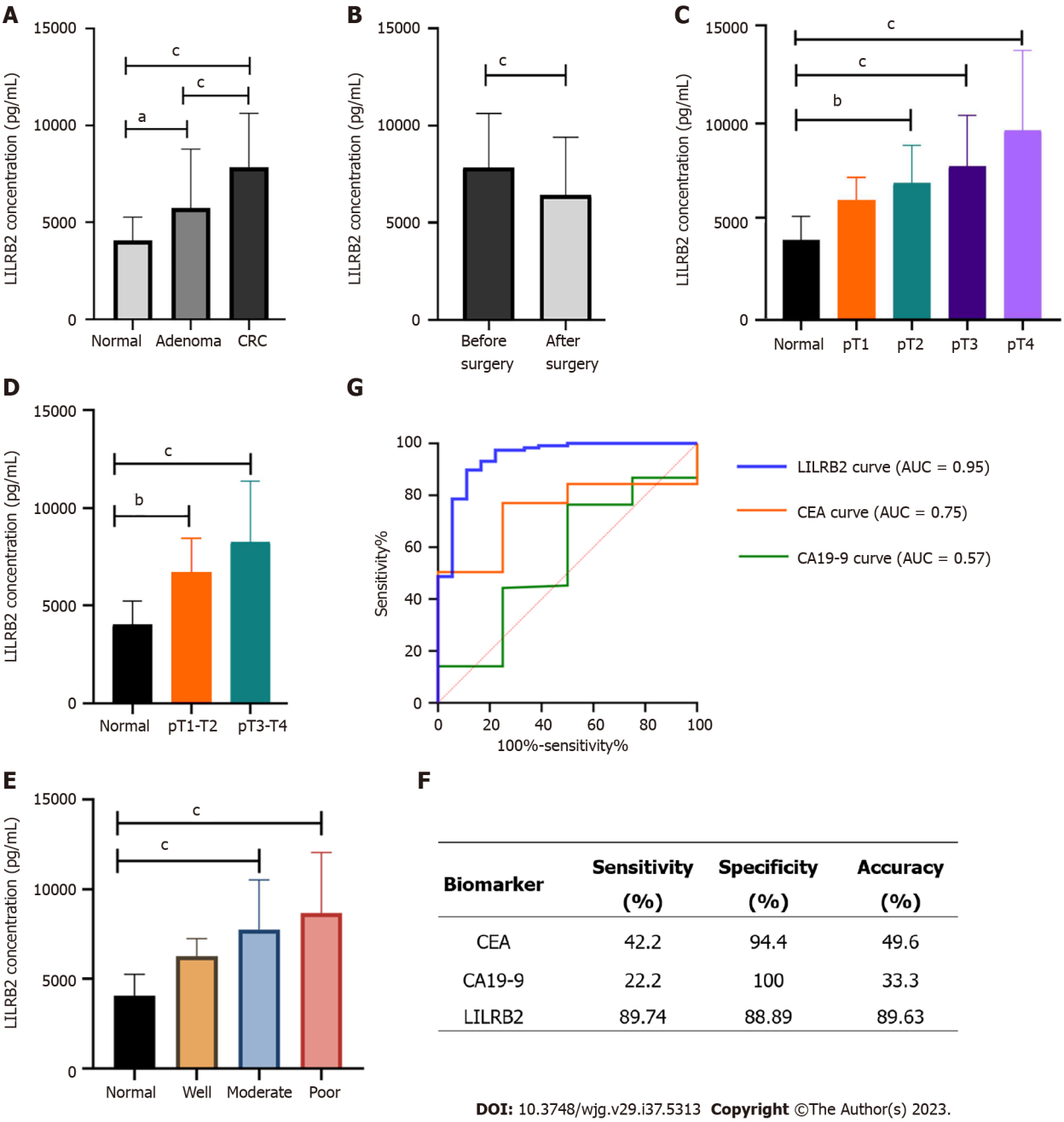
Figure 3 Association between leukocyte immunoglobulin-like receptor B2 and angiopoietin-like protein 2 expression and the prognosis of colorectal cancer. A: Angiopoietin-like protein 2 (*ANGPTL2*) mRNA expression and overall survival of colorectal cancer (CRC); B: *ANGPTL2* mRNA expression and progression-free survival of CRC; C: Leukocyte immunoglobulin-like receptor B2 (*LILRB2*) mRNA expression and overall survival of CRC; D: LILRB2 protein expression and overall survival of CRC. LILRB2: Leukocyte immunoglobulin-like receptor B2; ANGPTL2: Angiopoietin-like protein 2; CRC: Colorectal cancer.

and enhanced cell motility, thus conferring high invasive and metastatic potential to tumour cells[18]. On the other hand, in NSCLC cells, activation of epidermal growth factor receptor-protein kinase B and extracellular signal-related kinases 1 and 2 signalling induces LILRB2 production, and LILRB2 overexpression reprograms the TME, recruits tumour-associated macrophages of the M2-like phenotype, inhibits dendritic cell maturation and antigen presentation, and promotes the progression of NSCLC[13]. In addition, LILRB2 expression was also significantly increased in hepatocellular carcinoma and breast cancer, but the underlying mechanisms by which LILRB2 regulates the progression of hepatocellular carcinoma and breast cancer need to be further investigated[19,20].

In the last three years, several LILRB-targeted therapies have entered clinical trials, but most are in phase I and phase II clinical trials. JTX-8064 is a specific antibody targeting LILRB2 that exerts antitumour effects by blocking the binding of the receptor to MHC-I class molecules, ANGPTLs and other relevant ligands in the TME, and its combination therapy with the PD-1 inhibitor pablizumab has also been conducted in clinical trials[21]. IO-108 is a novel inhibitory antibody that specifically binds LILRB2 with high affinity and blocks the binding of the receptor to ligands in the TME while reprogramming immunosuppressed myeloid cells to exhibit a proinflammatory phenotype, thereby enhancing the antitumour effects of both intrinsic and adaptive immunity[22]. In addition to LILRB2, the targeting of other LILRB family members has been a hot topic of research in recent years. For example, BND-22, a new class of humanized immunoglobulin G4 monoclonal antibodies targeting the LILRB1 receptor, can be targeted for the treatment of solid tumours; this blocks immunosuppression caused by the binding of the LILRB1 receptor and HLA-G ligand, which in turn



Biomarker	Sensitivity (%)	Specificity (%)	Accuracy (%)
CEA	42.2	94.4	49.6
CA19-9	22.2	100	33.3
LILRB2	89.74	88.89	89.63

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Figure 4 Screening and diagnostic efficacy of serum leukocyte immunoglobulin-like receptor B2 concentration. A: Comparison of serum leukocyte immunoglobulin-like receptor B2 (LILRB2) concentrations among colorectal cancer (CRC) patients, adenoma patients and healthy controls; B: Comparison of serum LILRB2 concentrations before and after radical surgery; C and D: Comparison of serum LILRB2 concentrations between samples with different infiltration depths; E: Comparison of serum LILRB2 concentrations between samples with different degrees of differentiation; F: Sensitivity, specificity and accuracy of serum LILRB2, carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) for the diagnosis of CRC; G: The receiver operating characteristic curve and area under the curve of serum LILRB2, CEA and CA19-9 concentrations in the diagnosis of CRC. LILRB2: Leukocyte immunoglobulin-like receptor B2; CRC: Colorectal cancer; CEA: Carcinoembryonic antigen; CA19-9: Carbohydrate antigen 19-9; ROC: Receiver operating characteristic curve; AUC: Area under the curve. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

activates T-cell-mediated innate and adaptive immunity and acts as a tumour killer[23,24]. IO-202 is a monoclonal antibody targeting the inhibition of LILRB4 for the indications of AML and chronic granulocytic leukaemia and has been granted orphan drug status by the United States Food and Drug Administration for the treatment of AML[25]. In terms of the latest progress of several clinical trials, the indications are broad, and no drugs targeting LILRBs have had breakthrough therapeutic effects in tumours; the present study provides a theoretical basis for assessing LILRB2 expression in CRC to guide immune checkpoint blockade therapy to achieve early screening and precision medicine.

Based on this study, we proposed for the first time that serum LILRB2 concentration could be used as a noninvasive screening biomarker for CRC with better diagnostic efficacy than traditional serum tumour markers to facilitate early diagnosis and treatment of CRC[26,27]. We analysed the source of serum LILRB2, since LILRB2 molecules are expressed on the surface of myeloid cells and tumour cells, and we hypothesized that serum LILRB2 may originate from circulating exfoliated cells of CRC, inflammatory cells or immune cells associated with CRC. Postoperative serum LILRB2 concen-

trations were significantly lower than preoperative serum concentrations in CRC, indicating a strong association between serum LILRB2 source and CRC; to further verify the relationship, a comparison of paired serum from 50 patients before and after surgery was performed and revealed that serum LILRB2 concentrations were significantly lower after surgery than before surgery, confirming that serum LILRB2 level is associated with tumour burden in CRC. It was hypothesized that serum LILRB2 might originate from circulating tumour cells shed in the blood and that the decreased LILRB2 concentration is associated with a reduced CRC cell load in the blood after surgery. Another source is hypothesized to be CRC-associated immune cells because tumorigenesis is closely related to the chronic inflammatory state in the body and during tumour development, chemotaxis and infiltration of myeloid cells, including neutrophils, dendritic cells, and tumour-associated macrophages, which are common cell types with LILRB2 expression[28,29]. There are no relevant studies on the source of serum LILRB2 globally, this study can only make reasonable speculations on its source. However, considering the half-life of protein, the time is not long enough to detect the serum LILRB2 concentration only 24 h after operation, and this is also not long enough for the immune cells to disappear gradually from circulation. Therefore, follow-up after the surgical removal, including measurements of the serum LILRB2 concentration, circulating tumor cell load and immune cell infiltration, is necessary. It is more meaningful that the serum LILRB2 decreases after 2 wk or longer of follow-up and increases when the tumor progresses or recurs.

There are advantages but also limitations to this study. The advantages are as follows: First, this study is based on the results of preliminary high-throughput proteomics screening and further protein expression validation and correlation analysis, with a solid prior experimental foundation, rather than through mere database mining. Second, this study confirms that LILRB2 can be a novel therapeutic target for CRC with high potential for clinical translation, providing new therapeutic opportunities for CRC. Third, this study is the first to suggest that ELISA of serum LILRB2 concentration can be used as a noninvasive screening method for CRC, with better diagnostic efficacy than traditional serum tumour markers. The shortcomings of this study are that further testing and analysis of circulating tumour cell load and immune cell infiltration in the blood of CRC patients are needed in the future to clarify the source of serum LILRB2. Furthermore, LILRB2 levels could be confounded by the presence of other tumors concurrently, such as NSCLC, hepatocellular and breast cancer, which needs to be excluded. In addition, although the results of this study corroborate those of previous basic studies, the single center study type and small sample size are still limitations of this study, and more efforts are needed in the future to eventually achieve the goal of translation to clinical treatment.

CONCLUSION

In conclusion, LILRB2 protein is significantly highly expressed in CRC tissues, and high LILRB2 protein expression is significantly correlated with tumour progression and a poor prognosis. The LILRB2 receptor is synergistically expressed with the ANGPTL2 ligand, and their binding and interaction are closely related to CRC development. Serum LILRB2 concentration showed higher diagnostic efficacy than traditional markers, highlight its potential as an innovative screening marker for CRC. Therefore, LILRB2 protein is a potential novel therapeutic target and noninvasive screening biomarker, which could be beneficial for early screening and precise treatment and is important for the development of new therapeutic strategies for CRC.

ARTICLE HIGHLIGHTS

Research background

The incidence and mortality of colorectal cancer (CRC) have been rising continuously. CRC has become the second leading cause of cancer-related death worldwide, and the 5-year survival rate of patients with advanced CRC is below 20%.

Research motivation

Early screening and precise targeted therapy are very important to improve the prognosis of CRC patients. Therefore, identifying novel therapeutic targets and early screening biomarkers is urgent for clinical practice.

Research objectives

To explore whether leukocyte immunoglobulin-like receptor B2 (LILRB2) can be used as a potential therapeutic target and noninvasive screening biomarker for CRC.

Research methods

On the basis of previous proteomics, immunohistochemical staining was used to verify the expression of LILRB2 protein and its ligand angiopoietin-like protein 2 (ANGPTL2) protein in CRC cancer tissues and paired paracarcinoma tissues, and to explore the association between their expression and the clinicopathological features. Enzyme-linked immunosorbent assay was used to detect serum LILRB2 concentration and explore the diagnostic efficacy for CRC.

Research results

LILRB2 protein is significantly overexpressed in CRC cancer tissues and is closely associated with peritumoral infilt-

ration, distant metastasis and poor prognosis. LILRB2 may bind to the ligand ANGPTL2 protein, and the synergistic expression and interaction of the two proteins promotes CRC progression and metastasis. Serum LILRB2 concentration has high sensitivity, specificity and accuracy in screening CRC, which is better than traditional carcinoembryonic antigen and carbohydrate antigen 19-9.

Research conclusions

Therefore, LILRB2 protein can be used as a potential novel therapeutic target and noninvasive screening biomarker.

Research perspectives

Novel LILRB2 protein is beneficial for early screening and precise treatment, which provides new opportunities to improve the prognosis of CRC patients.

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FOOTNOTES

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META-ANALYSIS

Efficacy and safety of semaglutide in non-alcoholic fatty liver disease

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease. The prevalence and disease burden of NAFLD are projected to exponentially increase resulting in significant healthcare expenditures and lower health-related quality of life. To date, there are no approved pharmacotherapies for NAFLD or non-alcoholic steatohepatitis (NASH). Semaglutide has glycemic and weight loss benefits that may be advantageous for patients with NAFLD.

AIM

To investigate the efficacy and safety of semaglutide in patients with NAFLD.

METHODS

MEDLINE, CENTRAL, and EMBASE were searched from inception to May 1, 2023, to identify eligible randomized controlled trials (RCTs). Meta-analysis was performed using random effects model expressing continuous outcomes as mean differences (MD) or standardized MDs (SMD), and dichotomous outcomes as odds ratios (OR) with 95% confidence intervals (CI). Statistical heterogeneity was assessed using the Cochran's *Q* test and *I2* statistic.

RESULTS

Three RCTs involving 458 patients were included. Semaglutide increased the likelihood of NASH resolution (OR: 3.18, 95%CI: 1.70, 5.95; $P < 0.001$), impro-

vement in steatosis (OR: 2.83, 95%CI: 1.19, 6.71; P = 0.03), lobular inflammation (OR: 1.81, 95%CI: 1.11, 2.96; P = 0.02), and hepatocellular ballooning (OR: 2.92, 95%CI: 1.83, 4.65; P < 0.001), but not fibrosis stage (OR: 0.71, 95%CI: 0.15, 3.41; P = 0.67). Radiologically, semaglutide reduced liver stiffness (SMD: -0.48, 95%CI: -0.86, -0.11; P = 0.01) and steatosis (MD: -4.96%, 95%CI: -9.92, 0.01; P = 0.05). It also reduced alanine aminotransferase (MD: -14.06 U/L, 95%CI: -22.06, -6.07; P < 0.001) and aspartate aminotransferase (MD: -11.44 U/L, 95%CI: -17.23, -5.65; P < 0.001). Semaglutide led to improved cardiometabolic outcomes, including decreased HgA1c (MD: -0.77%, 95%CI: -1.18, -0.37; P < 0.001) and weight loss (MD: -6.53 kg, 95%CI: -11.21, -1.85; P = 0.006), but increased the occurrence of GI-related side effects (OR: 3.72, 95%CI: 1.68, 8.23; P = 0.001). Overall risk of serious adverse events was similar compared to placebo (OR: 1.40, 95%CI: 0.75, 2.62; P < 0.29).

CONCLUSION

Semaglutide is effective in the treatment of NAFLD while maintaining a well-tolerated safety profile. Future studies are required to evaluate its effects on fibrosis regression and different phases of NAFLD.

Key Words: Non-alcoholic fatty liver disease; Fatty liver; Metabolic-associated fatty liver; Semaglutide

Core Tip: Semaglutide demonstrates significant histologic improvements, with a higher likelihood of non-alcoholic steatohepatitis resolution and improved steatosis, lobular inflammation, and hepatocellular ballooning, but it does not significantly improve fibrosis stage compared to placebo. Furthermore, semaglutide results in radiologic improvements in liver stiffness and steatosis, liver enzymes, as well as cardiometabolic effects on body weight and HgA1c, while maintaining a well-tolerated safety profile.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease, with an estimated worldwide prevalence of 32.4%[1,2]. The prevalence and disease burden of NAFLD are projected to exponentially increase, with mathematical models forecasting a 168% increase in the incidence of decompensated cirrhosis and a 178% increase in NAFLD-related deaths between 2015 and 2030. These projections highlight the significant healthcare expenditures and lower health-related quality of life associated with the disease[3-5].

NAFLD is a spectrum of liver disease characterized by hepatic steatosis in the absence of excessive alcohol consumption[6]. The majority of patients with NAFLD have NAFL, of which approximately 20% will develop non-alcoholic steatohepatitis (NASH) and have a risk of further progression to cirrhosis, hepatocellular carcinoma, and end-stage liver disease[3]. Although lean NAFLD is increasingly recognized, the majority of patients with NAFLD have one or more components of metabolic syndrome, which is also independently strongly associated with fibrosis progression[7,8].

GLP-1 receptor agonists (RAs) offer promising therapeutic options in NAFLD due to their beneficial glycemic and weight loss effects. GLP-1 receptors have been detected on human hepatocytes, and it is hypothesized that their activation by GLP-1 RAs can have positive effects on hepatic steatosis, lipotoxicity, fatty acid oxidation, and cytokines involved in hepatic inflammation and fibrosis[9,10]. Moreover, GLP-1 RAs may have indirect hepatoprotective benefits through increased insulin secretion in response to hyperglycemia, decreased glucagon secretion, delayed gastric emptying, and significant weight loss[11,12].

Among the GLP-1 RAs, semaglutide has demonstrated the greatest glycemic and weight loss benefits[13]. In a recent phase three trial of patients with overweight or obesity, semaglutide showed a significant decrease in body weight by 14.9% compared to 2.4% with placebo[14]. Additionally, semaglutide has shown reduced rates of major adverse cardiovascular events and a lower risk of adverse renal outcomes in patients with type 2 diabetes (T2DM)[15]. It has since been approved for the treatment of T2DM and chronic weight management.

Several randomized clinical trials have also demonstrated the beneficial effects of semaglutide in patients with NAFLD. A previous systematic review with meta-analysis was conducted to assess the impact of semaglutide on biochemical and radiologic measures of NAFLD[16]. However, more than 85% of the study's patients had diabetes or obesity rather than confirmed NAFLD. Additionally, no histological outcomes were reported, which are considered the gold standard for diagnosing and managing NAFLD. Since its publication, a recent randomized controlled trial (RCT) by Loomba *et al*[17] has been performed, focusing on semaglutide in patients with NASH and compensated cirrhosis. The purpose of this systematic review and meta-analysis is to provide an updated review on the efficacy and safety of semaglutide, focusing on patients with NAFLD, in order to more specifically reflect the NAFLD population and expand the current

understanding of semaglutide in NAFLD.

MATERIALS AND METHODS

Search strategy and study selection

The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines and was registered prospectively on the PROSPERO database (ID: CRD42023422487). Two independent reviewers (K.Z. and R.K.) evaluated the titles and abstracts of all identified studies based on predetermined inclusion and exclusion criteria (Supplementary Table 1). Any discrepancies were resolved through discussion with a third reviewer (T.H.).

Multiple databases, including MEDLINE, CENTRAL, EMBASE, and grey literature sources such as Clinicaltrials.gov and the World Health Organization International Clinical Trials Registries, were searched from inception to May 1, 2023 using a predefined search strategy (Supplementary Table 2). Additionally, a forward and backward citation search was performed on eligible studies using CitationChaser.

Data extraction and outcome measures

Two reviewers (K.Z. and R.K.) independently extracted the data using predetermined data collection forms. Any discrepancies were resolved through consultation with a third reviewer (T.H.).

The primary outcomes of interest for this study were histological improvement in NAFLD activity score, resolution of NASH with no worsening of liver fibrosis, and improvement in liver fibrosis without worsening of NASH, as defined by the NASH Clinical Research Network Criteria (CRN).

The secondary outcomes of interest included radiologic improvement in liver stiffness and steatosis, measured using either magnetic resonance elastography (MRE) or Fibroscan; changes in liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)]; cardiometabolic parameters such as body weight, HgA1c, total cholesterol, non-HDL cholesterol, and LDL cholesterol; as well as adverse events, including gastrointestinal-related side effects and serious adverse events.

Quality assessment

The risk of bias was independently conducted by two reviewers (K.Z. and R.K.) using the Cochrane risk-of-bias 2 tool for randomized trials. Due to the limited number of included studies, a funnel plot was not generated to assess publication bias. Finally, the quality of the evidence was evaluated using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework.

Missing data

To address missing data, attempts were made to contact the study authors for the necessary information. In cases where the data could not be obtained, relevant values were extracted from figures, using the PlotDigitizer software, following Cochrane methodology. If the data was unavailable anywhere in the study, it was excluded from the analysis. Missing standard deviations (SD) for continuous outcomes were estimated using the available standard error, 95% confidence interval (CI), or P value. If these values were not available, SDs were imputed from other studies, as outlined by Furukawa *et al*[18].

Data synthesis and analysis

Continuous outcomes were presented as mean differences (MD) in change scores with corresponding 95%CI. The DerSimonian and Laird random-effects model was used given expected clinical and methodological diversity between the included studies. When outcomes were measured on different scales that could not be converted to a common scale, such as Fibroscan and MRE measurements, they were reported as standardized MDs (SMD) using Hedges' G. A correlation coefficient of $r = 0.4$, which is consistent with previous meta-analyses of liraglutide in NAFLD, was utilized [19].

Dichotomous outcomes were expressed as odds ratios (OR) with 95%CI. Statistical heterogeneity was assessed using Cochran's Q statistic and I^2 statistic, where $P < 0.10$ and $I^2 > 50\%$ were considered significant indicators of heterogeneity. The significance level for all other statistical tests was set at $P < 0.05$. RevMan version 5.4 (Copenhagen, Denmark) was used for all statistical analyses.

Sensitivity analysis was conducted using the leave-one-out method to evaluate the influence of each individual study on the overall estimate. Additional sensitivity analysis was performed comparing the outcomes using a fixed-effect model *vs* random-effect model. Subgroup analysis was performed by stratifying participants within the included studies based on their T2DM status. However, due to the limited number of included studies, additional sensitivity and subgroup analyses were not performed.

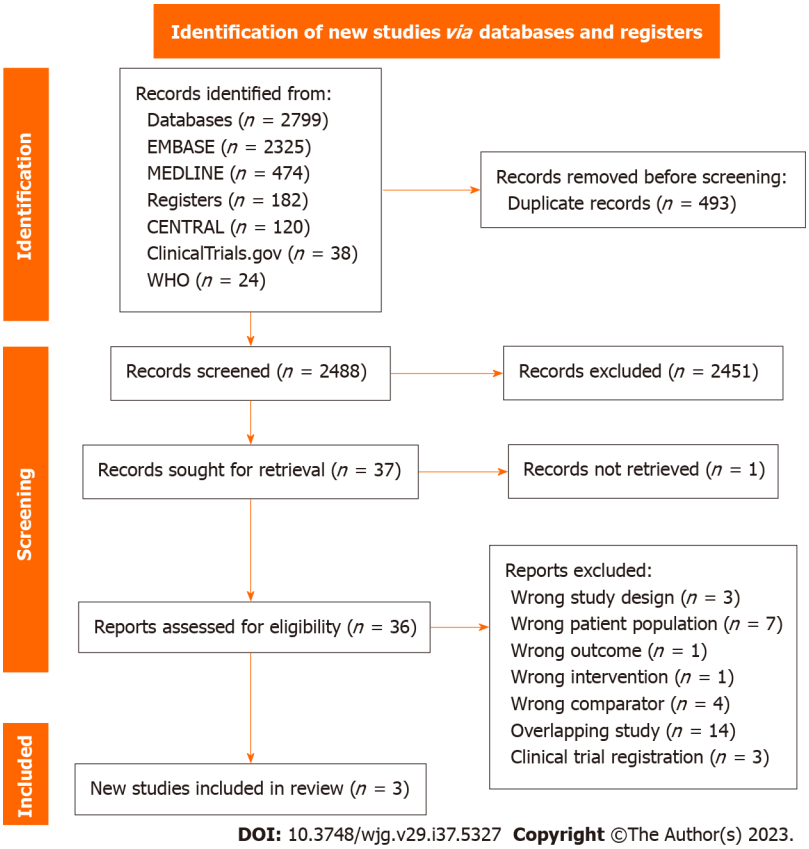

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Figure 1 PRISMA flow diagram of study selection. WHO: World health organization.

RESULTS

Search results

A total of 2981 potentially eligible studies were identified, of which 493 duplicates were removed prior to screening. An additional 2451 studies were excluded based on titles and abstracts screening. Full text was obtained for 36 out of the 37 eligible studies. From these, we identified three studies that met our inclusion criteria and were included in the meta-analysis[17,20,21] (Figure 1).

Study characteristics

The study characteristics of the included summaries are outlined in Table 1. A total of 458 patients were included, with 321 receiving semaglutide and 137 receiving placebo. All studies were RCTs ranging from 48 wk to 72 wk in duration. Various doses of semaglutide were utilized. Newsome *et al*[20] compared daily doses of 0.1 mg, 0.2 mg, and 0.4 mg of semaglutide, whereas Flint *et al*[21] and Loomba *et al*[17] used daily doses of 0.4 mg and weekly doses of 2.4 mg, respectively. Furthermore, Flint *et al*[21] focused exclusively on patients with NAFL, while the other two studies only included patients with biopsy confirmed NASH. Both Newsome *et al*[20] and Loomba *et al*[17] conducted histological assessments. All studies included patients with and without T2DM.

Risk of bias assessment

The methodological qualities of the studies are summarized in the appendix, Supplementary Figure 1. The trial of Newsome *et al*[20] was classified as having a low risk of bias, while the other trials raised some concerns. Specifically, the study of Loomba *et al*[17] had concerns related to randomization, as a higher proportion of patients in the semaglutide group had an Ishak fibrosis score of 6, while more patients receiving placebo had a score of 4 or 5. Additionally, baseline measurements of MRE, hepatic collagen proportion, liver enzymes, and pro-C3 were slightly higher in the semaglutide group. Flint *et al*[21] raised concerns regarding missing data, as seven out of 34 patients in the semaglutide group discontinued treatment, and no sensitivity analysis was performed, or analysis conducted to address the resulting bias. The quality of the evidence was evaluated using the GRADE framework as illustrated in Table 2.

Outcome evaluation

Effect of semaglutide on histological parameters: Two studies, involving 391 patients, evaluated histological outcomes (Figure 2). Semaglutide was associated with a significantly higher likelihood of NASH resolution with no worsening of liver fibrosis (OR: 3.18, 95%CI: 1.70, 5.95; I^2 = 0%) (Figure 2A). However, there was no significant improvement in liver fibrosis stage without worsening of NASH (OR: 0.71, 95%CI: 0.15, 3.41; I^2 = 80%) (Figure 2B). Significant improvements

Table 1 Baseline characteristics of included studies

Ref.	Location sponsor	Study design	Sample size (*n*)	Demographics (%)	Intervention/comparator(s)	Outcomes assessed
Newsome *et al*[20], 2021	16 countrie, 143 sites; Novo Nordisk	MC, DB, four-arm parallel-group RCT; duration: 72 wk; randomization: 3:3:3:1:1:1	320	Age (SD): 55.0 (10.6); male/female: 125(39)/195(61); T2DM: 199 (62)	Semaglutide: 0.1 mg SQ OD (*n* = 80); 0.2 mg SQ OD (*n* = 78); 0.4 mg SQ OD (*n* = 82); and placebo (*n* = 80)	Primary: Resolution of NASH; secondary: Liver fibrosis stage, total and component of NAS, ALT, AST, liver stiffness, liver steatosis, cardiometabolic parameters, adverse events
Flint *et al* [21], 2021	Germany, 2 sites; Novo Nordisk	Two-centre, DB, two-arm parallel-group RCT; duration: 72 wk; randomization: 1:1	67	Age (SD): 60.0 (9.3); male/female: 47(70)/20(30); T2DM: 49 (73)	Semaglutide: 0.4 mg SQ OD (*n* = 34); placebo (*n* = 33)	Primary: Liver stiffness MRE at week 48; secondary: Liver stiffness at week 24 and 72, liver steatosis, ALT, AST, cardiometabolic parameters, adverse events
Loomba *et al*[17], 2023	5 countries, 38 sites; Novo Nordisk	MC, DB, two-arm-parallel group RCT; duration: 48 wk; randomization: 2:1	71	Age (SD): 59.5 (8.0); male/female: 22(31)/49(61); T2DM: 53 (75)	Semaglutide: 2.4 mg SQ qw (*n* = 47); Placebo (*n* = 24)	Primary: Liver fibrosis stage; secondary: Liver stiffness, liver steatosis, NASH resolution, total and component of NAS, ALT, AST, cardiometabolic parameters, adverse events

MC: Multi-centre; DB: Double-blind; RCT: Randomized controlled trial; SD: Standard deviation; T2DM: Type 2 diabetes mellitus; SQ: Subcutaneous; OD: Once daily; qw: Weekly; NASH: Non-alcoholic steatohepatitis; NAS: NAFLD activity score; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; MRE: Magnetic resonance elastography.

were observed in all NAFLD Activity Score (NAS) components with OR 2.83 (95%CI: 1.19, 6.71; I^2 = 57%) for steatosis, 1.81 (95%CI: 1.11, 2.96; I^2 = 0%) for lobular inflammation, and 2.92 (95%CI: 1.83, 4.65; I^2 = 0%) for hepatocellular ballooning (Figure 2C-E). Significant heterogeneity was noted for improvement in liver fibrosis stage.

Effect of semaglutide on radiologic parameters: All three studies reported radiologic parameters and semaglutide demonstrated a significant reduction in liver stiffness on MRE or Fibroscan, with a standardized MD of -0.48 (95%CI: -0.86, -0.11; I^2 = 57%) (Figure 3A). Additionally, a significant reduction in liver steatosis on MRI proton density fat fraction (MRI-PDFF) was observed, with a MD of -4.96% (95%CI: -9.92, 0.01; I^2 = 64%), although significant heterogeneity was noted (Figure 3B).

Effect of semaglutide on liver enzymes: All three studies evaluated ALT and AST, which showed a significant reduction of 14.06 U/L (95%CI: -22.06, -6.07; I^2 = 0%) and 11.44 U/L (95%CI: -17.23, -5.65; I^2 = 0%), respectively, compared to placebo. No significant heterogeneity was observed (Figure 4).

Effect of semaglutide on cardiometabolic parameters: All three studies evaluated total body weight, which revealed a significant reduction of 6.53 kg (95%CI: -11.21, -1.85; I^2 = 0%) compared to placebo, with no significant heterogeneity observed (Figure 5A).

Overall, semaglutide also significantly decreased HgA1c by 0.77% (95%CI: -1.18, -0.37; I^2 = 75%) compared to placebo (Figure 5B). Subgroup analysis of participants within the studies, stratified by T2DM status, showed a significant reduction in HgA1c among patients with T2DM (MD: -1.10%, 95%CI: -1.48, -0.72; I^2 = 29%), but not among those without (MD: -0.37%, 95%CI: -0.79, 0.06; I^2 = 61%). Heterogeneity was no longer significant with the subgroup analysis.

Regarding lipid panel results, semaglutide was not associated with a significant difference in triglycerides (MD: -24.03 mg/dL, 95%CI: -60.94, 12.88; I^2 = 42%), total cholesterol (MD: -7.31 mg/dL, 95%CI: -51.66, 37.03; I^2 = 87%), non-HDL cholesterol (MD: -7.52 mg/dL, 95%CI: -49.32, 34.27; I^2 = 84%), and LDL cholesterol (MD: -4.72 mg/dL, 95%CI: -56.23, 46.79; I^2 = 92%), although significant heterogeneity was observed (Supplementary Figure 2).

Adverse events with semaglutide: Semaglutide was associated with a significantly higher occurrence of gastrointestinal-related side effects compared to placebo (OR: 3.72, 95%CI: 1.68, 8.23; I^2 = 49%) (Figure 6A). However, the overall risk of serious adverse events was comparable between the two groups (OR: 1.40, 95%CI: 0.75, 2.62; I^2 = 0%) (Figure 6B).

Sensitivity and subgroup analysis

Each article was individually excluded to examine the influence of each study on the overall effect-size estimate (Supplementary Table 3). Most of the outcomes remained unchanged; however, when Newsome *et al*[20] was removed, the effect size for resolution of NASH, steatosis, lobular inflammation, and hepatocellular ballooning became non-significant. The effect estimates for liver stiffness when restricted to MRE and excluding Fibroscan as used in the study by Newsome *et al*[20], also became non-significant. However, there was no longer any heterogeneity observed.

Regarding cardiometabolic outcomes, the significant effect of semaglutide on body weight was no longer sustained when the trial by Flint *et al*[21] was excluded. Furthermore, when Newsome *et al*[20] was removed, there was a significant

Table 2 Grading of recommendations assessment, development, and evaluation summary of findings table

Outcomes	Anticipated absolute effects[1] (95%CI)		Relative effect (95%CI)	Number of participants (studies)	Certainty of the evidence (GRADE)
	Risk with placebo	Risk with semaglutide			
Resolution of NASH with no worsening of liver fibrosis assessed with: Liver biopsy	183 per 1000	416 per 1000 (276 to 571)	OR 3.18 (1.70 to 5.95)	301 (2 RCTs)	+++O: Moderate[2]
Improvement in liver fibrosis stage without worsening of NASH assessed with: Liver biopsy	317 per 1000	248 per 1000 (65 to 613)	OR 0.71 (0.15 to 3.41)	301 (2 RCTs)	++OO: Low[2,3,4]
Liver stiffness assessed with: MRI-PDFF or Fibroscan	-	SMD 0.48 lower (0.86 lower to 0.11 lower)	-	350 (3 RCTs)	++++: High
Liver steatosis assessed with: MRE	The mean liver steatosis ranged from -0.57% to -2.57%	MD 4.96 % lower (9.92 lower to 0.01 higher)	-	138 (2 RCTs)	+++O: Moderate[3]
ALT	The mean ALT ranged from 1.90 U/L to -11.22 U/L	MD 14.06 U/L lower (22.06 lower to 6.07 lower)	-	458 (3 RCTs)	++++: High
AST	The mean AST ranged from 1.50 U/K to -5.76 U/K	MD 11.44 U/K lower (17.23 lower to 5.65 lower)	-	458 (3 RCTs)	++++: High
Serious adverse events	109 per 1000	147 per 1000 (84 to 244)	OR 1.40 (0.75 to 2.62)	456 (3 RCTs)	+++O: Moderate[2,4]

[1]The risk in the intervention group [and its 95% confidence interval (95%CI)] is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95%CI).
[2]Few events.
[3]Possibly substantial heterogeneity.
[4]Wide confidence interval including no effect and does not exclude appreciable benefit or harm.
GRADE Working Group grades of evidence. High certainty: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect. Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect. CI: Confidence interval; MD: Mean difference; OR: Odds ratio; SMD: Standardised mean difference; MRI-PDFF: MRI proton density fat fraction; MRE: Magnetic resonance elastography.

decrease in triglycerides, total cholesterol, and non-HDL cholesterol. Additionally, the exclusion of Newsome *et al*[20] led to a significant reduction in LDL-cholesterol, whereas the exclusion of Loomba *et al*[17] resulted in significant increases. No significant differences were observed in the sensitivity analysis when comparing the fixed-effect model to the random-effect model (Supplementary Table 4).

DISCUSSION

In this systematic review and meta-analysis, semaglutide demonstrated significant histologic improvements, with a higher likelihood of NASH resolution and improved NAS components, but it did not significantly improve fibrosis stage compared to placebo. Furthermore, semaglutide resulted in radiologic improvements in liver stiffness and steatosis, liver enzymes, as well as cardiometabolic effects on body weight and HgA1c, while maintaining a well-tolerated safety profile.

In the systematic review with meta-analysis on the impact of semaglutide on biochemical and radiologic measures of NAFLD conducted by Dutta *et al*[16], the majority of included patients did not have confirmed NAFLD. Out of the four RCTs included, only two involved patients with NAFLD. The other two trials focused on the cardiovascular outcomes of semaglutide in patients with type 2 diabetes and the efficacy of semaglutide in weight loss for patients with obesity. Since these two trials did not separately report outcomes for the NAFLD subgroup, they were not included in our systematic review and meta-analysis. Furthermore, the meta-analysis did not include any histological outcomes, which are considered the gold standard for NAFLD diagnosis and management. In contrast, our paper exclusively focuses on the NAFLD population, reports histological outcomes, and provides an updated review that includes the recent RCT conducted by Loomba *et al*[17].

Previous studies have demonstrated an association between histological resolution of NASH and decrease in NAS components with improvement in fibrosis stage[22,23]. However, despite improvements in other histologic outcomes, our meta-analysis did not observe an improvement in liver fibrosis. Of note, Loomba *et al*[17] had an imbalance in baseline characteristics, with a higher proportion of patients in the semaglutide group exhibiting higher grade fibrosis and non-invasive markers of inflammation compared to the placebo group. This imbalance may have reduced the treatment effect

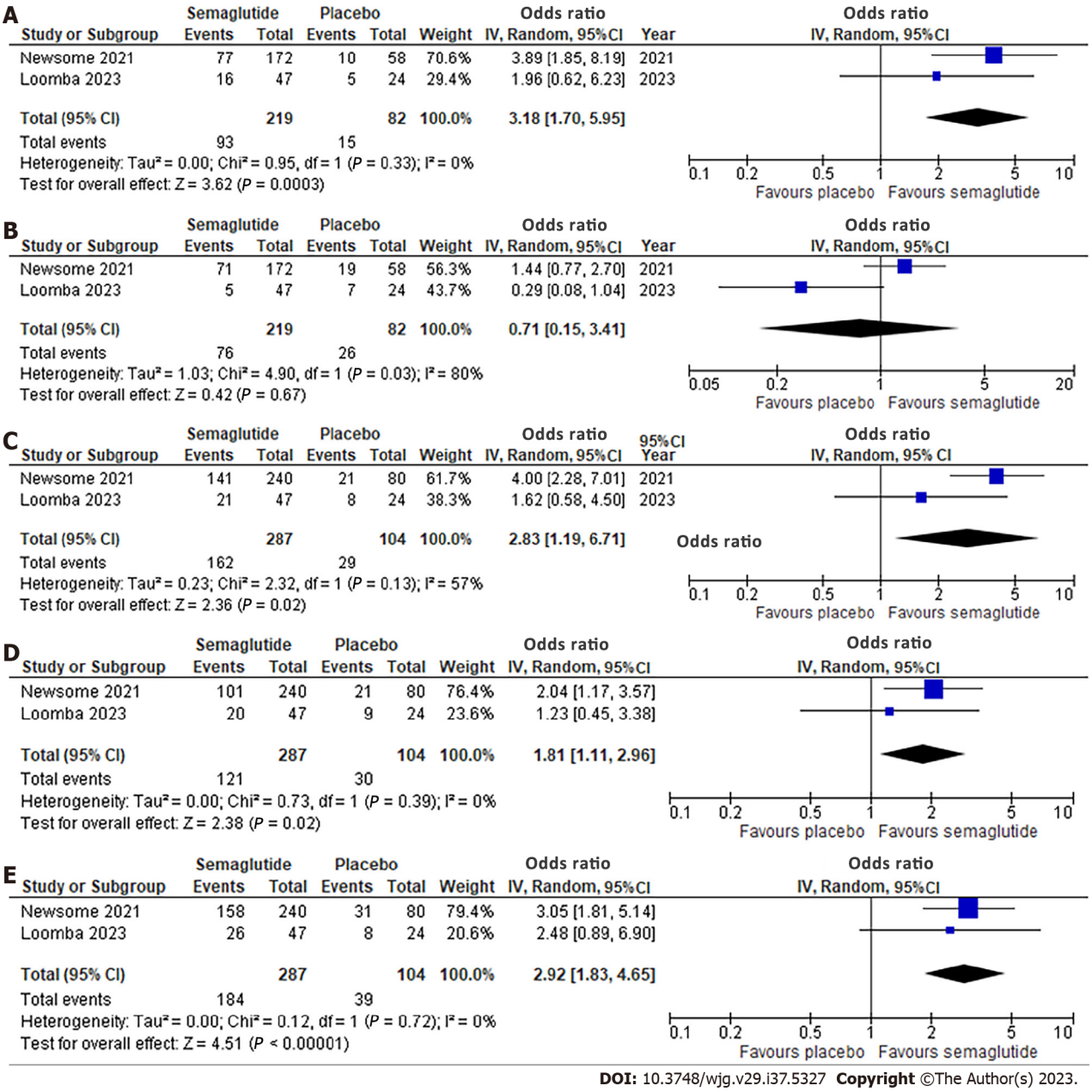

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Figure 2 Effect of semaglutide on histologic parameters. A: Resolution of non-alcoholic steatohepatitis (NASH) with no worsening of liver fibrosis; B: Improvement in liver fibrosis stage without worsening of NASH; C: Improvement in steatosis; D: Improvement in lobular inflammation; E: Improvement in hepatocellular ballooning. 95%CI: 95% confidence intervals.

estimate and resulted in the observed heterogeneity. Furthermore, the proportion of patients with improved fibrosis in the placebo group in the trial of Newsome *et al*[20] (33%) was higher than that reported in the LEAN trial (14%) or the pooled placebo outcomes of 23 RCTs involving patients with NAFLD (21%), possibly contributing to the non-significant treatment effect estimate[24,25]. Despite no difference in the improvement of fibrosis, Newsome's trial observed that a smaller proportion of patients in the semaglutide group (5%) experienced worsening of fibrosis compared to the placebo group (19%), suggesting a potential benefit[20]. It is possible that a longer follow-up time may be required to achieve improvements in fibrosis, particularly since most of the patients had advanced fibrosis, and the timeline for improvement in fibrosis remains unclear.

Although liver biopsy remains the gold standard for diagnosing and staging NAFLD, MRE and MRI-PDFF are effective non-invasive assessments of liver stiffness and steatosis, respectively. MRI-PDFF is significantly associated with histological NASH CRN steatosis grade, independent of age, sex, and other NASH parameters[26]. This correlation has been demonstrated in several other studies[27,28]. Both MRE and MRI-PDFF are considered more accurate than ultrasound-based transient elastography in detecting fibrosis and steatosis, respectively, and remain effective in patients with obesity, which is a common comorbidity in patients with NAFLD[29].

However, the accuracy of MRI-PDFF is limited by the extent of hepatic fibrosis. Permutt *et al*[30] demonstrated that the association between MRI and histology-determined steatosis remained relatively stable at fibrosis stages 0-3 but significantly dropped at stage 4. Similarly, Idilman *et al*[27] showed that the correlation between liver biopsy and MRI-

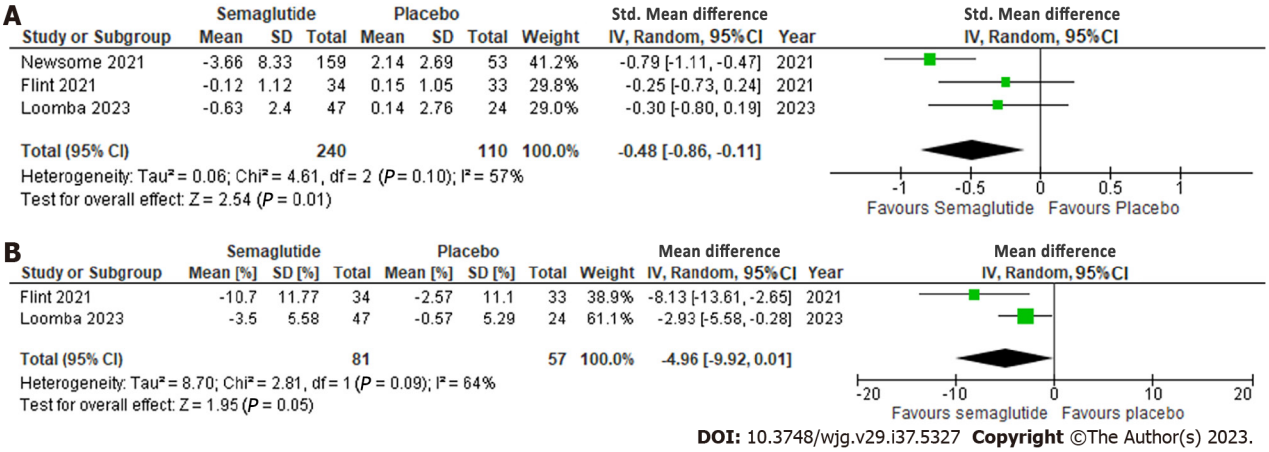

Figure 3 Effect of semaglutide on radiologic parameters. A: Liver stiffness assessed by magnetic resonance enterography or Fibroscan; B: Liver steatosis assessed by MRI proton density fat fraction. 95%CI: 95% confidence intervals.

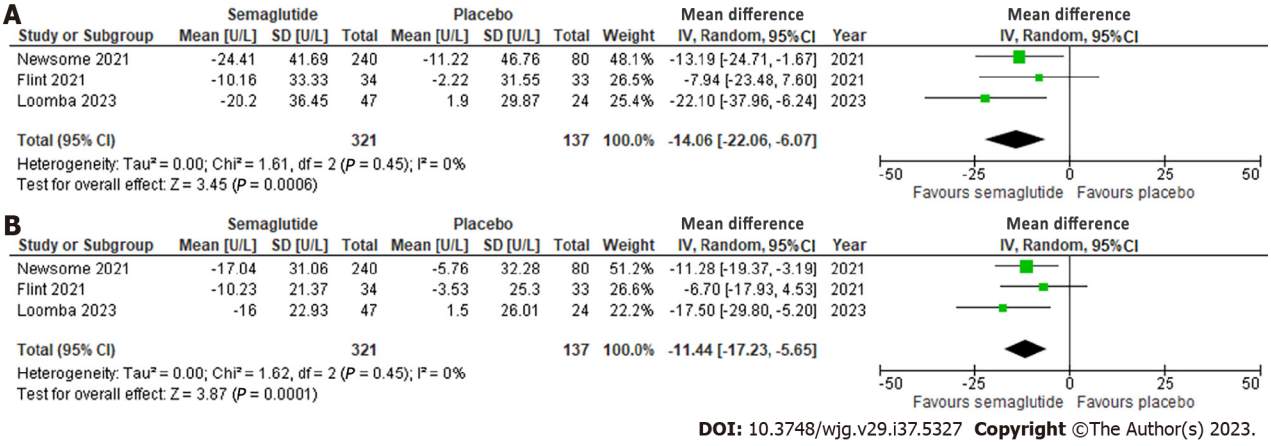

Figure 4 Effect of semaglutide on liver enzymes. A: Alanine aminotransferase; B: Aspartate aminotransferase. 95%CI: 95% confidence intervals.

determined steatosis was less pronounced when fibrosis was present (r = 0.60) than when fibrosis was absent (r = 0.86). In contrast, liver stiffness measured using MRE is less influenced by fibrosis and provides a more accurate prediction of liver fibrosis in patients with more advanced fibrosis[31]. The decreased reproducibility and accuracy of MRI PDFF in higher fibrosis stages may explain the substantial heterogeneity observed in liver steatosis in our study, especially considering that the population of the included studies had advanced fibrosis. Heterogeneity in liver stiffness, although not statistically significant, became negligible when Fibroscan was removed and only MRE was utilized to measure liver stiffness.

Semaglutide showed a significant association with moderate decreases in ALT and AST, which have previously been correlated with histologic response, fibrosis regression, and reduced progression in NAFLD[22,32]. However, both the LEAN trial and a meta-analysis of liraglutide in NAFLD did not observe significant reductions in liver enzymes[24,33]. Despite being in the same drug class with similar mechanisms of action, there may be intrinsic differences between effect of semaglutide and liraglutide. Furthermore, our study demonstrated a significant improvement in HgA1c and a reduction of 6.53 kg in body weight in the semaglutide compared to placebo group. The superior metabolic outcomes associated with semaglutide, compared to liraglutide, may lead to a more effective reduction in hepatocellular stress and injury, resulting in a significant decrease in liver enzymes. Vilar-Gomez *et al*[34] previously demonstrated that patients with weight losses ≥ 10% had the highest rates of NAS reduction, NASH resolution and fibrosis regression. Therefore, semaglutide appears to be particularly beneficial for patients with both NAFLD and features of metabolic syndrome.

The safety profile of semaglutide was comparable to that reported in the recent phase 3 trial with once-weekly dosing in patients with obesity and trials from the SUSTAIN (Semaglutide Unabated Sustainability in Treatment of Type 2 Diabetes) programs[14,35]. Gastrointestinal-related side effects, including nausea, constipation, vomiting, and abdominal pain, were significantly more prevalent in patients receiving semaglutide compared to placebo. These side effects are well-documented among the GLP-1 RA class. In all RCTs included in our review, adverse events mostly occurred during treatment initiation or the dose-escalation period. These symptoms become less pronounced with gradual up-titration and are often self-limiting, subsiding after a few weeks. Furthermore, the risks of serious adverse events were not different between semaglutide and placebo.

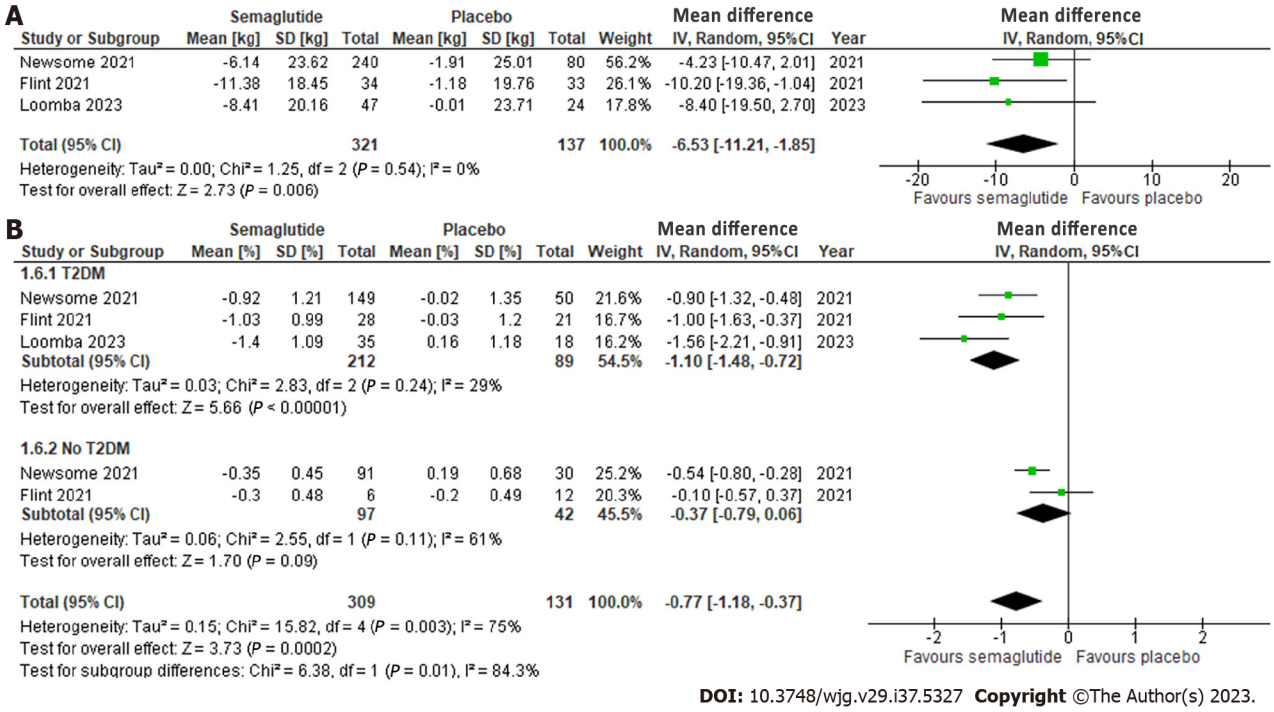

Figure 5 Effect of semaglutide on cardiometabolic parameters. A: Body weight; B: HgA1c in patients with type 2 diabetes (T2DM) *vs* without T2DM. 95%CI: 95% confidence intervals.

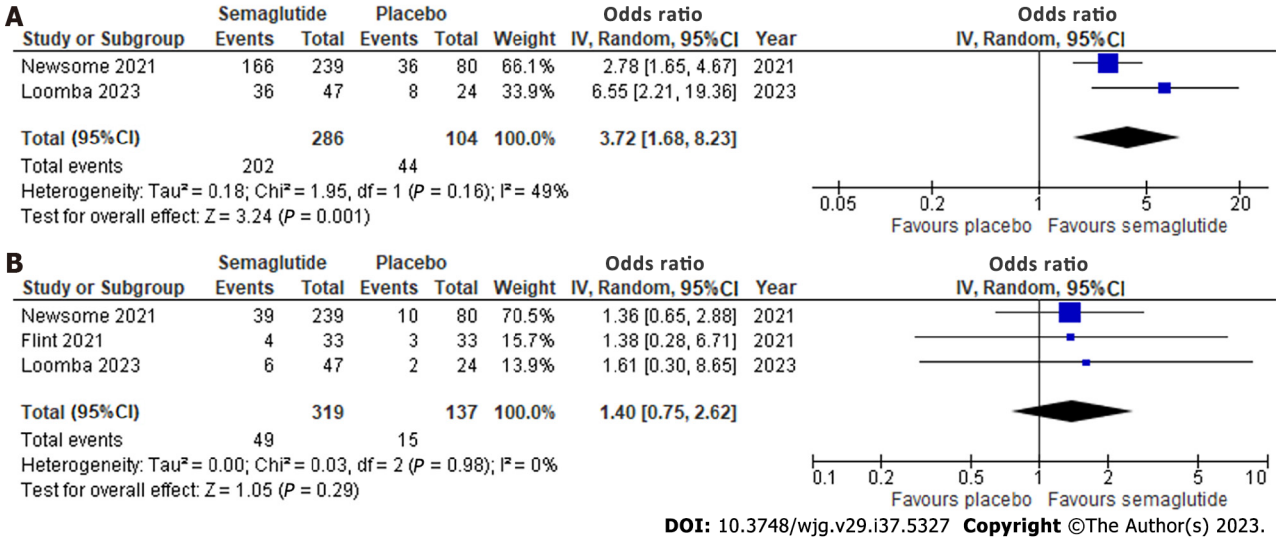

Figure 6 Adverse events with semaglutide. A: Gastrointestinal related side effects; B: Serious adverse events. 95%CI: 95% confidence intervals.

Our study has several limitations. The included RCTs are clinically heterogeneous from each other, with patients across the spectrum of NAFLD. Flint *et al*[21] recruited patients with NAFL, whereas Newsome and Loomba's trials involved patients with NASH and NASH-related cirrhosis, respectively. The heterogeneous patient population limits the applicability of the results, as treatment and response across the spectrum of NAFLD may differ. Additionally, a range of doses of semaglutide was used across the trials, including 0.1 mg, 0.2 mg, 0.4 mg once daily, and 2.4 mg weekly. However, this is less of a concern as once-weekly dosing has been shown to be comparably effective in the obesity population, and unpublished data suggests similar plasma concentrations to daily dosing[14,17]. Furthermore, histologic outcomes were only reported for patients with NASH or NASH-related cirrhosis, but not for patients with NAFL. Therefore, the effect of semaglutide on histologic outcomes in the NAFL population remains unclear. Lastly, the limited number of eligible studies restricted our ability to perform subgroup analysis comparing outcomes in NAFL, NASH, and NASH-related cirrhosis populations, or meta-regression to investigate heterogeneity and the effect of covariates on the effect sizes.

CONCLUSION

In conclusion, our meta-analysis of RCTs demonstrates that semaglutide has beneficial histologic, radiologic, liver enzyme, and cardiometabolic effects in patients with NAFLD, with a well-tolerated safety profile. Semaglutide is particularly beneficial for patients with NAFLD and features of metabolic syndrome, given its notable effects on lowering HbA1c and promoting weight loss. However, the results are limited by the small number of included studies and clinical heterogeneity, which restricts the generalizability these findings across the spectrum of NAFLD. Additional RCTs with larger sample sizes and longer durations are required to characterize the effects of semaglutide on fibrosis regression and its role in the different phases of NAFLD.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the leading cause of chronic liver disease. The prevalence and disease burden of NAFLD are projected to exponentially increase resulting in significant healthcare expenditures and lower health-related quality of life. To date, there are no approved pharmacotherapies for NAFLD or non-alcoholic steatohepatitis (NASH).

Research motivation

Several randomized clinical trials have demonstrated the beneficial effects of semaglutide in patients with NAFLD. Prior systematic review with meta-analysis assessing the impact of semaglutide did not report histological outcomes and were not focused on a NAFLD specific population.

Research objectives

This study aimed to review the efficacy and safety of semaglutide, focusing on patients with NAFLD, in order to more specifically reflect the NAFLD population and expand the current understanding of semaglutide in NAFLD.

Research methods

MEDLINE, CENTRAL, EMBASE, and grey literature sources were searched from inception to May 1, 2023, to identify eligible randomized controlled trials (RCTs) using a predefined search strategy. Predetermined outcomes were extracted, and quality assessment was performed using the Cochrane risk-of-bias 2 tool and GRADE framework. Meta-analysis was performed using random effects model expressing continuous outcomes as mean differences (MD) or standardized MDs (SMD), and dichotomous outcomes as odds ratios (OR) with 95% confidence intervals (CI). Statistical heterogeneity was assessed using the Cochran's Q test and I^2 statistic.

Research results

A total of three RCTs involving 458 patients were included. Semaglutide increased the likelihood of NASH resolution (OR: 3.18, 95%CI: 1.70, 5.95; $P < 0.001$), improvement in steatosis (OR: 2.83, 95%CI: 1.19, 6.71; $P = 0.03$), lobular inflammation (OR: 1.81, 95%CI: 1.11, 2.96; $P = 0.02$), and hepatocellular ballooning (OR: 2.92, 95%CI: 1.83, 4.65; $P < 0.001$), but not fibrosis stage (OR: 0.71, 95%CI: 0.15, 3.41; $P = 0.67$). Radiologically, semaglutide reduced liver stiffness (SMD: -0.48, 95%CI: -0.86, -0.11; $P = 0.01$) and steatosis (MD: -4.96%, 95%CI: -9.92, 0.01; $P = 0.05$). It also reduced ALT (MD: -14.06 U/L, 95%CI: -22.06, -6.07; $P < 0.001$) and AST (MD: -11.44 U/L, 95%CI: -17.23, -5.65; $P < 0.001$).

Semaglutide led to improved cardiometabolic outcomes, including decreased HgA1c (MD: -0.77%, 95%CI: -1.18, -0.37; $P < 0.001$) and weight loss (MD: -6.53 kg, 95%CI: -11.21, -1.85; $P = 0.006$), but increased the occurrence of GI-related side effects (OR: 3.72, 95%CI: 1.68, 8.23; $P = 0.001$). Overall risk of serious adverse events was similar compared to placebo (OR: 1.40, 95%CI: 0.75, 2.62; $P < 0.29$).

Research conclusions

Semaglutide demonstrated significant histologic improvements, with a higher likelihood of NASH resolution and improved NAS components, but it did not significantly improve fibrosis stage compared to placebo. Furthermore, semaglutide resulted in radiologic improvements in liver stiffness and steatosis, liver enzymes, as well as cardiometabolic effects on body weight and HgA1c, while maintaining a well-tolerated safety profile.

Research perspectives

Additional RCTs with larger sample sizes and longer durations are required to characterize the effects of semaglutide on fibrosis regression and its role in the different phases of NAFLD.

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FOOTNOTES

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SCIENTOMETRICS

Global trends and hotspots of treatment for nonalcoholic fatty liver disease: A bibliometric and visualization analysis (2010-2023)

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Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is chronic, with its progression leading to liver fibrosis and end-stage cirrhosis. Although NAFLD is increasingly common, no treatment guideline has been established. Many mechanistic studies and drug trials have been conducted for new drug development to treat NAFLD. An up-to-date overview on the knowledge structure of NAFLD through bibliometrics, focusing on research hotspots, is necessary to reveal the rational and timely directions of development in this field.

AIM

To research the latest literature and determine the current trends in treatment for NAFLD.

METHODS

Publications related to treatment for NAFLD were searched on the Web of Science Core Collection database, from 2010 to 2023. VOSviewers, CiteSpace, and R package "bibliometrix" were used to conduct this bibliometric analysis. The key information was extracted, and the results of the cluster analysis were based on network data for generating and investigating maps for country, institution, journal, and author. Historiography analysis, bursts and cluster analysis, co-occurrence analysis, and trend topic revealed the knowledge structure and research hotspots in this field. GraphPad Prism 9.5.1.733 and Microsoft Office Excel 2019 were used for data analysis and visualization.

RESULTS

In total, 10829 articles from 120 countries (led by China and the United States) and 8785 institutions were included. The number of publications related to treatment for NAFLD increased annually. While China produced the most publications, the

United States was the most cited country, and the United Kingdom collaborated the most from an international standpoint. The University of California-San Diego, Shanghai Jiao Tong University, and Shanghai University of Traditional Chinese Medicine produced the most publications of all the research institutions. The International Journal of Molecular Sciences was the most frequent journal out of the 1523 total journals, and Hepatology was the most cited and co-cited journal. Sanyal AJ was the most cited author, the most co-cited author was Younossi ZM, and the most influential author was Loomba R. The most studied topics included the epidemiology and mechanism of NAFLD, the development of accurate diagnosis, the precise management of patients with NAFLD, and the associated metabolic comorbidities. The major cluster topics were "emerging drug," "glucagon-like peptide-1 receptor agonist," "metabolic dysfunction-associated fatty liver disease," "gut microbiota," and "glucose metabolism."

CONCLUSION

The bibliometric study identified recent research frontiers and hot directions, which can provide a valuable reference for scholars researching treatments for NAFLD.

Key Words: Bibliometrics; Treatment; Therapy; Nonalcoholic fatty liver disease; Metabolic dysfunction-associated fatty liver disease; Historiography analysis

Core Tip: A total of 10829 articles published between 2010-2023 were identified through a bibliometric analysis to explore the trends and hotspots of treatment for nonalcoholic fatty liver disease (NAFLD). Replacing NAFLD/nonalcoholic steatohepatitis with metabolic dysfunction-associated fatty liver disease has been shown to greatly promote transformation of the treatment strategy of the disease. Research on gut microbiomes and traditional medicine will continue to be a short-term research hotspot. Obeticholic acid (phase 3 clinical validation) and semaglutide (under study) are likely to become the first approved drugs for NAFLD treatment.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) was first proposed by Ludwig *et al*[1] in 1980 to describe fatty liver disease without a history of alcoholism. NAFLD is further divided histologically into nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH)[2]. NASH can progress to fibrosis, eventually leading to cirrhosis and cirrhosis-related complications such as end-stage liver disease and hepatocellular carcinoma (HCC)[3]. In the United States, NASH cirrhosis is the leading indicator and fastest-growing cause of candidates waiting for liver transplantation (LT)[4] and is expected to become the prevalent indication for LT by 2030[5]. Currently, NAFLD is an epidemic among chronic diseases, threatening the health of nearly 1.7 billion people worldwide and placing a huge burden on individuals, families, and healthcare systems[6].

The clinical burden of NAFLD extends beyond liver-related morbidity and mortality as there is growing evidence suggesting its association with various metabolic comorbidities, including obesity, type 2 diabetes, cardiovascular disease, chronic kidney disease, hypertension, and hypercholesterolemia[7]. The global epidemics of obesity, diabetes, and metabolic syndrome (MetS) have led to an increasing prevalence of NAFLD[8]. The presence of multiple metabolic comorbidities increases the risk of NAFLD and the risk of progressive liver disease. The primary liver pathology in NAFLD affects liver structure and function, increasing the risk of morbidity and mortality associated with cirrhosis, end-stage liver disease, and HCC, with cardiovascular disease accounting for the most deaths in patients with NAFLD[9]. Therefore, Eslam *et al*[10], representing the International Consensus Group in 2020, recommended renaming NAFLD/NASH to metabolic dysfunction-associated fatty liver disease (MAFLD) to more accurately reflect its pathogenesis and accelerate the transition to novel therapeutics.

The most effective strategy for addressing chronic disease is to reduce the disease burden through prevention. However, this goal has not been achieved for NAFLD. Lifestyle changes focused on weight loss remain the cornerstone of NASH treatment, with a 10% weight loss able to resolve steatohepatitis or improve fibrosis and portal vein inflammation [11]. Nonetheless, achieving significant weight loss in clinical practice is challenging, as its effects are slow and can easily be reversed. Studies have shown that only 30% of patients can lose more than 5% of their weight through lifestyle changes after 13 mo[12].

New drug research and development for NAFLD treatment has been slow. Currently, clinical phase 2b and phase 3 studies have achieved certain efficacy. It is anticipated that new drugs will be available in the near future. However, no drugs have been approved yet, and the efficacy of the various compounds under development is not deemed satisfactory. The response rates for the investigational drugs in current studies range from 20%-40%, with no significant difference from the placebo response rate of 10%-20%[10].

Bibliometric analysis is a statistical method of literature analysis that examines the publication status of studies in a specific field from both quantitative and qualitative perspectives. It includes various factors such as authorship, country of origin, affiliated institutions, journal publications, and keywords[13]. Bibliometric tools such as VOSviewer, the R package "bibliometrix"[14], and CiteSpace[15] were used to visualize the results of this literature analysis, and these tools are gradually being used more frequently in the medical field. The application of bibliometrics to analyze research in the treatment of NAFLD/MAFLD is exceedingly helpful for identifying hotspots and research trends in this field of study, and in identifying those countries, institutions, and researchers that contribute the most. The advantages of this research methodology also include the possibility of analyzing and summarizing the evolution of research directions and content for a given institution or individual, as well as of quantitatively analyzing the collaboration between them. However, there are not many bibliometric statistical analyses in the field of liver disease, with few articles related to NAFLD[16-20]. In analyzing research that focuses on the possible causes of classical liver diseases, the research hotspots are mainly viral hepatitis and alcoholic liver disease. In recent years, with the overall decline in the prevalence of viral hepatitis and the annual increase in the incidence of NAFLD, there has been a growing interest in the latter. Indeed, the collective research and resultant advancement in knowledge of the disease from the proposal of "NAFLD" in 1980[1], to the proposed renaming of the disease to "MAFLD" in 2020[10], to the recent publication of the "multi-society Delphi consensus statement on new fatty liver disease nomenclature"[21] is serving as a signal to the end of the diagnosis and research into NAFLD and the entry into a brand new era of the more accurately termed "metabolic dysfunction-associated steatotic liver disease (MASLD)". Therefore, at the time of this new naming of the disease, it is necessary to analyze and summarize the research results for the treatment of this disease through the method of bibliometric statistical analysis, so as to accurately analyze the research hotspots and the trend of future development through a more objective approach and global perspective.

As mentioned earlier, NAFLD is widespread and harmful, and new treatment strategies are urgently needed to halt the progression of hepatic steatosis and fibrosis[22]. Since the renaming of NAFLD to MAFLD in 2020, a major shift has been observed in the direction of its treatment. With the publication of research results in the past 2 years, it is crucial to conduct a statistical analysis of relevant literature, identify key contributors and the current research status in this field, pinpoint current research areas of focus, and outline future research trends and development prospects.

MATERIALS AND METHODS

Search strategy

We conducted a literature search on the Web of Science Core Collection database (https://www.webofscience.com/wos/woscc/basic-search) on April 10, 2023 to collect all literature data related to therapy for NAFLD. We completed the search and retrieval of data within 1 d in order to reduce the bias caused by frequent database updates. The search formula was set to [TS = (nonalcoholic fatty liver) or (non-alcoholic fatty liver disease) or (NAFLD) or (MAFLD) or (metabolic dysfunction-associated fatty liver disease) or (metabolic associated fatty liver disease)] and TS = [(therapy) or (treatment)] and LA = (English). The type of documents was set to "articles" and "review" (editorials, proceeding papers, abstracts, and book chapters articles were excluded) with a timespan ranging from January 1, 2010 to April 12, 2023 (Figure 1).

Bibliometric analysis

VOSviewer (version 1.6.19) is a bibliometric analysis software released in 2010 by van Eck and Waltman[23]. VOSviewer can extract the key information and show the results of cluster analysis based on network data for generating and investigating maps[24,25]. Country and institution analysis, journal and co-cited journal analysis, author and co-cited author analysis, and author keywords co-occurrence analysis were performed by the VOSviewer software[26]. Of note, reference co-citation cluster and keyword co-occurrence analysis have the ability to recognize the knowledge structure and research hotspots in a field[27].

The R package "bibliometrix"[28] (version 3.2.1) (https://www.bibliometrix.org) was applied for a thematic evolution analysis and to construct a global distribution network of publications of therapy for NAFLD. CiteSpace (version 6.2.R2 Advanced) is the most popular and recognized bibliometric visualization tool[29] developed by Synnestvedt *et al*[15]. In our study, the CiteSpace map primarily completed the dual-map overlay of journals' analysis and the timeline cluster analysis of keywords. GraphPad Prism 9.5.1.733 and Microsoft Office Excel 2019 were used for data analysis and visualization.

RESULTS

Quantitative analysis of publication

According to our search strategy, there were a total of 10829 studies on therapy or treatment for NAFLD since 2010, including 7826 articles and 3003 reviews. The annual number of publication increased year over year, and it showed

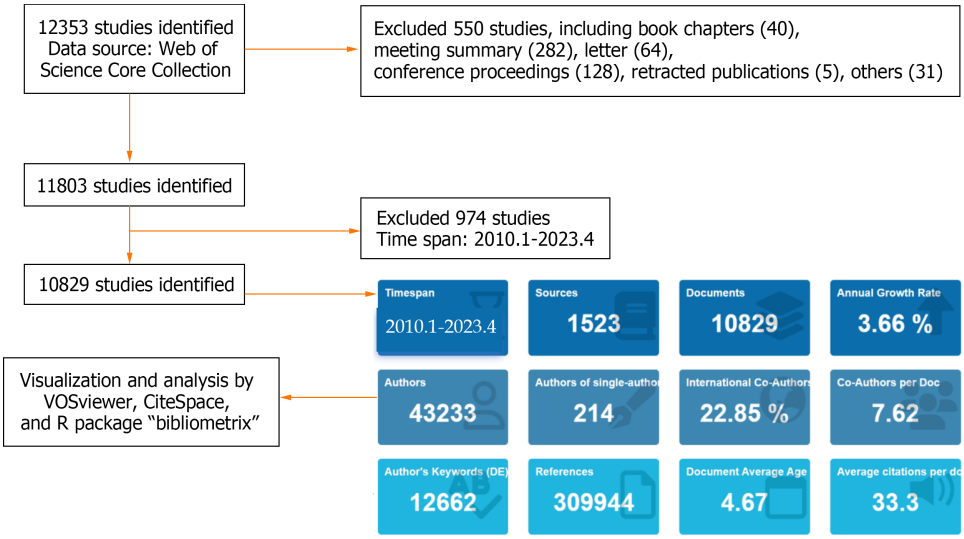

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Figure 1 Flowchart for publication screening.

explosive growth from 2019 to 2022. Although only a quarter of 2023 was available, there were more than 352 articles, suggesting a continuation of the upward trend (Figure 2A).

Country and institution analysis

These publications came from 120 countries and 8785 institutions. More than half of the publications came from China and the United States (54.9%). The United States had the most publications until 2020 when publications from China increased massively (Figure 2B and C). The top 10 countries were distributed in Europe, Asia, and North America (Figure 2D).

China had the highest single-country publications (SCP) ratio of all countries (total of 3160 papers; SCP: 2763; SCP ratio: 87.4%). The SCP ratio in the United States was 75.0%, with 1978 papers. Of note, there was an abundance of active cooperation between different countries and regions (Figure 2E). Germany and the United Kingdom placed the greatest emphasis on international cooperation; their multiple country publication ratios were 42.1% and 43.3%, respectively (Figure 2D).

Subsequently, we filtered and visualized 76 countries based on the minimum number of five publications and constructed a collaborative network based on the number and relationship of publications in each country. In 2018, the United States was the most important and biggest research center in the world, which was transferred to China in 2020 (Figure 2F).

Then, we selected 1173 institutions based on the minimum number of five publications for visualization and constructed a collaborative network based on the number and relationship of publications of each institution (Figure 2G). The top 10 institutions were located in two countries; seven of the institutions were located in China (Figure 2H). The three institutions that published the most relevant papers were University of California-San Diego, Shanghai Jiao Tong University, and Shanghai University of Traditional Chinese Medicine. University of California-San Diego was the leader in publication output and maintained a high level of publications in recent years (Figure 2I and J). In the past 3 years, Chinese institutions have increasingly published NAFLD research. In particular, Shanghai Jiao Tong University ranked second and Shanghai University of Traditional Chinese Medicine ranked third, publishing frequently since 2020 and showing expansive growth in 2022.

Journals and cited and co-cited journals

Publications related to therapy for NAFLD were published in 1523 journals. The International Journal of Molecular Sciences published the most papers (n = 281, 2.6%). The top three journals with the most citations were Hepatology (n = 21451), Journal of Hepatology (n = 20843), and the World Journal of Gastroenterology (n = 12454). Among the top 20 journals of total link strength, the journal with the highest impact factor (IF) was Nature Reviews Gastroenterology and Hepatology (IF = 73.082).

Among the top 20 co-cited journals, five journals were cited more than 10000 times. Hepatology (co-citation = 49058) was the most cited journal, followed by the Journal of Hepatology (co-citation = 26782), Gastroenterology (co-citation = 21448), Journal of Biological Chemistry (co-citation = 12220), and PLOS ONE (co-citation = 12083). In addition, the IF of The Lancet was the highest (IF = 202.731) followed by the New England Journal of Medicine (IF = 176.079).

The dual-map overlay of journals can show the citation relationships between citing journals on the left and the clusters of co-cited journals on the right through the colored paths[29]. As shown in Figure 3A, the orange path is the main citation path, which represents the studies published in molecular/biology/immunology journals primarily cited by literature in molecular/biology/gene and health nursing/medicine journals. The green path is the main document flow path, which represents the research published in molecular/biology/gene and health nursing/medicine journals

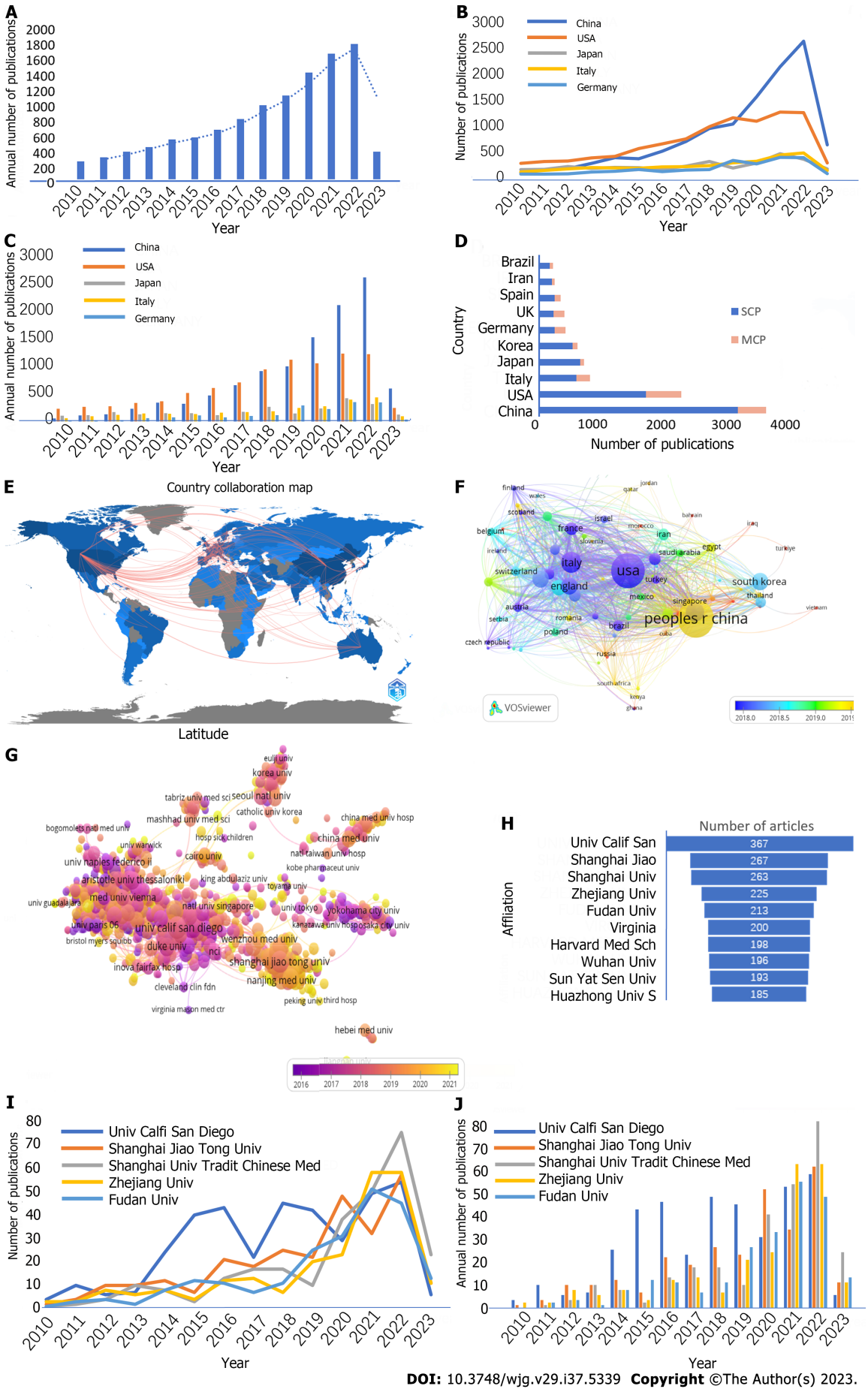

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Figure 2 Quantitative analysis of publication. A: Annual output of therapy for nonalcoholic fatty liver disease; B: Country production over time; C: Annual output by country; D: Corresponding authors' countries; E: Country collaboration world map; F: Visualization of countries; G: Visualization of institutions on nonalcoholic fatty liver disease research; H: Most relevant affiliations; I: Institution production over time; J: Annual output by institution. MCP: Multiple country publication; SCP: Single country publication.

primarily cited by studies published in medicine/medical clinical journals.

Historiography analysis

Through historiography analysis by R package "bibliometrix," we observed that the publications with the highest importance in chronological order in the dataset were Sanyal *et al*[30], Neuschwander-Tetri *et al*[31], Rinella[32], and Younossi *et al*[33] (Figure 3B and Table 1). Table 1 clearly showed that the vast majority of important studies (local cited documents and global cited documents were both high) were randomized controlled trials[30,31,34-36]. In addition, two important reviews in 2018 were "mechanisms of NAFLD development and therapeutic strategies"[37] and "global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention"[33].

Reference with citation bursts and cluster analysis

Reference with citation bursts refers to those references that are frequently cited by scholars in a certain field over a period of time. In our study, the details of 25 references with strong citation bursts by R package "bibliometrix" were listed in Table 2. As shown in Table 2, every bar represents a year, and the red bar represents strong citation burstiness. Citation bursts for references appeared as early as 2010 and as late as 2023. The burst strength of these 25 references ranged from 49.79 to 161.62, and endurance strength was from 2-5 years.

A total of eight major clusters (Q = 0.519; S = 0.8789; Q/S = 0.6532) were generated from the co-citation networks of references after cluster analysis by CiteSpace (Figure 3C). The cluster nomenclature may reflect the study hotspots and frontiers in treatment of NAFLD. The largest cluster was emerging drug, followed by glucagon-like peptide-1 (GLP-1) receptor agonist, liver diseases, MAFLD, gut microbiota, hepatologists points, glucose-induced glucagon-like peptide, and glucose metabolism.

Authors and cited and co-cited authors

A total of 51987 authors participated in research on the therapy for NAFLD. Among the top 10 authors, 5 (who were from the United States, the United Kingdom, Italy, and France) each published more than 50 papers (Figure 3D). We built a collaborative network based on authors whose number of published papers was ≥ 5 (Figure 3E). Sanyal *et al*[30], Loomba *et al*[38], and Ratziu *et al*[22] had the largest nodes due to publishing the most related publications. In addition, we observed close collaboration among multiple authors.

Sanyal *et al*[30] was considered one of the most distinguished scholars because of his prolific research output (Figure 3D) and the highest number of citations (Figure 3E). His research on fatty liver encompassed a multidisciplinary interactive approach, covering areas such as apoptosis, cancer research, obesity, and microRNA. He hypothesized that NASH was associated with altered liver microRNA expression and alterations in the human gut microbiome were associated with cirrhosis and its complications[39]. He has committed to the field of effective therapeutic development for NASH and has provided summaries of new drugs under development or key ongoing research[40]. These include highly anticipated treatments such as farnesoid X receptor agonists, peroxisome proliferator-activated receptor agonists, GLP-1 receptor agonists, C-C chemokine receptor type 2 and 5 inhibitors, caspase inhibitors, lysyl oxidase-like 2 inhibitors, galectin-3 inhibitors, and others.

According to the H-index, which is a measure used to assess the scientific productivity and impact of researchers, the most influential scientists in this field of NAFLD are Loomba *et al*[38], Sanyal *et al*[30]. Loomba *et al*[38] has focused on various aspects of NAFLD, including aging, epidemiology, genetic and environmental susceptibility, natural history, and the treatment of NASH. His research has also explored the efficacy of metformin[40], ezetimibe[41], obeticholic acid (OCA)[31], sitagliptin[42], cenicriviroc[43], selonsert[44], polyethylene glycol[45], and semaglutide[46] in the treatment of NAFLD.

Among the 163234 co-cited authors, five authors were co-cited more than 2000 times. The most co-cited author was Younossi *et al*[33] (n = 4007) followed by Chalasani *et al*[3] (n = 2363) and Sanyal *et al*[30] (n = 2088). Younossi *et al*[33] concluded that lifestyle modifications to achieve weight loss remains a first-line intervention for patients with NAFLD [47]. He also found that a dosage of 25 mg of OCA significantly improved fibrosis and NASH disease activity scores in patients with NASH[34,48]. Chalasani *et al*[3] suggested that bariatric surgery may be a viable option for patients with cirrhosis and extreme obesity[49]. Furthermore, Chalasani *et al*[3]was involved in research on the treatment of NASH using saroglitazar[50], belapectin[51], and OCA[34,48].

Co-cited references

There are 309771 co-cited references on research on the therapy for NAFLD over the past 12 years. In the top 10 co-cited references, all references were co-cited more than 550 times. We selected references with co-citation ≥ 20 for the construction of the co-citation network map (Figure 3F). Four articles occupied an important position and became the center of the co-citation network diagram. They were Kleiner *et al*[52], Younossi *et al*[33], Sanyal *et al*[30], and Chalasani *et al*[3]. The most cited country is the United States (Figure 3G).

Table 1 Reported clinical results of neoadjuvant immunotherapy for resectable esophageal squamous cell carcinoma

Ref.	Year	Title	LCS	GCS
Musso *et al*[93]	2010	A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease	213	410
Sanyal *et al*[30]	2010	Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis	986	2063
Promrat *et al*[35]	2010	Randomized controlled trial testing the effects of weight loss on nonalcoholic steatohepatitis	426	832
Ratziu *et al*[22]	2010	A position statement on NAFLD/NASH based on the EASL 2009 special conference	245	731
Lavine *et al*[94]	2011	Effect of vitamin E or metformin for treatment of nonalcoholic fatty liver disease in children and adolescents: the toninc randomized controlled trial	363	711
Musso *et al*[105]	2012	Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomised trials	224	435
Mudaliar *et al*[96]	2013	Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease	234	631
Neuschwander-Tetri *et al*[31]	2015	Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial	586	1416
Rinella *et al*[32]	2015	Nonalcoholic fatty liver disease: A systematic review	403	1443
Buzzetti *et al*[97]	2016	The multiple-hit pathogenesis of NAFLD	538	1386
Boursier *et al*[98]	2016	The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota	213	717
Cusi *et al*[106]	2016	Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: A randomized trial	277	525
Ratziu *et al*[107]	2016	Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and-δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening	350	642
Armstrong *et al*[36]	2016	Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study	529	1006
Friedman *et al*[37]	2018	Mechanisms of NAFLD development and therapeutic strategies	550	1526
Younossi *et al*[33]	2018	Global burden of NAFLD and NASH: Trends, predictions, risk factors and prevention	739	2379
Younossi[56]	2019	Non-alcoholic fatty liver disease-a global public health perspective	243	906
Younossi *et al*[6]	2019	Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis	254	808
Younossi *et al*[34]	2019	Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial	263	541

EASL: European association for the study of the liver; GCS: Global cited documents; LCS: Local cited documents; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

Keywords with citation bursts and clustered network

Keywords with citation bursts refers to those keywords or topics that are frequently discussed in a certain field over a period of time. In our study, 25 keywords with strong citation bursts were identified by R package "bibliometrix" (Figure 4A). Judging from the top 25 keywords with the strongest citation bursts, the whole period can be divided into two: Period I (2010-2017) and period II (2019-2023). Keywords in period I primarily included MetS, insulin resistance, lifestyle intervention, and focus on pathogenesis such as lipid peroxidation. Subsequently, keywords in period II primarily included traditional Chinese medicine, network pharmacology, lifestyle modification, autophagy, and gut microbiota. Cluster analysis of the keyword timeline graph built by CiteSpace showed the evolution of high-frequency keywords (Figure 4B).

Co-occurrence analysis of author keywords

Through the co-occurrence analysis of author keywords (n = 12665), we could quickly capture research hotspots in a certain field. Table 3 showed the top 20 high-frequency keywords excluding the name of the disease (such as NAFLD, MAFLD, NASH, *etc*) for publications regarding therapy for NAFLD. Among these keywords, obesity, insulin resistance, inflammation, and MetS appeared more than 500 times, which represented the main research direction of therapy for NAFLD. We filtered keywords with the number of occurrences ≥ 20 and performed cluster analysis through VOSviewer (Figure 4C). We obtained five clusters representing five research directions. The keywords in red clusters consisted of inflammation, oxidative stress, lipid metabolism, high-fat diet, lipogenesis, macrophages, *etc*. The keywords in yellow clusters consisted of gut microbiota, probiotics, bile acids, *etc*. The keywords in blue clusters consisted of vitamin E, weight loss, diet, *etc*. The keywords in purple clusters consisted of obesity, MetS, insulin resistance, metformin, adiponectin, diabetes, liraglutide, *etc*. The keywords in green clusters consisted of HCC, fibrosis, cirrhosis, live

Table 2 Top 25 references with strong citations

Rank	Ref.	Year	Primary research content	Strength	Beginning	Ending
1	Sanyal *et al*[30]	2010	Vitamin E showed improvement in nonalcoholic steatohepatitis in adults without diabetes	161.62	2010	2015
2	Chalasani *et al*[3]	2018	The diagnosis and management of nonalcoholic fatty liver disease practice guideline	150.14	2013	2017
3	Neuschwander-Tetri *et al*[31]	2015	Obeticholic acid improved the histological features of nonalcoholic steatohepatitis	102.84	2016	2020
4	Vernon *et al*[111]	2011	Systematic review: The epidemiology and natural history of NAFDL and NASH in adults	97.78	2012	2016
5	Chalasani *et al*[2]	2012	A practice guideline of the diagnosis and management of NAFLD	87.88	2013	2017
6	Williams *et al*[116]	2011	Prevalence of NAFLD was higher than estimated, especially in hispanics and diabetes patients	82.64	2012	2016
7	Promrat *et al*[35]	2010	Weight reduction achieved through lifestyle intervention led to improvements in liver histology in NASH	80.62	2011	2015
8	Vilar-Gomez *et al*[11]	2015	NASH resolution/fibrosis regression occurred in patients with ≥ 10% weight loss induced by lifestyle changes	77.63	2016	2020
9	Lavine *et al*[94]	2011	Neither vitamin E nor metformin was effective in reducing ALT in patients with pediatric NAFLD	76.39	2012	2016
10	Rinella *et al*[32]	2015	A systematic review of NAFLD	73.68	2016	2020
11	Cohen *et al*[101]	2011	NAFLD is strongly associated with obesity and insulin resistance	72.23	2012	2016
12	Angulo *et al*[110]	2015	Fibrosis stage was associated with long-term overall mortality, liver transplantation, and liver-related events	71.71	2016	2020
13	Ratziu *et al*[22]	2010	Collaboration between hepatologists and specialists in the endocrine, nutritional, and cardiology fields should be encouraged to optimize clinical management	62.53	2011	2015
14	Targher *et al*[7]	2010	NAFLD was associated with an increased risk of cardiovascular disease	58.16	2011	2015
15	Byrne *et al*[5]	2015	NAFLD is a multisystem disease, affecting extrahepatic organs and regulatory pathways	57.94	2016	2020
16	Ekstedt *et al*[108]	2015	Fibrosis stage predicted both overall and disease-specific mortality	57.38	2016	2020
17	Wong *et al*[109]	2015	NASH has been predicted to become the leading indication for liver transplantation	54.86	2016	2020
18	Anstee *et al*[112]	2013	NAFLD is associated with liver-related morbidity and mortality and an increased risk of developing cardiovascular disease and T2DM	50.86	2014	2018
19	Younossi *et al*[53]	2016	As the global epidemic of obesity fuels metabolic conditions, the clinical and economic burden of NAFLD will become enormous	125.36	2018	2021
20	Loomba *et al*[114]	2013	The epidemiology and demographic characteristics of NAFLD vary worldwide	63.19	2015	2018
21	Tilg *et al*[115]	2010	Many parallel hits derived from the gut and/or the adipose tissue may promote liver inflammation. endoplasmic reticulum stress and related signaling networks, (adipo) cytokines, and innate immunity are emerging as central pathways that regulate key features of NASH	58.39	2012	2015
22	Buzzetti *et al*[97]	2016	The multiple-hit pathogenesis of NAFLD	49.79	2018	2021
23	Eslam *et al*[102]	2020	A diagnosis of MAFLD requires positive criteria	75.68	2021	2023
24	Eslam *et al*[10]	2020	Renaming NAFLD to MAFLD was proposed to accelerate the translational path to new treatments	68.10	2021	2023
25	Newsome *et al*[103]	2021	Treatment with semaglutide resulted in a higher percentage of NASH resolution than placebo	55.16	2021	2023

ALT: Alanine aminotransferase; MAFLD: Metabolic dysfunction-associated fatty liver disease; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; T2DM: Type 2 diabetes mellitus.

transplantation, immunotherapy, *etc*.

Trend topic analysis

The trend topic analysis of the keywords showed that from 2010 to 2016, the research focused on diet, lifestyle intervention, vitamin E, *etc*. Since 2017, researchers have begun to focus on MetS and actively explore the precise

Table 3 Top 20 high-frequency keywords (excluding the name of disease)

Rank	Keyword	Occurrence
1	Obesity	898
2	Insulin resistance	684
3	Inflammation	670
4	Metabolic syndrome	547
5	Oxidative stress	444
6	Steatosis	418
7	Fibrosis	414
8	Hepatocellular carcinoma	306
9	Lipid metabolism	304
10	Gut microbiota	260
11	Diabetes	258
12	Cirrhosis	241
13	Treatment	177
14	Autophagy	165
15	High-fat diet	141
16	Bariatric surgery	124
17	Cardiovascular disease	123
18	Metformin	121
19	Apoptosis	118
20	Lipogenesis	118

diagnosis and effective treatment for NAFLD. Since the suggestion of renaming NAFLD to MAFLD in 2020, the main keywords were insulin resistance, obesity, inflammation, microbiome, OCA, *etc*. It is worth noting that gut microbiota, gut-liver axis, and traditional Chinese medicine are the latest trending topics (Figure 4D).

DISCUSSION

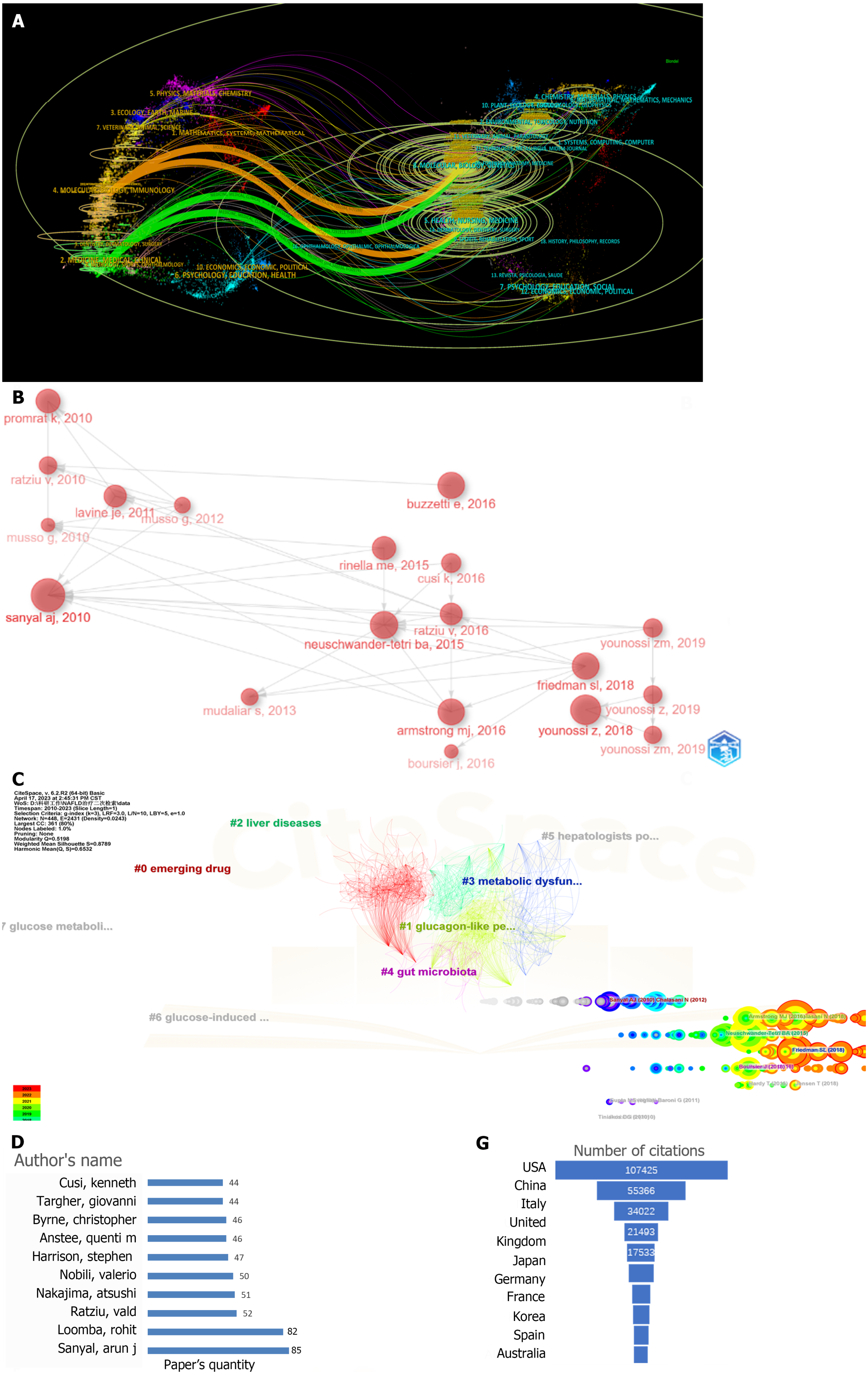
General information

Since 2010, the annual publications regarding NAFLD treatment have increased, particularly in the past 5 years. The number of related studies has also grown rapidly, indicating that research on NAFLD treatment is in an explosive period, and China and the United States are the leading countries studying the treatment of NAFLD. The United States has been the largest center for publications on the treatment of NAFLD worldwide. However, 2020 was a turning point when the number of publications from China exploded. The cumulative number of publications from China surpassed those from the United States in 2022. China accounts for 70% of the top 10 research institutions regarding publication volume, and the remaining institutions are in the United States. University of California San Diego has been a leader in the NAFLD field; however, research institutions in China have developed rapidly in recent years.

China is developing rapidly in the NAFLD field, surpassing the United States in the number of related publications. There are several reasons for this, which we analyzed below.

The first reason is that the incidence of NAFLD is increasing in China. The global prevalence of NAFLD is 25.2%[53] and 29.2% in China. This has increased rapidly (2008-2010: 25.4%; 2015-2018: 32.3%)[54]. Over the past 20 years, the economic development, urbanization, and dietary patterns in China have changed (*e.g.*, increased consumption of animal-derived foods, refined grains, and highly processed, high-sugar, and high-fat foods)[55]. This has led to sedentary lifestyles and overnutrition, undergirding obesity and the NAFLD epidemic[56].

The second reason is that the burden of NAFLD in China will continue to increase. With the development of the global economy, the global prevalence of NASH will increase by 15%-56%, and the highest prevalence of NAFLD is expected to occur in China[6]. The Chinese population is large, and the burden of the disease increases exponentially[57,58]. In a NAFLD disease burden modeling analysis, it was estimated that by 2030, the NASH population in China is expected to increase by 48% compared to that in 2016 (48.26 million), with an 86% increase in HCC (14090)[59].


A
5. PHYSICS, MATERIALS, CHEMISTRY
3. ECOLOGY, EARTH, MARINE
1. MATHEMATICS, SYSTEMS, MATHEMATICAL
4. MOLECULAR, BIOLOGY, IMMUNOLOGY
2. MEDICINE, MEDICAL, CLINICAL
10. ECONOMICS, ECONOMIC, POLITICAL
6. PSYCHOLOGY, EDUCATION, HEALTH
4. CHEMISTRY, MATERIALS, PHYSICS
1. SYSTEMS, COMPUTING, COMPUTER
5. HEALTH, NURSING, MEDICINE
7. PSYCHOLOGY, EDUCATION, SOCIAL
12. ECONOMICS, ECONOMIC, POLITICAL
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#4 gut microbiota
#6 glucose-induced ...
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Targher, giovanni 44
Byrne, christopher 46
Anstee, quenti m 46
Harrison, stephen 47
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Italy 34022
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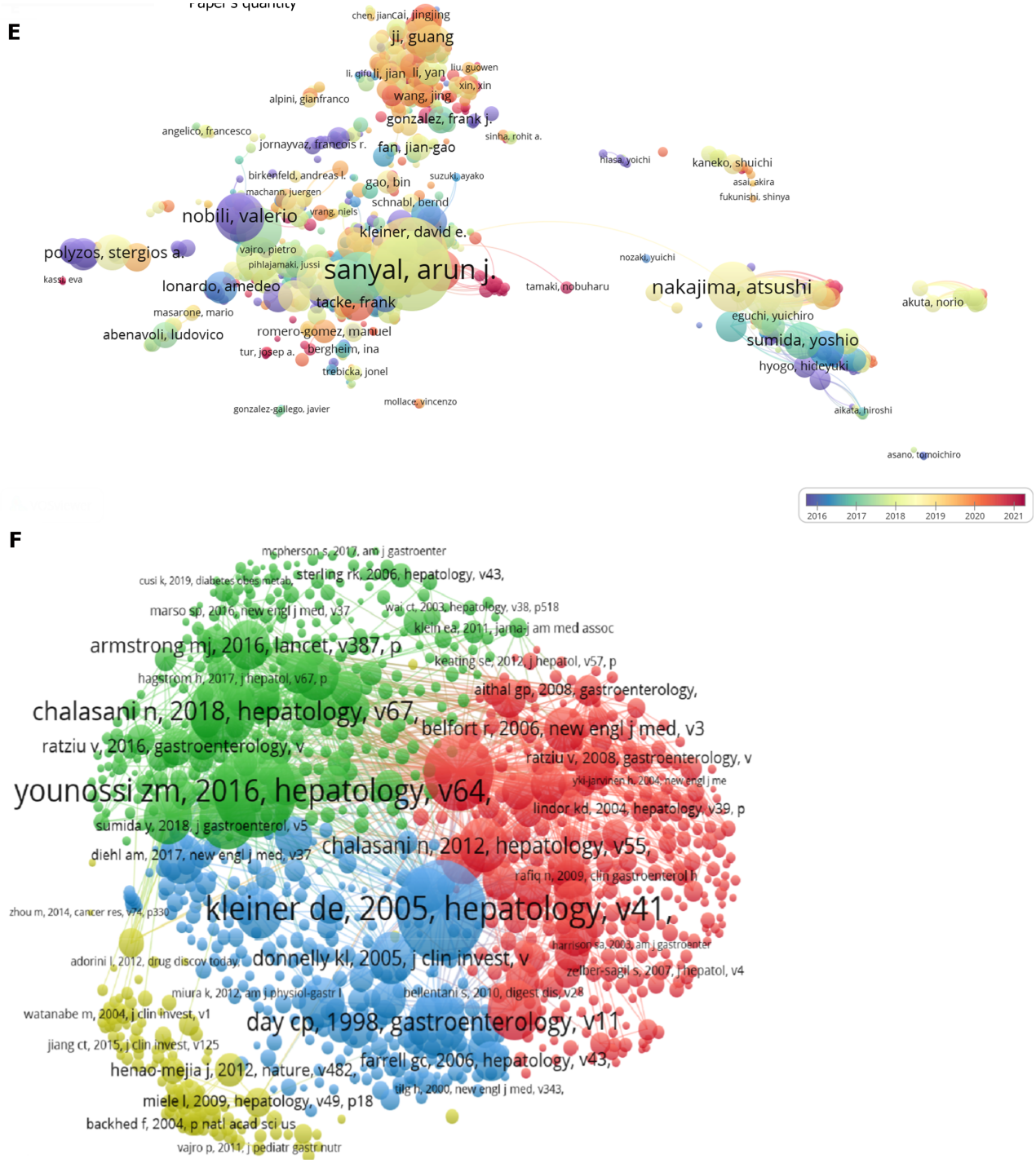

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Figure 3 Research trends. A: Dual-map overlay of journals publishing studies on the therapy for nonalcoholic fatty liver disease; B: Historiograph; C: Co-citation network of reference cluster analysis; D: Paper's quantity of most relevant authors; E: Visualization of relevant authors; F: Visualization of co-cited references; G: Most cited country.

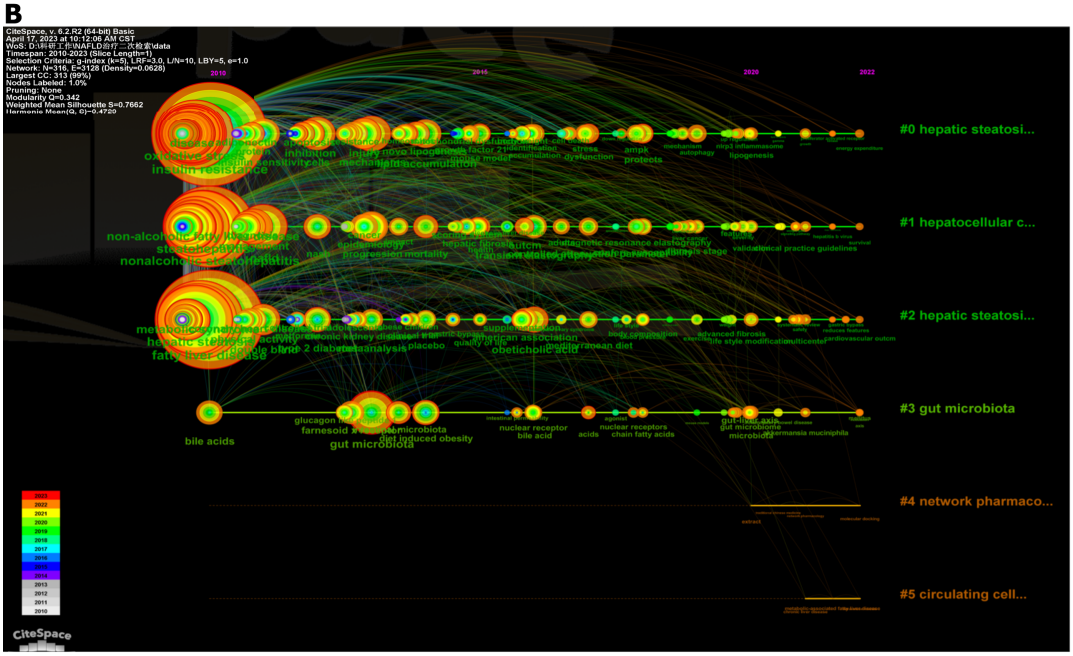
The third reason is that MetS is a significant public health problem in China[60]. MetS is the strongest risk factor for NAFLD and NASH. The association between NAFLD and MetS may be bidirectional, particularly for diabetes and systemic hypertension, while traditional Chinese culinary culture (high in fat, salt, and fiber) adversely affects these diseases. Given the two-way causal relationship between NAFLD and type 2 diabetes mellitus, a rapid increase in diabetes and obesity rates directly leads to an increase in the prevalence of NAFLD[61].

The fourth reason is that the prevalence of hepatitis B in China has decreased. China has the highest prevalence of hepatitis B. However, government control measures, effective hepatitis B virus vaccination, and standardized anti-hepatitis B virus treatment have had a positive impact on decreasing the prevalence. Hepatitis B in China has been largely controlled, making chronic liver disease caused by NAFLD increasingly prominent.

A

Keywords	Year	Strength	Begin	End	2010-2023
Metabolic syndrome	2010	63.88	2010	2013	
Placebo controlled trial	2010	35.06	2010	2016	
Natural history	2010	31.34	2010	2015	
Term follow up	2010	23.89	2010	2016	
Life style intervention	2010	23.74	2010	2014	
Risk factors	2010	22.99	2010	2015	
Randomized controlled trial	2010	20.1	2010	2017	
Alanine aminotransferase	2010	16.9	2010	2013	
Necrosis factor alpha	2010	16.51	2010	2016	
Chronic hepatitis C	2010	15.92	2010	2016	
Coronary heart disease	2010	15.55	2010	2015	
Vitamin E	2010	15.44	2010	2014	
Cryptogenic cirrhosis	2010	15.35	2010	2016	
Gamma glutamyl transferase	2010	15.35	2010	2016	
Follow up	2010	15.24	2010	2016	
Activated protein kinase	2010	14.62	2010	2016	
Nonalcoholic steatohepatitis	2010	14.39	2010	2011	
Ursodeoxycholie acid	2010	12.86	2010	2014	
Fatty liver	2010	19.87	2011	2013	
Magnetic resonancs clastography	2018	13.27	2018	2020	
Gut microbiota	2013	23.85	2021	2023	
Traditional chinese medicine	2021	13.75	2021	2023	
Network pharmacology	2021	13.59	2021	2023	
Obeticholic acid	2016	12.88	2021	2023	
Clinical practice guidelines	2021	12.82	2021	2023	

B



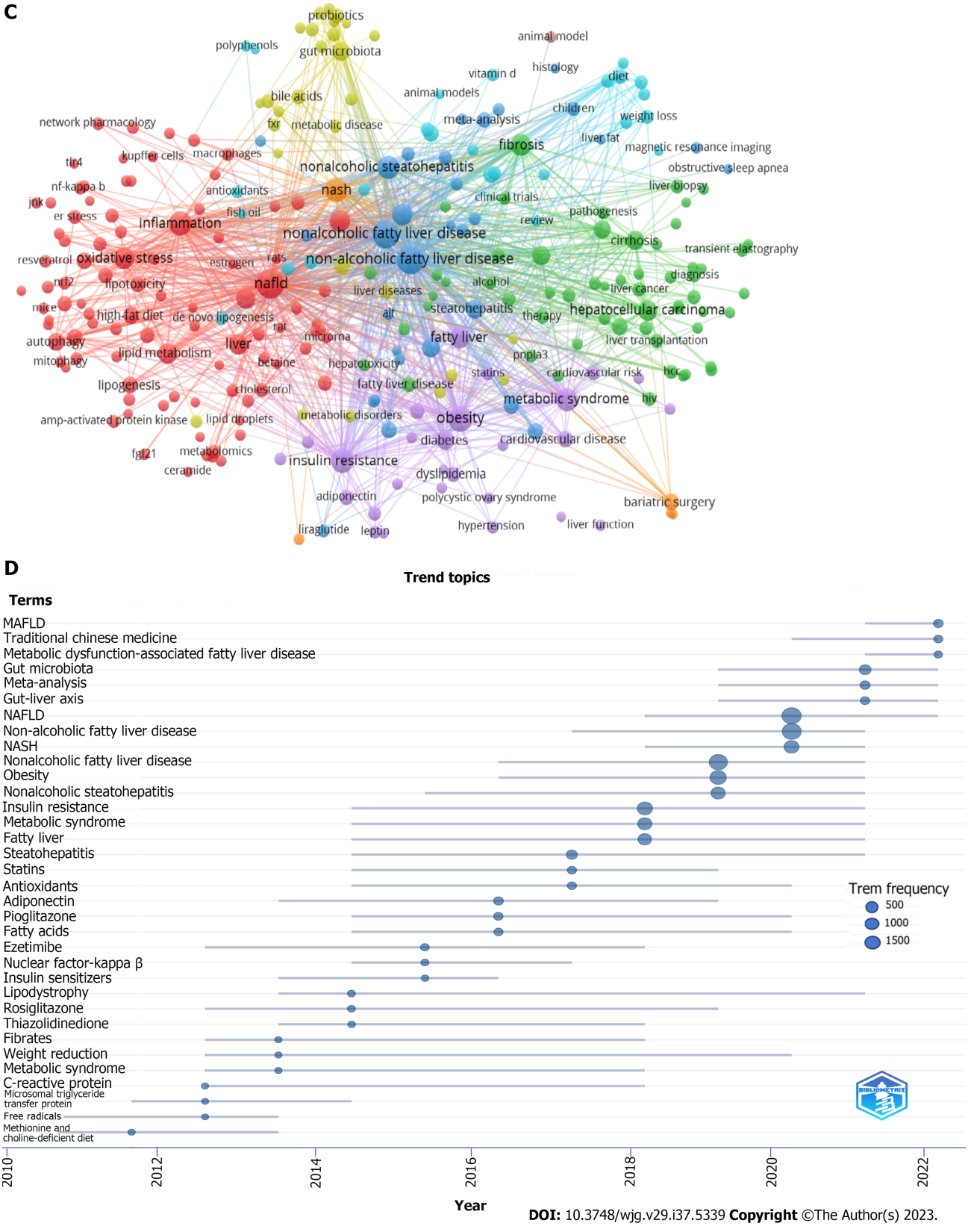


Figure 4 Research hotspots. A: Keywords with the strongest burst strength; B: Timeline cluster analysis; C: Co-occurrence author keyword; D: Trend topics.

The fifth reason is that the government attached importance to scientific research and improved the level of scientific research. In 2010, China spent less than half the amount on research and development as the United States (208280000 USD *vs* 444709000 USD) (https://data.oecd.org/rd/gross-domestic-spending-on-r-d.htm). Recently, due to economic development and robust government support, China has become the world's second-largest industry research and development center, with its total research and development expenditure reaching 80% of that of the United States' research and development expenditure (631845000 USD) in 2019 (https://www.oecd.org/sti/msti-highlights-march-

2021.pdf). Over the years, basic and clinical research on liver disease in China has been listed as a priority investment area by the National Natural Science Foundation of China (NSFC). From 2010 to 2022, the total number of research projects related to fatty liver funded by the NSFC was 7515, with a total funding of 535000000 USD. The number of projects and funding provided by the NSFC has increased annually. During the same period the National Institutes of Health in the United States funded 3304 projects (1267000000 USD) in the field of fatty liver disease. Although the investment gap between China and the United States is still significant, the number of NSFC-funded projects has surpassed that of the United States.

The final reason is the emphasis on traditional medicine. The NSFC has funded 960 projects related to traditional Chinese medicine and integrated traditional Chinese medicine and western medicine, while there are few funded projects related to traditional medicine in the United States. This also reflects the advantages and characteristics of the fatty liver research field in China. For example, Shanghai University of Traditional Chinese Medicine systematically and comprehensively analyzes the multitarget mechanism of action of traditional Chinese medicine compounds[62-64] and explores the metabolite-target-disease network of traditional Chinese medicine[65-67] in the treatment of NAFLD. The concept of multitarget therapy with traditional Chinese medicine compounds is consistent with the multiple pathogeneses of NAFLD. It is challenging for a single drug to address the diversity of the pathogenesis of NAFLD. Therefore, traditional Chinese medicine and compound multitarget active ingredients may usher in a new era in the treatment of NAFLD.

Although the number of publications in China has grown rapidly in recent years, the citation volume is still relatively low. Possible causes are: (1) Insufficient international cooperation in China. China's SCP ratio was as high as 87.4%, the highest among all countries; (2) the international discourse of China in this field needs to be improved. The top experts in this field are all American scholars who have decades of experience in the field. Their research directions are focused and deep, which allows the publication of their research in top journals. Conversely, researchers in China lack international academic authority and have few articles published in top journals; and (3) the quality and innovation of publications needs to be improved. Research in China is primarily basic, and there is a lack of multicenter randomized controlled trials and large-sample prospective cohort studies with high clinical evidence levels.

Although Chinese scientists are not ranked high regarding personal influence, they are ranked second only to the United States in the ranking of cited countries, which indicates that the overall scientific research strength in China cannot be underestimated. The top 30 cited Chinese studies focused on epidemiology[59,61,68,69], basic research, particularly pathogenesis[70-76], the search for therapeutic targets[77-89], in vitro tests[76,82,86], animal experiments[87-89], and review articles[81,83]. At present, most data on drug treatment originate from foreign research, and there is a lack of research on the Chinese population. This is an important direction that needs to be addressed in future research on NAFLD treatments in China.

Most studies on NAFLD treatment have been published in the International Journal of Molecular Sciences (IF = 6.208, second quartile), indicating that it is currently the most popular journal in this field of research. Among the top 20 journals, Nature Reviews Gastroenterology and Hepatology (IF = 73.082) had the highest IF. For co-cited journals, we found that most were high-impact top quartile journals. These high-quality international journals provide research support for the treatment of NAFLD. Among them, Hepatology and Journal of Hepatology are the most popular co-cited journals, indicating the high quality of the journal in the field of NAFLD research. Additionally, the research results in molecular/biology/immunology journals mainly flow to molecular/biology/genetic and health care/medical journals, which also indicates that the development of therapeutics is inseparable from basic research fields such as molecular biology, genetics, and immunity. The articles in the field of NAFLD treatment research have been cited by medical/clinical journals, which illustrates the clinical application value of treatment research.

Historiography analysis

Co-cited references are regarded as the basis of research in a certain field, and the development and evolutionary dynamics of a discipline can be explored by studying co-cited networks[90,91]. We selected the 10 most co-cited articles to determine the research basis for the treatment of NAFLD. Among the 309771 cited publications, the design and validation of the histology scoring system for NAFLD published by Kleiner *et al*[52] in 2005 was the most cited study. The top 10 citations covered five main topics: diagnosis; epidemiology[33,53]; pathogenesis; therapeutic drug development[30,31]; and review of NAFLD[92]. Epidemiology is the exploration of disease prevalence and incidence[33], global burden trend prediction and prevention[53], and the overall understanding of the disease. The standardization of diagnostic methods [3,52] is a prerequisite for disease management and guiding treatment. The exploration of pathogenesis[37,92] is conducive to the development of new drugs and individualized and precise treatment. Vitamin E, pioglitazone[30], and OCA[31] are the most promising NAFLD therapeutics in development. These main topics also reflect the evolution of NAFLD drug research directions.

Historiography analysis is commonly described as "the history of history" and seeks to explain origins and evolution providing a clearer understanding of the future. Results from the first randomized controlled trial in 2010 of adult NASH patients using lifestyle modification as an active therapeutic intervention suggested that lifestyle changes focused on diet, exercise, and behavioral changes could successfully improve overall histological activity, degree of steatosis, and liver chemistry of NASH[35]. Weight loss, physical activity, reduction of a sedentary lifestyle, and dietary changes should be treated as the first-line treatment of NAFLD/NASH and assessed after 6 mo. If this is not effective, then additional treatment options, such as medication, may be considered[30].

Simultaneously, it was hypothesized that oxidative stress dealt a second "blow" to the liver, and there was a strong correlation between the severity of NASH and the degree of oxidative stress. However, the results from antioxidant treatment are inconsistent in the treatment of NAFLD[93]. Only vitamin E, through inhibiting fatty acid oxidation, is superior to placebo in the treatment of NAFLD[30]. NAFLD/NASH is closely related to the global diabetes and obesity epidemics. Some hypoglycemic drugs, such as metformin, can improve aminotransferase levels, and pioglitazone can

improve steatohepatitis (recommended NASH regimen: pioglitazone or vitamin E combined with high-dose ursodeoxycholic acid)[30]. However, in 2011, it was found that neither vitamin E nor metformin was superior to placebo for the primary outcome of a sustained reduction in alanine aminotransferase levels in children with NAFLD[94]. A 2012 meta-analysis noted that vitamin E improved histological indices after 2 years of use, but increased insulin resistance and plasma triacylglycerol were observed. Long-term use may increase the risk of prostate cancer, and there is a lack of efficacy in reducing liver fibrosis[37]. In patients who did not respond to lifestyle interventions, pioglitazone improved histological disease activity, slowed fibrosis progression, and broadly improved the cardiometabolic endpoints[95]. However, the use of thiazolidinediones has been limited by adverse effects, such as weight gain, fluid retention, increased risk of fractures (particularly in older women), and bladder cancer.

OCA is a semi-synthetic derivative of human cholic acid, which is a natural agonist of farnesoid X receptors. Farnesoid X receptors are nuclear hormone receptors that regulate glycolipid metabolism, can block the conversion of cholesterol to bile acids, increase serum cholesterol concentration, and promote the reverse transport of cholesterol from tissues. A phase 2 trial in 2013[96] and a multicenter randomized controlled trial in 2015 confirmed that OCA was well tolerated in the treatment of NAFLD, increased insulin sensitivity and weight loss, and reduced markers of liver inflammation and fibrosis[31]. An interim analysis of a phase 3 clinical trial in 2019 concluded that 25 mg/day of OCA resulted in significant histological improvements in NASH[34] and unfortunately in itching and a moderate increase in low-density lipoprotein cholesterol.

The 2016 "two-hit" hypothesis became obsolete, and the "multiple blows" hypothesis more accurately explained the pathogenesis of NAFLD, which includes insulin resistance, hormones secreted by adipose tissue, nutritional factors, gut microbiota, and genetic and epigenetic factors[97]. In 2016, Buzzetti *et al*[97] evaluated the association between intestinal dysbiosis and liver fibrosis in human NAFLD and found that animal diets favor the accumulation of branched-chain fatty acids of *Bacteroides*, thereby promoting insulin resistance and increasing the risk of NASH. The diet of agricultural societies is rich in fiber, starch, and plant polysaccharides, which promotes the abundance of *Prevobacterium*, and its abundance decreases with an increase in liver lesions. A gut microbiota analysis provided a theoretical basis for NAFLD patients to regulate diet structure and intestinal microbial preparations[98].

In 2018, probiotics containing endotoxin antibodies and bovine colostrum were evaluated for their efficacy in the treatment of NASH by enhancing brown adipose tissue to burn glucose and ameliorate obesity and glucose abnormalities [37]. Insulin resistance has long been considered an important component of the pathogenesis of NAFLD and worsens as the disease progresses. GLP-1 analogs reduce hepatic steatosis, liver enzyme concentrations, and insulin resistance by inducing insulin secretion and reducing glucagon secretion in a glucose-dependent manner. Armstrong *et al*[36] reported the effects of GLP-1 analogs on liver histology in patients with NASH in a randomized, placebo-controlled trial. Liraglutide was well tolerated in this study, improving several key components of MetS, including weight and glycemic control, and improving NASH histology.

The pathogenesis of NAFLD involves multiple drivers, and no more than 40% of patients in clinical trials have shown benefits from monotherapy, which is likely insufficient to prompt regulatory approval of monotherapy for long-term treatment of NAFLD. Current research favors combination therapies including drug combinations, single-agent searches for multitarget effects[99], and comorbidities in patients with NAFLD. We hypothesize that NAFLD has progressed to become the most common cause of chronic liver disease worldwide[57]. The global burden of NAFLD and NASH is growing rapidly. Future research should focus on accurate non-invasive measurement of biomarkers and clarification of pathogenic pathways, which will facilitate the development and effective evaluation of the efficacy of new drugs. Simultaneously, we continue to actively explore effective treatments, including the development of effective treatments for patients with NASH and prevention methods for individuals at high risk of progression[56].

Research trends and hotspots

Citation bursts represent emerging topics in a particular field of study[100]. Based on the main research content[5,30,97,100,101] of the strongly cited burst references (Table 2), we found that renaming NAFLD to MAFLD[10,102] and the development of semaglutide for NAFLD treatment[103] may represent the main hotspots of current NAFLD treatment research. Both of these topics were cited in 2021. As shown in Table 2, 16 of the 25 citations were reviewed, and high-quality reviews reflected understanding of the disease at a certain time. With the exploration of the pathogenesis of NAFLD, the naming and diagnosis of the disease have been gradually updated and standardized[104], and its treatment strategy has gradually evolved from vitamin E, weight loss, and lifestyle interventions[105] for the management of metabolism-related diseases[106,107].

In addition to citation bursts, keywords can also quickly capture the distribution and evolution of hot topics in our research field. The top keywords from the last 2 years included gut microbiota, traditional Chinese medicine, network pharmacology, OCA, and clinical practice guideline. In keyword trend topic analysis, it was evident that MAFLD, traditional Chinese medicine, gut-liver axis, and gut microbiota were the most frequently discussed keywords. Interestingly, insulin sensitizers such as pioglitazone for the treatment of fatty liver were popular in 2012, reached their peak from 2014 to 2016, and gradually faded in 2020.

Due to the complex pathogenesis of NAFLD, it is challenging to treat[108-110]. Effective treatment requires precise localization based on the patients' phenotype[111] and genetic background[10]. The study of treatment strategies[112,113], epidemiological knowledge[114], pathogenesis[115], and precise diagnosiss[116] (including non-invasive diagnostic techniques) may all be integrated into the consideration of heterogeneity in disease treatment. The current mainstream view is to replace the diagnosis of NAFLD/NASH with MAFLD. Hence, this name change has greatly promoted the transformation of the treatment strategy of the disease. The previous treatment plan mainly included two directions: (1) Correction of insulin resistance and reduction of fat mass with a focus on lifestyle changes for weight loss, including physical activity, diet, insulin sensitizers, and anti-obesity surgery; and (2) prevention/reversal of lipotoxicity-induced

hepatocellular damage by inhibiting lipid peroxidation and oxidative stress or by using anti-inflammatory, anti-apoptotic, or other hepatoprotective agents.

Future therapeutic research on NAFLD/MAFLD should focus on comprehensive therapies tailored to individual patients, considering the above two therapeutic directions, and targeting multitarget and multiaction mechanisms[29]. Research on gut microbiomes and traditional medicine will continue to be a short-term research hotspot. With the official name change to MASLD[117], more emphasis was given to the management of associated comorbid, particularly metabolic abnormalities. OCA, which has entered phase 3 clinical validation, and semaglutide, which is currently under study, are likely to become the first approved drugs for the treatment of NAFLD. A clinical trial called Semaglutide treatment in the real-world for fibrosis due to NAFLD in obesity and type 2 diabetes mellitus (SAMARA) to evaluate the efficacy of semaglutide in improving liver scarring associated with NAFLD is currently ongoing in the US. Once SAMARA is completed, a larger multi-centred global trial may follow.

Advantages and shortcomings: This study had several advantages. First, we systematically analyzed the research on NAFLD/MAFLD treatment through bibliometrics, which provided comprehensive guidance for scholars who are interested in related research. Second, we used three bibliometric tools simultaneously for the survey, hence our data analysis process was objective. Third, bibliometric analysis provided a more complete insight into hot topics and frontiers than traditional reviews.

This study also had some shortcomings. First, the data for this study was extracted from the Web of Science Core Collection database only. Other databases were not searched, and some relevant studies may have been missed. This may increase the risk of bias in the selection of the literature. Second, we filtered studies published in English, which means that non-English language papers were underestimated. In addition, the 2023 publication data was not fully included, resulting in insufficient statistics for 2023. The research directions of NAFLD/MAFLD therapeutic research, cluster analysis, and trend topic analysis may not be comprehensive and may not cover marginal and emerging topics. Last but not least, bibliometric analysis is an analytical method of bibliometrics that focuses on the analysis of measures such as the number of articles issued, cited and quoted, with particular consideration of research countries, institutions and individuals, but pays less attention to the content of the articles themselves; as such, it may not be able to detect and temporally count research for a new type of topic, especially for the beginning stage of a certain research field that may become a hot spot in the future.

CONCLUSION

The treatment of NAFLD has important research value and application prospects, as indicated by the rapidly increasing number of documents. The study of treatments and therapies for NAFLD, particularly NASH, is highly valued worldwide. The leading countries publishing NAFLD research are the United States and China. NAFLD research in China is developing rapidly, with their number of published articles quickly surpassing the United States in recent years. However, it is worth noting that most of the top scientists in the field are from the United States. Conducting an in-depth study of the pathogenesis can foster the development and research of new drugs, while accurate diagnosis, specifically through non-invasive diagnostic technology, contributes to better patient management and efficacy evaluation. The multiple-hit pathogenesis of NAFLD and the renaming of NAFLD to MAFLD require enhanced multidisciplinary and multicenter cooperation. Mechanism studies provide guidance for therapeutic strategies, while clinical application and treatment research represent the transformation of basic research.

ARTICLE HIGHLIGHTS

Research background

Nonalcoholic fatty liver disease (NAFLD) is a chronic disease that threatens the lives of numerous people globally. However, there are limited bibliometric statistical analyses on NAFLD. In this study, we conducted a bibliometric analysis to examine previous research on NAFLD, aiming to identify key contributors and assess the current research status in the field of liver health. Moreover, we identified prospects for future research trends and development.

Research motivation

The progression of NAFLD leads to liver fibrosis and end-stage cirrhosis. However, no treatment has been established. Many mechanistic studies and drug trials have been undertaken for the development of new drugs for NAFLD treatment. In particular, NAFLD was renamed metabolic dysfunction-associated fatty liver disease (MAFLD), and there was a major shift in treatment strategy. It is necessary to understand the knowledge structure of NAFLD through bibliometrics, focusing on research hotspots in order to explore the direction of development in this field.

Research objectives

The treatment of NAFLD has important research value and application prospects. It is anticipated that drugs may become available in the near future. However, no drugs are currently approved. Clinical phase 2b and phase 3 studies have achieved certain efficacy. While trends and hotspots can be clearly studied through bibliometric analysis, clues can reveal possible future therapeutic strategies for scholars in the field.

Research methods

Bibliometric analysis was applied to provide a comprehensive understanding of the knowledge structure of a research field, and visualization analysis was used to visualize the results. Historiography analysis, bursts and cluster analysis, co-occurrence analysis, and trend topic analysis were also utilized to reveal the knowledge structure and research hotspots in this field.

Research results

The bibliometric study identified recent research frontiers and hotspot directions, which will provide a valuable reference for scholars researching treatments for NAFLD. The leading countries publishing NAFLD research are the United States and China. The NAFLD research field in China has developed rapidly in the past 3 years.

Research conclusions

Research on the treatment and therapeutics of NAFLD, especially nonalcoholic steatohepatitis, is highly valued by the global academic community. It is likely that obeticholic acid, which has entered phase 3 clinical validation, and semaglutide, which is currently under study, will become the first approved drugs for the treatment of NAFLD.

Research perspectives

The multiple-hit pathogenesis of NAFLD and the renaming of NAFLD to MAFLD requires enhanced multidisciplinary and multicenter cooperation. More clinical trials are needed to verify the safety and efficacy of drugs and to discover new ones.

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FOOTNOTES

Author contributions: Zhang ZH and Dai JJ conceived and designed the study; Dai JJ performed the research, analyzed the data and wrote the manuscript; Zhang ZH and Zhang YF supervised the study; All authors have read and approved the final manuscript as submitted.

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