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EDITORIAL

From non-alcoholic fatty liver disease to metabolic-associated steatotic liver disease: Rationale and implications for the new terminology

Stephen David Howard Malnick, Doron Zamir

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Abstract

Non-alcoholic fatty liver disease (NAFLD) was the term first used to describe hepatic steatosis in patients with the metabolic syndrome who did not consume excess amounts of alcohol. Alcoholic liver disease (ALD) has many similarities to NAFLD in both pathogenesis and histology. This entity is now the most prevalent chronic liver disease worldwide as a consequence of the epidemic of obesity. Attempts to incorporate the importance of the metabolic syndrome in the development of steatosis resulted in the renaming of NAFLD as metabolic-associated fatty liver disease. This new term, however, has the disadvantage of the use of terms that may be perceived as derogatory. The terms fatty and non-alcoholic have negative connotations in many cultures. In addition, non-alcoholic is not usually a term applicable to pediatric cases of hepatic steatosis. Recently, an international collaborative effort, with participants from 56 countries, after a global consultation process, recommended to change the nomenclature to steatotic liver disease -including metabolic dysfunction- associated steatotic liver disease, metabolic-associated steatohepatitis and metabolic dysfunction-associated ALD. The new terminology is consistent with most of the previously published epidemiological studies and will have a major impact on research into diagnosis, prognosis and treatment.

Key Words: Non-alcoholic fatty liver disease; Steatosis metabolic-associated steatotic liver disease; Nomenclature

Core Tip: Due to the epidemic of obesity, there has been an increase in the prevalence of the metabolic syndrome and hepatic steatosis. The term non-alcoholic fatty liver disease (NAFLD) was given to describe the hepatic manifestation of the metabolic syndrome. Recently the nomenclature has been changed to metabolic dysfunction- associated steatotic liver disease. This removes stigmata associated with the term fatty and include a new entity reflecting both the metabolic syndrome and alcohol as causes of steatosis. These new terms do not alter the inclusion criteria for most of the published studies on NAFLD and will facilitate future studies.

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INTRODUCTION

The excess consumption of alcohol and food was first described in biblical times. Noah, after leaving the ark at the end of the flood, planted a vineyard, consumed too much of the wine he produced and lay naked in his tent (Genesis 9: 21-27). The two daughters of Lot, fearing that there were no men left in the world to provide them with children, made their father inebriated and then became pregnant by him (Genesis 19: 30-38). The Bible also warns against the excess consumption of food and alcohol, "Do not join those who drink too much wine or gorge themselves on meat (Proverbs 23: 19-21). Our failure to comply with these biblical words of advice, has resulted in an epidemic of obesity and alcohol consumption worldwide. As a consequence of this there is now a large increase in obesity and the associated metabolic syndrome resulting in an increase in hepatic steatosis.

STEATOTIC LIVER DISEASE

Steatosis of the liver, or fatty liver, with macrovesicular steatosis and Mallory bodies was described by Addison in 1836 [1]. Connor[2] and Chaikoff *et al*[3] found a link between steatosis and either alcohol consumption or diabetes in 1938. In the 1980s Ludwig *et al*[4] described patients who had a similar feature but denied alcohol consumption. The initial nomenclature was non-alcoholic fatty liver disease (NAFLD). The metabolic syndrome is based on the presence of hypertension, impaired glucose tolerance, elevated triglyceride levels, low high-density lipoprotein and increased abdominal circumference, with some differences between different ethnic groups[5].

Subsequently, NAFLD was noted to be the hepatic manifestation of the metabolic syndrome[6]. NAFLD is now the major cause of chronic liver disease worldwide and one of the main indications for liver transplantation[7]. The definition of non-alcoholic is 30 G per day for a male and 20 g per day for a female. However, many patients who are considered to have NAFLD do in fact have significant alcohol consumption[8].

The pathogenesis of steatotic liver disease involves the development of a dysbiosis, resulting in endogenous alcohol production, increased intestinal permeability, the production of endotoxins, which reach the liver by way of the hepatic portal vein. The insulin resistance that is associated with the metabolic syndrome results in an increase in intrahepatic fat, increased gluconeogenesis and increased free fatty acid levels[9]. In both alcoholic and non-alcoholic steatosis the liver injury is in the parenchyma. Progressive liver injury results in an evolution from simple steatosis, to steatohepatitis, fibrosis and cirrhosis. Lifestyle modifications can decrease and even reverse the disease progression[9].

As a result of this a new nomenclature was proposed: metabolic dysfunction-associated fatty liver disease (MAFLD) [10]. This term was felt to reflect the importance of metabolic factors in the etiology of MAFLD and to assist in providing a better understanding of this entity by patients.

There are, however, problems with this nomenclature as well. For example, not all cases of MAFLD are seen in patients with obesity. This is reflected in the term lean NAFLD[11]. In many of these patients a history of drinking sweet sugary beverages or soda with artificial sweeteners may be present. Some patients with cirrhotic liver disease suspected to be caused by MAFLD may not have fat in the liver when assessed by biopsy. The change to MAFLD has been suggested to have been premature[12].

Treatment for MAFLD is mainly based on lifestyle changes. Clinical trials with anti-obesity treatments, lipid-lowering agents and insulin sensitizers in the main were not successful. There is concern that concentrating just on metabolic factors could impede the development of other therapeutic mechanisms[13]. In addition, in many trials the end-point selected was a decrease in the NASH score and not in the more important degree of fibrosis or clinical end-points including cardiovascular events or death[14]. Other factors affecting the development of steatosis include the intestinal microbiome, genetic factors including PNPLA3 mutations[15], and sarcopenia.

Recently, the name has been revised once more. A global consultation process using the well-established Delphi technique was performed from 2020 to 2023[16-18]. This involved 236 participants from 56 countries. The terms non-alcoholic and fatty were rejected for use since they were associated with a stigma in terms of patient understanding. Steatosis was chosen to replace “fatty”. The new nomenclature to be recommended was metabolic dysfunction-associated steatosis liver disease (MASLD).

The presence of at least one of the five cardiometabolic risk factors is essential to make the diagnosis. The previous diagnosis of non-alcoholic steatohepatitis (NASH) was recommended to be replaced by metabolic dysfunction associated steatohepatitis (MASH). Cryptogenic steatotic liver disease was coined to cover those cases with no clear cause.

Metabolic dysfunction-associated ALD (MetALD) is a new term to describe those cases with excessive alcohol consumption. This entity has not been previously defined and will now have to be investigated further in appropriate clinical trials.

CONSEQUENCES OF THE CHANGE IN NOMENCLATURE

The change in nomenclature preserves the existing data on natural history of the disease, biomarkers and clinical trials, which is very important. An analysis of the European NAFLD registry cohort has found that 98% of the participants would fulfill the new criteria for MASLD[19]. In addition, a study of 1333 patients from Hong Kong with NAFLD found that only 4 (0.3%) of patients did not meet the new MASLD criteria[20].

The diagnosis of MASLD has an advantage of removing the stigma associated with the term fatty, and patient advocacy groups were consulted as part of the process. The importance of this effect differs between different cultures [21].

Steatotic liver disease reflects the range of causes of hepatic steatosis and permits characterization of fibrotic severity – for example MASH with stage 3 fibrosis. It is to be noted that disease stage and severity are not changed in this new definition and will still be valid with the increasing use of non-invasive testing for determining these parameters.

There is a strong similarity between the metabolic criteria for diagnosing MASLD and those previously suggested for MAFLD. The new definition of MASLD is based on simple and accessible clinical criteria and biological measurements without the need for specialized testing for insulin resistance such as homeostasis model assessment of insulin resistance. There may be a place for subsequent testing in the minority of patients with hepatic steatosis in the absence of cardiometabolic risk factors.

The new classification also enables subgroups to be included within the umbrella term steatotic liver disease. This includes medications and rare genetic diseases in children. The newly defined term MetALD reflects the importance of alcohol consumption in the development of steatosis, even in the absence of metabolic factors.

CONCLUSION

This new nomenclature for the most common liver disease worldwide is important for developing a cohesive definition between groups worldwide which will enable extensive data collection with implications for the development of personalized medical therapeutic and enabling incorporation of existing data sets. The public health implications of this are likely to be very significant.

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Immunological crossroads: The intriguing dance between hepatitis C and autoimmune hepatitis

Jonathan Soldera

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Abstract

Delving into the immunological crossroads of liver diseases, this editorial explores the dynamic interplay between hepatitis C virus (HCV) and autoimmune hepatitis (AIH). While HCV primarily manifests as a viral infection impacting the liver, previous studies unveil a captivating connection between HCV and the emergence of AIH. The dance of the immune system in response to HCV appears to set the stage for an intriguing phenomenon—an aberrant autoimmune response leading to the onset of AIH. Evidence suggests a heightened presence of autoimmune markers in individuals with chronic HCV infection, hinting at a potential overlap between viral and autoimmune liver diseases. Navigating the intricate terrain of viral replication, immune response dynamics, and genetic predisposition, this editorial adds a layer of complexity to our understanding of the relationship between HCV and AIH. In this immunological crossroads, we aim to unearth insights into the complex interplay, using a compelling case where AIH and primary sclerosing cholangitis overlapped following HCV treatment with direct-acting antivirals as background.

Key Words: Liver diseases; Hepatitis C virus; Autoimmune hepatitis; Primary sclerosing cholangitis; Inflammatory bowel disease

Core Tip: This editorial delves into the dynamic interplay between hepatitis C virus (HCV) and autoimmune hepatitis (AIH), tracing the historical progression from the era of non-A non-B hepatitis to the discovery of HCV and the advent of direct-acting antiviralagents (DAAs). A recent case highlights the emergence of an overlap between AIH and primary sclerosing cholangitisfollowing successful DAA treatment for HCV. The case underscores the potential risks associated with rapid viral clearance and emphasizes the need for vigilance regarding the development of autoimmune liver diseases post-DAA treatment. This complex relationship warrants further exploration for refined treatment approaches.

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INTRODUCTION

The landscape of hepatitis posed a real challenge in the past, persisting into contemporary times, as cases of unexplained jaundice perplexed clinicians. The turning point occurred approximately five and a half decades ago when researchers such as Holland, Schmidt, Purcell, Walsh, and Alter delved into the study of "transfusion-associated hepatitis" in 1969[1]. It would take another two decades until the identification of the infectious agent, hepatitis C virus (HCV), in 1989[2]. Reflecting on the hepatology field during the era when non-A non-B hepatitis dominated discussions, instances of unexplained post-transfusion hepatitis were commonplace and intrigued physicians. Furthermore, numerous cases of "nosocomial" hepatitis[3] and cryptogenic hepatitis added complexity, compounded by a lack of specific diagnostic tests. Importantly, this period witnessed the misdiagnosis of many patients with acute and chronic hepatitis as having autoimmune hepatitis (AIH) prior to the identification of HCV[4,5]. The journey to unravel this intricate problem spanned 39-14 years from the identification of non-A non-B hepatitis to the discovery of HCV, and an additional 25 years from the discovery of HCV in 1989 to the approval of Sofosbuvir in 2013, one of the most prescribed and most efficacious direct-acting antiviral agents (DAAs) for HCV treatment[6]. In retrospect, these 39 years reveal a marvelous progress for the medical sciences, from the early clinical and epidemiologic studies by Alter and others to the present-day, marked by the creative pharmacological approach involving the design of DAAs inhibiting HCV infection through the blockade of viral assembly and replication[6], and a global initiative, led by the member countries of the World Health Organization, to eliminate HCV *via* treatment and prevention by the year 2030[7].

Intriguingly, the progress made from those early clinical and epidemiologic studies has now led us to a new chapter in the dance between HCV and AIH. I have read with great attention and interest a recent case reported by Morihisa *et al*[8], in which the authors present the case of a 74-year-old woman with chronic HCV infection who, after successful DAA treatment, developed an overlap of AIH and primary sclerosing cholangitis (PSC). This case stands out as the first reported instance of the overlap of AIH and PSC following DAA treatment for HCV, highlighting the intricate interplay between viral clearance and subsequent autoimmune liver diseases.

Moreover, this case underscores the potential risks associated with the restoration of host immunity following rapid viral clearance, emphasizing the need to consider the development of autoimmune liver diseases after DAA treatment[8]. While DAAs have become a mainstay in HCV treatment due to their high efficacy and minimal adverse events, cases such as this urge us to be vigilant about potential consequences. It is worth noting that this case is not isolated. Other reports describe instances of AIH occurring after HCV treatment with DAAs[9-12]. These cases collectively suggest a complex relationship between HCV, DAA treatment, and the subsequent development of autoimmune liver diseases.

The intricate interplay at the crossroads of HCV and AIH forms a complex and intriguing dance. Long-standing beliefs about a dynamic interconnection between these seemingly distinct entities raise questions about possible overlaps, therapeutic implications, and prognostic significance.

One compelling aspect focuses on the potential shared pathways in treating both HCV and AIH. Published data has highlighted that successful DAA treatment can bring about comprehensive improvement in patients concurrently dealing with HCV[13-16]. Furthermore, the presence of autoimmune markers in HCV infection has been extensively studied, with a prevalence above 10% of serological markers of autoimmunity in chronic hepatitis C patients[17-20], which could be a marker of more severe chronic hepatitis C[21]. This raises more questions about the accurate delineation between HCV-related and autoimmune-related liver pathology, since interface hepatitis is present in both HCV and AIH[22,23]. An intriguing discovery emerges from studies indicating a positive response to corticosteroid and ursodeoxycholic acid therapy in patients presenting both HCV and positive AIH markers. These findings challenge conventional expectations surrounding the role of these medications in the context of HCV infection[24,25]. There is more to the dynamic nature of HCV and its potential to trigger autoimmune responses, as other triggers for AIH after HCV treatment have been described[26,27].

As we navigate through the discussion the case published by Morihisa *et al*[8], it is crucial to reiterate the link between PSC and inflammatory bowel diseases (IBD), particularly emphasizing the connection with Ulcerative Colitis[28]. Even in overlap syndromes involving AIH and PSC, a systematic review has reported a prevalence of IBD to be 45.3%[29]. While PSC is a severe condition known for its poor response to treatment, there appears to be an association of the overlap syndrome with a comparatively low mortality rate and a favorable response to treatment[29]. While the reported case

discussed in this editorial does not explicitly mention the performance of a colonoscopy, it is crucial to emphasize to readers that, in accordance with the EASL guidelines for treating PSC, the undertaking of a colonoscopy with random biopsies is paramount. This recommendation holds especially true for patients without a known history of inflammatory bowel disease[30].

CONCLUSION

The intricate interplay between HCV and AIH unveils a multifaceted relationship that extends beyond conventional classifications. The presence of autoantibodies in chronic hepatitis C challenges our understanding of immune responses in viral infections and prompts a reevaluation of diagnostic and therapeutic paradigms. While studies offer glimpses into the complex web of interactions, numerous questions persist, beckoning researchers to unravel the mysteries that shroud this intriguing intersection of liver diseases. The nuances of the interactions between AIH and HCV necessitate continued research and clinical awareness to enhance our understanding and refine treatment approaches. Additionally, liver specialists could consider screening for autoantibodies and immunoglobulin G levels before and during DAA therapy to monitor for potential immune-related diseases and facilitate timely diagnosis and treatment.

FOOTNOTES

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EDITORIAL

Sarcopenia and metabolic dysfunction associated steatotic liver disease: Time to address both

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Abstract

Sarcopenia and metabolic dysfunction associated steatotic liver disease (MASLD) are closely intertwined. Sarcopenia, traditionally a disease of the older adult and chronic disease population, has been closely studied as one of the pathophysiologic conditions at play in the development of MASLD. They share similar risk factors of insulin resistance and physical inactivity. Given similar pathophysiology along the liver-muscle axis, sarcopenia has been studied as a risk factor for MASLD, and vice versa. Current research suggests a bidirectional relationship. Given the chronicity of MASLD as a chronic inflammatory liver disease, it can break down muscle mass and lead to sarcopenia, while sarcopenia promotes intramuscular lipid accumulation that releases cytokines that can aggravate inflammation in the liver. However, for the longest time, a lack of consensus definition for MASLD and sarcopenia made it difficult to study their relationship and outcomes. A recent nomenclature update to diagnosing MASLD has made it easier for researchers to identify cohorts for study. However, no gold standard technique to measure muscle mass or consensus sarcopenia definition has been identified yet. Future studies are needed to reach a consensus and reduce diagnostic variation. With similar pathophysiology and shared risk factors between the two diseases, future research may also identify potential therapeutic targets along the liver-muscle axis that would benefit both sarcopenia and MASLD in order to maximize their outcomes.

Key Words: Sarcopenia; Steatotic liver disease; Metabolic dysfunction; Insulin resistance; Liver-muscle axis

Core Tip: Sarcopenia and metabolic dysfunction associated steatotic liver disease (MASLD) share a bidirectional relationship along the liver-muscle axis. With similar pathophysiology and shared risk factors, MASLD is a risk factor for sarcopenia, and vice versa. Early identification and adequate diagnosis are important. However, lack of consensus definition made it difficult to research outcomes. With the recently updated consensus definition for MASLD, researchers may now better identify proper cohorts for study. Consensus on gold standard techniques and muscle mass cutoffs to define sarcopenia are still needed. Future research may identify potential therapeutic targets along the shared liver-muscle axis that would improve outcomes for both sarcopenia and MASLD.

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INTRODUCTION

With increasing incidence of metabolic dysfunction associated steatotic liver disease (MASLD) and the increasing age of the population, sarcopenia has been closely studied as one of the pathophysiologic conditions at play in the development and progression of MASLD. A comprehensive review published by Viswanath *et al*[1] closely examines the relationship between these two diagnoses. It highlights numerous shared risk factors, such as elevated lipid profile, physical inactivity, and diabetes mellitus, that suggest a bidirectional relationship between MASLD and sarcopenia. It also proposes pathogenic pathways along the liver-muscle axis that may serve as potential therapeutic targets for further research, such as insulin resistance, hormonal changes, and reduced levels of myokines.

However, for many years, lack of consensus definitions for MASLD and sarcopenia has made it challenging to research their relationship, their outcomes, and to develop targeted therapies. Prior to 2020, the spectrum of steatotic liver disease had been under the dichotomy of "nonalcoholic" and "alcoholic" fatty liver disease. Consensus groups began to meet to develop new nomenclature that would not rely on exclusionary confounder terms and use of stigmatizing language. There were many pros and cons to shifting from a binary, more widely familiar, potentially stigmatizing nomenclature to a more comprehensive, inclusive, less stigmatizing nomenclature to diagnose steatotic liver disease[2]. Ultimately in December 2023, the global Nonalcoholic Fatty Liver Disease Nomenclature Consensus Group published a new nomenclature that better defined criteria for steatotic liver disease, including MASLD, to facilitate diagnose and awareness of the disease[3]. MASLD has many shared diagnostic criteria with diabetes and cardiovascular disease so this new consensus definition better captures the metabolic component in an affirmative nonstigmatising way.

Sarcopenia is classically defined as the loss of skeletal muscle mass and muscle function, which can be part of the physiologic aging process, but can also be accelerated by malnutrition, low physical activity, or inflammatory conditions [4,5]. It is associated with negative outcomes including frailty, chronic disease, limited mobility, and premature mortality [4]. Just as MASLD is expected to increase in parallel to the rising prevalence of obesity globally, sarcopenia is expected to increase as the older adult population grows. Given the chronicity of MASLD as a chronic inflammatory liver disease, researchers have been interested in the impact of MASLD on sarcopenia, and vice versa.

SHARED PATHOPHYSIOLOGY

Both MASLD and sarcopenia are closely intertwined, as Viswanath *et al*[1] summarizes. Pathophysiologically, insulin resistance is believed to play a common role in the development of both diseases. Insulin typically targets the skeletal muscle and can promote muscle protein synthesis while inhibiting muscle protein catabolism[6]. Therefore, insulin resistance can induce muscle attenuation leading to sarcopenia by decreasing protein synthesis in skeletal muscle, increasing protein catabolism, or stimulating skeletal muscle autophagy[7]. Insulin resistance in adipose tissue increases *de novo* lipogenesis and increases release of free fatty acids that generate hepatic fat accumulation and a proinflammatory environment: A critical pathogenesis pathway for MASLD[8]. Myosteatosis, infiltration of fat into skeletal muscle, on the other hand, increases in insulin resistance and has been associated with early metabolic-associated steatohepatitis (MASH)prior to the onset of sarcopenia[9]. Fat accumulation in muscle tissue also promotes a proinflammatory cascade and oxidative stress, leading to impaired insulin signaling and muscle atrophy. The decreased muscle mass promotes insulin resistance, further exacerbating muscle atrophy. In investigating the muscle-liver-adipose tissue pathway, it remains a question to which disease ultimately comes first: Whether sarcopenia is a consequence of MASLD or a part of the disease natural history, as summarized in Figure 1.

SARCOPENIA AS A RISK FACTOR FOR MASLD

Sarcopenia is an independent risk factor for steatohepatitis and fibrosis[10,11]. One group measured the appendicular

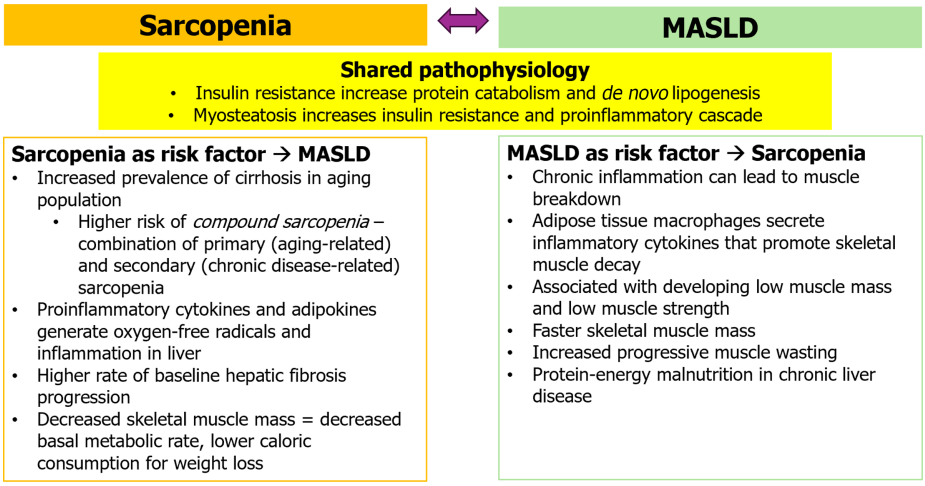

Figure 1 Summary of relationship between sarcopenia and metabolic dysfunction associated steatotic liver disease. MASLD: Metabolic dysfunction associated steatotic liver disease.

skeletal muscle mass in a biopsy-proven MASLD cohort and found that the prevalence of significant fibrosis (> or = F2) was higher in patients with sarcopenia than in those without[12]. Patients with sarcopenia at baseline have a higher rate of hepatic fibrosis progression[13]. Conversely, an increase in skeletal muscle mass has been shown to exert a positive effect in slowing down the progression of MASLD[14]. This effect may be due to increase in skeletal muscle mass causing elevated basal metabolic rate and improvement of insulin resistance[15]. Another study found that low skeletal muscle mass index and central obesity were associated with increased risk of MASLD and cardiovascular disease[16]. The proposed pathophysiological mechanism is through hormonal and cytokine changes found in sarcopenia. Intramuscular lipid accumulation can cause release of proinflammatory cytokines and adipokines that generate oxygen-free radicals and aggravate inflammation in the liver[12,17]. Among older adults with cirrhosis, the combination of primary (aging-related) and secondary (chronic disease-related) sarcopenia, also called compound sarcopenia, is associated with higher odds of mortality and worse outcomes than those without compound sarcopenia[18].

MASLD AS A RISK FACTOR FOR SARCOPENIA

There are different types of sarcopenia. Primary sarcopenia is caused solely by aging. Secondary sarcopenia is caused by other factors, such as chronic disease, physical inactivity, or poor or malnutrition. MASLD is a chronic liver disease that can cause chronic inflammation, that can lead to muscle breakdown, and the development of sarcopenia[19]. Adipose tissue macrophages are thought to secrete inflammatory cytokines, which then promote protein decay in skeletal muscle [5]. The presence of MASLD has been associated with increased risk of developing low muscle mass and low muscle strength, with greater impact on low muscle strength than on low muscle mass[20]. One cohort of 52815 patients found that patients with MASLD had faster skeletal muscle mass loss than those without MASLD[21]. There is also increased risk of progressive muscle wasting in patients with MASH even before cirrhosis, and that this sarcopenia worsens with progression to cirrhosis[22]. Patients with advanced cirrhosis also suffer from protein-energy malnutrition, which contributes to risk of developing sarcopenia[23]. Overall, sarcopenia in patients with cirrhosis is a poor prognostic risk factor for cirrhosis complications and higher mortality[24,25]. Studies have shown that sarcopenia can independently increase overall mortality and cardiac mortality in patients with MASLD[26,27].

CHALLENGES OF DIAGNOSIS

A significant challenge to further clarifying research in the relationship between sarcopenia and MASLD has been finding a consensus for the definition of sarcopenia[28,29]. There are various consensus groups who have published different operational cutoffs for low muscle mass, low muscle strength, and low physical performance, making it difficult to study relevant outcomes. One of the first sarcopenia definitions developed by the European Working Group on Sarcopenia (EWGSOP) recently revised their clinical algorithm to combine low muscle mass and low muscle strength to confirm diagnosis, with physical performance as a marker of severity[30]. This update was done to promote early detection of treatment of sarcopenia in hopes of preventing or delaying adverse health outcomes. However, there remains limited agreement between cutoff measures for each component, as well as diagnostic variation on how to best measure skeletal muscle mass. Computed tomography and magnetic resonance imaging can evaluate for whole-body muscle, but no standardized imaging protocol exists, and image acquisition settings may vary between studies[31,32]. Dual-energy X-ray absorptiometry can assess lean muscle mass, particular appendicular skeletal muscle mass, but may have limited availability. Bioelectrical impedance analysis is noninvasive, convenient to use, but may not be as high yield for skeletal

muscle mass, as it measures different body compartments, with limitations in obese or cachectic patients from body mass disproportion[33]. Future studies will be needed to assess criterion validity in order to find the best consensus definition for sarcopenia in both clinical and research settings.

CHALLENGES OF TREATMENT AND POTENTIAL THERAPY TARGETS

Given the close relationship between MASLD and sarcopenia, a multipronged approach to management is important. The mainstay therapy for MASLD is weight loss, which can be achieved through diet and regular physical activity, and improve hepatic steatosis and liver stiffness[34-36]. The treatment for sarcopenia similarly involves nutritional supplementation and regular physical activity to prevent deterioration of muscle mass, maintain muscle strength, and mitigate mobility disability[37,38]. Both MASLD and sarcopenia can benefit from physical activity. However, its impact may be limited in later stages of disease due to increased frailty[39]. One retrospective study found that moderate to vigorous physical activity (exercise average 4-5 times/wk, 555 min/wk) was associated with less risk for sarcopenia based on the measurement of higher skeletal muscle index and hand grip strength[40]. Prospective studies investigating the direct benefit of physical activity on progressive frailty, decline in muscle function, or muscle mass in the late stages of MASLD and sarcopenia are needed in the future.

Based on current pathophysiological knowledge, potential therapy targets along the liver-muscle axis may include testosterone supplementation, strength resistance training, and high protein diet. Testosterone is an anabolic hormone that helps maintain muscle mass and function, and can decrease with normal aging[41]. Testosterone can induce muscle fiber hypertrophy and may be protective against skeletal muscle catabolism by suppressing accumulation of inflammatory transcription factors[42]. There is also an association between MASLD and lower testosterone, as men with MASLD on average have lower testosterone than men without MASLD[43]. Current studies have suggested that testosterone can have a dose-dependent effect to achieve clinically significant gains in muscle mass without adversely affecting cardiovascular risk, but it varies by subject, dosage, and route of administration[41,44,45]. Many current studies were done in healthy young men with low to low-normal testosterone levels, so testosterone may not have as similar effect in frail older adults or patients with chronic illness and warrants further investigation for sarcopenia-directed therapy.

Resistance strength training has been suggested as an alternative therapy target. Physiologically, heavy resistance exercise can trigger the release of various anabolic hormones, including testosterone[46]. Resistance training typically involves repeated, systematic, regular exercises aimed at improving a patient's physical ability and muscle strength, with both upper and lower body exercises[47-49]. However, resources and engagement may be limited for patients in the community, with a large degree of variability in the implementation of physical activity programs, making it difficult to maintain any muscle mass or strength gains. Further high-quality clinical trials are needed to develop optimal resistant training parameters.

While optimal diets for MASLD are still being explored, high protein intake (1.0-1.2 g/kg/d) has been recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) for older adults to potentially counteract sarcopenia[50]. Aging patients typically develop anabolic resistance, requiring greater amounts of protein to stimulate the same response of muscle protein synthesis compared to younger adults. Therefore, it has been suggested that if anabolic resistance can be overcome by diet, muscle atrophy may be prevented. However, studies have shown variable effect based on type of protein (plant *vs* animal), with some even suggesting that higher protein intake is associated with sarcopenia rather than protective[51,52]. One longitudinal study showed that patients had increased risk of sarcopenia despite adequate protein intake[53]. High protein diet also has detrimental effects to other diseases that affect older adults, including coronary artery disease, bone and calcium metabolism, and cancer, so must be implemented with caution[54]. Additional research is needed to explore the effect of different protein sources and clarify optimal protein intake requirements.

Despite ongoing research on the promising effects of these therapies, further research is needed to validate these therapeutic interventions and their long-term effects on sarcopenia and MASLD.

CONCLUSION

Both sarcopenia and MASLD are associated with significant health risks, as pointed out by Viswanath *et al*[1] in their review. Therefore, early identification and adequate diagnosis are important. The recently updated consensus definition for MASLD may allow researchers to better identify proper cohorts for study, while gold standard techniques to measure muscle mass are needed in consensus. Given similar pathophysiology and shared risk factors, future research may optimize treatment strategies to target both of these complex diagnoses to maximize the outcome.

FOOTNOTES

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EDITORIAL

Importance of the gut microbiota in the gut-liver axis in normal and liver disease

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Abstract

The gut microbiota is of growing interest to clinicians and researchers. This is because there is a growing understanding that the gut microbiota performs many different functions, including involvement in metabolic and immune processes that are systemic in nature. The liver, with its important role in detoxifying and metabolizing products from the gut, is at the forefront of interactions with the gut microbiota. Many details of these interactions are not yet known to clinicians and researchers, but there is growing evidence that normal gut microbiota function is important for liver health. At the same time, factors affecting the gut microbiota, including nutrition or medications, may also have an effect through the gut-liver axis.

Key Words: Gut microbiota; Liver; Gut-liver axis; Immunity; Non-alcoholic fatty liver disease

Core Tip: The gut microbiota plays an important immune and metabolic role in the body both under physiologic conditions and in the development of various liver diseases. The gut microbiota is involved in the production of various substances such as short-chain fatty acids, which play an important role in linking the gut to other organs. The composition of the gut microbiota may change in various liver diseases, and this relationship is two-way.

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INTRODUCTION

The gut microbiota is a subject of increasing interest to clinicians and researchers due to the growing body of new data on its important metabolic and immune roles in the organism[1-4]. The human gastrointestinal tract is continuously colonized by a vast array of microorganisms, including bacteria, archaea, fungi, and viruses, and the number of these microorganisms is estimated to be comparable to or significantly greater than the number of human cells[5-7]. It is estimated that the microbiota's collective genome may contain 100 times more genes than the human genome[8-10]. Of course, these microorganisms are closely related to the human body. The gut microbiota population is known to be dominated by anaerobic bacteria, with 90% of the gut microbiota consisting of Bacteroidetes (Gram-negative) and Firmicutes (Gram-positive), followed by Actinobacteria (Gram-positive) and Proteobacteria (Gram-negative)[11,12].

The relationship between macroorganisms and microbiota has deep evolutionary roots and is well-known in animals. This symbiotic relationship serves many important physiological purposes, including the participation of bacteria in the digestion of food substances that are inaccessible to macroorganism digestive enzymes, as well as the synthesis of substances required by the macroorganism and participation in immune mechanisms. The gut microbiota produces metabolites such as short-chain fatty acids (SCFAs)[13,14]. The three primary SCFAs are acetate, propionate, and butyrate. These SCFAs are formed from dietary fibers such as resistant starch, cellulose, and pectin. SCFAs serve as an energy source for the intestinal epithelium and also enter the bloodstream through the portal vein, where they participate in various processes, including immune mechanisms. SCFA play a role in regulating immune cell activation in various organs, including the liver[13]. Butyrate, for instance, promotes the functional maturation of liver-resident natural killer cells in the liver by acting through hepatocytes and Kupffer cells[15]. Furthermore, the gut microbiota has been linked to liver regeneration[16]. During hepatectomy, the gut microbiota is associated with hepatocyte proliferation through the activity of CD1d-dependent natural T-killer and Kupffer cells[17,18].

The gut microbiome of mammals and humans is highly diverse, consisting of thousands of known species. Dietary patterns significantly impact this diversity. Studies have demonstrated that alterations in dietary patterns, both in humans and animals, can modify the composition of the gut microbiota. Research has shown that mice fed a Western diet experienced a decrease in Bacteroidetes and an increase in Firmicutes in their gut compared to normal mice[19,20]. Similarly, captive great apes have a different microbiota profile compared to their wild counterparts, which is similar to the microbiome of humans from non-urbanized societies. Furthermore, obesity has been linked to changes in the composition of the gut microbiota. The proportion of Bacteroidetes has been found to decrease in obese individuals compared to those who are lean. However, this proportion has been shown to increase with weight loss on two types of low-calorie diets[21]. It is worth noting that bariatric surgery also impacts the composition of the gut microbiota, which is an area of increasing interest[22].

The human body has mechanisms that promote microbial colonization of the gut and mechanisms to control this microbial population. The intestinal microbiome is a complex regulated system that is closely related to the human body. For example, goblet cells produce mucus that contains mucins and has two layers that separate bacteria from underlying intestinal epithelial cells[11]. The layers have a comparable protein composition, which includes the gel-forming mucin Muc2 as a significant structural component[23]. Additionally, the inner mucus layer is tightly packed, immobile relative to the epithelium, and free of bacteria, while the outer mucus layer is mobile, colonized by bacteria, and has a larger volume due to proteolytic cleavage of Muc2 mucin. The spatial separation of bacteria and the intestinal epithelium is maintained by antibacterial substances secreted by the epithelium and secretory immunoglobulin A[24,25]. The composition of the colonic mucosal barrier is determined by the microbiota[26]. Symbiotic bacteria use various strategies to circumvent the intestinal immune system, including actively suppressing epithelial proinflammatory signaling pathways[25].

The gut microbiota has a close bidirectional relationship with the liver[27]. The liver is connected to the gut through the portal vein system, which carries blood from the gastrointestinal tract. This blood contains various substances, including bacterial lipopolysaccharide (LPS), that can significantly impact the liver's structure and function. The liver can also affect the gut microbiota through the enterohepatic circulation of bile acids[28]. Therefore, the gut-liver axis is a bidirectional communication and its disruption has important clinical implications. Alcoholic liver disease (ALD), non-ALD, and biliary tract diseases have been linked to changes in the gut microbiome. Alterations in the gut microbiome can affect the development and severity of liver steatosis, inflammation, and fibrosis through multiple interactions with immune and other cells[29].

In recent years, there has been an increased interest in the role of gut microbiota as a key factor in the development of liver steatosis. Metabolic-associated fat liver disease or non-alcoholic fatty liver disease (NAFLD) is an important medical problem and is of growing interest to clinicians and researchers[30,31]. NAFLD is characterized by the accumulation of excessive fat in the liver (steatosis) in patients who do not consume significant amounts of alcohol. The disease is thought to be caused by a complex interplay of internal and external factors, including nutritional disorders and disturbances in the gut microbiota structure. Changes in the gut microbiota have been associated with NAFLD and are dependent on the clinical stages of the disease[32,33]. The study found that disease progression corresponded with a decrease in microbiota diversity and an increase in Gram-negative bacteria, and a decrease in Gram-positive bacteria[33-35]. The development of a pro-inflammatory and metabolically toxic gut microbiota environment leads to disruption of the intestinal barrier, which increases the liver's exposure to negative nutritional and microbiota-related factors[33,36]. Increased permeability of the intestinal barrier to substances is a contributing factor in metabolic dysfunction-associated steatotic liver disease patients with fibrosis[37]. It is worth noting that bacterial DNAs were detected in the liver tissue of patients with NAFLD [38]. In patients with morbid obesity, Bacteroidetes and Firmicutes were more prevalent, while in patients without morbid obesity, mainly Proteobacteria were present. Additionally, the presence of Proteobacteria DNA was associated with lobular and portal inflammation scores[39].

Patients with ALD have increased intestinal permeability and elevated systemic levels of gut-derived microbial products[40]. Bacterial LPS contributes to inflammation in ALD through activation of Toll-like receptor 4[41,42].

The gut microbiota plays an important role in the progression of liver cirrhosis and the development of disease decompensation, including through bacterial translocation and inflammation[20,43]. The use of antibiotics is considered a treatment for complications of liver cirrhosis[20,44].

Dysbiosis of gut microbiota is also known to be associated with cholelithiasis[45]. Progression of cholelithiasis is characterized by changes in the bacterial community of bile, including a decrease in the number of Proteobacteria and an increase in the number of Firmicutes and Bacteroidota in groups of patients compared to healthy controls[46]. Bile acids may promote the growth of some bacteria that metabolize bile acids and at the same time inhibit the growth of other bacteria sensitive to bile[47]. In turn, the gut microbiota regulates the expression of several of the enzymes involved in bile acid formation, including CYP7A1, CYP7B1, and CYP27A1[47,48].

Thus, a growing body of evidence demonstrates the important role of the gut microbiota, making it a therapeutic target [49]. As diet is one of the key factors influencing changes in gut microbiota composition, dietary modification is an important component of the treatment of many diseases. In addition, exercise has a favorable effect on the gut microbiota profile[50-52]. There is a growing number of studies on fecal transplants, but their therapeutic potential is still unclear [53]. Studies on the potential use of probiotics for the prevention and treatment of liver disease are also of interest[54-56]. In general, despite a fairly large body of accumulated knowledge, there is currently no sufficiently convincing data on effective effects on the gut microbiota to achieve the goals of treatment and prevention of liver disease.

CONCLUSION

Thus, the gut microbiota is an important metabolic and immune "organ" that has close bidirectional links to liver function. The gut microbiota has extensive biological functions, many details of which are only beginning to be understood. Active study of the function of the gut microbiota in recent years has led to a better understanding of its role in the development and progression of various liver and biliary diseases. Obviously, studying only changes in the structure of the microbiota in various diseases and conditions is not able to answer all questions, since data on the ratio of bacteria do not provide an understanding of their functional activity. In addition, it may also be relevant that bacteria may change their activity in response to patients taking different medications. In this regard, pharmacomicrobiomics represents a promising new area of research[57,58]. It should also be noted that the therapeutic potential of the gut microbiota has limited application to date and is mainly related to dietary modification. In this regard, new studies of the gut microbiota-liver axis may be useful to improve approaches to the treatment of liver diseases.

FOOTNOTES

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EDITORIAL

Cold ischemia time in liver transplantation: An overview

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Abstract

The standard approach to organ preservation in liver transplantation is by static cold storage and the time between the cross-clamping of a graft in a donor and its reperfusion in the recipient is defined as cold ischemia time (CIT). This simple definition reveals a multifactorial time frame that depends on donor hepatectomy time, transit time, and recipient surgery time, and is one of the most important donor-related risk factors which may influence the graft and recipient's survival. Recently, the growing demand for the use of marginal liver grafts has prompted scientific exploration to analyze ischemia time factors and develop different organ preservation strategies. This review details the CIT definition and analyzes its different factors. It also explores the most recent strategies developed to implement each timestamp of CIT and to protect the graft from ischemic injury.

Key Words: Cold ischemia time; Liver transplantation; Organ donation; Donation after cardiac death; Warm ischemia time; Machine perfusion

Core Tip: Many variables affect liver transplantation outcomes. Among these variables, cold ischemia time (CIT), defined as the time from the cold flushing of the donor organ until the graft is removed from ice to be implanted into the recipient, is the one of the most important and is incorporated into many predictive scoring systems. CIT is a multifactorial variable that depends on donor hepatectomy time, transit time and recipient surgery time and can only be calculated retrospectively.

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INTRODUCTION

The outcome of liver transplantation (LT) has markedly improved in recent decades with patient survival rates of > 90%, 85% and 70% at 1-, 5-, and 10-years post-LT[1,2]. Such good results have been achieved thanks to improvements in the management of many variables that affect LT outcomes. Among these variables, cold ischemia time (CIT), defined as the time from the cold flushing of the donor organ until the graft is removed from ice to be implanted into the recipient, is the one of the most important factors that affects organ and patient survival. Indeed, several reports have documented that prolonged CIT is an independent risk factor for the development of delayed graft function and primary non-function[3]. Moreover, many of the predictive models proposed to estimate survival after LT incorporate CIT into their scoring systems[4-6]. However, CIT is a multifactorial variable that depends on donor hepatectomy time, transit time and recipient surgery time and can only be calculated retrospectively. Therefore, to reduce CIT and improve the results of transplantation, attention must be focused on each modifiable timestamp associated with CIT (Figure 1). In this editorial we analyze each step of CIT and describe ongoing research about this important LT variable.

DEFINITION OF ISCHEMIA TIME

Organ ischemia time is divided into cold and warm ischemia time (WIT). WIT is a term used to describe ischemia of cells and tissues under normothermic conditions[7]. In the transplant setting, WIT is used to describe two physiologically distinct periods of ischemia: (1) Ischemia during organ procurement, from the time of cross clamping (or of asystole in non-heart-beating donors), until cold perfusion or normothermic regional perfusion are commenced; and (2) Ischemia during implantation, from removal of the organ from ice until portal reperfusion[8]. CIT is defined as the time from cold perfusion of the graft until the removal of the graft from ice to be implanted. During this period, static cold storage lowers the temperature of the graft between 0 °C and 4 °C.

PATHOPHYSIOLOGY OF CIT

Hypothermia has been considered key to successful graft preservation since 1960 when Colins demonstrated that a kidney could be preserved for 30 h safely before transplantation[9]. During hypothermia, cellular metabolism is slowed (the coenzyme Q10 effect[10]), which limits the need for adenosine triphosphate (ATP). However, the beginning of CIT when organs are flushed with cold preservation solution causes inflammation and injury due to sodium-potassium membrane pump dysfunction, resulting in cellular edema with free calcium influx, and subsequent activation of enzyme cascades leading to cellular death. Once the graft is perfused by recipient blood at the end of WIT, there is a restoration of circulation and ATP breakdown with xanthine oxidase generation of free radicals. This causes lipid peroxidation with cellular destruction named ischemia-reperfusion (IR) injury[11,12]. This graft damage is exacerbated by prolonged CIT and it is responsible of primary non-function, arterial thrombosis, biliary complications and recipient mortality[13,14].

Therefore, organs with prolonged CIT are often deemed unsuitable for LT[15]. Many studies have attempted to define the ideal duration of CIT but there is no absolute consensus, and the only recommendation is that it should be as short as possible. Initially, some investigators considered acceptable a CIT of up to 18 h, while recipient survival was shown to be adversely affected by CIT over 12 h in a European survey and over 10 h in a United States survey[16,17].

Today, a CIT between 8 and 10 h is tolerable[18,19], demonstrating significant differences in complications compared with liver grafts implanted after this period. Recently Lozanovski *et al*[20], reported that each additional hour of CIT was associated with a 3.4% increase risk of liver graft loss and the extent of negative impact depended on underlying disease. Indeed, patients with hepatitis C virus (HCV)-cirrhosis demonstrated the highest risk of graft loss due to prolonged CIT, probably because during the hepatocyte proliferation in the IR injury, HCV infiltrates into the proliferating cells, leading to early HCV recurrence[21]. Additionally, Pan *et al*[22] demonstrated that risk of prolonged hospital stays (PLOS, > 30 d) steadily increased with increasing CIT, reaching the greatest odds ratio (OR) for PLOS with 13-14 h [odds ratio (OR) = 2.05; 95% confidence interval (CI): 1.57-2.67] and 15-16 h (OR = 2.06; 95%CI: 1.27-3.33) of CIT.

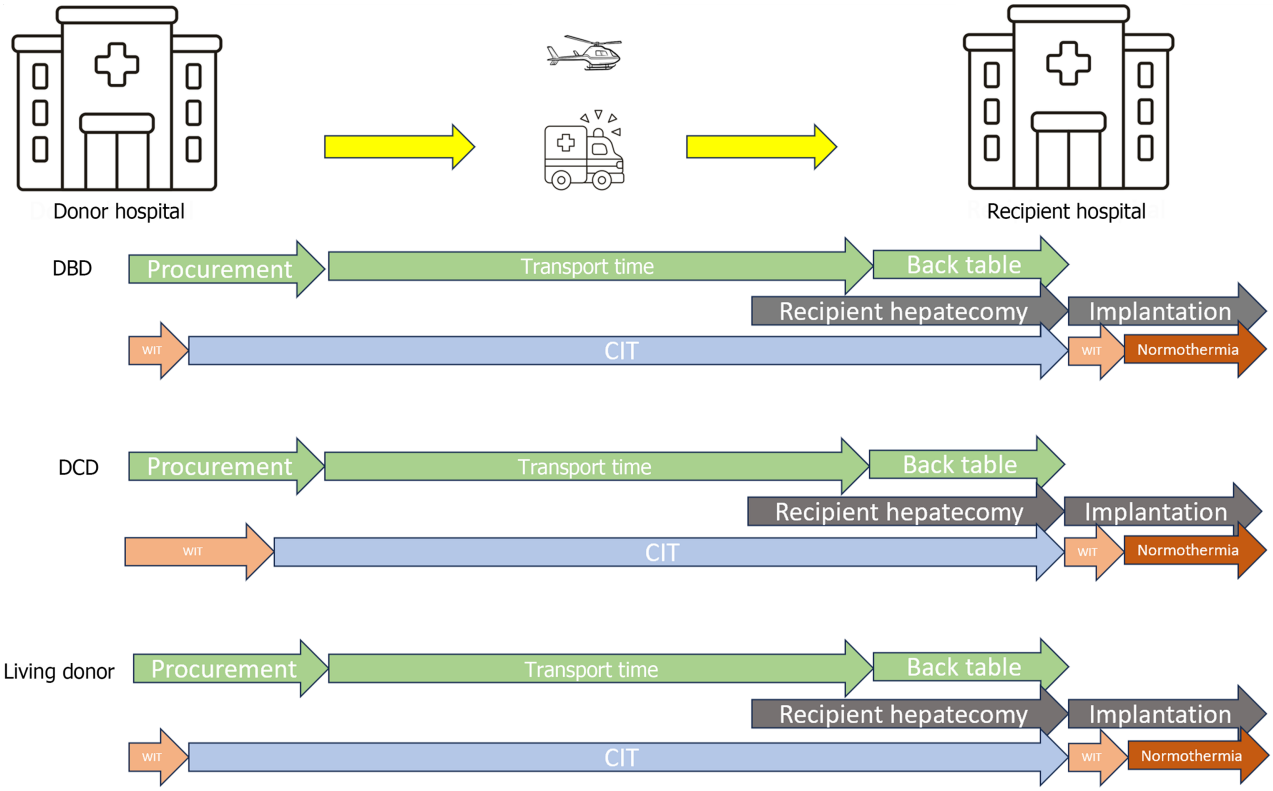

Figure 1 The different phases during liver procurement and transplantation. In donation after brain death donor, warm ischemia time (WIT) is very short, and it starts at the time of cross clamping until flush of cold perfusion. Donation after circulatory death donor is more complex: WIT starts when either SpO_2 or blood pressure drop below a certain threshold and lasts until the start of cold perfusion or the start of normothermic regional perfusion. For every donor, cold ischemia time (CIT) starts at cold perfusion, after aortic cross-clamp, and continues during graft removal, graft transportation and back table preparation until removal from ice for implantation in the recipient. Transport time is a part of CIT unless the organ undergoes some form of normothermic organ perfusion. CIT: Cold ischemia time; DBD: Donation after brain death; DCD: Donation after circulatory death.

DIFFERENTIAL INFLUENCE OF CIT

The impact of prolonged CIT varies between donation types, ages and graft steatosis severity. In donation after circulatory death (DCD) LT, successful outcomes hinge on events occurring during organ procurement and prolonged organ ischemia (donor WIT and CIT) dramatically impact LT results, so every step of procurement aims to minimize ischemic times. Paterno *et al*[23] reported that CIT cut-off > 4 h in DCD LT is associated with increased risk for graft loss, longer post-transplant hospital stays, higher rate of primary non-function, and hyperbilirubinemia. The elderly liver graft has a diminished regenerative response to partial resection and a reduction in the capacity to generate an acute phase protein response[24,25] so prolonged CIT results in an increase of substrate for reactive oxygen species potentiating the effects of IR injury[26]. It is unclear why steatotic livers demonstrate increased susceptibility to IR injury. Proposed mechanisms include impaired hepatic microcirculation[27,28] and mitochondrial dysfunction[29]. Macrovesicular steatosis leads to increased hepatocyte volume causing obstruction of the adjacent sinusoid space and increased hepatic microcirculation vascular resistance[30,31]. This can impair oxygen and nutrient delivery following reperfusion to an already susceptible organ. Increased lipid levels in steatotic livers result in the formation of reactive oxygen species which may lead to mitochondrial dysfunction[32]. The interruption of crucial mitochondrial processes disrupts normal cellular bioenergetics, impairs cellular function, and leads to cell necrosis or apoptosis[33]. Kupffer cell dysfunction and impaired leukocyte adhesion may also increase steatotic liver susceptibility to IR injury[34].

CIT STEPS

Donor CIT starts when organs are flushed with cold preservation solution. This is the donor cross-clamp timestamp. In the case of multiorgan donors, liver procurement begins after heart and lung retrieval. The surgical technique[35] is divided into warm and cold dissections according to the tissue perfusion time. Warm dissection has the advantage of perfusion after confirming the vascular structures and it is mandatory in case of living donor and recommended in case of *in situ* split LT[36,37] where hepatic vessels and parenchymal dissection should be performed before cross clamp to reduce CIT. Contrarily, cold dissection can reduce operative donor time and organ damage by rapid organ procurement. Both warm and cold dissection end with donor hepatectomy, with duration influencing early outcomes after LT. There are likely independent factors influencing donor hepatectomy time, as surgical technique and the surgeon's experience,

but duration > 60 min is associated with early allograft dysfunction[38]. European studies have demonstrated that donor hepatectomy time is an independent risk factor for graft loss and development of ischemic cholangiopathy[39-41]. Moreover, every 10-min increase in donor hepatectomy time has a detrimental effect on early allograft dysfunction similar to a 1-h increase in CIT[42]. Conversely, no significant interaction between donor hepatectomy time and donor type (donation after brain death *vs* DCD) has been observed, indicating that DCD is equally susceptible to the effect of donor hepatectomy time. A particular type of procurement is the super-rapid technique[43]. Utilized in DCD donors in case of absence of normothermic regional perfusion, it initiates in less than 4 min after skin incision to reduce the WIT. The supraceliac aorta is cross-clamped and the intrapericardial inferior vena cava is vented to avoid organ engorgement. The inferior mesenteric vein is then cannulated to perfuse the portal system. Once the liver becomes cold and free of blood, *en bloc* hepatectomy is performed expeditiously. However, it is strongly recommended that this technique be performed by experienced donor surgeons.

To avoid graft ischemia entirely, Gül *et al*[44] and He *et al*[45] proposed the "ischemia-free" LT techniques. The first technique proposed to perform a liver graft procurement without cold preservation, based on the setting of an in-house donor and a consecutive *in situ* preparation of the liver to be able to perform an immediate warm-ischemia-only LT without cold preservation. In the second method liver grafts are procured, preserved, and implanted under continuous normothermic machine perfusion. Both studies reported good results in 6 and 38 cases respectively. However, the risk of extrahepatic organ loss, technical difficulties, the need of an in-house donor and the cost of machine perfusion limited the consideration of such novel techniques as the gold standard in liver procurement.

Transit time

Once procured, the donor liver graft is preserved for transport, either by static cold storage on ice or recently in machine perfusion. Transportation of the graft requires seamless coordination between the donor hospital, the national transplant organization or the Organ Procurement Organization, and the recipient's transplant surgeons. Ambulances, helicopters, and airplanes could be utilized depending on the distance involved and the national allocation policy. Allocation policies have evolved in the last few decades from prioritizing local centers first to a priority national assignment of model for end-stage liver disease-sodium score[46] that resulted in reduced waitlist mortality and increased graft utilization in the United States. However, the allocation process is complex[47], and CIT can be managed by optimizing internal organization and regional allocation when estimated CIT exceeds acceptable limits. Moreover, it is universally accepted that there is no difference in clinical transplant outcomes for local and imported liver allografts[48] and with the ready availability of modern private jet travel and careful coordination, CIT could be minimized. To date, there are only two studies about the relationship between transport time and CIT in the LT setting. In 2002, Totsuka *et al*[49] reported that increased CIT decreases liver graft survival rate in case of organ transportation for a long distance (> 200 m) so they recommended avoiding long-distance graft transportation. Conversely, during the World Transplant Congress in 2014, Gentry *et al*[50] reported in a larger cohort that: (1) Graft survival rate is not affected by distance; (2) The transport time explains < 15% of variation in CIT; and (3) As for kidney transplantation[51] CIT is not dominated by transport factors. More specifically, these results indicated for the first time that CIT is the amount of time which accumulates between organ recovery and organ transplantation and is not only impacted by transit time. Although further investigations are required to clarify the detailed factors involved in CIT, literature reports new transport technology that make organs able to be transported thousands of miles from donor to recipient. In 2019, Scalea *et al*[52] tested an innovated unmanned aircraft system, or drone, in kidney and liver graft transport from the donor hospital to the recipient hospital. This modern technology which would not rely on inconvenient commercial flight times or prohibitively expensive charter flights could allow organs to be transplanted more expeditiously. Moreover, given the background of the era of COVID-19 when travel of in-person procurement surgeons has been discouraged and the 27 documented fatalities in 7 aircraft crashes while traveling on an organ procurement flight[53], real improvements in organ transportation with clinicians and pilot safety are needed. Alternatively, organs can be transported using a normothermic perfusion option. Indeed, portable normothermic perfusion enabling initiation of perfusion from the donor hospital until the recipient hospital allows maximal reduction of CIT and extended periods of preservation and observation relative to cold storage. With normothermic perfusion, organs are maintained under close-to-physiologic conditions. This technology also allows transplant centers to monitor organ function for an extended period and lowers the rate of IR injury, an approach that is particularly advantageous for marginal and older donor organs[54]. In the PROTECT trial, the use of normothermic machine perfusion resulted in a significant reduction in CIT (175.4 min compared with 338.8 min) and led to superior short-term and midterm clinical outcomes, with significant reduction of early allograft dysfunction[55]. However, this approach has not been widely implemented due to complexity and the cost.

Recipient surgery

Back table preparation and recipient hepatectomy are the last timestamp of CIT in LT. Theoretically, in the ideal logistical strategy both procedures should be performed simultaneously and synchronized. However recipient hepatectomy can be challenging due to local conditions such as portal hypertension and perihilar inflammation so it can delay the liver graft reperfusion in the recipient. Median recipient hepatectomy times reported vary widely from 45 min[56,57] to 131 min[58] but previous decompensated cirrhosis with variceal bleeding and/or ascites, higher body mass index, previous abdominal surgery, and surgeon experience are independently associated with prolonged recipient hepatectomy (> 131 min) which is associated significantly with CIT. Back table preparation can be performed in the recipient hospital or in the donor hospital immediately after the removal of the graft to avoid any additional CIT. It consists in checking for organ or vessel injuries and in a very thorough and meticulous vessel dissection to minimize the risk of allograft congestion and bleeding during implantation. However, surgical procedure could be modified and may prolong CIT. For example, in case of split LT or living donor, vascular conduits could be lengthened by patch, venoplasty or arterial graft interposition,

to prevent vessel narrowing and optimize arterial supply or venous drainage. The procedure needs two surgeons and duration varies depending on the procurement technique: Extensive dissection[59] or the *en bloc* procurement technique [60]. In 2022, Song *et al*[61] proposed a magnetic anchoring traction assisted system able to assist the surgeon in the vascular exposure and dissection of liver graft back table. This system replaced the assistant surgeon in the back table preparation and reduced significantly (P = 0.019) the time taken for the procedure (55 min *vs* 85 min). However, the study did not report if this system impacted CIT but only the feasibility, safety, and effectiveness of the traction system. To our knowledge, none of the large transplant registries collect information on back-table time, although this might be a valuable variable to investigate[62].

CIT AND PRESERVATION SOLUTION

The impacts of CIT could be also influenced by preservation solution. The most widely used preservation solutions have been Euro Collins[63], University of Wisconsin Solution (UW)[64], Institut Georges Lopez-1 (IGL-1), Celsior solution[65] and histidine-tryptophan-ketoglutarate (HTK) preservation solution. The "optimal" preservation solution should prevent graft damage by minimizing cellular changes during CIT and decrease IR injury to the organ after restoration of the blood supply in the recipient. A recent meta-analysis from the European Liver Transplant Registry compared results of LT in relation to liver preservation using four different preservation solutions (UW, IGL-1, Celsior solution, and HTK) and concluded that better results in LT could be achieved using UW and IGL-1, especially in the setting of prolonged CIT[66]. Other studies comparing HTK with UW, report no difference in the occurrence of common complications or necessity of operative revisions after LT, confirmed also in subgroup analyses for living donor and paediatric transplantation and cases with prolonged CIT[67,68]. Moreover, HTK could have an economically superior profile due to low cost. Thus, it remains unclear whether one of the solutions is preferable in situations with extensively prolonged CIT.

DYNAMIC PRESERVATION

Dynamic preservation should be included among the prospects to minimize CIT and improve graft outcomes. This innovative strategy could not only replace static cold storage preservation and avoid CIT, but also offering a platform for viability assessment[69]. Liver allografts can be preserved through hypothermic machine perfusion, normothermic machine perfusion, and subnormothermic machine perfusion. Their use has the potential advantage of improving clinical results in LT especially in extended criteria donor allografts[70]. Although associated with increased costs, techniques employing machine perfusion of liver allografts have been considered clinically feasible but not yet well defined. Thus, hypothermic and normothermic approaches may have complementary applications depending on the clinical situation [71]. With the aim of reducing CIT, portable normothermic machine perfusion seems to be more effective since it allows ischemia free procurement (as mentioned above), supports safe organ transportation, extends preservation times, enables graft viability testing and improves recovery of injured liver graft[71].

CONCLUSION

In conclusion, CIT is one of the most important factors impacting graft and recipient survival and it is the result of the accumulated time between organ procurement and organ transplantation. Because of the complicated network involved, each step (donor, transit, recipient) should be considered to minimize CIT duration. Reducing donor hepatectomy, recipient hepatectomy and back table time by novel procurement techniques and adequate fellow training, and improving transportation with modern technologies maintaining organ (and surgeon) safety are the keys for a successful reduction of CIT. Portable donor liver machine perfusion offers an effective method to reduce CIT and mitigate the effects of CIT on graft injury, thereby expanding the liver donor pool and reducing waiting list mortality.

FOOTNOTES

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EDITORIAL

Milestones to optimize of transjugular intrahepatic portosystemic shunt technique as a method for the treatment of portal hypertension complications

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Abstract

This editorial describes the milestones to optimize of transjugular intrahepatic portosystemic shunt (TIPS) technique, which have made it one of the main methods for the treatment of portal hypertension complications worldwide. Innovative ideas, subsequent experimental studies and preliminary experience of use in cirrhotic patients contributed to the introduction of TIPS into clinical practice. At the moment, the main achievement in optimize of TIPS technique is progress in the qualitative characteristics of stents. The transition from bare metal stents to extended polytetrafluoroethylene–covered stent grafts made it possible to significantly prevent shunt dysfunction. However, the question of its preferred diameter, which contributes to an optimal reduction of portal pressure without the risk of developing post-TIPS hepatic encephalopathy, remains relevant. Currently, hepatic encephalopathy is one of the most common complications of TIPS, significantly affecting its effectiveness and prognosis. Careful selection of patients based on cognitive indicators, nutritional status, assessment of liver function, *etc.*, will reduce the incidence of post-TIPS hepatic encephalopathy and improve treatment results. Optimize of TIPS technique has significantly expanded the indications for its use and made it one of the main methods for the treatment of portal hypertension complications. At the same time, there are a number of limitations and unresolved issues that require further randomized controlled trials involving a large cohort of patients.

Key Words: Liver cirrhosis; Portal hypertension; Gastroesophageal variceal bleeding; Prevention; Management; Transjugular intrahepatic portosystemic shunt

Core Tip: Transjugular intrahepatic portosystemic shunt (TIPS) is an interventional radiological procedure that significantly reduces portal pressure, prevents decompensation and improves survival of cirrhotic patients. This editorial describes the milestones to optimize of TIPS technique, which have made it one of the main methods for the treatment of portal hypertension complications worldwide.

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INTRODUCTION

Portal hypertension is the main syndrome characteristic of liver cirrhosis significantly affecting its prognosis. It is defined as a pathological increase in portal pressure, the gold-standard method of clinical evaluation of which is the measurement of the hepatic venous pressure gradient (HVPG). The normal range of HVPG is 1–5 mmHg, whereas a values of ≥ 10 mmHg indicates the presence of clinically significant portal hypertension (CSPH)[1]. The development of CSPH is an important event in the natural history of liver cirrhosis, since it leads to life-threatening complications that decrease the median survival rate to 2-4 years[2].

Transjugular intrahepatic portosystemic shunt (TIPS) is an interventional radiological procedure that significantly reduces portal pressure[3], prevents decompensation and improves survival of cirrhotic patients[4]. During TIPS placement, direct portal pressures are measured and used to calculate the portosystemic pressure gradient (PSPG). The PSPG is the pressure difference between the portal vein and the inferior vena cava (the right atrium). The treatment goal of the TIPS is a PSPG of ≤ 12 mmHg or its reduction by at least 50% of the baseline. Modern guidelines recommend TIPS to cirrhotic patients for preventing recurrent gastroesophageal variceal bleeding with the ineffectiveness of the combined use of non-selective β-blockers and endoscopic band ligation. It is also indicated for acute gastroesophageal variceal bleeding with the ineffectiveness of pharmacotherapy with vasoactive drugs (terlipressin, somatostatin, octreotide) and endoscopic band ligation. In addition, select cirrhotic patients Child-Turcotte-Pugh (CTP) class B (> 7 points) or CTP class C (< 14 points) with active gastroesophageal variceal bleeding at initial esophagogastroduodenoscopy or having the highest risk for rebleeding (HVPG) > 20 mmHg at the time of hemorrhage) can be performed early or pre-emptive TIPS within 72 h of admission (ideally on the first day)[5]. Other indications for its use are refractory ascites and hepatic hydrothorax[6]. The results of the conducted studies indicate the prospects of TIPS in portal vein thrombosis and Budd-Chiari syndrome[7], as well as in cirrhotic patients with CSPH before high-risk surgery[8]. In addition, the possibility of its use in porto-sinusoidal vascular disorders[9] and hepatocellular carcinoma[10] is being discussed.

This editorial describes the milestones to optimize the TIPS technique, focusing on the role of innovative ideas and technical solutions that have made this interventional radiological procedure an important method for the treatment of portal hypertension complications.

INNOVATIVE IDEAS THAT CONTRIBUTE TO THE IMPLEMENTATION OF TIPS IN CLINICAL PRACTICE

The TIPS technique was experimentally developed in the late 1960s at the Department of Diagnostic Radiology at the University of California, Los Angeles. During the research on the possibility of detecting biliary tract obstruction by means of transjugular cholangiography, unintentional hits of a modified Ross needle into the intrahepatic branches of the portal vein suggested a new diagnostic method-transjugular portography, and later-TIPS. To create an intrahepatic portosystemic shunt in experimental animals (5 dogs), after transjugular portography, a channel was formed in the liver parenchyma by Dotter's coaxial Teflon thin-wall angioplasty catheters of different sizes conducted through the superior vena cava, right atrium, inferior vena cava and left hepatic vein. To keep it open, initially a radiopaque rigid Teflon tube 6-10 cm long with an inner diameter of 6 mm was installed in it, which functioned well, but had the property of shifting cranially into the inferior vena cava, and in one case even into the right atrium[11]. A spring coil tubing coated with silicone-based copolymer with an inner diameter of 6 mm, placed with the distal end in the portal vein and the proximal end in the inferior vena cava, was stable and offered better results. Such a shunt diverted a substantial fraction and sometimes all portal blood into the systemic circulation and remained patent for two weeks. Later, usually at the caudal end of the tubing, where it came in contact with the wall of the portal vein, a blood clot began to form, which eventually led to shunt thrombosis[12]. Thus, these initial experimental studies on dogs, as well as human specimens of normal and cirrhotic liver, established the prospects of TIPS, but the question of the material for creating a shunt remained open.

In the early 1980s, Colapinto *et al*[13], the Grüntzig dilatation catheter with a balloon at least 9 mm in diameter and 4-6 cm long was used as a shunt. In their research, they placed it in a channel that was formed in the liver parenchyma between the hepatic and portal veins in six patients with decompensated liver cirrhosis, who had massive gastroesophageal variceal bleeding. All of them immediately experienced a decrease in portal pressure by 10-15 mmHg, and angiography performed 12 h after the procedure showed shunt patency, which was also confirmed in three of the four surviving patients six weeks later.

Somewhat later, Palmaz *et al*[14], in experimental animals (12 dogs) a specially made expandable tubular woven mesh of stainless steel wire was used as a stent to create an intrahepatic portosystemic shunt. It was placed in the channel formed in the liver parenchyma between the anterior surface of the inferior vena cava and the bifurcation of the portal vein, and then expanded by inflating an angioplasty balloon. In eight of the nine surviving dogs, the shunt functioned for nine months after installation. Subsequently, a similar model was used in nine dogs with chronic Portal hypertension induced by intraportal injections of polyvinyl alcohol (Ivalon). A shunt patency rate of 100% was observed up to 48 wk. Low portal pressure and high shunt flow accounted for the good results[15].

In 1989, Richter *et al*[16] presented the first clinical description of TIPS using two Palmaz stents in cirrhotic patients CTP class C with CSPH. Despite a decrease in PSPG from 38 to 18 mmHg and a significant improvement in the clinical status of the patient, he died on day 11 after the procedure because of sudden onset of acute respiratory distress arising from acute nosocomial fungus and cytomegalovirus infection. The autopsy revealed a fully patent shunt without superficial thrombus. Microscopically, a thin endothelial layer on the inner shunt surface was found to be present. A year later, the same authors published a paper reporting the successful outcome of TIPS using the Palmaz stent in nine cirrhotic patients with CSPH and histories of multiple life-threatening gastroesophageal variceal bleeding[17].

These optimistic results were the impetus for the introduction of TIPS into clinical practice. However, two main TIPS-related problems immediately arose, namely shunt dysfunction and post-TIPS hepatic encephalopathy.

EVOLUTION OF TECHNICAL REFINEMENTS OF TIPS STENTS

TIPS stents should contribute to an effective reduction in portal pressure and have special mechanical properties, including high elasticity, strength to withstand liver stiffness, wear resistance and good biocompatibility to reduce the risk of thrombosis and intimal hyperplasia, which can lead to shunt dysfunction, as well as an optimal diameter to prevent post-TIPS hepatic encephalopathy[18].

The first-generation TIPS stents were mainly represented by bare metal stents (BMSs) made of biomedical metals or alloys. For example, the Palmaz® stainless steel stent (Cordis, Miami, FL, United States), known for its high mechanical strength and corrosion resistance, had insufficient flexibility and its use was accompanied by a high incidence of complications. The nitinol (nickel–titanium alloy) stents such as the Zilver® (Cook Medical, Bloomington, IN, United States), Luminexx® (Bard Inc. New Jersey, United States), Smart Control® (Cordis, Miami, Fl, United States), *etc.* have shown good biocompatibility and corrosion resistance results, and the unique shape memory and superelasticity properties that allow them to expand themselves have contributed to widespread use in TIPS[19]. Nevertheless, in the era of BMSs, shunt dysfunction was a frequent and dangerous complication of TIPS, which, as a rule, was a consequence of its acute thrombosis, pseudo-intimal hyperplasia as a result of bile leakage from damaged bile ducts into the stent lumen and hyperplasia of the hepatic vein intima[20]. The gradual development of stenosis or occlusion of the shunt reduced the effectiveness of TIPS, largely limiting its use to "rescue therapy" or "bridge" to liver transplantation[21].

To solve this problem, several experimental and clinical studies have focused their efforts on the development of covered stent grafts. Their use should have significantly improved the long-term shunt patency due to the absence of pseudo-intimal hyperplasia. After studying various materials, it turned out that the best results were shown by polytetrafluoroethylene-covered stent grafts (PTFE-SGs)[22]. An important condition for PTFE-SGs installation is to ensure their sufficient length: so that they are located in the right or left branch of the portal vein at a distance of at least 1-2 cm from its bifurcation (uncovered part), passes through the channel formed in the liver parenchyma and then along the hepatic vein to the confluence with the inferior vena cava (covered part). At the same time, the distance of the cranial stent end to the inferior vena cava should be no more than 1 cm[23]. It is also necessary to completely cover the intraparenchymal channel to prevent bile from entering the stent lumen and the development of pseudo-intimal hyperplasia[24].

In the late 1990s, PTFE-SGs, and later extended PTFE-SGs (ePTFE-SGs), with diameters of 8, 10 and 12 mm were introduced into clinical practice and showed a significant advantage over BMSs. This, in particular, was demonstrated in a meta-analysis including of six studies involving 1.275 cirrhotic patients with CSPH (346-TIPS with PTFE-SGs and 929-TIPS with BMSs), in which the use of PTFE-SGs contributed to better primary shunt patency [hazard ratio (HR): 0.28, 95% confidence interval (CI): 0.20-0.35], lower incidence of HE (HR: 0.65, 95%CI: 0.45-0.86) and less mortality (HR: 0.76, 95%CI: 0.58-0.94)[25].

Since the early 2000s, self-expanding ePTFE-SGs have become available. In 2004, W.L. Gore & Associates in Phoenix AZ, United States developed self-expanding nitinol ePTFE-SG VIATORR® specifically for TIPS (Viatorr TIPS Stent-VTS), which was the first to receive approval from the United States Food and Drug Administration. The uncovered part of its self–expanding nitinol skeleton (2 cm) is located in the portal vein, whereas the covered part (5-8 cm) is located in the intraparenchymal channel and hepatic vein. The stent graft is available in diameters of 8, 10, and 12 mm[26]. In a retrospective single-center study by Geeroms *et al*[27] including 285 cirrhotic patients with CSPH treated with TIPS using VTS stent grafts, the 1-, 2- and 5-year primary shunt patency was 91.5%, 89.2% and 86.2%, respectively, with no new shunt dysfunctions after 5 years' follow-up. Shunt revision was performed more often in ascites patients (P = 0.02). The 1-, 4- and 10-year survival rates were 69.2%, 52.1% and 30.7%, respectively. In a retrospective single-center study by Li *et al* [28] including 59 cirrhotic patients with CSPH, elective TIPS implantation using VTS stent grafts contributed to a decrease in PSPG from 21 mmHg (interquartile range: 19-25) to 13 mmHg (interquartile range: 10-16). The cumulative rate of overall mortality was 34.2% at five years. The cumulative rates of shunt dysfunction and gastroesophageal variceal rebleeding were 11.0% and 28.3% at five years, respectively. The cumulative four-year post-TIPS hepatic encephalopathy free rate was 48.6%.

As an alternative to VTS stent grafts, non-dedicated ePTFE-SGs like the Fluency® (Angiomed GmbH, a subsidiary of C.R. Bard, Inc.), primarily designed for treating peripheral vascular diseases, were adapted for TIPS. Unlike VTS stent grafts, it is completely covered and does not have an uncovered part on the portal vein side. In a retrospective single-center study by Luo *et al*[29], including 495 cirrhotic patients with CSPH treated with TIPS using Fluency stent grafts, early procedure-related complications occurred in 67 patients (13.5%). TIPS creation resulted in an immediate decrease in mean PSPG from 23.4 ± 7.1 mmHg to 7.6 ± 3.5 mmHg. The 1- and 3-year primary shunt patency was 93%, and 75.9%, respectively. The 1- and 3-year survival rates were 93.4% and 77.2%, respectively. The 1- and 3-year probability of remaining free of gastroesophageal variceal bleeding rates were 94.2% and 71.4%, respectively.

A meta-analysis of four randomized controlled trials (RCTs) has been performed to compare the outcomes of self-expanding ePTFE-SGs *vs* BMSs for TIPS. VTS stent grafts alone, Fluency stent grafts alone, and VTS stent grafts plus Fluency stent grafts were employed in one, two, and one RCT, respectively. It was demonstrated that the self-expanding ePTFE-SGs group had significantly higher probabilities of overall survival (HR: 0.67, 95%CI: 0.50-0.90, $P = 0.008$) and shunt patency (HR: 0.42, 95%CI: 0.29-0.62, $P < 0.0001$) than the BMSs group. Additionally, the self-expanding ePTFE-SGs group might have a lower risk of post-TIPS hepatic encephalopathy than the BMSs group (HR: 0.70, 95%CI: 0.49-1.00, $P = 0.05$)[30].

In 2017, a new dedicated ePTFE-SG known as the VIATORR® TIPS Endoprosthesis with Controlled Expansion (VCX) (W.L. Gore & Associates, Phoenix, AZ, United States) was introduced. Its diameter from 8 to 10 mm is adjustable regardless of the possible passive dilation. This makes it possible to accurately calibrate the shunt and monitor the PSPG throughout the entire postoperative period, ensuring a good clinical outcome with a fairly low complication rate[31].

Thus, over the past quarter century, there has been significant progress in the qualitative characteristics of TIPS stents, among which the use of PTFE-SGs has proved to be the most successful. If all the rules of their installation are followed, shunt dysfunction is currently rare. However, the problem of post-TIPS hepatic encephalopathy does not lose its relevance.

TECHNICAL SOLUTIONS AIMED AT PREVENTING POST-TIPS HEPATIC ENCEPHALOPATHY

Hepatic encephalopathy is one of the most common complications of TIPS and occurs in 23%-55% of patients during the first year after surgery. Its pathophysiology is difficult and consists of a complex interaction of hyperammonemia and systemic inflammation. Simply put, it is caused by hyperammonemia-related neurotoxicity. In the context of the development of post-TIPS hepatic encephalopathy, two factors must be considered. First of all, this is a "steal" phenomenon, when ammonia-enriched blood from the intestine bypasses the liver and is not included in the urea cycle, which exacerbates hyperammonemia. Secondly, portocaval shunting (including TIPS) leads to hyperactivity of phosphate-activated glutaminase in the intestine, contributing to an increase in gut-derived ammonia[32]. Many predictors of post-TIPS hepatic encephalopathy are well described and presented in Figure 1, and some of them are directly related to the technical characteristics of stents and the method of their placement[33]. This should be taken into account, since routine prophylactic therapy by administration of rifaximin and lactulose may be ineffective in this case [34].

Optimal stent diameter

In this regard, the question of the optimal diameter and degree of expansion of PTFE-SGs for effective reduction in portal pressure without the risk of developing post-TIPS hepatic encephalopathy remains relevant[35]. Obviously, a larger stent diameter leads to a greater reduction in portal pressure. However, the stent diameter does not correlate with PSPG, which cannot be predicted by pre-TIPS hemodynamic variables, but depends on individual conditions[36].

Given the experience of using BMSs, TIPS with 12 mm self-expanding PTFE-SGs were initially employed. However, the high incidence of post-TIPS hepatic encephalopathy forced them to be abandoned[37]. Indeed, in a retrospective single-center study by Habash *et al*[38] including 360 cirrhotic patients with CSPH treated with TIPS using 12 mm VTS stent grafts, percentage of patients with symptoms of post-TIPS hepatic encephalopathy were 34.4%, 42.9%, and 49.5% at 3, 6, and 12 months, respectively. In a systematic review and meta-analysis including of five RCTs or observational studies involving 489 cirrhotic patients with CSPH treated with TIPS using 8 or 10 mm PTFE-SGs, the 8 mm PTFE-SGs group had higher efficacy regarding one-year or three-year overall survival [odds ratio (OR): 2.88, $P = 0.003$] and (OR: 1.81, $P = 0.04$) and lower post-TIPS hepatic encephalopathy (OR: 0.69, $P = 0.04$) compared with 10 mm PTFE-SGs group. There were no significant differences in gastroesophageal variceal rebleeding rate (OR: 0.80, $P = 0.67$). However, shunt dysfunction was lower in 10 mm PTFE-SGs group (OR: 2.26, $P = 0.003$)[39]. In a retrospective single-center study by Kloster *et al*[40] including 33 cirrhotic patients with CSPH treated with TIPS using 8 mm and 10 mm (85% and 15% patients, respectively) VCX stent grafts, mean final PSPG was 6 mmHg. Cumulative post-TIPS hepatic encephalopathy incidence was 61%. 1-, 3-, 6-, and 12-month post-TIPS hepatic encephalopathy rates were 24%, 30%, 53%, and 61% over 247-d median follow-up.

A significant proportion of patients who develop severe post-TIPS hepatic encephalopathy have a fairly low PSPG (5-10 mmHg). Therefore, in order to achieve good results when choosing the optimal diameter of ePTFE-SGs, it is necessary to keep within a very narrow therapeutic framework of PSPG, namely less than 12 mmHg to control gastroesophageal variceal bleeding and above 10 mmHg for preventing post-TIPS hepatic encephalopathy. In practice, it is difficult to achieve this goal when using self-expanding ePTFE-SGs. Indeed, after the initial ePTFE-SGs expansion to 8 mm and a drop in PSPG, for example, to the required 10 mmHg, it cannot be excluded that further ePTFE-SGs expansion to 10 mm will not lead to a subsequent decrease in PSPG, increasing the risk of developing post-TIPS hepatic encephalopathy[41].

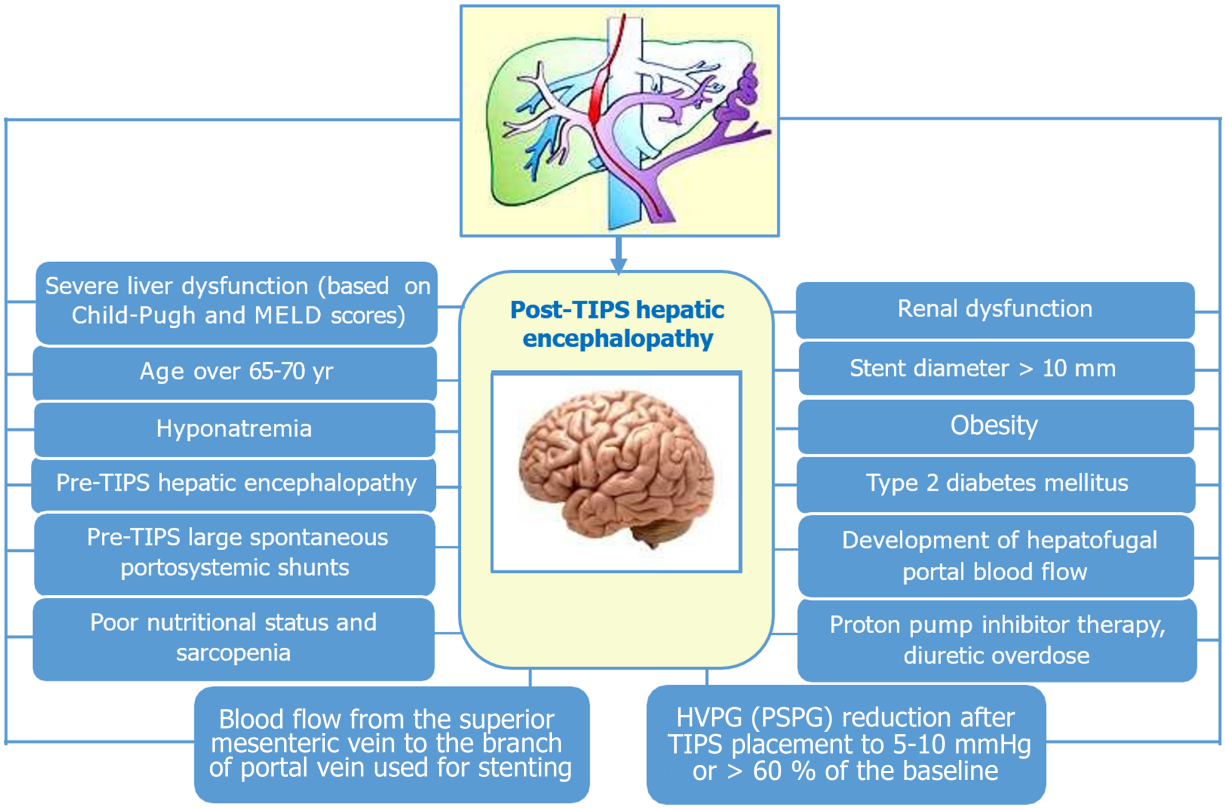

Figure 1 Individual predictors for post-transjugular intrahepatic portosystemic shunt hepatic encephalopathy. HVPG: Hepatic venous pressure gradient; PSPG: Portosystemic pressure gradient; TIPS: Transjugular intrahepatic portosystemic shunt.

For example, in a retrospective single-center study by Borghol *et al*[42] including 16 cirrhotic patients with CSPH treated with TIPS using 10 mm VTS stent grafts, that was underdilated (*i.e.*, 8 mm) at the time of stent placement, angiography showed its expansion from 8.96 mm ± 1.12 (SD) to 10 mm ± 1.45 (SD) after 6 months ($P = 0.04$) with no further significant changes over time after 12 months (10.28 mm ± 1.9 mm), 18 months (9.93 ± 1.51 mm) and 24 months (9.92 ± 0.9 mm) after TIPS. In a prospective study by Pieper *et al*[43], including 20 cirrhotic patients with CSPH treated with TIPS using VTS stent grafts, two-dimensional (2D) and three-dimensional (3D) ultrasonography showed an expansion of underdilated self-expanding stent grafts from 8 mm to 8.8 ± 0.24 mm (2D) and 8.7 ± 0.27 mm (3D) ($P < 0.001$) after 1 wk and to 9.4 ± 0.15 mm (2D) and 9.4 ± 0.11 mm (3D) ($P < 0.001$) 6 wk after TIPS.

Schepis *et al*[44] performed a prospective, non-randomized study of 42 unselected cirrhotic patients with CSPH treated with TIPS using VTS stent grafts, which received underdilated self-expanding stent-grafts (7 and 6 mm) and 53 patients which received self-expanding stent-grafts of 8 mm or more (controls). Post-TIPS hepatic encephalopathy developed in a significantly lower proportion of patients with underdilated self-expanding stent-grafts (27%) than controls (54%) during the first year after the procedure ($P = 0.015$), but the proportions of patients with recurrent gastroesophageal variceal bleeding or ascites did not differ significantly between groups. VTS stent grafts dilatation above 6 mm, PSPG below 10 mmHg or a decrease in PSPG after TIPS by more than 50% were independently associated with one-year post-TIPS hepatic encephalopathy. In a prospective case-control study by Praktiknjo *et al*[45] including 114 cirrhotic patients with CSPH treated with TIPS using 10 mm VCX stent grafts underdilated to 8 mm and 10 mm VTS stent grafts underdilated to 8 mm, VCX stent grafts diameter was 8.0 (7.8-9.2) mm at a median time of 359 (87-450) d, compared with VTS stent grafts at 9.9 (9.7-10.0) mm ($P < 0.001$). PSPG immediately after TIPS procedure and after 7 d did not change significantly in VCX stent grafts [mean 9.4 (± 0.8) *vs* 10.4 (± 0.7) mmHg, $P = 0.115$)]. The lack of passive expansion to nominal diameter of underdilated VCX stent grafts contributed to a reduction in hospital readmissions due to post-TIPS hepatic encephalopathy (23% *vs* 51% for VCX stent grafts and VTS stent grafts ($P < 0.001$), respectively.

To date, the question of the optimal diameter and degree of expansion of PTFE-SGs for effective reduction in portal pressure without the risk of developing post-TIPS hepatic encephalopathy remains open. It is obvious that for its prevention, the target reduction in PSPG should be less than was required in the era of BMSs, but there is still not enough concrete data to make scientifically sound decisions.

Choosing a branch of the portal vein for stenting

TIPS stents are usually placed *via* access from the central part of the right hepatic vein to the right branch of the portal vein. The lower anatomical location and horizontal course of the right branch of the portal vein ensure relatively safe and easy penetration of the needle into its lumen[46]. At the same time, there is a theory that blood from the splenic and superior mesenteric veins in portal hypertension does not mix completely in the main portal vein, but enters the left and right branches of the portal vein separately, that is, blood from the superior mesenteric vein mainly enters the right

branch, while blood from the splenic vein mainly enters the left branch. It is assumed that the use of the left branch of the portal vein for stenting is more appropriate, since it can reduce the risk of developing post-TIPS hepatic encephalopathy [47]. In a study by Deng *et al*[48], including 15 cirrhotic patients with CSPH, blood samples were collected from the left branch, right branch and main trunk of the portal vein during TIPS. The plasma ammonia concentration was 96.4 ± 17.6 mmol/L, 113.5 ± 18.4 mmol/L and 106.9 ± 38.7 mmol/L ($P > 0.05$), respectively, without any statistically significant differences ($P > 0.05$). In a retrospective single-center study by Yang *et al*[49] including 243 cirrhotic patients with CSPH treated with TIPS, it was found that in about three quarters of cases, blood from the splenic and superior mesenteric veins was completely mixed in the intrahepatic portal system under portography, this was accompanied by a comparable risk of post-TIPS hepatic encephalopathy. For the rest of the patients, in about three quarters of the cases, the right branch of the portal vein received blood from the splenic vein, and the left branch of the portal vein received blood from the superior mesenteric vein. If the right branch of the portal vein received blood from the superior mesenteric vein, and the left branch of the portal vein received blood from the splenic vein, this was accompanied by an increased risk of post-TIPS hepatic encephalopathy.

In studies where BMSs was used for TIPS creation the frequency of hepatic encephalopathy in stenting of the left branch of the portal vein was lower than in stenting of the right branch of the portal vein[50-52], however, in the case of using VTS stent grafts, it occurred approximately the same[53]. In a retrospective single-center study by Miraglia *et al*[54] including 193 cirrhotic patients with CSPH, compared the outcome of TIPS using VCX stent grafts placed in the left branch (37 patients - group 1) and the right branch (156 patients-group 2) of the portal vein. The median follow-up was 9.6 months. PSPG after TIPS was 6.2 ± 2.2 mmHg in group 1 *vs* 6.3 ± 2.8 mmHg in group 2 ($P = 0.839$). The stent was dilated to 8-mm in 95% of patients in group 1 *vs* 77% of patients in group 2 ($P = 0.015$). The incidence of post-TIPS hepatic encephalopathy was 13% of patients in group 1 and 24% of patients in group 2 ($P = 0.177$).

The results of these studies do not allow us to draw a definitive conclusion, and further multicenter prospective RCTs are needed to find out whether the occurrence of post-TIPS hepatic encephalopathy may be associated with the choice of a specific branch of the portal vein for stenting.

TRANS-TIPS ANTEGRADE TRANSVENOUS OBLITERATION OF GASTROESOPHAGEAL VARICES

Maintaining hepatofugal blood flow, namely the gastroesophageal pathway of portosystemic shunting, despite a decrease in PSPG after TIPS, is a significant risk factor for gastroesophageal variceal rebleeding. Indeed, the determining factor in formation of gastroesophageal varices is the type of hepatofugal blood flow, and a gastroesophageal pathway of portosystemic shunting is the most important in this situation. The left gastric vein plays the main role in this pathway. It drains blood from both surfaces of the stomach, ascends from right to left along the lesser curvature of the stomach into the lesser omentum to the esophageal hiatus, where it receives esophageal veins. The left gastric vein then turns backward and passes from left to right behind the omental bursa and flows into the portal vein. Anastomoses between the left and right gastric veins and the left and short gastric veins, respectively indicated by terms “coronary vein” and “posterior gastric vein”, have clinical significance only in portal hypertension, because they are involved in the formation of gastroesophageal varices and related with them paraesophageal varices[55]

Trans-TIPS antegrade transvenous obliteration of gastroesophageal varices should be considered as a therapeutic option with high blood flow in them and the presence of large afferent gastric veins or shunts under portography[56]. The procedure can be used as an adjunct to retrograde transvenous obliteration of gastric varices or as an alternative in the absence of gastrorenal shunts. If multiple afferent gastric veins are present, the largest vein (usually the left gastric vein) is left for balloon occlusion, while the small veins are occluded using coils or vascular plugs. The advantage of this approach is that it does not require new access, which minimizes the risk of vascular and biliary injury, while the disadvantages include a time-consuming invasive pathway consists in prolonged and indirect access to afferent gastric veins[57].

A recent systematic review and meta-analysis of 11 studies involving 1,075 cirrhotic patients with CSPH showed that the combination of TIPS with antegrade transvenous obliteration of gastroesophageal varices is accompanied by fewer gastroesophageal variceal rebleeding than after TIPS alone [relative risk (RR): 0.59, 95%CI: 0.43-0.81, $P = 0.001$], and better results were obtained with the use of PTFE-SGs (RR: 0.56, 95%CI: 0.36-0.86, $P = 0.008$). At the same time, there were no differences in the frequency of shunt dysfunction (RR: 0.88, 95%CI: 0.64-1.19, $P = 0.40$), post-TIPS hepatic encephalopathy (RR: 0.84, 95%CI: 0.66-1.06, $P = 0.13$) and mortality (RR: 0.87, 95%CI: 0.65-1.17, $P = 0.34$). The authors draw an important conclusion about an individual approach in determining the indications for the combined use of TIPS with antegrade transvenous obliteration of gastroesophageal varices, taking into account the balance of risk and benefit[58].

CONCLUSION

The worldwide experience of using TIPS has made it an important method of treating complications of portal hypertension, which has actually replaced open surgical interventions. This interventional radiological procedure has gone through a complex, almost half-century-long path of evolution from innovative ideas to original technical solutions. The transition from BMSs to ePTFE-SGs made it possible to significantly prevent shunt dysfunction. However, the question of its preferred diameter, which contributes to an optimal reduction of portal pressure without the risk of developing post-TIPS hepatic encephalopathy, remains relevant. Whether the choice of a specific branch of the portal vein for stenting plays a role in its occurrence should be studied in the future. Currently, hepatic encephalopathy is one of the

most common complications of TIPS, significantly affecting its effectiveness and prognosis. Careful selection of patients based on cognitive indicators, nutritional status, assessment of liver function, *etc.*, will reduce the incidence of post-TIPS hepatic encephalopathy and improve treatment results. Trans-TIPS antegrade transvenous obliteration of gastroesophageal varices can be considered with high blood flow in them and the presence of large afferent gastric veins as a therapeutic option aimed at preventing gastroesophageal variceal rebleeding. Optimize of TIPS technique has significantly expanded the indications for its use and made it one of the main methods for the treatment of portal hypertension complications. At the same time, there are a number of limitations and unresolved issues that require further RCTs involving a large cohort of patients.

FOOTNOTES

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Abstract

Achievement of a 'clinical cure' in chronic hepatitis B (CHB) implies sustained virological suppression and immunological control over the infection, which is the ideal treatment goal according to domestic and international CHB management guidelines. Clinical practice has shown encouraging results for specific patient cohorts using tailored treatment regimens. These regimens incorporate either nucleos(t)ide analogs, immunomodulatory agents such as pegylated interferon α, or a strategic combination of both, sequentially or concurrently administered. Despite these advancements in the clinical handling of hepatitis B, achieving a clinical cure remains elusive for a considerable subset of patients due to the number of challenges that preclude the realization of optimal treatment outcomes. These include, but are not limited to, the emergence of antiviral resistance, incomplete immune recovery, and the persistence of covalently closed circular DNA. Moreover, the variance in response to interferon therapy and the lack of definitive biomarkers for treatment cessation also contribute to the complexity of achieving a clinical cure. This article briefly overviews the current research progress and existing issues in pursuing a clinical cure for hepatitis B.

Key Words: Chronic hepatitis B; Clinical cure; Polyethylene glycol interferon; Treatment strategies; Research progress

Core Tip: Clinical cure has become an ideal goal pursued by chronic hepatitis B (CHB) patients. To enable more patients to achieve this goal, immunotherapy targets, the development of drugs targeting the viral life cycle, gene editing technologies, and the application of other methods have promoted the achievement of a clinical cure for CHB; however, this topic warrants continuous exploration.

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INTRODUCTION

Chronic hepatitis B (CHB) is an important disease posing a severe threat to human health. According to World Health Organization estimates, nearly 1/3 of the world's people have been infected with hepatitis B virus (HBV). There are more than 86 million HBV-infected people in China, which ranks first in the world in the number of infected people[1,2]. In recent years, approximately 50%-80% of hepatocellular carcinoma (HCC) cases worldwide have been caused by chronic HBV infection, and more than half of the new HCC cases and deaths worldwide occurred in China[3], specifically more than 84% caused by HBV infection[4]. Moreover, the risk of HCC in HBV-infected patients is 10-30 times that of the uninfected population[5]. Therefore, treating and managing CHB, delaying the progression of liver cirrhosis, and reducing the incidence of HCC are the current clinical priority issues to be addressed[6,7]. Since 2015, the Chinese guidelines for the prevention and treatment of CHB have proposed the idea of pursuing a clinical cure for CHB, and many scholars at home and abroad have started active exploration. Reports on research projects related to clinical cures were readily available and showed promising results. The clinical cure for hepatitis B has also been constantly updated from the 2015 edition to the 2022 edition in China. The clinical cure for CHB has substantially advanced from theoretical to practical. While these results are very encouraging, many patients still do not respond well to treatments, and many difficulties must be overcome to achieve a good treatment effect.

CLINICAL CURE: AN IDEAL GOAL FOR PATIENTS WITH CHB

Proposal of the concept of clinical cure

The clinical cure for hepatitis B must start with the antiviral treatment of hepatitis B. As is well known, the antiviral treatment for hepatitis B mainly includes two types of drugs: Nucleos(t)ide analogs (NAs) and interferons. The advent of NAs has brought remarkable changes to the anti-viral treatment of hepatitis B. The use of nucleoside drugs has marked a new approach to the antiviral treatment of hepatitis B, and the treatment of CHB has since entered the era of antiviral treatment. After nearly 30 years of development, current NAs exhibit various advantages, including well-established therapeutic efficacy, low resistance, minimal long-term adverse reactions, and affordable pricing. Most CHB patients can achieve long-term virological suppression during the medication period, thus improving long-term outcomes[8,9]. However, upon summarizing a substantial amount of clinical data on NAs, it was found that their clearance effect on hepatitis B surface antigen (HBsAg) is extremely limited, with an annual clearance rate of < 1%[10,11]. Nevertheless, even with such a low clearance rate, it ignites hope among scholars in the field of liver disease for the clinical cure of hepatitis B. In fact, achieving clearance of covalently closed circular DNA (cccDNA) solely with NAs would require at least 30 to 40 years[12], indicating that patients would need to take NAs for their entire lifetime.

In 2013, Block *et al*[13] initiated a discussion on the concept of "cure" for CHB, finding that even individuals who spontaneously cleared acute HBV infection were still at a higher risk of death from HCC compared to those who have never been infected with HBV[13]. The term "cure" used alone to describe the treatment outcomes of CHB is limited. Therefore, these scholars also pioneered the "functional cure (apparent virological cure)" concept, which entails clearance of HBsAg and sustained suppression of HBV DNA. Consequently, the types of CHB cures are categorized into complete cure, also known as virological cure, and functional cure, also known as clinical cure or immunological cure[14]. The former type refers to serum HBsAg clearance, intrahepatic and serum HBV DNA clearance (including intrahepatic cccDNA and integrated HBV DNA), and persistent positive serum anti-HBc, with or without developing anti-HBs. The cccDNA structure is stable and persistent, and the HBV genome can be integrated into host DNA. Currently, there is a lack of specific targeted drugs for cccDNA and integrated HBV DNA; therefore, it is challenging to achieve a complete cure. In contrast, a clinical cure is easier to achieve. First-line antiviral drugs such as NAs and interferon both have the potential to achieve clinical cure; thus, clinical cure has gradually become the ideal treatment goal for patients with CHB.

Use of interferon accelerates clinical cure process for CHB

Interferon therapy has been used in the treatment of CHB for over 50 years, during which a substantial body of clinical experience and evidence-based medical data have been accumulated. The initial therapeutic goal of standard interferon-alpha or pegylated interferon alpha (Peg-IFNα) treatment was to achieve a sustained virological response. The primary treatment objectives for HBeAg-positive patients with CHB are HBeAg clearance and serological conversion; for HBeAg-negative patients, the key goal is sustained HBV DNA suppression. The treatment duration for Peg-IFNα, determined by these primary objectives, is 48 wk, followed by a 24-wk observation period post-treatment. In 2016, Marcellin *et al*[15] reported for the first time research outcomes where the primary goal was HBsAg clearance or serological conversion, showing that 48 wk of Peg-IFNα combined with tenofovir disoproxil fumarate (TDF) treatment could achieve an HBsAg clearance rate of 9.1%[15]. This highlighted the feasibility of aiming for a clinical cure as a treatment goal, garnering significant attention and interest among clinicians. Subsequently, studies with the primary goal of achieving HBsAg clearance or associated serological conversion have gradually increased. An increasing number of studies have focused

on Peg-IFNα treatment strategies to enhance clinical cure rates, meeting the crucial requirements of current clinical practice.

The sustained suppression of HBV DNA by NAs has laid the foundation for the clinical cure of CHB. The prudent use of Peg-IFNα on top of NAs therapy can significantly enhance the clinical cure rate of CHB. Interferons exert dual immunomodulatory and antiviral effects through mechanisms such as enhancing immune cell functions, promoting cytokine expression, inducing the production of interferon-stimulated genes (ISGs), and encoding various antiviral proteins *via* the interferon signaling pathway, which impact crucial biological processes like HBV replication and transcription. Compared to NAs, the course of Peg-IFN therapy is limited rather than long-term, with higher serological responses and more sustained effects. However, Peg-IFN is effective only for a subset of patients and lacks the robust tolerability seen with NAs.

Extensive clinical research has confirmed that combination therapy with NAs and Peg-IFN represents the most promising treatment strategy for achieving clinical cure by integrating potent viral suppression with the host immune response restoration. In clinical practice, the combined NA and Peg-IFN treatment regimens have accumulated numerous successful cases of HBsAg seroconversion and safe discontinuation of NAs, supported by evidence-based medical data. Currently, combined treatment approaches primarily include initial combination therapy and sequential combination therapy, the latter encompassing 'switch' strategies (from NAs to Peg-IFN) and 'add-on' strategies (NAs in addition to Peg-IFN). In addition, prolonging treatment duration and intermittent therapy are popular research directions for achieving clinical cure in current CHB management.

Combination therapy capitalizes on the different antiviral mechanisms of NAs and Peg-IFNα. Rational concurrent use can restore the host's immune response against the potent viral suppression, yielding synergistic and complementary effects and making it the most viable available treatment strategy for achieving ideal therapeutic goals[14]. Moreover, numerous clinical studies have confirmed that sequential or combined treatment regimens with NAs and Peg-IFNα achieve favorable outcomes in specific subpopulations that benefit from interferon therapy. Prospective studies of Peg-IFNα treatment in individuals with CHB have shown that the HBsAg clearance rate in patients receiving combined Peg-IFNα and TDF therapy surpasses that in patients treated with either agent alone[16,17].

Sequential therapy: In their study, Huang *et al*[18] included 43 patients undergoing sequential peg-IFN therapy following NAs. Their results indicated that sequential peg-IFNα treatment for 48 wk achieved a 32.6% HBsAg clearance rate and 27.9% serological conversion rate[18]. In contrast, no cases of HBsAg loss were observed in the NAs monotherapy group. Japanese researchers conducted a study involving 23 patients treated with NAs for over a year who achieved HBeAg clearance and had HBsAg levels < 1500 IU/mL. These patients were additionally treated with peg-IFNα for 48 wk, followed by a follow-up. At 72 wk, a significant increase in efficacy was observed, with a 37.4% HBsAg clearance rate and 29.7% serological conversion rate compared to the group treated with NAs alone[19]. Many other studies, including those conducted by our team, have similarly confirmed that adding peg-IFNα in NAs-treated patients with CHB and HBsAg levels < 1500 IU/mL can significantly increase the HBsAg clearance rate[20]. Overall, for NAs-experienced patients with low HBsAg levels and sustained HBV suppression, sequential or combination therapy of NAs with Peg-IFNα can significantly increase the rates of HBsAg seroclearance and seroconversion (Table 1)[21-35].

Extended treatment: In a multicenter clinical study with HBsAg clearance as the primary treatment goal reported by Hu *et al*[26], patients who had been on NAs treatment for over 2 years and had undergone HBeAg seroconversion were switched to Peg-IFNα therapy for either 48 or 96 wk. The results revealed that the HBsAg clearance rates at 48 wk and 96 wk were 14.4% (22/153) and 20.7% (31/150), respectively. For patients with baseline HBsAg < 1500 IU/mL and HBsAg < 200 IU/mL at 24 wk of treatment, the HBsAg clearance rates at 48 wk and 96 wk were 51.4% and 58.7%, respectively. These results proved that extending the Peg-IFN treatment duration in the NAs-treated population can increase the clinical cure rate.

Intermittent treatment: Extending the course of Peg-IFNα may enhance clinical cure rates; however, this strategy should not be adopted indefinitely. Instead, an extended overall treatment duration with phased intermittent therapy could be more advantageous. Such staged intermittent treatment strategy for long-term antiviral therapy aims to ultimately achieve clinical cure objectives, involving "treat-interrupt-retreat" cycles of Peg-IFNα on top of ongoing NA therapy, which has been clinically proven effective. Long-term treatment with Peg-IFNα may lead to CD8+ T-cell exhaustion, failing to sustain an immunological response. Therefore, pausing Peg-IFNα treatment allows for the recovery of host immune function while maintaining NA therapy, which promotes the rebuilding of specific immunity under sustained HBV DNA control, creating an opportunity for subsequent Peg-IFNα re-administration[36,37].

Therefore, these results are very promising, and they anticipate the definite attainment of a clinical cure for CHB. The table below lists some representative clinical cure studies of different Peg-IFNα-based treatment strategies in recent years (Table 1).

The Asia-Pacific region exhibits a high prevalence of HBV infection, prompting numerous clinical investigations centered on interferon-based therapies within this geographic area. Consequently, an ongoing international debate persists regarding the therapeutic efficacy of interferon in patients with CHB. Notably, various nations in Europe and America have also conducted comprehensive clinical assessments to ascertain the efficacy of interferon-based treatments for CHB patients within their respective populations.

The Hepatitis B Research Network has enrolled adults and children suffering from CHB in multiple medical centers in the United States and Canada and published a series of studies. For instance, investigations led by Perrillo *et al*[38] examined CHB patients in the immune-tolerant phase positive for the e antigen. Notably, while the proportion of adults and children experiencing alanine aminotransferase (ALT) elevation was comparable following the addition of interferon to NAs, a significantly higher proportion of children achieved a > 1 Log10 IU/mL decline in HBsAg (39% *vs* 22%, P <

Table 1 Studies in which chronic hepatitis B patients were cured after pegylated interferon alpha-based treatment

Treatment option	Ref.	Duration of interferon use	HBsAg negative conversion rate (%)
"NA plus Peg-IFNα" strategy	Marcellin *et al*[21], 2016	48 wk	9.1
	Hagiwara *et al*[22], 2018	48 wk	10.4
	Hu *et al*[23], 2021	48 wk	11.5
"Switching NA to Peg-IFNα" strategy	Han *et al*[24], 2016	48 wk	8.5
	Wu *et al*[25], 2019	48 wk	9.3
	Hu *et al*[26], 2018	48 wk	14.4
"NA plus Peg-IFNα" strategy	Bourlière *et al*[27], 2017	48 wk	7.8
	Lim *et al*[28], 2019	48 wk	9.0
	Li *et al*[29], 2015	48 wk	18.5
	Wu *et al*[20], 2020	72 wk	37.4
"Peg-IFNα monotherapy" strategy (IHCs)	Cao *et al*[30], 2017	48 wk	29.8
	Wu *et al*[31], 2021	72 wk	47.9
	Chen *et al*[32], 2021	48 wk	55.6
"Extended treatment" strategy	Hu *et al*[26], 2018	96 wk	20.7
	Li *et al*[33], 2011	81.32 ± 39.36 wk	21.7
	Yan *et al*[34], 2018	96 wk	29.0
"Intermittent treatment" strategy	Li *et al*[35], 2022	/	19.4

HBsAg: Hepatitis B surface antigen; NA: Nucleos(t)ide analog; Peg-IFNα: Pegylated interferon alpha; IHCs: Inactive Hepatitis B surface antigen carrier status.

0.05)[38]. Additionally, Terrault *et al*[39] conducted a randomized (1:1) trial involving the administration of TDF over 192 wk, with or without Peg-IFNα administered during the initial 24 wk. Subsequently, TDF was withdrawn at week 192, followed by a 48-wk off-treatment follow-up period until week 240. Noteworthy findings revealed a higher HBsAg clearance rate in the combination therapy group during the treatment phase. However, by week 240, no statistically significant disparity in HBsAg clearance rates was observed between the two groups, underscoring the nuanced implications of combining Peg-IFNα with TDF therapy and subsequent TDF discontinuation[39].

In conclusion, these studies underscore the complexity and variability of therapeutic responses to interferon-based regimens across diverse patient populations, necessitating further exploration and refinement of treatment strategies in the management of CHB.

Practice of clinical cure of CHB: Status of cure in different populations

Clinical cure problem for dominant population and non-dominant population of interferon treatment: Currently, the overall clinical cure rate achieved by Peg-IFNα in CHB patients is only close to 10%, much higher than the 1% with NAs; however, these results are still unsatisfactory. Studies have found that in some populations, after treatment with Peg-IFNα, the clinical cure rate of CHB patients was significantly higher than that of other CHB patients. The advantageous population for Peg-IFNα treatment mainly refers to patients treated with NAs for > 1 year, with low levels of HBsAg (especially ≤ 1500 IU/mL), negative for HBeAg, and with HBV DNA < 2000 IU/mL. Current studies in China, such as the "Mount Everest Engineering Project" and "Hepatitis B Uncapping Project", have all been focusing on the Peg-IFNα dominant population, and the clinical cure rate can reach > 30%[20,40]. For patients with inactive HBsAg carrier status (IHC), the Asia-Pacific, European, and American guidelines unanimously advise against treatment[41-43]. In recent years, studies have found that the domestic IHC population has exceeded 30 million, and with age, the IHC population is at higher risk of HBV reactivation, liver cirrhosis, or even HCC. A study from Taiwan that enrolled 1965 IHC patients and followed them up for an average of 11.5 years showed that 16% had HBV reactivation, and for patients with HBV reactivation, the cumulative incidence of liver cirrhosis during the 20 years reached 46%, and the 25-year cumulative incidence of liver cirrhosis reached 15% in all subjects[44]. In addition, previous studies have found that 14%-24% of IHC patients may develop HBeAg-negative CHB, and nearly 20% may reverse to HBeAg-positive CHB; 25%-62% of IHC patients tend to have moderate to severe liver fibrosis, and 3.3%-62% moderate to severe liver inflammation, while approximately 10% may even have liver cirrhosis[45]. Ethnic differences may be the main reason for the widely different study results between China, Europe, and the United States. Consequently, significant changes have occurred in treating IHC patients in recent years. The definition of the IHC population fits with the category of the Peg-IFNα dominant population, so numerous studies have used Peg-IFNα for IHC patients and achieved significant results. Among 142 Peg-IFNα-naive CHB patients with low HBsAg quantification enrolled in our center, the HBsAg clearance rate reached 47.9%

after 72 wk of Peg-IFNα use[31]. A meta-analysis revealed that the overall HBsAg clearance rate at 48 wk in the IHC population could reach an encouraging 47%[46], also showing that selecting appropriate CHB patients for Peg-IFNα-containing treatment can maximize the advantage of interferon.

Another issue to be addressed is the clinical pathway for individuals who are not suitable candidates for interferon therapy to achieve clinical cure. Currently, many researchers are trying to broaden the study scope of the clinically cured population in pursuit of the maximum clinical cure for CHB patients. Therefore, many researchers have also begun studying nondominant populations' clinical cure. Clinical cure projects such as the "Voyage Project," "Leadership Project," and "Oasis Project" are also underway among non-ideal populations. For instance, in the "Voyage Project," > 40% of patients classified as immunotolerant achieved a reduction in HBsAg to < 3000 IU/mL following Peg-IFNα therapy, which suggests that individuals within the interferon non-ideal population may gradually transition to ideal candidates through proactive treatment, thereby presenting an opportunity to attain the higher goal of clinical cure[47]. In addition, although the clinical cure rate of HBeAg-positive treatment-naive CHB patients using PEG-IFNα-based treatment is lower than that of patients with a predominance of NA treatment, the result is significantly better than that of NA monotherapy[48]. Whether there are better treatment strategies to improve the cure rate for these non-ideal populations in clinical practice, such as setting the goal but the course of treatment and the intermittent treatment strategy, is also being actively explored.

Clinical cure of hepatitis B in children: Studies of clinical cure for children CHB patients have also made a great breakthrough. The HBsAg clearance rate of CHB patients after antiviral and Peg-IFNα treatment is significantly increased, and younger age at starting treatment has been associated with a higher clinical cure rate[49]. It was reported that children tolerated Peg-IFNα well during treatment; this series of study results further strengthened confidence in the clinical cure of CHB. In their research from 2006, Kansu *et al*[50] indicated that the HBsAg clearance rates were 12.5% and 4.6%, respectively, when comparing the initial combination of lamivudine (LAM) with IFN and the sequential combination of LAM with IFN after 2 mo ($P > 0.05$)[50]. The high clinical cure rate observed in children may be attributed to the lower absolute quantity of liver cells and vigorous proliferation, shorter HBV infection duration, and smaller cccDNA reservoir [51,52]. However, the specific mechanisms underlying these factors remain unclear. Subsequently, numerous real-world clinical studies have also demonstrated that pediatric CHB patients exhibit a high response rate to antiviral therapy, particularly younger children, who possess advantageous factors for clinical cure. The earlier the initiation of antiviral therapy, the higher the cure rate[53]. Research conducted by Zhang *et al*[54] found that the clearance rates of HBV DNA, seroconversion rates of HBeAg, and clearance rates of HBsAg in children aged 1-7 years with CHB were higher than those in children aged 7-16 years with CHB[54]. The antiviral efficacy and clinical cure rate of CHB in children are encouraging, which brings hope for more clinical cures of CHB.

Clinical cure of hepatitis B in parturients: HBV-infected parturients are often in a particular immune activation state after delivery. Recently, there has been an increasing number of clinical studies on this group of patients. For example, a domestic study that included HBeAg-positive parturients who received antiviral treatment in the third trimester according to their wishes and for whom the treatment was discontinued immediately after delivery showed that, whether antiviral or not, the ALT level was significantly increased in the postpartum period of pregnancy, and 40% of the parturients had acute inflammation of the liver[55]. Another study that enrolled 45 parturients with CHB showed that 24.4% had an acute increase in ALT and 11.8% had HBeAg clearance; HBeAg-positive parturients may experience immune activation after delivery[56], which may explain the postpartum increase in ALT and the development of hepatitis in the puerpera. With the delivery of the fetus and the release of maternal immunosuppression, CD8+ T cells, their memory T-cell subsets, and effector T-cell subsets in patients with hepatitis activation and the increased expression levels of perforin and granzyme B may have an important role in breaking immune tolerance and causing hepatitis[57]. Thus, the postpartum stage may be a good opportunity to achieve a clinical cure for CHB mothers, and combined with Peg-IFNα treatment, it is expected to further improve the clinical cure rate.

Urgency to explore clinical cure biomarkers: With the expansion of various multicenter clinical studies, the use of Peg-IFNα in the CHB patient population has become more widespread. However, due to the existence of some adverse reactions associated with Peg-IFNα, it is urgent to identify specific indicators to predict the efficacy of Peg-IFNα therapy. On the one hand, this approach can instill more confidence in CHB patients and help identify individuals suitable for Peg-IFNα treatment. On the other hand, it can facilitate timely treatment discontinuation in cases of poor efficacy, thereby maximizing patient benefit. Although HBV DNA can be used as an evaluation indicator for antiviral treatment, it cannot predict HBsAg clearance well. Three early predictive indicators have been identified: Low baseline HBsAg levels, rapid decline in HBsAg at 12/24 wk of treatment, and more than a twofold increase in ALT at 12 wk of treatment. In recent years, various novel serum markers have been used to predict the efficacy of CHB treatment. HBV RNA is transcribed from cccDNA and, to some extent, can reflect the transcriptional activity level of cccDNA within the liver[58]. It has been found that HBV RNA levels were lower in CHB patients who achieved HBeAg clearance, and HBV RNA levels at 12/24 wk could effectively predict HBeAg clearance. A strong positive correlation between HBV RNA and HBeAg has also been established[59]. In current clinical studies on the treatment of CHB, HBV RNA has been identified as a predictive factor for the clinical response to Peg-IFNα treatment at 12 wk[60]. A small number of patients who experience HBsAg clearance may encounter virological relapse after treatment discontinuation, which may be due to the continued production of HBsAg from residual cccDNA. Therefore, HBV RNA might also serve as a biomarker for predicting the timing of discontinuation of Peg-IFNα treatment following therapy.

Limitations of interferon-based clinical cure scheme for hepatitis B

The drawbacks of Peg-IFN include the necessity for rigorous patient selection based on indications, administration *via* subcutaneous injection, and the potential for numerous adverse reactions. Due to the risk of liver decompensation, Peg-IFN is contraindicated in patients with decompensated liver cirrhosis, pregnant women, and those with polyethylene glycol interferon intolerance[61]. Some studies suggest that extending the treatment duration beyond 48 wk is beneficial, particularly in HBeAg-negative patients, but this is challenging in practice due to poor patient tolerability[62,63].

Furthermore, long-term use of interferon may lead to many psychiatric issues such as depression, irritability, anxiety, agitation, loss of appetite, fatigue, sleep disturbances, and impaired cognitive function, significantly impacting patients' quality of life and work capacity[64]. Therefore, a thorough assessment of patients' mental status is necessary before initiating interferon therapy, further narrowing the target patient population. Interferon-associated depression warrants particular attention in clinical practice, emphasizing the necessity of monitoring the mental health status of patients undergoing interferon therapy.

PROSPECTS AND EXPECTATIONS FOR CLINICAL CURE OF HEPATITIS B: NEW DRUG RESEARCH PROJECTS IN FULL SWING

Immune pathogenesis of HBV infection may be a breakthrough in expanding clinical cure

As research progresses, achieving clinical cure through Peg-IFNα inhibition and clearance of HBV has become closely associated with the antiviral immune response of the host. The indicators with an ability to predict cure can be summarized into three aspects: Virological indicators, biochemical indicators, and immunological indicators. Currently, virological indicators include HBsAg levels and declines, HBeAg, HBV DNA, HBcrAg levels and declines, and baseline HBV RNA levels. Biochemical indicators mainly include elevated ALT levels. Immunological indicators encompass natural killer cells, dendritic cells (DCs), B cells, follicular helper T cells, and T regulatory cells. CHB results from complex interactions between host immunity and viral replication involving innate and adaptive immune cells. The mechanisms involved are intricate. Peg-IFNα has been demonstrated to activate both host innate and adaptive immune responses, which is beneficial for increasing the clearance rate of HBsAg in patients. Scholars have achieved remarkable progress in improving immune responses by combining Peg-IFNα with other immune strategies. Studies on mouse liver cancer models have found that combining Peg-IFNα with PD-1 antibodies can restore or even enhance the cytotoxic effects of CD8+ T cells, demonstrating synergistic anti-tumor effects[65]. Evidence confirmed that PD-1+CXCR5+CD8+ T cells possess the functions of traditional CD8+ T cells and can activate B cells. PD-1 does not exhibit an exhausted phenotype in CXCR5+CD8+ T cells. Furthermore, CXCR5+CD8+ T cells have a particular role in controlling viremia in CHB patients [66]. The above-mentioned immune cell subsets may provide potential immunotherapeutic targets for HBV cure. It is believed that with continuous exploration of the immune mechanisms and the discovery of new immune targets, strategies for CHB cure will become more diverse. In conclusion, numerous factors in clinical settings can influence the response to Peg-IFNα. Identifying the factors influencing the response to Peg-IFNα, especially the impact of host immune mechanisms, will be significant for the clinical application of Peg-IFNα.

In 2019, Gane *et al*[67] first attempted to use the PD-1 inhibitor nivolumab in HBeAg-negative CHB patients. Their results showed that at 12 wk of treatment, patients receiving nivolumab at 0.3 mg/kg, regardless of whether HBV therapeutic vaccine was added, had an average decrease in HBsAg of 0.3 Log10 IU/mL compared to baseline. At 24 wk of treatment, patients receiving nivolumab at 0.3 mg/kg had an average decrease in HBsAg of 0.48 Log10 IU/mL compared to baseline[67]. This pilot study provided the first evidence of the efficacy and safety of immune checkpoint inhibitors (ICIs) in non-tumor chronic HBV-infected individuals. It also demonstrated that ICIs can restore HBV-specific immune responses in patients with chronic HBV infection. Some studies have suggested that long-term use of IFN may also induce the expression of PD-L1, which could further suppress T cell function[68,69]. Both IFN-α/γ can induce the expression of PD-L1 in liver cancer cells and liver cells. The IFN-α/γ-STAT1-PD-L1 pathway significantly mediates T cell hyporesponsiveness and the inactivation of liver-infiltrating T cells in the hepatic microenvironment. This effect, known as "adaptive resistance," is a self-protective mechanism for the body. However, this mechanism is not conducive to the clearance of HBV. Therefore, some scholars have proposed the combination of a PD-1 inhibitor and Peg-IFNα to achieve the goal of HBV clearance, relieve immunosuppression, and enhance the scavenging effect of HBsAg. Mouse studies have found that anti-PDL1-IFNα chimeric dimers can enhance the function of DCs, breaking immune tolerance[70]. Combined treatment with the hepatitis B vaccine can induce persistent anti-HBs immune responses in CHB mice, potentially achieving long-term HBsAg clearance. However, there are no clinical studies on combining ICIs with Peg-IFNα in CHB patients. Nevertheless, based on the foundation laid by the above research, it is believed that ICIs combined with Peg-IFNα may achieve more effective clinical cures for CHB, building upon the effects of Peg-IFNα.

Due to the close association between HBV clearance and T cell function, there has been increasing use of T cell engineering and manipulation in the clinical cure of CHB in recent years. One notable approach is the adoptive transfer of engineered T cells. This technique involves obtaining T cells from the patient's blood, modifying them with HBV-specific receptor gene transfer technology to engineer HBV-specific receptors on the surface of T cells, expanding these engineered T cells *in vitro*, and then reinfusing them into the patient's body to exert immunological effects[71]. Currently, there are two main types of engineered T cells used for HBV therapy: Chimeric antigen receptor T cells and T cells modified with specific T cell receptors[72-74]. Studies have found that leukemia patients with concurrent CHB who received bone marrow transplants from donors with HBV-specific T cell responses (vaccinated or spontaneous HBV clearers) experienced HBV clearance[75,76]. Similar studies have detected HBV clearance in recipients with HBV-specific

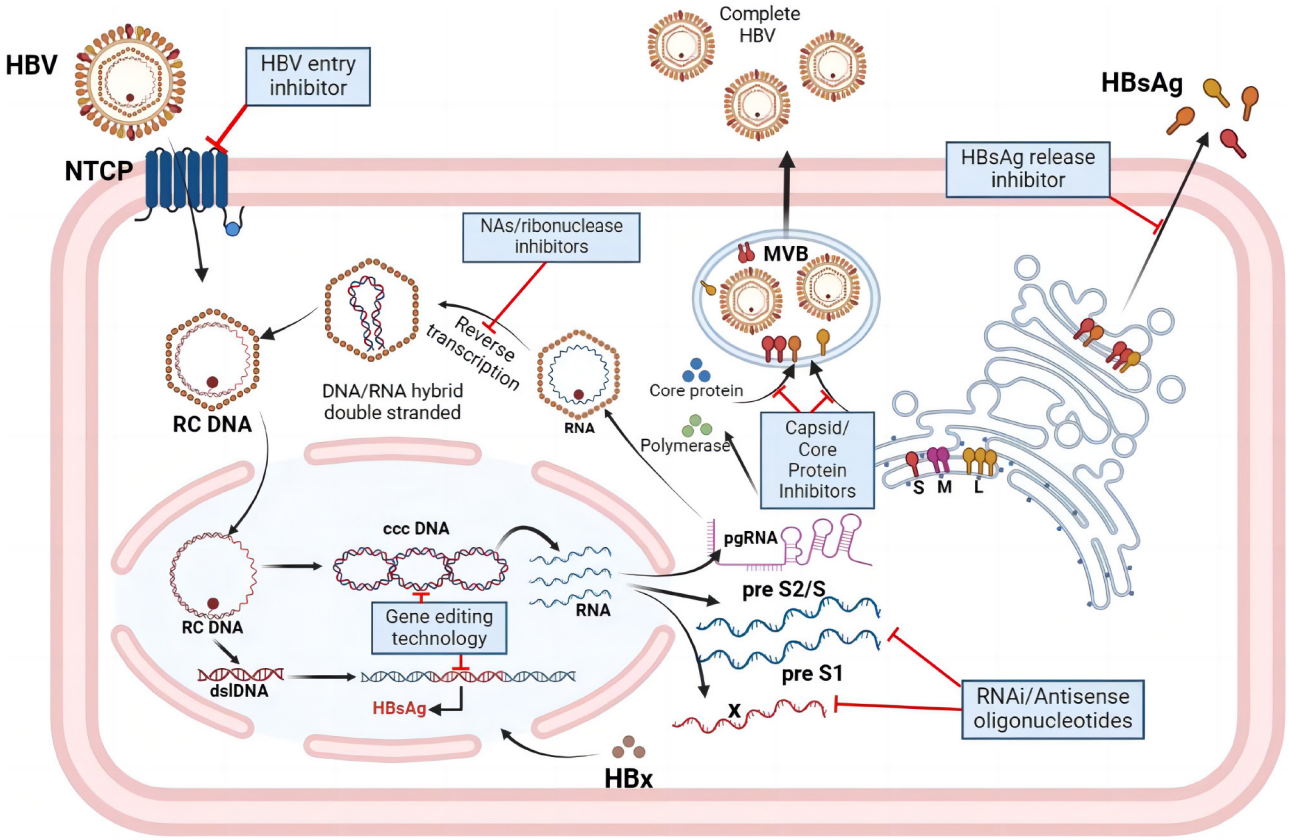

Figure 1 Inhibition of release of HBsAg from infected liver cells. HBV: Hepatitis B virus; NTCP: Sodium taurocholate cotransporter peptide; cccDNA: Covalent, closed, circular DNA; RC DNA: Relaxed circular DNA; dslDNA: Double-stranded linear DNA; pgRNA: Pregenomic RNA; MVB: Polyvesicle; HBsAg: Hepatitis B surface antigen; HBx: Hepatitis B virus x protein.

adaptive immune responses after liver transplantation from HBsAg-positive donors, with increased HBV-specific cellular and humoral immune responses[77]. These studies collectively indicate that the adoptive transfer of engineered HBV-specific T cells for immunological restoration therapy could represent a breakthrough in achieving clinical cure in CHB patients.

Development of drugs targeted at viral life cycle to accelerate clinical cure of CHB

Currently available drugs still cannot completely eliminate cccDNA and integrated host genes containing HBV DNA, while the efficacy of existing antiviral therapies for CHB in clinical use is limited. Therefore, research projects and developing new drugs for HBV treatment are actively underway. With a deeper understanding of the HBV lifecycle, drug development has begun to target the entire process of HBV replication. Anti-HBV drugs currently under development primarily aim to block and inhibit HBV infection and replication in liver cells, exerting antiviral effects by targeting the virus lifecycle. They mainly operate through the following mechanisms: (1) HBV entry inhibitors: Blocking HBV entry into liver cells; (2) Nucleic acid polymers: Inhibiting the production of HBsAg; (3) NAs: Interfering with the DNA polymerase required for HBV DNA replication, commonly used as oral antiviral medications; (4) Post-transcriptional regulation interference: Interfering with or inhibiting HBV RNA, preventing virus translation. Drugs targeting this mechanism mainly include RNA interference and antisense oligonucleotides; (5) Capsid inhibitors: Interfering with the protein capsid of HBV and preventing virus assembly; and (6) HBsAg release inhibitors: Inhibiting the release of HBsAg from infected liver cells (Figure 1).

How far are we from clinical cure to complete cure?

Peg-IFNα can exert antiviral activity at multiple stages of HBV replication. For example, during the reverse transcription of pgRNA into HBV DNA, Peg-IFNα can enhance the expression of APOBEC3 cytidine deaminase and ISGs, inducing widespread G-to-A mutations and cccDNA degradation, thereby blocking HBV DNA replication and depleting the cccDNA pool[78,79]. It can also induce downregulation of HBV RNA synthesis and promote the degradation of pgRNA [80,81]. These mechanisms indicate that the use of Peg-IFNα can somewhat promote the complete cure of CHB. However, the core of a complete cure for HBV infection lies in eradicating all HBV genes, including intrahepatic cccDNA and integrated HBV DNA. Although Peg-IFNα has some effect on cccDNA, its impact remains limited. In recent years, the development of gene editing technology has shown promising perspectives for the complete cure of HBV. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is a repeat sequence in prokaryotic genomes, primarily functioning to excise viral DNA fragments integrated into prokaryotic genomes through an enzyme called Cas9. Initially discovered in bacteria, CRISPR-Cas9 quickly became a breakthrough gene-editing tool for treating various diseases,

including HBV infection. By designing guide RNA complementary to the target DNA sequence, the CRISPR-Cas9 system can selectively and specifically cleave the target DNA genome, resulting in site-specific double-strand breaks. The broken ends induce error-prone non-homologous end-joining, often resulting in frameshift mutations, producing non-functional truncated proteins, and ultimately inactivating the target DNA genome[82,83]. However, before CRISPR-Cas9 technology can be used in clinical applications, potential challenges must be addressed. In the face of these challenges, continuous improvement of CRISPR-Cas9 technology is underway, and utilizing CRISPR-mediated gene editing technology to completely eradicate all HBV genomes to achieve a complete cure may also be a powerful tool in conquering chronic HBV infection.

CONCLUSION

There are still many urgent problems to be solved regarding CHB clinical cohort studies, basic research, new drug research and development, dominant and nondominant populations, efficacy prediction and evaluation indicators, combined antiviral and immune regulation treatment regimens, and individualized treatment. In the future, a complete cure for hepatitis B is expected to require a combination of various drugs. However, the clinical cure for CHB is an achievable ideal treatment goal that researchers should continue to address.

FOOTNOTES

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ORIGINAL ARTICLE

Observational Study

In-hospital outcomes in COVID-19 patients with non-alcoholic fatty liver disease by severity of obesity: Insights from national inpatient sample 2020

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) increases the risk of cardiovascular diseases independently of other risk factors. However, data on its effect on cardiovascular outcomes in coronavirus disease 2019 (COVID-19) hospitalizations with varied obesity levels is scarce. Clinical management and patient care depend on understanding COVID-19 admission results in NAFLD patients with varying obesity levels.

AIM

To study the in-hospital outcomes in COVID-19 patients with NAFLD by severity of obesity.

METHODS

COVID-19 hospitalizations with NAFLD were identified using International Classification of Disease -10 CM codes in the 2020 National Inpatient Sample database. Overweight and Obesity Classes I, II, and III (body mass index 30–40) were compared. Major adverse cardiac and cerebrovascular events (MACCE) (all-cause mortality, acute myocardial infarction, cardiac arrest, and stroke) were compared between groups. Multivariable regression analyses adjusted for sociodemographic, hospitalization features, and comorbidities.

RESULTS

Our analysis comprised 13260 hospitalizations, 7.3% of which were overweight, 24.3% Class I, 24.1% Class II, and 44.3% Class III. Class III obesity includes younger patients, blacks, females, diabetics, and hypertensive patients. On multivariable logistic analysis, Class III obese patients had higher risks of MACCE, inpatient mortality, and respiratory failure than Class I obese patients. Class II obesity showed increased risks of MACCE, inpatient mortality, and respiratory failure than Class I, but not significantly. All obesity classes had non-significant risks of MACCE, inpatient mortality, and respiratory failure compared to the overweight group.

CONCLUSION

Class III obese NAFLD COVID-19 patients had a greater risk of adverse outcomes than class I. Using the overweight group as the reference, unfavorable outcomes were not significantly different. Morbid obesity had a greater risk of MACCE regardless of the referent group (overweight or Class I obese) compared to overweight NAFLD patients admitted with COVID-19.

Key Words: Non-alcoholic fatty liver disease; Obesity; Obese; Body mass index; Major adverse cardiac and cerebrovascular events; Mortality; Acute myocardial infarction; Cardiac arrest; Stroke

Core Tip: Non-alcoholic fatty liver disease (NAFLD) has become a major cause of morbidity and mortality from liver disease and is the most common cause of chronic liver disease, with a global prevalence of 25% and a 50.4% increase in prevalence in the last three decades. To the best of our knowledge, this is the largest study investigating the comorbidities and outcomes of coronavirus disease 2019 hospitalizations with NAFLD and different levels of obesity.

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INTRODUCTION

With the global surge in the obesity pandemic, non-alcoholic fatty liver disease (NAFLD) has become increasingly prevalent and a major contributor to morbidity among liver diseases[1]. There seems to be a bidirectional association between NAFLD and obesity. NAFLD is estimated to have a global prevalence of 25%–30% and up to 90% in morbidly obese individuals[2]. However, these numbers may be underestimated, as NAFLD is usually asymptomatic in the early stages of the disease. Inversely, obese individuals constitute a high proportion of patients with NAFLD[3]. Numerous studies have elucidated the correlation between both coronavirus disease 2019 (COVID-19) and obesity[4] as well as

NAFLD[5], highlighting the adverse outcomes in this patient population. It is widely acknowledged that people falling within the various classes of obesity experience an elevated risk of severe outcomes from COVID-19[6]. The intricate and multifaceted mechanisms linking COVID-19, NAFLD, and obesity encompass systemic inflammation, immune dysregulation, and impaired metabolic pathways. There is a scarcity of data regarding disease progression and prognosis in individuals encompassing all three disease groups. It is crucial to better analyze the outcomes to guide management in this patient population. Given the established relationship between obesity and NAFLD, our study aims to investigate the in-hospital outcomes of NAFLD patients with COVID-19 with varying degrees of obesity categorized by body mass index levels.

MATERIALS AND METHODS

Source of data

The Healthcare Cost and Utilization Project (HCUP), sponsored by the Agency for Healthcare Research and Quality, utilizes the National Inpatient Sample (NIS) databases (2020) as its data sources (Table 1). The NIS is the country's largest publicly accessible all-payer inpatient healthcare dataset. With an average of 7 million unweighted discharges annually or more than 35 million weighted countrywide discharges, it comprises discharge data from roughly 20% of United States hospitals in more than 48 states. One primary diagnosis and up to 39 secondary discharge diagnoses are associated with each inpatient admission to the NIS. Since the NIS contains de-identified data, IRB approval was not required. More details regarding the database are available on the HCUP website.

Study population

Using the International Classification of Disease (ICD-10) diagnostic codes and NIS database for the year 2020, hospitalizations with COVID-19 were identified in patients with NAFLD. The NAFLD patients were divided into four groups based on their obesity class: overweight [body mass index (BMI) 25 or higher], Class I (BMI 30–34.9), Class II (BMI 35–39.9), and Class III (BMI 40 or higher). Baseline characteristics, comorbidities, and in-patient outcomes like respiratory failure, mechanical ventilation, and major adverse cardiac and cerebrovascular events (MACCE) [all-cause mortality, acute myocardial infarction (AMI), cardiac arrest, and stroke] were compared between obesity classes. Multivariable regression analyses were adjusted for sociodemographic, hospitalization characteristics, and comorbidities.

Outcomes of interest

The primary outcomes of interest in the study were the in-hospital outcomes of COVID-19 admissions among NAFLD patients stratified by obesity class. These outcomes included respiratory failure, the need for mechanical ventilation, and MACCE. MACCE was defined as the occurrence of all-cause mortality, AMI, cardiac arrest, or stroke. Individual components of MACCE were included as secondary outcomes. Baseline characteristics and comorbidities were also assessed and compared between obesity classes.

Statistical analyses

To compare baseline demographics and hospital characteristics, the Pearson Chi-square test was used to look at categorical variables, and the Mann-Whitney *U* test was used to look at continuous variables, since the distribution of continuous data across obesity classes was not normal. Continuous variables were presented as the median, whereas categorical variables were expressed as percentages. A two-tailed *P* value of less than 0.05 was considered statistically significant. National estimates were generated using complex sample modules, strata or cluster designs, and discharge weights (DISCWT). Multivariable regression analyses were performed to assess the relationship between obesity class and in-hospital outcomes while controlling for confounding factors and keeping overweight and Class I obesity as referent groups individually. This model factored in age, gender, race, elective vs. non-elective admission type, median household income, length of stay, insurance payer, hospital bed size, ownership, location/teaching status of the hospital, as well as clinically relevant comorbid conditions. To report the logistic regression results, adjusted odds ratios (OR), 95% confidence intervals (CI), and *P* values were calculated. IBM SPSS Statistics 25.0 (IBM Corp., Armonk, NY, United States) software was used for all statistical analyses.

RESULTS

There were a total of 13260 admissions for COVID-19 among patients with NAFLD who had a BMI of over 25, and out of those patients, 7.3% were categorized as overweight, 24.3% were categorized as Class I, 24.1% were categorized as Class II, and 44.3% were categorized as Class III. Overall, the entire study group contained more individuals who were white (42.6%) compared to blacks (14%). In total, 6.2% of total admissions died during hospitalization, and 9.1% suffered MACCE events (AMI: 2.5%, Acute ischemic stroke: 0.4%). 77% had routine dispositions; 12.2% required home health care; 8.6% were transferred to a skilled nursing facility; and 2.2% were transferred to a short-term hospital (Table 2).

Class III contained younger individuals than the overweight sub-group (39.0% *vs* 18.7%); more people were black (14.0% *vs* 7.9%), fewer Hispanics (35.6% *vs* 43.8%), and fewer Asians (2.8% *vs* 5.1%). In terms of comorbidities, Class III had lower hypertension rates compared to the overweight subgroup (58.4 *vs* 61.1%) and a lower rate of chronic kidney disease (9.3% *vs* 11.4%), while having higher rates of chronic pulmonary disease (22.3% *vs* 15.5%). Patients who were

Table 1 Baseline characteristics of coronavirus disease 2019 hospitalizations in non-alcoholic fatty liver disease patients with different levels of obesity: Insights from national inpatient sample 2020

Variables		Overweight, %	Class I, %	Class II, %	Class III, %	*P* value
Age (yr) at admission	Mean	58	53	52	48	< 0.001
Sex	Male	58.5	59.7	52	43.3	< 0.001
	Female	41.5	40.3	48	56.7	< 0.001
Race	White	42.1	41.6	45	49.5	< 0.001
	Black	7.9	11.8	14.9	15.8	< 0.001
	Hispanic	43.8	41.4	36.3	30.9	< 0.001
	Asian/PI	5.1	4.5	1.8	2.2	< 0.001
	Nat. American	< 0.1	0.8	2	1.6	< 0.001
Payer	Medicare	34.1	26.4	26.6	19.9	< 0.001
	Medicaid	26.7	21.8	15.1	20.7	< 0.001
	Private	33	44.8	49.1	50.9	< 0.001
	Self-pay	5.1	6.7	8.7	8.1	< 0.001
Admission type	Non-elective	99	98.8	98.4	97.7	< 0.001
Comorbidities	Hypertension	61.1	56.4	58.4	59.1	0.024
	Diabetes	46.6	46.7	48.8	50.6	< 0.001
	Hyperlipidemia	45.1	42.5	41.6	32.3	< 0.001
	PVD	3.1	3.6	5.3	2.2	< 0.001
	Tobacco use disorder	19.2	21.6	20.3	17.6	< 0.001
	Prior MI	3.1	2	1.6	1.6	0.007
	Prior TIA/Stroke	3.1	2.8	3	1.7	< 0.001
	Prior VTE	2.6	4	3.6	4.2	0.090
	Cancer	5.2	2.5	1.7	2.5	< 0.001
	CKD	11.4	9.1	9.9	8.7	0.029
	Drug abuse	2.1	1.2	1.7	1.8	0.161
	Cannabis use disorder	< 0.1	0.8	0.6	0.9	0.029
	Depression	7.3	10.1	11.4	13.1	< 0.001
	Chronic pulmonary disease	15.5	18.3	21.4	26.1	< 0.001
	Hypothyroidism	10.9	9.1	10.5	13.9	< 0.001
	Bariatric surgery	< 0.1	0.9	1.9	1.5	< 0.001
Discharge	Routine	66.7	77.4	78.9	77.3	< 0.001
	Transfer to short term Hospital	< 0.1	3	2	2	< 0.001
	Other: SNF, ICF, *etc.*	16.7	6.9	7.5	9%	< 0.001
	Home health care	15.5	12.7	11.6	11.7	< 0.001
Length of stay (d)	Median	10	8	8	8	< 0.001

Overweight BMI 25-29.9; Class I Obesity BMI 30-34.9; Class II Obesity BMI 35-39.9; Class III Obesity BMI 40 or higher. PI: Pacific Islander, MI: Myocardial Infarction; PVD: Peripheral vascular disease; VTE: Venous thromboembolism; SNF: Skilled nursing facility; ICF: Intermediate care facility; MACCE: Major adverse cardiovascular and cerebrovascular events.

Table 2 Multivariable odds of in-hospital outcomes in obese coronavirus disease 2019 hospitalizations with non-alcoholic fatty liver disease

	Obesity II *vs* I	**Obesity III *vs* I**	**Obesity I *vs* overweight**	
Outcomes	aOR [LL-UL]	aOR [LL-UL]	aOR [LL-UL]	*P* value
MACCE	1.19 [0.78-1.81]	1.82 [1.22-2.73]	1.66 [0.92-2.98]	0.016
Death during hospitalization	1.10 [0.69-1.76]	1.96 [1.23-3.11]	1.59 [0.84-3.02]	0.015
Acute myocardial infarction	1.91 [0.77-4.72]	2.32 [0.92-5.88]	1.72 [0.53-5.57]	0.346
Cardiac arrest	1.14 [0.49-2.67]	1.25 [0.56-2.80]	0.60 [0.13-2.78]	0.782
Acute ischemic stroke	0.23 [0.04-1.28]	0.36 [0.06-2.02]	5.52 [0.55-55.56]	0.130
Respiratory failure	1.27 [0.99-1.61]	1.35 [1.07-1.70]	0.97 [0.67-1.41]	0.049
Mechanical ventilation	0.90 [0.63-1.31]	1.34 [0.97-1.85]	1.01 [0.58-1.75]	0.095
	I *vs* overweight	**II *vs* overweight**	**III *vs* overweight**	
Outcomes	aOR [LL-UL]	aOR[LL-UL]	aOR[LL-UL]	*P* value
MACCE	0.60 [0.34-1.08]	0.72 [0.39-1.32]	1.10 [0.62-1.95]	0.016
Death during hospitalization	0.63 [0.33-1.19]	0.69 [0.35-1.35]	1.23 [0.66-2.30]	0.015
Respiratory failure	1.04 [0.71-1.51]	1.28 [0.88-1.88]	1.40 [0.96-2.04]	0.057
Mechanical ventilation	0.99 [0.57-1.72]	0.90 [0.51-1.59]	1.33 [0.78-2.27]	0.095

Overweight BMI 25-29.9; Class I Obesity BMI 30-34.9; Class II Obesity BMI 35-39.9; Class III Obesity BMI 40 or higher. Multivariable regression models were adjusted for age at admission, sex, race, income quartile for patient's ZIP code, type of admission, hospital location/teaching status, hypertension, diabetes, hyperlipidemia, peripheral vascular disease, tobacco use disorder, prior MI, prior TIA/Stroke, cancer, CKD and Bariatric surgery status. aOR: Adjusted odds ratio; LL: Lower limit, UL: Upper limit; MACCE: Major adverse cardiac and cerebrovascular events.

overweight had higher rates of MACCE (13% compared to 7.4%, 8.3%, and 9.8%), more inpatient mortality (9.8% *vs* 5.6%, 5.6%, and 7.4%), and higher rates of mechanical ventilation (13% *vs* 11%, 9.7%, and 12.9%). Patients who were overweight also had higher rates of inpatient mortality (9.8% *vs* 5.6%, 5.6%, and 7.4%). This finding, however, did not seem to be significant for MACCE (aOR: 1.66, CI: 0.922-2.98, $P > 0.05$), for inpatient mortality (aOR: 1.59, CI: 0.84–3.02, $P > 0.05$), or mechanical ventilation (aOR: 1.01, CI: 0.58–1.75, $P > 0.05$). The multivariate analysis proved this by showing that there was no significant relationship between these factors and the outcome.

In Class III, more people were younger than there were in Class I (39% against 25.3%), more people were black (15.8% *vs* 11.8%), more people had hypertension (59.1% versus 56.4%), more diabetics (50.6% *vs* 46.7%), more people had chronic pulmonary disease (26.1% *vs* 18.3%), more females (56.7% *vs* 40.3%), and fewer men (11.2% *vs* 22.6%). On Multivariable logistic analysis, Class III obese patients, when compared with Class I individuals, had higher odds of MACCE (aOR: 1.82, CI:1.22-2.73, $P = 0.016$), higher odds of inpatient mortality (aOR:1.96, CI: 1.23-3.11, $P = 0.015$), and higher odds of respiratory failure (aOR: 1.35, CI: 1.07-1.70, $P = 0.049$), while for the need for mechanical ventilation, Acute ischemic stroke, AMI, and cardiac arrest, both groups did not differ.

DISCUSSION

To the best of our knowledge, this is the largest study investigating the comorbidities and outcomes of COVID-19 hospitalizations with NAFLD and different levels of obesity. We report the following key points of the study: (1) The overwhelming majority of our study population were whites, even though African Americans have the highest prevalence of obesity; (2) Compared to the overweight and Class I obesity groups, the Class III obesity group were more frequently younger; (3) The overweight group had higher rates of MACCE and in-hospital mortality than all other groups; and (4) Class III obese patients had higher risks of MACCE, inpatient mortality, and respiratory failure than Class I obese patients. However, compared to the overweight group, all other obesity classes had non-significant risks of MACCE, inpatient mortality, and respiratory failure.

In recent times, NAFLD has become a major cause of morbidity and mortality from liver disease. It is the most common cause of chronic liver disease, with a global prevalence of 25% and a 50.4% increase in prevalence in the last three decades[7]. These rates coincide with the increasing pandemic of obesity and type 2 diabetes mellitus (T2DM). Obesity is a global health problem affecting over 2 billion people. BMI is often used to gauge obesity and correlates with cardiovascular diseases, diabetes, hypertension, and certain cancers. The severity of NAFLD also increases with worsening obesity, which is probably why a majority of the patients hospitalized with NAFLD and COVID-19 in our study belonged to the Class III obesity group[8]. There is a rising trend of obesity and overweight in young adults due to increased consumption of calorie-rich foods like fast foods, a lack of exercise, and other socioeconomic and psychological

factors. It was interesting to note that the Class III group had younger individuals but fewer elderly individuals than the overweight group. This is in accordance with the latest NCHS data on obesity[9]. Although the prevalence of obesity among blacks is the highest among any other race, in our study, whites contributed to the majority of the study population. Multiple studies have shown that African American races are protected from NAFLD despite having a higher prevalence of diabetes and obesity, possibly due to fundamental ethnic differences in lipid hemostasis[10]. However, compared to the overweight population, the Class III obesity group more frequently consisted of blacks, coinciding with the most recent national NCHC data on obesity[9].

NAFLD is closely related to metabolic syndrome and hence shares many risk factors with cardiovascular disease (CVD), strengthening the association between the two. There is a strong bidirectional association between the two, which suggests the need for clinical interventions to modify metabolic risk factors like T2DM, dyslipidemia, hypertension, and obesity in this population. NAFLD, obesity, and COVID-19 are highly inflammatory states and are independent risk factors for CVD and associated worse outcomes. NAFLD is associated with a pro-atherogenic lipid profile[11,12] and increased circulating levels of pro-inflammatory cytokines like interleukin (IL)-6[13]. Liver damage is one of the important aspects of COVID-19[14,15]. COVID-19 infection has been linked to elevated levels of serum IL-6, a cytokine with pro-atherogenic properties that can contribute to unfavorable outcomes in individuals with pre-existing coronary artery disease. Furthermore, elevated IL-6 causes a pro-inflammatory state, which has been associated with obesity as well[16]. Logically speaking, patients with worse obesity should have worse outcomes. However, several studies have shown that there is no necessary linear relationship between increasing BMI and worse outcomes. This finding is further highlighted in our study. It was interesting to note that Class III obesity had decreased rates of MACCE, in-patient mortality, and AMI compared to the overweight group but higher rates than the Class I obesity group. Many studies have shown cardiovascular diseases to have better outcomes in overweight and obese patients compared to their leaner counterparts[17], and this phenomenon is termed the "obesity paradox". This paradox, however, is most likely true for patients with Class 1 obesity[18]. The risk of worse outcomes with increasing obesity follows a U-shaped curve. Class III had worse outcomes than Class I in our study. Although the obesity paradox exists, it may not apply to the morbidly obese[19]. Compared to overweight patients, all other obesity classes had no significant risk for MACCE, inpatient mortality, cardiorespiratory failure. The reasons for this could be multiple. Metabolically "obese" normal-weight individuals (MONW) are those who are on the leaner side but metabolically unhealthy. Current obesity guidelines, which are based on BMI, fail to distinguish between MONW and metabolically healthy obese[20]. This could explain the non-linear relationship between BMI and adverse outcomes. Moreover, leaner individuals have a low pre-test probability and may present at a more advanced stage of the disease, which could lead to an overall worse prognosis.

The NIS helped us achieve nationwide estimates using weighted discharge records. However, there are a few limitations to be considered. These include over- or under-coding errors due to the administrative nature of data collection, no follow-up information, and a lack of medication data. The research uses codes for diagnosis to detect NAFLD, which could result in an estimation of the prevalence of NAFLD, particularly in individuals with less severe forms of the condition. This potential bias in diagnosis might affect how we perceive the severity of NAFLD among obesity categories. Even though multivariable regression analyses have been used to account for factors, there is still a possibility of confounding. There might be unconsidered variables that could affect the reported connections between obesity classes and COVID-19 outcomes. The cross-sectional design of the study makes it challenging to determine a cause-and-effect relationship between NAFLD, different levels of obesity, and COVID-19 outcomes. Moreover, we cannot dismiss the possibility of reverse causation, meaning that COVID-19 impacts outcomes related to obesity. It is important to validate and consider the implications of this study in relation to patient care, taking into account the broader clinical context.

CONCLUSION

In this nationwide analysis, Class III obese NAFLD COVID-19 patients had a greater risk of adverse outcomes than Class I. Using the overweight group as a reference, unfavorable outcomes were not significantly different. Morbid obesity had a greater risk of MACCE regardless of the referent group (overweight or class I obese) compared to overweight NAFLD patients admitted with COVID-19. The presence of morbid obesity consistently amplifies the occurrence of cardiovascular events underscoring the need for personalized care, within this particular high-risk subgroup of COVID-19 population diagnosed with NAFLD.

FOOTNOTES

Author contributions: Srikanth S and Desai R contributed to resources; Srikanth S, Garg V, Subramanian L, Verma J, Klair H, Kavathia S, Teja J, Vasireddy N, Kumar A, Dhanush K, Bodhankar S, Hashmi S, and Desai R contributed to writing-original draft; Garg V, Subramanian L, Verma J, Sharma H, Klair H, Kavathia S, Teja J, Vasireddy N, Kumar A, Dhanush K, Bodhankar S, Hashmi S, Chauhan S, and Desai R contributed to writing - review & editing; Srikanth S and Sharma H, and Desai R contributed to visualization; Sharma H and Chauhan S contributed to supervision; Chauhan S and Desai R contributed to conceptualization, methodology; Desai R contributed to software, formal analysis; All authors have read and approved the final manuscript.

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ORIGINAL ARTICLE

Observational Study

Liver histological changes in untreated chronic hepatitis B patients in indeterminate phase

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Abstract

BACKGROUND

Studies with large size samples on the liver histological changes of indeterminate phase chronic hepatitis B (CHB) patients were not previously conducted.

AIM

To assess the liver histological changes in the indeterminate phase CHB patients using liver biopsy.

METHODS

The clinical and laboratory data of 1532 untreated CHB patients were collected, and all patients had least once liver biopsy from January 2015 to December 2021. The significant differences among different phases of CHB infection were compared with *t*-test, and the risk factors of significant liver histological changes were analyzed by the multivariate logistic regression analysis.

RESULTS

Among 1532 untreated CHB patients, 814 (53.13%) patients were in the indeterminate phase. Significant liver histological changes (defined as biopsy score ≥ G2 and/or ≥ S2) were found in 488/814 (59.95%) CHB patients in the indete-rminate phase. Significant liver histological changes were significant differences among different age, platelets (PLTs), and alanine aminotransferase (ALT) subgroup in indeterminate patient. Multivariate logistic regression analysis indicated that age

≥ 40 years old [adjust odd risk (aOR), 1.44; 95% confidence interval (CI): 1.06-1.97; P = 0.02], PLTs ≤ 150 × 10^9/L (aOR, 2.99; 95%CI: 1.85-4.83; P < 0.0001), and ALT ≥ upper limits of normal (aOR, 1.48; 95%CI: 1.08, 2.05, P = 0.0163) were independent risk factors for significant liver histological changes in CHB patients in the indeterminate phase.

CONCLUSION

Our results suggested that significant liver histological changes were not rare among the untreated CHB patients in indeterminate phase, and additional strategies are urgently required for the management of these patients.

Key Words: Chronic hepatitis B; Indeterminate phase; Gray-zone; Liver biopsy; Pathological histology; Risk factors

Core Tip: In this study, we investigated liver histological changes in 814 chronic hepatitis B patients in the indeterminate phase, in which significant liver histological changes (defined as biopsy score ≥ G2 and/or ≥ S2) were found in 488/814 (59.95%) patients, which suggested that additional strategies are urgently required for management of these patients.

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INTRODUCTION

Hepatitis B virus (HBV) infection is an important public health concern worldwide, and also one of the leading causes of liver inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)[1-3]. At present, chronic hepatitis B (CHB) infection is mainly divided into four phases, including immune tolerant phase, HBeAg-positive immune active phase, inactive phase, and HBeAg-negative immune active phase[1-3]. The concept of CHB phase is clinically important to determine the history and risk of developing CHB- related complications, as well as assisting clinicians in the therapy of CHB. Antiviral therapy is currently recommended for CHB patients in HBeAg- positive and -negative immune active phases to reduce the risk of cirrhosis and HCC, while regular monitoring of CHB patients in inactive and immune tolerant phases is essential[1-4].

Nevertheless, there are still some "gray-zone" patients beyond the definition of CHB phase[5]; for instance, corresponding to CHB patients in HBeAg-positive immune tolerant phase whose alanine aminotransferase (ALT) level is normal and serum HBV-DNA level > 10^6 IU/m, patients with normal ALT level and serum HBV-DNA level ≤ 10^6 IU/L that would be beyond the clinical criteria of tolerant phase, these "gray-zone" patients cannot be assigned into none of the four phases and allocated into indeterminate phase, and the main management strategy for them is monitoring[1,6-8]. Recently, studies showed that the disease progression of these patients was inconsistent. For instance, a study performed in the United States showed that Caucasian patients with indeterminate phase CHB without antiviral treatment had a good outcome in the long-term follow-up, and antiviral therapy could be safely avoided or delayed[8]. Nevertheless, studies found that untreated indeterminate CHB patients were at a higher risk of HCC than the immune tolerant and inactive CHB patients, although majority of those patients remained indeterminate during follow-up, had a higher 10-year cumulative HCC incidence and a higher risk of HCC than those remained in the inactive phase[9,10]. Therefore, it remains controversial whether these patients deserve long-term monitoring or require further positive intervention. Therefore, the present study was conducted to explore liver histological changes in the untreated indeterminate phase CHB patients by liver biopsy.

MATERIALS AND METHODS

Patients

A total of 3658 CHB patients who underwent liver biopsy in the Third People's Hospital of Shenzhen (Shenzhen, China) from January 2015 to December 2021 were enrolled. These adult patients (older than 18), with HBsAg positive more than 6 months and never received antiviral therapy. Patients had a stable state of hepatitis B related antigen, and antibodies, HBV-DNA level, and ALT level within 6 months before liver biopsy. Patients with the following conditions were excluded: Co-infection with other viruses, including hepatitis A virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, Epstein-Barr virus, cytomegalovirus, herpes virus infection, human immunodeficiency virus infection; co-existence of decompensated liver cirrhosis, HCC, and other malignant liver tumors; concurrent with other chronic liver diseases, such as autoimmune hepatitis, alcoholic liver disease, primary biliary cirrhosis, nonalcoholic fatty liver disease; immune phase were unstable within 6 months before liver biopsy; and a history of undergoing liver transplantation before the

enrolment. Finally, 1532 CHB patients were met the inclusion criteria and included in the study (Figure 1). All data were collected from electronic medical record system of our hospital, including age, gender, disease history, albumin (ALB), ALT, aspartate aminotransferase (AST), glutamate aminotransferase (GGT), total bilirubin (TBIL), white blood cell (WBC), platelet (PLT), HBsAg, HBeAg, HBeAb, HBcAb, HBV-DNA level, imaging findings and liver biopsy report. The present study was approved by the Ethics Committee of the Third People's Hospital of Shenzhen (Approval No. 2022-003), and patients' informed consent was waived due to the retrospective nature of the study.

Definitions

According to the clinical staging criteria presented by the American Association for the Study of Liver Diseases (AASLD) guideline (ver. 2018)[1], patients with CHB were divided into the four phases, including immune tolerant phase, HBeAg-positive immune active phase, inactive phase, and HBeAg-negative immune active phase. The upper limits of normal (ULN) of ALT was 35 U/L for males and 25 U/L for females based on the AASLD guideline (ver. 2018)[1,11].

Patients who did not meet the clinical criteria of four phases were classified as indeterminate phase. According to the different statuses of HBeAg, and the levels of ALT and HBV-DNA, corresponding to the four-phase, patients in the indeterminate phase were further divided into 4 subgroups: The indeterminate A phase (patients with HBeAg-positive, normal ALT level, and serum HBV-DNA level ≤ 10^6 IU/mL); the indeterminate B phase (patients with HBeAg-positive, ALT ≥ 2 ULN and serum HBV- DNA level < 20000 IU/mL, or patients with HBeAg-positive, ALT equal to 1-2 ULN, regardless of HBV-DNA level); the indeterminate C phase (patients with HBeAg- negative, normal ALT level, and serum HBV-DNA level ≥ 2000 IU/mL); and indeterminate D phase (patients with HBeAg-negative, ALT ≥ 2 ULN and serum HBV-DNA level < 2000 IU/mL, or patients with HBeAg-negative, ALT equal to 1-2 ULN, regardless of HBV- DNA level (Supplementary Table 1)[5].

Prediction models for liver histology

In the present study, aspartate aminotransferase-to-PLT ratio index (APRI), fibrosis-4 (FIB-4) index, and gamma-glutamyl transpeptidase to PLT ratio (GPR) were used to assess liver histological changes. Studies have shown that APRI < 1.0, FIB-4 < 1.45, and GPR ≤ 0.32 were the critical values for excluding patients with advanced fibrosis and liver cirrhosis[1-4,12,13].

Liver biopsy and histological assessment

Liver tissues were obtained by percutaneous liver biopsy using a 16-gauge disposable needle. The inflammation and fibrosis of the liver were assessed by the Scheuer scoring system. The histological grading of hepatic inflammation ranged from G0 to G4, and fibrosis from S0 to S4. Liver inflammation ≥ G2 was defined as significant inflammation, liver fibrosis ≥ S2 was defined as significant fibrosis, and liver inflammation ≥ G2 and/or liver fibrosis ≥ S2 was defined as significant liver histological changes. Liver samples were evaluated and reviewed by two experienced pathologists who were blinded to patients' biochemical data.

Statistical analysis

Data were presented as frequency or percentage for categorical variables, and as mean ± SD or median interquartile range for continuous variables. Univariate and multivariate logistic regression analyses were performed to determine factors associated with liver histological changes. Odds ratios (ORs) and 95% confidence intervals (CIs) with *P*-values were presented. A two-tailed $P < 0.05$ was considered statistically significant. Data were analyzed using SPPS 24.0 software (IBM, Armonk, NY, United States), the R 3.1.2 statistical package (R Foundation for Statistical Computing, Vienna, Austria), and EmpowerStats software (X&Y Solutions, Inc., Boston, MA, United States).

RESULTS

Patients' characteristics

The mean age of patients was 38.09 ± 9.07 years, 69.97% of patients were male, and 118 (7.70%), 197 (12.86%), 317 (20.69%), and 86 (5.61%) patients were in the immune tolerant phase, HBeAg-positive immune active phase, immune inactive phase, and HBeAg-negative immune active phase, respectively. Moreover, 814 (53.13%) patients were assigned into the indeterminate phase (Figure 2A). The characteristics of all patients were shown as Table 1. There were 62 (7.62%), 160 (19.66%), 304 (37.35%), and 288 (35.38%) patients in the indeterminate A, B, C, and D phases, respectively (Figure 2A). The characteristics of the indeterminate phase patients as shown in Table 2. Patients in the indeterminate C and D phases were older than those in the indeterminate A and B phases. The levels of ALT, AST, TBIL, HBsAg, and HBV-DNA in the indeterminate B phase were higher than those in other indeterminate phases, while the levels of ALB, GGT, WBC and PLT had no significant.

Noninvasive model

APRI, FIB-4, and GPR scores are presented in Supplementary Tables 2 and 3. The proportions of APRI < 1, FIB-4 < 1.45, and GPR ≤ 0.32 in the indeterminate patients were 94.96%, 81.33% and 61.06%, respectively. Similarly, APRI < 1 and FIB-4 < 1.45 had the highest proportions in the different indeterminate sub-phases.

Table 1 Clinical characteristics of chronic hepatitis B patients in the different phases, *n* (%)

Phase	Immune-Tolerant CHB	HBeAg-positive immune active CHB	Inactive CHB	HBeAg-negative Immune Active CHB	Indeterminate	*P* value
N	118 (7.70)	197 (12.86)	317 (20.70)	86 (5.61)	814 (53.13)	
Age, yr	33.11 ± 7.52	32.41 ± 7.15	40.13 ± 9.37	40.43 ± 9.48	39.15 ± 8.73	< 0.001
Male	53 (44.92)	131 (66.50)	222 (70.03)	68 (79.07)	598 (73.46)	< 0.001
HBsAg, log10, IU/mL	4.62 ± 0.59	4.18 ± 0.73	2.38 ± 1.26	3.28 ± 0.65	3.18 ± 1.02	< 0.001
HBeAg						< 0.001
Negative	0 (0.00)	0 (0.00)	317 (100.00)	86 (100.00)	592 (72.73)	
Positive	118 (100.00)	197 (100.00)	0 (0.00)	0 (0.00)	222 (27.27)	
HBV-DNA, log10, IU/mL	8.03 ± 0.54	7.60 ± 1.01	2.47 ± 0.56	5.68 ± 1.40	4.71 ± 1.73	< 0.001
ALT, (IQR), U/L	19.00 (15.00-23.00)	138.00 (88.00-233.00)	18.00 (14.00-25.00)	106.50 (79.00-245.00)	33.00 (23.00-45.00)	< 0.001
AST, (IQR), U/L	20.00 (17.00-22.75)	74.00 (50.00-129.00)	20.00 (17.00-23.00)	57.00 (42.25-108.00)	26.00 (21.00-33.00)	< 0.001
GGT, (IQR), U/L	15.00 (12.00-20.00)	52.00 (29.00-89.00)	18.00 (14.00-27.00)	50.50 (34.25-80.25)	25.00 (17.00-40.00)	< 0.001
TBIL, (IQR), umol/L	11.25 (8.20-14.47)	15.20 (10.90-21.60)	12.40 (9.30-16.70)	15.60 (11.83-21.20)	12.75 (9.90-17.30)	< 0.001
ALB, g/L	44.53 ± 3.42	42.52 ± 4.26	44.85 ± 3.23	43.57 ± 5.68	44.50 ± 3.91	< 0.001
WBC, 10^9/L	5.62 ± 1.24	5.55 ± 1.59	5.69 ± 1.44	5.85 ± 1.97	5.85 ± 1.60	0.062
PLT, 10^9/L	228.04 ± 58.84	198.12 ± 54.38	204.09 ± 57.04	200.98 ± 65.66	205.85 ± 59.42	< 0.001
Stage of Inflammation						< 0.001
0	2 (1.69)	0 (0.00)	11 (3.47)	0 (0.00)	14 (1.72)	
1	90 (76.27)	31 (15.74)	271 (85.49)	23 (26.74)	506 (62.16)	
2	26 (22.03)	113 (57.36)	33 (10.41)	48 (55.81)	263 (32.31)	
3	0 (0.00)	51 (25.89)	2 (0.63)	15 (17.44)	26 (3.19)	
4	0 (0.00)	2 (1.02)	0 (0.00)	0 (0.00)	5 (0.61)	
Degree of fibrosis						< 0.001
0	3 (2.54)	5 (2.54)	14 (4.42)	1 (1.16)	17 (2.09)	
1	82 (69.49)	72 (36.55)	181 (57.10)	28 (32.56)	390 (47.91)	
2	29 (24.58)	65 (32.99)	80 (25.24)	32 (37.21)	219 (26.90)	
3	4 (3.39)	31 (15.74)	25 (7.89)	8 (9.30)	96 (11.79)	
4	0 (0.00)	24 (12.18)	17 (5.36)	17 (19.77)	92 (11.30)	
≥ G2	26 (22.03)	166 (84.26)	35 (11.04)	63 (73.26)	294 (36.12)	< 0.001
≥ S2	33 (27.97)	120 (60.91)	122 (38.49)	57 (66.28)	407 (50.00)	< 0.001
G2/S2	44 (37.29)	171 (86.80)	129 (40.69)	71 (82.56)	488 (59.95)	< 0.001

IQR: Interquartile range; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Glutamate aminotransferase; TBIL: Total bilirubin; ALB: Albumin; WBC: White blood cell; PLT: Platelet; CHB: Chronic hepatitis B.

Patients' histopathological characteristics

Of 1532 CHB patients, significant inflammation, fibrosis, and liver histological changes were found among 584 (38.12%), 739 (48.24%), and 903 (58.94%) patients, respectively. The proportions of significant inflammation in the immune tolerant, HBeAg-positive immune active, inactive, HBeAg-negative immune active, and indeterminate phases were 22.03%, 84.26%, 11.04%, 73.26%, and 36.12%, respectively; the proportions of significant fibrosis were 27.97%, 60.91%, 38.49%, 66.28%, and 50.00%, respectively; and the proportions of significant liver histological changes were 37.29%, 86.80%, 40.69%, 82.56%, and 59.95%, respectively (Table 1 and Figure 2B). The proportions of significant inflammation in

Table 2 Clinical characteristics of different indeterminate phase chronic hepatitis B, *n* (%)

Phase	Indeterminate-A	Indeterminate-B	Indeterminate-C	Indeterminate-D	*P* value
N	62 (7.62)	160 (19.66)	304 (37.35)	288 (35.38)	
Age, yr	36.42 ± 7.56	34.30 ± 7.70	40.93 ± 8.08	40.56 ± 9.05	< 0.001
Male	46 (74.19)	111 (69.38)	211 (69.41)	230 (79.86)	0.019
HBsAg, log10, IU/mL	3.58 ± 0.81	4.12 ± 0.79	2.99 ± 0.73	2.77 ± 1.08	< 0.001
HBeAg					< 0.001
Negative	0 (0.00)	0 (0.00)	304 (100.00)	288 (100.00)	
Positive	62 (100.00)	160 (100.00)	0 (0.00)	0 (0.00)	
HBV-DNA, log10, IU/mL	4.33 ± 1.22	7.03 ± 1.72	4.28 ± 0.79	3.88 ± 1.40	< 0.001
ALT, (IQR), U/L	24.00 (18.25-28.00)	45.00 (38.00-55.00)	21.00 (16.00-26.00)	43.00 (37.00-57.25)	< 0.001
AST, (IQR), U/L	23.00 (20.00-26.38)	32.00 (27.80-40.00)	21.00 (18.00-24.00)	29.50 (25.00-38.00)	< 0.001
GGT, (IQR), U/L	20.00 (16.00-32.80)	32.15 (18.00-53.25)	19.00 (14.00-25.23)	32.00 (21.75-49.25)	0.167
TBIL, (IQR), umol/L	13.10 (8.98-16.71)	13.55 (10.50-17.42)	12.55 (9.70-16.35)	12.90 (10.07-17.72)	< 0.001
ALB, g/L	43.83 ± 4.28	43.96 ± 4.05	44.71 ± 3.65	44.73 ± 3.98	0.144
WBC, 10^9/L	6.14 ± 1.64	5.70 ± 1.49	5.76 ± 1.61	5.96 ± 1.62	0.085
PLT, 10^9/L	207.32 ± 69.37	203.07 ± 59.53	210.12 ± 55.75	202.57 ± 60.84	0.477
APRI stage					< 0.001
< 1	59 (95.16)	145 (90.62)	301 (99.01)	268 (93.06)	
2	2 (3.23)	9 (5.62)	3 (0.99)	6 (2.08)	
> 2	1 (1.61)	6 (3.75)	0 (0.00)	14 (4.86)	
FIB-4 stage					0.136
< 1.45	49 (79.03)	131 (81.88)	257 (84.54)	225 (78.12)	
1.45- 3.25	10 (16.13)	21 (13.12)	43 (14.14)	47 (16.32)	
> 3.25	3 (4.84)	8 (5.00)	4 (1.32)	16 (5.56)	
GPR stage					< 0.001
≤ 0.32	42 (67.74)	75 (46.88)	247 (81.25)	133 (46.18)	
> 0.32	20 (32.26)	85 (53.12)	57 (18.75)	155 (53.82)	
Stage of inflammation					< 0.001
0	4 (6.45)	2 (1.25)	4 (1.32)	4 (1.39)	
1	23 (37.10)	80 (50.00)	231 (75.99)	172 (59.72)	
2	28 (45.16)	67 (41.88)	68 (22.37)	100 (34.72)	
3	7 (11.29)	10 (6.25)	0 (0.00)	9 (3.12)	
4	0 (0.00)	1 (0.62)	1 (0.33)	3 (1.04)	
Degree of fibrosis					< 0.001
0	2 (3.23)	2 (1.25)	6 (1.97)	7 (2.43)	
1	23 (37.10)	81 (50.62)	164 (53.95)	122 (42.36)	
2	12 (19.35)	34 (21.25)	89 (29.28)	84 (29.17)	
3	8 (12.90)	16 (10.00)	33 (10.86)	39 (13.54)	
4	17 (27.42)	27 (16.88)	12 (3.95)	36 (12.50)	
≥ G2	35 (56.45)	78 (48.75)	69 (22.70)	112 (38.89)	< 0.001
≥ S2	37 (59.68)	77 (48.12)	134 (44.08)	159 (55.21)	0.019

G2/S2	41 (66.13)	105 (65.62)	156 (51.32)	186 (64.58)	0.002

IQR: Interquartile range; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Glutamate aminotransferase; TBIL: Total bilirubin; ALB: Albumin; WBC: White blood cell; PLT: Platelet; APRI: Aspartate aminotransferase-to-platelet ratio index; FIB-4: Fibrosis-4 index; GPR: Gamma- glutamyl- transpeptidase to platelet ratio.

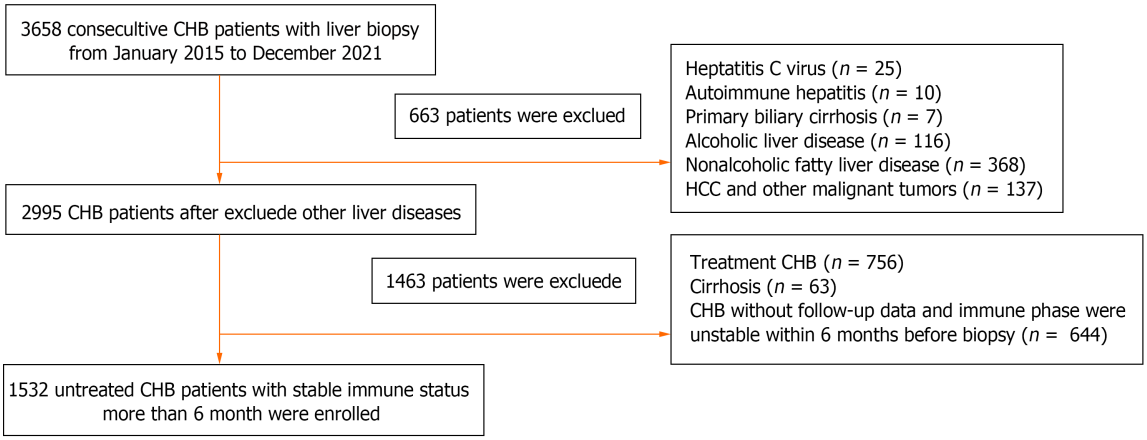

Figure 1 Flow diagram of patient selection. CHB: Chronic hepatitis B; HCC: Hepatocellular carcinoma.

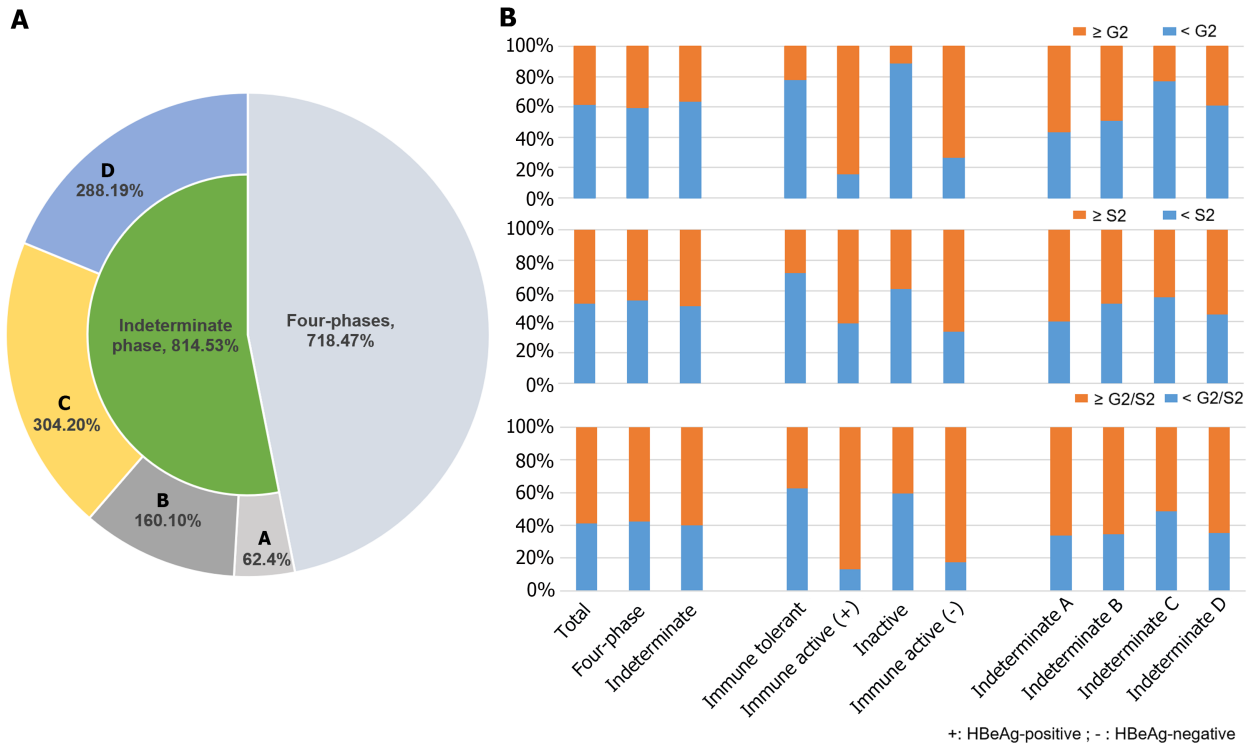

Figure 2 Liver histological changes in different clinical phases. A: Proportions of chronic hepatitis B patients in different clinical phases; B: The liver histological changes in different clinical phases using liver biopsy. Four phase including including immune tolerant phase, HBeAg-positive immune active phase, inactive phase, and HBeAg-negative immune active phase. ≥ G2 was defined by significant necroinflammation, ≥ S2 defined by significant fibrosis, and ≥ G2/S2 defined by significant histopathological changes.

indeterminate A, B, C, and D phases were 56.45%, 48.75%, 22.70%, and 38.89%, respectively; the proportions of significant fibrosis in indeterminate A, B, C, and D phases were 59.68%, 48.12%, 44.08%, and 55.21%, respectively; and the proportions of significant liver histological changes in indeterminate A, B, C, and D phases were 66.13%, 65.62%, 51.32%, and 64.58%, respectively (Table 2 and Figure 2B).

Age, PLT, and ALT subgroup liver histopathology comparison in the indeterminate patient

Liver histological changes of the indeterminate patient were also analyzed by different levels of age, PLT, and ALT. The proportion of significant liver histological changes in patients with age ≥ 40 years old, PLT ≤ 150 × 10^9/L, and ALT ≥ 1ULN were significantly higher than that in age < 40 years old, PLT > 150 × 10^9/L, and ALT < 1ULN groups, respectively, as shown in Figure 2A. There was no significant difference in inflammation between the patients who were younger and older than 40 years, and no discernible difference in the significant fibrosis between patients with ALT ≥ 1ULN and < 1ULN (Figure 3A).

Patients were further categorized by levels of PLT and ALT in the different age subgroups (Figure 3B and C). Regardless of age was ≥ 40 or < 40 years, individuals with PLT ≤ 150 × 10^9/L had considerably greater proportions of significant liver fibrosis, inflammation, and histological changes than those with PLT > 150 × 10^9/L (Figure 3B). When compared with the ALT ≥ 1ULN and < 1ULN groups, the proportion of significant liver inflammation, significan fibrosis, and significant liver histological changes in the patients with ALT ≥ 1ULN was higher than those with ALT < 1ULN group when age was ≥ 40 years (Figure 3C).

Risk factors associated with liver histological changes

The analysis of risk factors of liver histological changes in all patients showed that age ≥ 45 years old, HBeAg-positive, high HBV-DNA level (≥ 3 × log10 IU/mL), PLT ≤ 150 × 10^9/L, and abnormal ALT level were independent risk factors of significant inflammation and significant liver histological changes (Supplementary Table 4).

According to the results of the multivariate logistic regression analysis of indeterminate patients (Table 3), age ≥ 40 years old adjust odd risk (aOR), 1.44; 95%CI: 1.06-1.97; P = 0.02), PLT ≤ 150 × 10^9/L (aOR, 2.99; 95%CI: 1.85-4.83; P < 0.0001), and ALT ≥ 1 ULN (aOR, 1.48; 95%CI: 1.08, 2.05, P = 0.0163) were independent risk factors for significant liver histological changes. Age ≥ 40 years old and PLT ≤ 150 × 10^9/L were independent risk factors for significant inflammation and fibrosis; HBeAg-positive and ALT ≥ 1ULN were also positively correlated with significant inflammation.

Risk factors of liver histological changes in the subgroups of indeterminate patient by different status of HBeAg were also analyzed (Supplementary Tables 5 and 6). Age ≥ 40 years old and PLT ≤ 150 × 10^9/L were both of independent risk factors for significant inflammation and significant liver histological changes in the HBeAg positive indeterminate patients (Supplementary Table 5). In the HBeAg positive indeterminate patients, both PLT ≤ 150 × 10^9/L and ALT ≥ 1 ULN were independent risk factors for significant inflammation, fibrosis and liver histological changes, and age ≥ 40 years was the risk factor for significant necroinflammation (Supplementary Table 6). These results were similar to the results of the overall indeterminate patients. In addition, the results suggested that different gender had different risks in different HBeAg status populations, which is worthy of further exploration in later studies.

DISCUSSION

The available CHB management guidelines have provided detailed diagnostic and treatment criteria, in which timely antiviral therapy was recommended for patients in the immune active phase, and monitoring of ALT level and liver histological changes were found essential for patients in the immune tolerant and inactive phases[1,2,4]. However, patients in the indeterminate phase are beyond the definition of four phases, and no histological evidence to confirm the necessity of antiviral therapy. The monitoring of ALT level and liver histological changes was recommended by the relevant guidelines. Our results showed that patients had a high rate of histological progression, and over half of patients (59.9%) had G2 inflammation and/or S2 fibrosis; even in CHB patients in indeterminate A and C phases whose ALT level was normal, the proportions of liver histological changes in patients reached 66.13% and 51.32%, respectively, suggesting the majority of patients in the indeterminate phase were in disease progression.

Recently, a large sample size cohort performed in the United States showed that among patients with cirrhosis, 9% of patients were in the indeterminate phase[10]. Another study from the United States assessed the phenotype of HBV in 1390 adult participants enrolled in the Hepatitis B Research Network Cohort Study, of whom 524 patients were in the indeterminate phase, 88 (19%) and 5 (1%) patients obtained APRI scores > 0.50-2.0 and > 2, and 78 (17%) and 13 (3%) patients achieved FIB-4 scores equal to 1.45–3.25 and > 3.25, respectively[14]. Hsu *et al*[15] evaluated 198 untreated Asian-American CHB patients with a mean follow-up time of 21 months, and using the modified ALT criteria, it was revealed that 43 (28.1%) patients had phase-based changes, of whom 31/43 (72.1%) patients were shifted from phase 1 and indeterminate phase to phases 2 and 4, as being more active CHB phases. However, our histological findings showed higher percentages of inflammation and fibrosis than the above studies. Our study showed 294 (36.12%) and 407 (50.00%) patients were ≥ G2 and ≥ S2, respectively; 20 (2.46%) and 21 (2.58%) patients obtained APRI scores of 1-2 and > 2, respectively; 4 (3.39%) and 31 (3.81%) patients achieved APRI scores of 121 (14.86%) and > 3.25, respectively. Recently, Chinese scholars investigated liver histological changes of 106 HBeAg-negative CHB patients with normal ALT level and HBV-DNA level ≥ 2000 IU/mL, equal to patients in the indeterminate C phase, and the proportions of significant inflammation, significant fibrosis, and significant liver histological changes were found in 58.5%, 67.9% and 74.5% of patients, respectively[16]. The differences between liver biopsy and APRI/FIB-4 scores could be mainly attributed to the low diagnostic accuracy of APRI/FIB-4 scores. Several studies have found that the diagnostic accuracy of APRI and FIB-4 for significant liver fibrosis in CHB patients was low[13,17-20] and not ideal substitutes for liver biopsy[21].

To date, few studies have concentrated on the HBeAg-positive indeterminate CHB patients. In the present study, we found that 50.90%, 51.35% and 57.60% of HBeAg-positive indeterminate CHB patients had significant inflammation, fibrosis, and liver histological changes, respectively. In the indeterminate A phase, with HBeAg-positive, normal ALT level, and low HBV-DNA level, more than 50% of patients had significant inflammation and significant fibrosis.

Table 3 Predictors of liver histopathology changes in the indeterminate patients

	Univariable		Multivariable[1]	
Significant necroinflammation	**OR, (95%CI)**	***P* value**	**Adjusted OR, (95%CI)**	***P* value**
Age, yr				
< 40	Referent		Referent	
≥ 40	1.29 (0.97, 1.72)	0.0846	1.75 (1.25, 2.44)	0.001
Sex				
Male	Referent		Referent	
Female	0.76 (0.54, 1.06)	0.1007	0.75 (0.52, 1.07)	0.1098
HBeAg				
-	Referent		Referent	
+	2.36 (1.72, 3.24)	< 0.0001	2.55 (1.77, 3.68)	< 0.0001
HBV-DNA log10, IU/mL				
< 3	Referent		Referent	
≥ 3	0.98 (0.63, 1.53)	0.9258	1.10 (0.67, 1.80)	0.7017
PLT, 10^9/L				
> 150	Referent		Referent	
≤ 150	3.16 (2.14, 4.66)	< 0.0001	2.77 (1.83, 4.19)	< 0.0001
ALT, U/L				
< ULN	Referent		Referent	
≥ ULN	1.85 (1.38, 2.49)	< 0.0001	1.60 (1.15, 2.25)	0.006
Significant fibrosis	OR, (95%CI)	*P* value	Adjusted OR, (95%CI)	*P* value
Age, yr				
< 40	Referent		Referent	
≥ 40	1.42 (1.07, 1.87)	0.0136	1.36 (1.00, 1.85)	0.049
Sex				
Male	Referent		Referent	
Female	0.88 (0.65, 1.20)	0.4272	0.97 (0.70, 1.35)	0.8752
HBeAg				
-	Referent		Referent	
+	1.08 (0.79, 1.47)	0.6367	1.14 (0.80, 1.62)	0.4734
HBV-DNA log10, IU/mL				
< 3	Referent		Referent	
≥ 3	0.85 (0.56, 1.31)	0.4636	0.93 (0.58, 1.49)	0.7537
PLT,10^9/L				
> 150	Referent		Referent	
≤ 150	4.30 (2.75, 6.71)	< 0.0001	3.92 (2.49, 6.17)	< 0.0001
ALT, U/L				
< ULN	Referent		Referent	
≥ ULN	1.27 (0.96, 1.68)	0.0907	1.15 (0.84, 1.59)	0.3758
Significant liver histological changes	OR, (95%CI)	*P* value	Adjusted OR, (95%CI)	*P* value
Age, yr				
< 40	Referent		Referent	

≥ 40	1.37 (1.03, 1.82)	0.0308	1.44 (1.06, 1.97)	0.0214
Sex				
Male	Referent		Referent	
Female	0.97 (0.71, 1.33)	0.8423	1.05 (0.76, 1.47)	0.7551
HBeAg				
-	Referent		Referent	
+	1.41 (1.03, 1.95)	0.0347	1.40 (0.97, 2.01)	0.0689
HBV-DNA log10, IU/mL				
< 3	Referent		Referent	
≥ 3	0.97 (0.63, 1.49)	0.8813	1.13 (0.70, 1.81)	0.626
PLT,10^9/L				
> 150	Referent		Referent	
≤ 150	3.34 (2.09, 5.35)	< 0.0001	2.99 (1.85, 4.83)	< 0.0001
ALT, U/L				
< ULN	Referent		Referent	
≥ ULN	1.57 (1.19, 2.09)	0.0016	1.48 (1.08, 2.05)	0.0163

[1]Adjusted for age, sex, alanine aminotransferase level, HBeAg statue, platelet level, and hepatitis B virus DNA level.
HBV: Hepatitis B virus; ALT: Alanine aminotransferase; GGT: Glutamate aminotransferase; PLT: Platelet; ULN: Upper limits of normal.

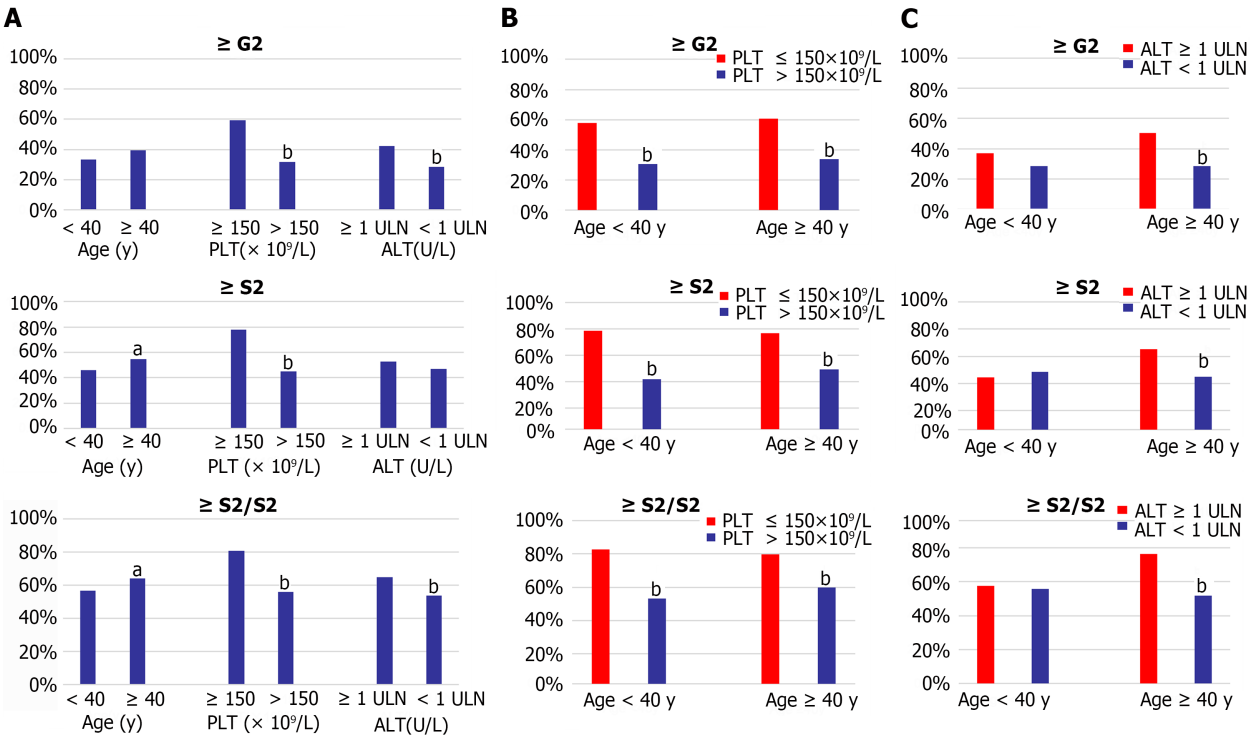

Figure 3 Age, platelet count, and alanine aminotransferase subgroup liver histopathology comparison in indeterminate patient. A: Distribution of significant liver inflammation (≥ G2), fibrosis (≥ S2), and histological changes (≥ G2/S2) in subgroup of indeterminate patient according to different age, platelet count (PLT), and alanine aminotransferase (ALT) levels; B: Distribution of ≥ G2, ≥ S2, ≥ G2/S2 in different age and PLT groups; C: In different age and ALT groups. [a]$P < 0.05$, [b]$P < 0.001$. ALT: Alanine aminotransferase; PLT: Platelet count; ULN: Upper limits of normal.

Additionally, our study revealed that more than 60% of patients had significant liver histological changes in the indeterminate B and D phases with a mild abnormal ALT level (more than 1 ULN, while lower than 2 ULN), and HBV-DNA level was relatively low in HBeAg-positive or -negative patients.

Some studies have assessed risk factors for liver inflammation and fibrosis in indeterminate CHB patients. In our study, the multivariate logistic regression analysis revealed that age ≥ 40 years old, PLT ≤ 150 × 10^9/L, and ALT level ≥ ULN were independent risk factors for significant fibrosis and liver histological changes in the indeterminate CHB patients. Previous studies have suggested that age was a risk factor for long-term prognosis in patients with chronic HBV infection, and the incidence of adverse events increased with age[22,23]. A study on HBeAg-negative CHB patients with persistently normal ALT level showed that older age was an independent predictor of significant liver fibrosis and liver histological damage[24]. Additionally, another study revealed that age ≥ 40 years old was a risk factor for HCC in indeterminate patients[9]. Numerous studies showed that abnormal ALT level was related to liver fibrosis and cirrhosis, and the traditional normal range of ALT level has remained controversial[11,25]. Studies have demonstrated that ULN of lower ALT could better reflect the liver histological changes[11,25]. A Korean study suggested that compared with ALT level < 20 U/L, the risk of inflammation in patients with ALT levels of 20-29 and 30-39 U/L significantly increased[26]. A study on CHB patients with persistently normal ALT level also revealed that ALT level > 0.5 ULN (20 U/L) was associated with liver fibrosis and inflammation[16]. In our study, according to ALT level of 35 IU/L in male and 25 IU/L in female patients, which were lower than the reference levels, an abnormal ALT level was found as a risk factor for significant inflammation and fibrosis in indeterminate CHB patients. Therefore, a lower ALT level used as a reference level could be more beneficial to the management of CHB patients. In addition, previous studies demonstrated that PLT was an independent predictor of liver fibrosis and cirrhosis in the CHB patients, regardless of undergoing treatment[17,19,27,28]. Our study further supported this finding in the indeterminate phase patients. Portal hypertension and hypersplenism are factors leading to thrombocytopenia, accompanied by the development of liver fibrosis and cirrhosis. Other studies on patients undergoing liver transplantation have suggested that thrombocytopenia was associated with the progression of liver fibrosis, leading to the decreased production of thrombopoietin by hepatocytes[27,28].

Previous studies suggested that CHB patients in the indeterminate phase had a better outcome and with a lower risk of development to adverse prognoses, such as cirrhosis and HCC[8,29]. But, these studies mainly concentrated on Caucasians with relatively small sample size and the follow-up time was no longer enough. Recently, some studies have shown that indeterminate phase patients had a higher risk of development to HCC during follow-up[9,10,30]. A retrospective study that included 3336 untreated CHB patients from the United States and Taiwan showed that, after 10 years of follow-up, the cumulative incidence of HCC in 1303 indeterminate phase patients was 4.5-fold higher than those in inactive patients (2.7% *vs* 0.6%), and the cumulative incidence of HCC in patients who remained indeterminate after follow-up was 9 times higher than those remained inactive (4.6% *vs* 0.5%)[9].

Our study have some limitations. This was a cross-sectional and single-center study, and long-time multicenter follow-up data were absent, thus, we could not determine the results of disease progression with inclusion of cirrhosis and HCC. Multicenter and well-designed prospective studies are therefore required to confirm the findings of the present study. In addition, the initial liver biopsy for the majority of patients in the indeterminate phase was conducted between 2020 and early 2021. Due to the impact of the coronavirus disease 2019 pandemic, many of these patients were unable to undergo a second paired liver biopsy, and as a result, the dynamic changes in liver pathology are not addressed in this article. This is another limitation of our research. Our research team has initiated relevant paired clinical studies to offer more robust evidence for future clinical practice.

CONCLUSION

In summary, this is the largest cohort study that enrolled CHB patients who were in all the phases of indeterminate using liver biopsy. Our study indicated that about 60% of indeterminate CHB patients had histopathological changes. Age ≥ 40 years old, PLT ≤ 150 × 10^9/L, and ALT ≥ ULN were independent risk factors for significant liver fibrosis or significant histological changes in the indeterminate CHB patients. Liver biopsy should be recommended for indeterminate patients to evaluate liver histopathological changes, especially in those patients with ≥ 40 years old, or PLT ≤ 150 × 10^9/L. More optimal ALT cutoff value in CHB patients may still need to be adjusted to identify more populations in need of treatment. Antiviral therapy of indeterminate patients is worthy of consideration in the management of CHB patients.

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FOOTNOTES

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Diagnostic and prognostic role of LINC01767 in hepatocellular carcinoma

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Abstract

BACKGROUND

Hepatocellular carcinoma (HCC) is a primary contributor to cancer-related mortality on a global scale. However, the underlying molecular mechanisms are still poorly understood. Long noncoding RNAs are emerging markers for HCC diagnosis, prognosis, and therapeutic target. No study of LINC01767 in HCC was published.

AIM

To conduct a multi-omics analysis to explore the roles of LINC01767 in HCC for the first time.

METHODS

DESeq2 Package was used to analyze different gene expressions. Receiver operating characteristic curves assessed the diagnostic performance. Kaplan-Meier univariate and Cox multivariate analyses were used to perform survival analysis. The least absolute shrinkage and selection operator (LASSO)-Cox was used to identify the prediction model. Subsequent to the validation of LINC01767 expression in HCC fresh frozen tissues through quantitative real time polymerase chain reaction, next generation sequencing was performed following LINC01767 over expression (GSE243371), and Gene Ontology/Kyoto Encyclopedia of Genes

and Genomes/Gene Set Enrichment Analysis/ingenuity pathway analysis was carried out. *In vitro* experiment in Huh7 cell was carried out.

RESULTS

LINC01767 was down-regulated in HCC with a log fold change = 1.575 and was positively correlated with the cancer stemness. LINC01767 was a good diagnostic marker with area under the curve (AUC) [0.801, 95% confidence interval (CI): 0.751-0.852, P = 0.0106] and an independent predictor for overall survival (OS) with hazard ratio = 1.899 (95%CI: 1.01-3.58, P = 0.048). LINC01767 nomogram model showed a satisfied performance. The top-ranked regulatory network analysis of LINC01767 showed the regulation of genes participating various pathways. LASSO regression identified the 9-genes model showing a more satisfied performance than 5-genes model to predict the OS with AUC > 0.75. LINC01767 was down-expressed obviously in tumor than para-tumor tissues in our cohort as well as in cancer cell line; the over expression of LINC01767 inhibit cell proliferation and clone formation of Huh7 *in vitro*.

CONCLUSION

LINC01767 was an important tumor suppressor gene in HCC with good diagnostic and prognostic performance.

Key Words: Hepatocellular carcinoma; LINC01767; Multi-omics analysis; GSE243371; Cell proliferation; Clone formation

Core Tip: LINC01767 was down-regulated based on The Cancer Genome Atlas and GSE dataset. It was positively with the cancer stemness in hepatocellular carcinoma (HCC) based on the single cell sequence data. LINC01767 demonstrate a good diagnostic and diagnostic performance. The 9-gene model demonstrated better performance than the 5-gene model in predicting the overall survival of HCC patients using least absolute shrinkage and selection operator regression, with an area under the curve greater than 0.75. LINC01767 was down regulated obviously in tumor than para-tumor tissues in our cohort. LINC01767 was down regulated in cancer cell line comparing with LO2; the over expression of LINC01767 inhibit the cell proliferation and impede the clone formation of Huh7 *in vitro*.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the leading liver cancer and ranks fifth among all cancers, resulting in more than 600000 deaths each year globally[1,2]. Especially in Asia, HCC was more prevailing for hepatitis B virus infection. In Western countries, HCC is linked to nonalcoholic fatty liver disease as well[3]. Due to the subtle progression of HCC, the majority of patients are diagnosed at advanced stages[4]. Chemotherapy, radiotherapy, and trans-catheter arterial chemo embolization are the conventional options for treating advanced HCC[5], yet the underlying mechanisms remain largely unknown[6].

Only about 2.3% of the human genome is made up of protein-coding RNAs[7-9]. The majority of primary transcripts consist of noncoding RNAs (ncRNAs), which play a crucial role in the maintenance of normal physiological functions and the development of human cancers[9,10]. Long ncRNAs (lncRNAs) (> 200 nts)[11,12] is a type of regulatory ncRNAs. LncRNAs serve as guides, decoys, scaffolds, and competitive endogenous RNAs, for other molecules, to control the expression of numerous genes and ncRNAs[13].

Numerous lncRNAs have been discovered to have significant functions in the pathology of HCC. Elevated levels of Malat1 have been associated with unfavorable outcomes in different cancer types, such as bladder[14], lung[15], gallbladder[13,16] , and liver cancers[12,17-19]. LncRNAs are utilized in diagnosing HCC and could be targeted for therapy[20,21]. Research indicates that XIST controls the expression of PTEN and enhances the advancement of HCC[22]. Systematic identification of non-coding pharmacogenomic potential in cancer address the importance of lncRNAs[23].

It was part of the diagnostic profile for HCC in a prior investigation[24]. LINC01767, also identified as CRML1 (colorectal metastatic long non-coding RNA, RP4-710M16.2), exhibited increased expression in metastatic colorectal cancer (CRC) and played a crucial part in the spread of CRC to the liver[25]. Nevertheless, there have been no multi-omics assessments or *in vitro* experiments conducted on it in HCC. Therefore, we conducted a multi-omics analysis to explore the functions of LINC01767 in HCC.

MATERIALS AND METHODS

Data collection and preprocessing

Data from RNA sequencing and patient clinical details were obtained from The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/). A total of 422 cases were extracted including 50 normal tissues and 372 HCC patients. Informed certifications were all stated in the TCGA projected. The Gene Expression Omnibus (GEO) data were analyzed *via* LNCAR website [lnCAR: A comprehensive resource for lncRNAs from cancer arrays (renlab.org)]. Drug sensitivity prediction of regulation related genes was based GSCALite web, and the predicting drug sensitivity details information was listed in previous article.

Sample collection and preparation

RNA quantification and qualification for RNA-sequencing: RNA purity was checked using the NanoPhotometer® spectrophotometer (IMPLEN, CA, United States). NEBNext® UltraTM RNA Library Prep Kit for Illumina® (NEB, United States) was utilized to create sequencing libraries, adhering to the manufacturer's guidelines. Index codes were incorporated to assign sequences to individual samples. Index-coded samples were clustered on a cBot Cluster Generation System with TruSeq PE Cluster Kit v3-cBot-HS (Illumia) following the manufacturer's guidelines. Details can be accessed *via* genechem company https://www.genechem.com.cn/.Raw DATA is accessible on GSE243371.

Techniques utilized include cell culture, RNA silencing, and quantitative polymerase chain reaction: American Type Culture Collection (Rockville, MD, United States) provided the LO2 normal liver cell line and the HepG2, Hep3B, PLC, SK, 7721, and 293T human liver cancer cell lines. The cell line acquired in August 2022 was tested and authenticated by STR before being used for experiments, with additional testing conducted in March 2023. Subsequently, the cells were cultured in DMEM/DME/F-12/1640 (Invitrogen), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cell lines were cultured in a 37 °C and 5% CO_2 humid environment. The growth media, reagents, and supplements were acquired from the Gibco corporation.

Cells were transfected with 40 nM small interfering RNAs (siRNAs) for RNA interference as directed by the manufacturer. Total RNA was extracted 72 h after treatment with TRIZAL for quantitative real time polymerase chain reaction (RT-qPCR) analysis; cDNAs were generated from 0.5 μg of total RNA using a High-Capacity cDNA Reverse Transcription Kit (Dongyangfang, #FSQ101). The QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems) was used to conduct RT-qPCR using Power SYBR Green PCR Master Mix (MonAmp™ ChemoHS qPCR Mix, #MQ00401S). The relative expression of genes was calculated using the ΔΔCt method and normalized to GAPDH.

The siRNAs (sense, antisense) were used as previous described[22]. The primer sequences used for RT-qPCR included LINC01767 forward (GCTAAGGGATTTGCCACTGC) and reverse (GGTTGGAGGATGGACGTTGA), as well as GAPDH forward (GGTGAAGGTCGGAGTCAACG) and reverse (TGGGTGGAATCATATTGGAACA).

Cell proliferation assay: The Thiazolyl Blue (MTT) experimental assay was utilized to generate cell growth curves. 1000 cells were plated in 96-well dishes and incubated for six days to measure cell survival. The experiments were conducted three times separately.

Transwell migration and invasion assays: Huh7 cells were placed in the top compartments of a 24-well transwell plate from Corning in Beijing, China. Matrigel was applied to the membranes during the invasion assay conducted by BD Biosciences. Cells were placed in the upper chamber with serum-free media. The bottom compartments were filled with the culture medium containing 10% FBS. After being treated with 4% paraformaldehyde for 15 min, the cells were then exposed to crystal violet following a 48-h incubation period. Five randomly chosen microscopic fields per filter were used to evaluate the cell counts, which were then observed using microscopy.

Wound-healing assay: Huh7 cells were cultured for 48 h until they reached 80% confluence, at which point a linear artificial wound was created using a 200 μL pipette tip. The cell migration ability was measured by photographing the distance at 0 h and 24 h.

Clone formation assay: After transfection, the 400-1000 cells/well (according to the cell generation) were seeded in each experimental group in 6-well plate culture plates. The seeded cells are cultured in the incubator for 14 d or the number of cells is greater than 50 cells in most individual clones change the fluid every 3 d and observe the cell state. Before the experiment is terminated, photograph the cell clone under fluorescence microscopy and wash the cells once with phosphate buffered saline (PBS). Add 1 mL of 4% paraformaldehyde per well, fix the cells for 30-60 min, and wash the cells once with PBS. Apply 1000 μL of pure crystal violet staining solution for 10-20 min. ddH_2O was used to wash the cells, then microscopy was used to take pictures, and count the clones.

Cell cycle and apoptosis assay: PI-FACS cell cycle assay experimental was carried out as the procedures from the (PI Sigma P4170, Triton X-100 Sigma SLBT4524, RNase A Thermo Fisher Scientific EN0531). For adherent cells, once the 6 cm dish cells in each experimental group reach a coverage rate of approximately 80% (before entering the growth plateau), the cells will be digested and resuspended into a cell suspension, ensuring that the number of cells on the machine is adequate (≥ 10^6/group). Spin at 1300 r/min for 5 min, remove the liquid above the sediment, and rinse the cells with PBS (pH = 7.2-7.4) chilled at 4 degrees Celsius. Repeat the precipitation process once. Centrifuge at 1300 r/min for 5 min. Prepare the cell staining solution. Stain the cells by adding an appropriate volume of the staining solution (0.6-1 mL) based on the cell quantity and resuspending. The cell throughput rate is 300-800 cells/s. Apoptosis assay test using Annexin V-APC mono staining method was carried out as instruction of eBioscience kit (88-8007).

Development of a predictive signature associated with LINC01767 using the least absolute shrinkage and selection operator Cox regression model: In our study, the LINC01767 solely and its regulated pathway genes were used as candidate biomarkers. Using the 'glmnet' package in R, we applied least absolute shrinkage and selection operator (LASSO) Cox regression analysis to develop a precise prognostic signature for LIHC samples with the selected biomarkers. The risk score for each sample was determined by calculating the relative expression of each prognostic factor in the LINC01767 gene signature and its corresponding coefficient. The signature's risk score was determined by summing the products of each gene's relative expression and its corresponding LASSO coefficient.

Patients from the TCGA datasets were evenly split into two groups, low risk and the high risk, determined by the risk score of the gene signature associated with LINC01767. Next, we utilized time-sensitive receiver operating characteristic (ROC) curve assessment to determine the accuracy of our model's prediction by calculating the area under the curve (AUC) for overall survival (OS) at 1 year, 3 years, and 5 years. This analysis was conducted using the 'survivalROC' tool in R.

Data and statistical analysis

The RNA-sequence data from TCGA were normalized using TPM (Transcripts Per Million). Then different genes expression was analyzed by DESeq2 Package (Limma.R). To compare the expression levels of LINC01767 between the tumor and para-tumor groups using the two tailed unpaired *t* test, and between the different characteristic subgroup using ANOVA. Adjusted *P* values were calculated using Benjamini and Hochberg's method to control the false rate. DESeq2 identified genes as differentially expressed if they had an adjusted *P* value < 0.05 and a log fold change (FC) > 1.5. Perform enrichment analysis based on differential expressed genes (DEGs) using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. The Cluster Profiler R package was used to conduct GO enrichment analysis on DEGs. An analysis using Gene Set Enrichment Analysis (GSEA) was performed to evaluate the pathways that were enriched in the LINC01767-low and LINC01767-high groups.

ROC curves were employed to assess the diagnostic performance for HCC and the predictive ability of the nomogram and LASSO model. When AUC is greater than 0.5, the closer AUC is to 1, the more effective this variable is in predicting outcomes. AUC exhibits decreased accuracy between 0.5 and 0.7, demonstrates a satisfactory level of accuracy between 0.7 and 0.9, and achieves exceptional accuracy when exceeding 0.9. When AUC is equal to 0.5, the variable has no diagnostic value.

Kaplan-Meier analysis and Cox proportional hazards models were utilized to identify significant prognostic factors. Survival curves were generated with the Kaplan-Meier technique, and curves were compared using the log-rank test. A multivariate Cox regression analysis was conducted on the variables that achieved a significance level of $P < 0.05$ in the initial univariate analysis. When a notable impact was seen in the Cox model, independent predictors of prognosis were identified ($P < 0.05$). The final model's variables were chosen through a stepwise process of backward regression based on the Akaike information criterion. The nomogram model was built using the final Cox proportional hazard regression model and the rms package in R version 3.8.1 (http//www.r-project.org/). The nomogram underwent internal verifications, with an evaluation of the model's discrimination and calibration. Discrimination assessment in this article relied on the concordance index (C-index). A C-index value of 0.5 signified that the model did not have any predictive impact. A C-index of 1 showed that the model's predicted outcomes matched perfectly with the observed outcomes. A higher C-index value indicates more accurate predictions from the model. ROC curve was plotted to indicate the precision of prediction of 1-year, 3-year, 5-year OS rate. AUC greater than 0.7 stand a promising prediction performance. All data were processed with R 3.8.1 and MedCalc software 19.4.2.0. A *P* value less than 0.05 indicates statistical significance.

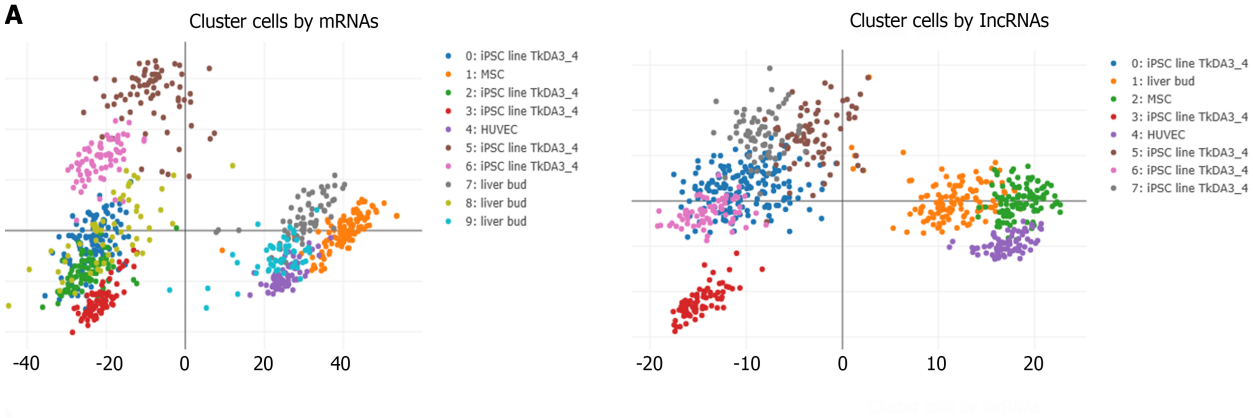
RESULTS

Single-cell sequencing analysis via the Color cell web based on GSE81252

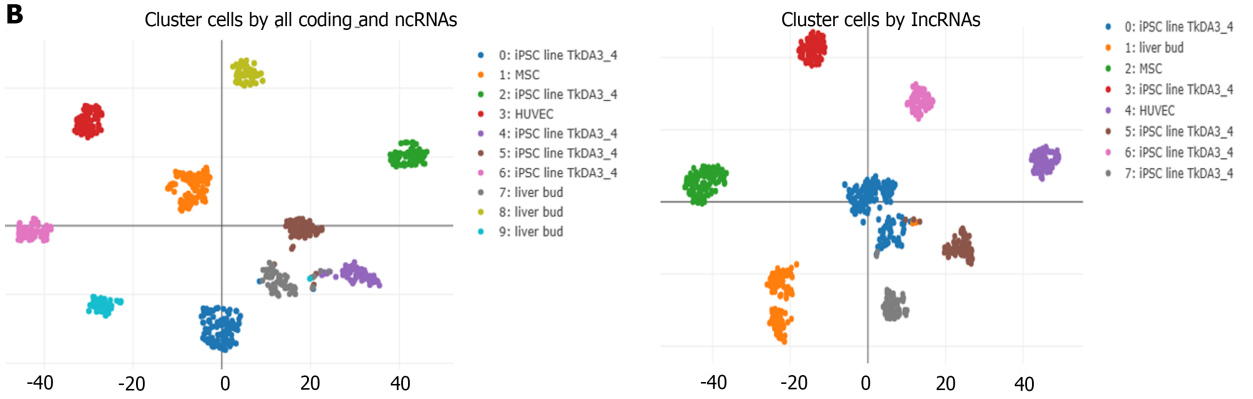
We analyze the expression of LINC01767 in single-cell sequencing data GSE81252 from web (https://rna.sysu.edu.cn/colorcells/index.php). Principal components analysis results showed that the 9-cluster cell clustered by all coding and ncRNAs and 7-cluster cell by ncRNAs solely were identified Figure 1A. The t-SNE Nonlinear dimensionality reduction showed the corresponding dimensionality reduction Figure 1B. The differential genes among different cell-cluster were shown in Figure 1C. The 9-clusters cell subgroups were classified as below: (1) Cluser 0/4/5/7 - cancer stem cell; (2) Cluser 1 - myofibroblast; cluster 2 - stem cell; (3) Cluser 3 - liver bud hepatic called cancer stem cell; (4) Cluster 6 - exhausted CD8+ T cell; (5) Cluster; (6) 8-regulatory T cell; and (7) Cluster 9 - endothelial precursor cell (Figure 1D). The expression of LINC01767 in different cluster was shown in Figure 1F, it was up regulated in cancer stem cell (cluster 7). And the differential gene enrichment of cluster 7 was shown in Figure 1E. In our analysis of liver carcinoma, we utilized the Stemness group's DNA methylation-based Stemness Scores, specifically the Stem cell signature probes (219 probes), to assess the correlation between LINC01767 and the stem score using Pearson correlation. We found a notable correlation in LIHC ($n = 366$, $r = 0.213$, $P = 0.000039$). Additionally, the RNAss, which represents the RNA-based Stemness Scores from the Stemness group, confirmed the link between LINC01767 and cancer stem cells. there was a significant positive association in LIHC ($n = 366$, lR = 0.221, $P = 0.0000206$) (Figure 1F). All the above results showed that the LINC07167 was positively correlated with the cancer stem cell in liver cancer.

GSE78160 (serum lncRNAs) data showed that the serum LINC01767 were up regulated in HCC patients with a logFC 0.699, $P = 0.026524$ (Figure 1G). Besides, the GO/KEGG analysis from lnCAR website showed that the LINC01767 is associated with the metabolic Figure 1G. The LINC01767 was expressed in liver and bile duct with a significantly higher

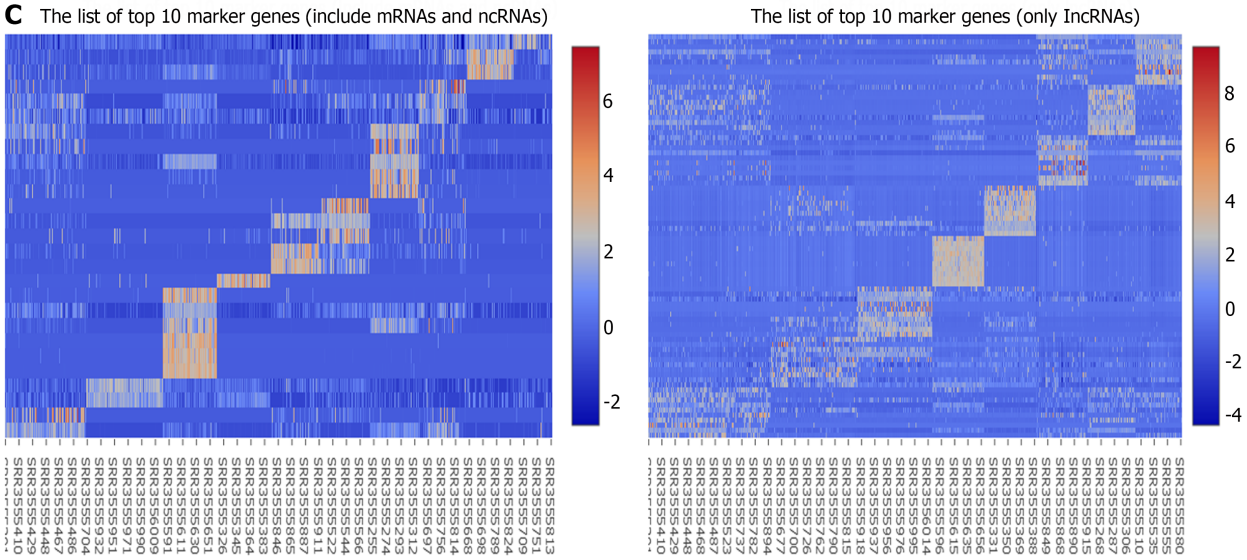
A



B



C



D

ColorCells

Cluster	Tissue	Cells	*P* value
0	liver(UBERON_0002107)	Cancer stem cell(None)	0.00177
1	liver(UBERON_0002107)	Myofibroblast(CL_0000186)	0.00616537
2	liver(UBERON_0002107)	Stem cell(CL_0000034)	0.00375892
3	liver(UBERON_0002107)	Liver bud hepatic cell(CL_0002321)	0.00000000403063
4	liver(UBERON_0002107)	Cancer stem cell(None)	0.00024544
5	liver(UBERON_0002107)	Cancer stem cell(None)	0.00550421
6	liver(UBERON_0002107)	Exhausted CD8+ T cell(CL_0000625)	0.00654602
7	liver(UBERON_0002107)	Cancer stem cell(None)	0.0008835
8	liver(UBERON_0002107)	Regulatory T (Treg) cell(CL_0000815)	0.000128354
9	liver(UBERON_0002107)	Endothelial precursor cell(CL_0002619)	0.00294153

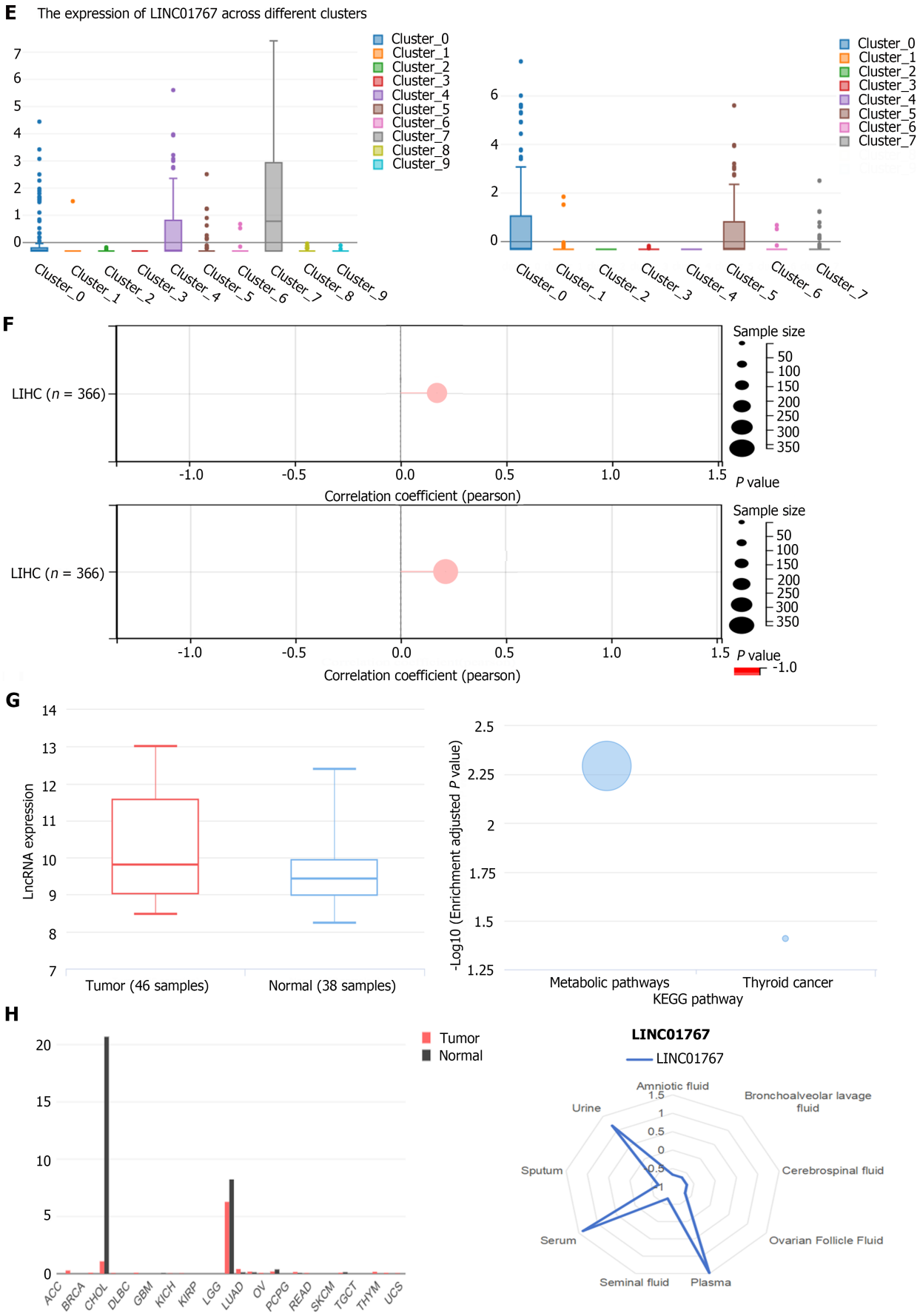


Figure 1 Single-cell sequencing analysis *via* the color cell web based on GSE81252 and validation. A: The principal components analysis results showed that the 9-cluster cell by all coding and non-coding RNAs and 7-cluster cell by noncoding RNAs solely were identified; B: The t-SNE Nonlinear dimensionality reduction showed the corresponding dimensionality reduction; C: The differential genes among different cell-cluster; D: The 9-clusters cell subgroups; E: The

expression of LINC01767 was upregulated in cluster 7 which was classified into cancer stem cell; F: DNAss (up) and RNAss (down) is associated in LIHC (n = 366, P < 0.001); G: GSE78160 (serum long noncoding RNAs) data showed that the serum LINC01767 was up regulated in hepatocellular carcinoma patients with a log fold change 0.699, P = 0.026524; LINC01767 is associated with the metabolic; H: LINC01767 was expressed in liver and bile duct with a significantly high level high level than other tissues LINC01767 demonstrate a high level in serum, urine, and plasma. ncRNA: Non-coding RNA; lncRNA: Long non-coding RNA.

level than other tissues Figure 1H. LINC01767 demonstrate a high expression level of serum, urine, and plasma LINC01767 (Figure 1H). Therefore, investigating the significance of LINC01767 in liver cancer was crucial.

Different expression of lncRNAs in HCC

A total of 3529 up regulated and 250 down regulated lncRNAs was identified in TCGA liver cancer. Among them, the previous identified lncRNAs (Figure 2A), such as HULC (upregulation), FENDRR (downregulation) were included as well. Additionally, we were intrigued by the LINC01767 due to its logFC of -1.575 and P < 0.0001 in Figure 2B. Furthermore, we confirmed the expression of LINC01767 in the GEO data set, revealing down regulation in GSE76297, GSE84005, GSE58043, and GSE51701 with fold changes between -0.815 and -2.196 (Figure 2C-F).

The diagnostic performance of LINC01767

ROC curves were used to evaluate the diagnostic accuracy of LINC01767 in our study. The findings indicated that LINC01767 exhibited excellent diagnostic accuracy, with an AUC of 0.801 [95% confidence interval (CI): 0.751-0.852, P = 0.0106], specificity of 94.00% (95%CI: 83.5-98.7), and sensitivity of 61.19% (95%CI: 56.0-66.2), at the optimal threshold of TPM 2.6729 (see Figure 2G). Higher LINC01767 was correlated with higher alpha-fetoprotein (AFP) (P = 0.038), but it had no significant correlation with other characteristics (Supplementary Table 1). LINC01767 could be a potentially sensitive diagnostic marker for HCC better than AFP.

The prognostic performance of LINC01767 by univariate and Cox regression

After omitting the dead cases within 30 d a total of 342 cases from TCGA were included. The study cohort had OS rates of 85.09% at 1 year, 71.64% at 3 years, and 66.67% at 5 years, along with a median OS of 20.745 months. The cohort had disease-free survival (DFS) rates of 65.44%, 47.65%, and 44.30% at 1, 3, and 5 years, with a median DFS of 14.45 months. The clinical and pathological viable influencing OS and DFS are listed in Table 1. In univariate viables survival analysis using Kaplan-Meier plot, significant predictors of OS were body mass index (BMI) (P = 0.008), risk factors (P = 0.007), the surgery method (P < 0.001), American Joint Committee on Cancer (AJCC) 7th tumor-node-metastasis (TNM) classification (P_T < 0.0001, P_N = 0.0325, P_{stage} < 0.0001), margin status (P = 0.0002), vascular invasion (P = 0.0326). While the LINC01767 expression level stands as a predictor for OS (P = 0.016) (Figure 2H). Significant predictors of DFS were BMI (P = 0.0295), risk factors (P = 0.007), surgery method (P = 0.001), AJCC 7th TNM classification (P_T < 0.0001, P_{stage} < 0.0001), vascular invasion (P = 0.0004). And LINC01767 expression level did not showed as the predictor for DFS (P = 0.7114), and the margin status did not either (with borderline significance, P = 0.0806).

Further, we conduct the multiple factor analysis using Cox regression. There were 373 samples in the original data, 182 samples with missing variable information, and the final number of samples was 191. LINC01767 was an independent predictor for OS in HCC when included the above factors in Cox regression with hazard ratio (HR) = 1.899 (95%CI: 1.01-3.58, P = 0.048) (Table 2).

OS prediction nomogram based on LINC01767

A predictive nomogram was created using important factors identified in the Cox analysis to predict the outcome of HCC, such as T/N/Residual tumor, vascular invasion, BMI, and LINC01767 shown in Figure 2I. In this case, a patient who had a BMI greater than 5 (which earned 2 points), underwent extensive surgery (earning 0 points), and the postoperative pathology revealed no lymph node metastasis (also earning 0 points), Elevated levels of LINC01767 expression (44 points) was calculated. The overall score is 46 points, with a 1-year survival rate of approximately 95%, a 3-year survival rate of around 85%, and a 5-year survival rate of roughly 75%. Internal validation demonstrated the nomogram's ability to effectively predict OS, with a C-index of 0.620 (0.573-0.667), indicating strong agreement. The calibration graph indicated a strong agreement between the projected and actual outcomes for 1-year and 3-year OS rates (Figure 2J). ROC was used to assess prediction performance, yielding an AUC of 0.59 (95%CI: 0.75-0.43) for 1-year accuracy, 0.71 (95%CI: 0.82-0.60) for 3-year accuracy, and 0.67 (95%CI: 0.79-0.55) for 5-year accuracy. These results indicate good predictive performance, as shown in Figure 2K. In Figure 2L, the TCGA high-risk group had a poorer prognosis with a median survival time of 2398 d compared to 2752 d in the low-risk group, with a HR of 2.71 (95%CI: 1.51-4.89, P = 5.6e-4). The nomogram model demonstrated superior DCA performance compared to LINC01767 alone, with a value above the line 0 in Figure 2M-O. Internal validation confirmed the nomogram's ability to accurately predict the C-index of OS, which was 0.620 (95%CI: 0.573-0.667) with strong concordance. ROC was used to assess the prediction performance, yielding an AUC of 0.59 (95%CI: 0.75-0.43) for 1-year accuracy, 0.71 (95%CI: 0.82-0.60) for 3-year accuracy, and 0.67 (95%CI: 0.79-0.55) for 5-year accuracy, indicating strong predictive capabilities. Cox regression is applied on the premise that the independent variables are required to meet the proportional risk hypothesis (P > 0.05), and if the GLOBAL satisfies P > 0.05, it can be considered that the multi-factor model satisfies the proportional risk hypothesis in Supplementary Table 2. The VIF can be utilized to examine variables within a model for problems related to multicollinearity. It is generally believed that when 0 < VIF < 10, there is no multicollinearity. The findings indicated that the LINC01767 nomogram's Cox model met the PH hypothesis (P > 0.05) and the VIF confirmed the independence of these

Table 1 univariate overall survival analysis and disease-free survival analysis in hepatocellular carcinoma

Variable	Number	Event/total	MST months	*P* value	Number	Event/total	MST months	*P* value
DFS					OS			
Gender				0.429				0.227
Male	208	116/208	21.55 (17.64-33.05)		233	74	81.67 (53.29-NA)	
Female	90	57/90	18.59 (14.85-35.84)		109	49	48.95 (37.29-80.68)	
Age				0.644				0.488
≤ 40	25	17/25	12.91 (8.61-NA)		29	9	NA (20.11-NA)	
40-60	113	61/113	25.3 (14.85-67.58)		128	44	69.51 (40.37-NA)	
> 60	100	55/100	24.77 (18.59-47.04)		1113	35	80.68 (55.35-NA)	
> 70	52	35/52	21.16 (14.59-33.05)		64	34	45.53 (33.02-70.01)	
Race				0.145				0.392
White	146	96	19.19 (14.956-23.424)		168	71	5.117 (35.861-55.919)	
Asian	133	69	27.2 (4.977-49.423)		148	42	NA	
Black	11	4	67.58 (NA)		15	5	16.286 (5.829-69.671)	
BMI				0.029[a]				0.008[a]
< 18.5	16	9	13.07 (9.13-NA)		19	6	107.03 (20.8-NA)	
18.2-23	77	50	12.84 (9.49-29.66)		89	40	35.74 (22.77-NA)	
23-25	49	22	50.03 (21.16-NA)		55	12	81.67 (81.67-NA)	
25-30	78	40	21.62 (16.13-NA)		88	28	70.01 (37.68-NA)	
> 30	57	36	25.3 (15.7-NA)		64	20	69.51 (45.89-NA)	
Gene expression				0.711				0.016[a]
High	133	72	28.88 (19.45-47.73)		168	103	53.29 (39.75-107.03)	
Low	132	67	16.49 (13.07-23.62)		167	122	69.51 (51.25-NA)	
Family history of cancer				0.515				0.439
Yes	91	53	18.33 (13.14-37.12)		104	39	69.51 (43.47-95.55)	
No	168	99	23.95 (18.59-35.58)		194	71	55.65 (33.384-77.916)	
Risks of HCC				0.724				0.007[a]
No	60	35	18.59 (12.514-24.66)		83	44	33.02 (23.447 42.593)	
Yes	219	125	23.030 (16.229-29.831)		241	73	80.68 (50.109-111.251)	
AFP				0.736				0.698
< 400 ng/mL	183	99	27.2 (20.99-36.7)		199	56	80.68 (55.35-NA)	
≥ 400 ng/mL	51	29	15.7 (8.64-NA)		61	21	81.67 (33.02-NA)	
CHIl-phu grade				0.658				0.220
A	187	101	23.03 (18.59-42.04)		204	52	102.66 (70.01-NA)	
B	17	10	23.95 (8.64-NA)		20	8	33.02 (19.74-NA)	
C	1	1	35.58 (NA-NA)		1	1	53.35 (NA-NA)	
Surgery				0.0001[a]				< 0.0001[a]
1	121	79	13.14 (9.72-19.25)		129	48	39.75 (30.58-NA)	
2	18	12	15.41 (6.24-NA)		25	14	37.29 (17.58)	
3	75	34	47.04 (28.8-71.06)		81	13	81.67 (69.51)	

4	68	39	29.96 (18.33-71.91)		79	32	70.01 (46.75)	
5	1	0	NA (NA-NA)		1	0	NA (NA-NA)	
6	15	9	19.65 (8.28-NA)		25	14	19.58 (4.24-NA)	
Tumor histological grade				0.656				0.693
G1	47	25	29.66 (19.45-73.62)		53	17	69.51 (46.75-NA)	
G2	138	80	20.99 (16.36-28.88)		161	58	55.65 (41.75-NA)	
G3	99	59	15.74 (12.61-47.04)		111	39	53.29 (45.07-NA)	
G4	10	5	7.36 (4.17-NA)		12	5	NA (13.47-NA)	
T-stage				< 0.0001[a]				< 0.0001[a]
T1	151	65	42.02 (33.06-71.06)		168	41	80.68 (69.51-NA)	
T2	73	49	15.7 (11.17-23.62)		84	28	60.84 (37.75-NA)	
T3	62	49	9.94 (8.54-14.85)		74	43	25.3 (18.27-58.84)	
T4	9	8	7.85 (5.91-NA)		13	10	18.33 (8.94-NA)	
Lymph node status				0.357				0.033[a]
Negative	211	118	23.03 (16.49-40.37)		239	2	83.51 (60.84-NA)	
Positive	3	2	8.64 (6.6-NA)		3	77	33.02 (9.86-NA)	
X	84	53	14.13-28.88		99	43	37.68 (27.5-55.65)	
Margin				0.0806	302	104	69.51 (53.29-102.66)	0.0002[a]
R0	270	154	21.62		15	8	37.29 (27.5-NA)	
R1	13	11	15.6		1	1	7.33 (NA-NA)	
Vascular invasion				0.0004[a]				0.0326[a]
Negative	164	77	35.84 (28.88-67.58)		187	54	80.68 (55.65-NA)	
Positive	73	47	13.63 (10.81-24.77)		27	27	81.67 (45.89-NA)	
NA	13	9	8.74 (4.14-NA)		8	8	48.95 (13.96-NA)	
Adjacent hepatic tissue inflammation				0.3787				0.772
No	100	56	23.95 (18.59-40.37)		112	35	80.68 (55.65-NA)	
Mild	86	48	21.55 (18.17-53.55)		93	25	NA (45.53-NA)	
Severe	15	10	18.33 (9.72-NA)		17	5	NA (35.74-NA)	
Stage				< 0.0001[a]				< 0.0001[a]
1	145	62	40.37 (29.96-71.91)		161	37	83.18 (70.01-NA)	
2	66	43	18.17 (9.72-29.3)		77	24	60.84 (45.53-NA)	
3	67	52	9.76 (8.34-14.22)		80	45	25.3 (20.11-58.84)	
4	2	2	7.49 (5.49-NA)		3	3	18.33 (7.33-NA)	

[a]$P < 0.05$.

DFS: Disease free survival; OS: Overall survival; MST: Mean survival time; NA: Not available; BMI: Body mass index; HCC: Hepatocellular carcinoma.

factors. These results suggest that the LASSO model of LINC01767 demonstrated a strong prognostic ability (Supplementary Table 3).

RNA-sequencing and bioinformatics analysis of over expression of LINC01767 in Huh7 cell line

After transfecting of Huh7 cell lines with lentivirus - LINC017670-OE plasmid, RNA transcriptome sequencing were performed for preliminary DEGs analysis (Figure 3A), the sequencing data quality was valid, the differential expression volcano plot is presented using the logFC > 1.5, and $P < 0.05$ (Figure 3B and C). The colors green and red indicate genes that are down regulated and up regulated, respectively.

Table 2 Multivariate overall survival disease-free survival analysis in hepatocellular carcinoma

Characteristics	Beta	HR	95%CI	*P* value
Pathologic T stage				
T1		Reference		
T2	0.085	1.089	0.455-2.603	0.848
T3	0.608	1.838	0.857-3.943	0.118
T4	0.364	1.440	0.1684-12.317	0.739
Pathologic N stage				
N0		Reference		
N1	1.413	4.108	0.528-31.958	0.177
Pathologic M stage				
M0		Reference		
M1	1.580	4.855	0.378-62.357	0.225
BMI				
≤ 25		Reference		
> 25	-0.013	0.987	0.532-1.830	0.966
Residual tumor				
R0		Reference		
R1	0.289	1.334	0.172-10.360	0.782
R2	0.0602	1.062	0.0340-33.231	0.972
Vascular invasion				
No		Reference		
Yes	0.556	1.743	0.829-3.664	0.143
LINC01767				
Low		Reference		
High	0.642	1.89944998718678	1.006-3.585	0.048[a]

[a]$P < 0.05$.

BMI: Body mass index; HR: Hazard ratio; CI: Confidence interval.

The GO/KEGG/GSEA analysis to demonstrate the potential mechanism of LINC01767 in Huh7

GO and KEGG pathway analysis for deferentially expressed genes was performed. The top 10 most significantly enriched GO (Figure 3D) and KEGG terms (Figure 3G) were presented, respectively. The GO analysis identified 498 GO categories that were significantly enriched, including 'carbohydrate biosynthetic process', 'gluconeogenesis', and 'cellular carbohydrate metabolic process' shown in Figure 3D. 21 KEGG pathways were identified with *P* and *Q*-values less than 0.05. GSEA analysis indicated that the go term of LINC01767 is associated with positive regulation of lymphocyte apoptosis, nucleotide kinase activity, and more other pathways (Figure 3E and F). Additionally, KEGG pathway analysis revealed that LINC01767 is linked to P53 signaling, RAP1 signaling, and other significant pathways (Figure 3H and I).

The ingenuity pathway analysis showed the potential regulation network related to LINC01767

The $P < 0.05$ and the differential gene of $|FC| > 2$ was adopted for ingenuity pathway analysis (IPA) analysis. Classical path analysis in pathogenesis of LINC01767 based on IPA shows the significant enrichment of differential genes in disease and function. Figure 3J shows the interaction between genes, regulators and functions in the dataset. The top-ranked regulatory network shows that the regulation net involved in regulators 1810019D21Rik, E2f, TNF superfamily member 12 (TNFSF12) through C1q and TNF related 12 (C1QTNF12), collagen type VI alpha 1 (COL6A1), COL6A2, COL9A3, CXC motif chemokine ligand 2 (CXCL2), CXCL8, Jagged2 (JAG2), KCNH2, methylenetetrahydrofolate dehydrogenase-cyclohydrolase 1 (MTHFD1), NOTCH3, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PPARGC1A), ribonucleotide reductase regulatory subunit M2 (RRM2), S100A9, solute carrier 2A4 (SLC2A4), SLC7A11, TP53, and other genes can activate Benign lesion, extraintestinal functional disorder, glucose metabolism disorder, organ degeneration, and inhibit metabolism of nucleic acid component or derivative. Figure 3K and L showed the protein-protein interaction of the LINC01767 related genes.

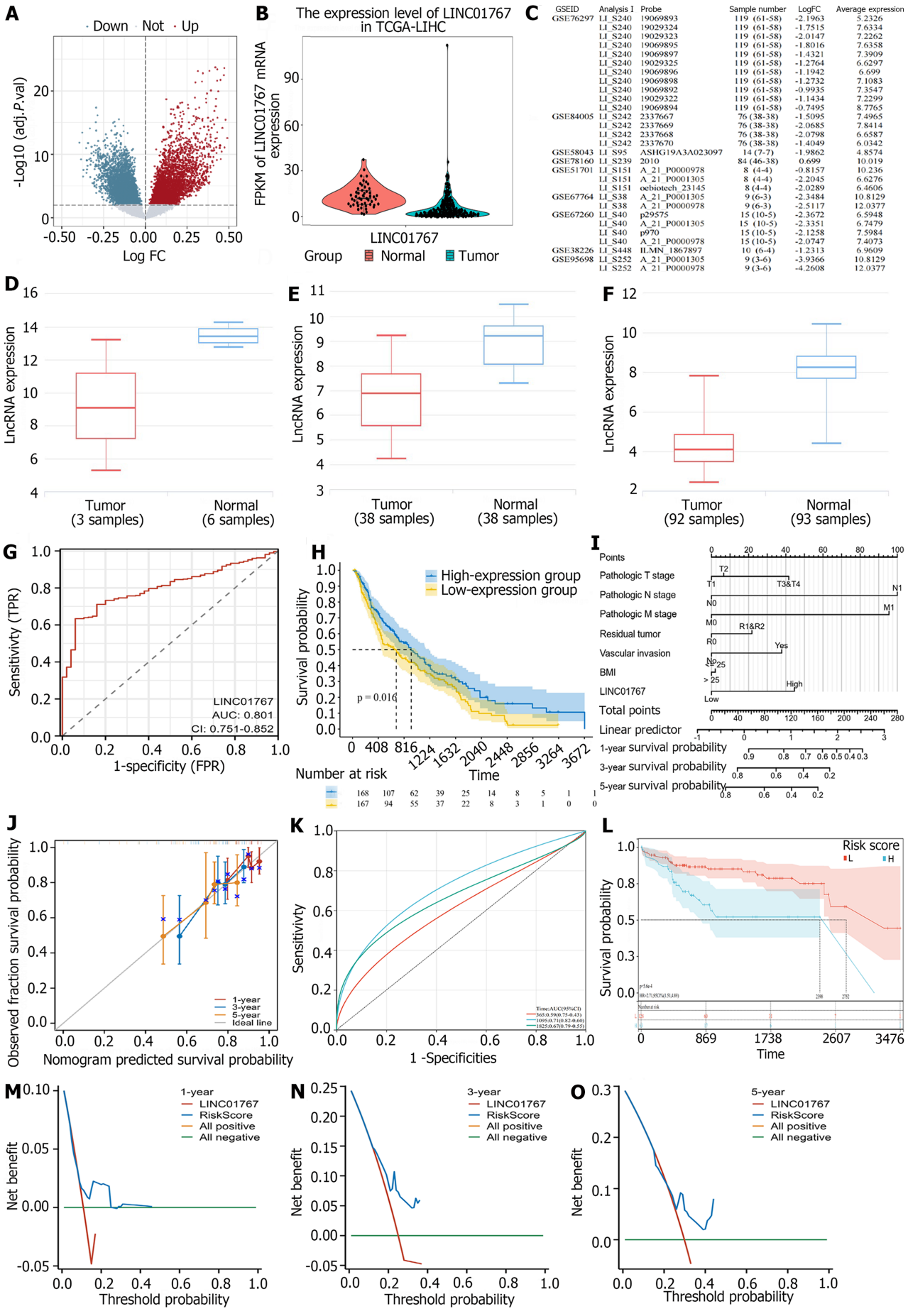


Figure 2 LINC01767 was downregulated and was potential diagnostic and prognostic marker in hepatocellular carcinoma. A: A total of 3529

up regulated and 250 down regulated long noncoding RNAs were identified; B: The LINC01767 caught our attention with a log fold change -1.575 and $P < 0.0001$; C: Deferential expression of LINC01767 in Gene Expression Omnibus dataset; D: LINC01767 downregulated in GSE78160; E: LINC01767 downregulated GSE58043; F: LINC01767 downregulated GSE84005; G: Receiver-operating characteristic (ROC) curves of LINC01767 in The Cancer Genome Atlas (TCGA); H: The LINC01767 expression level stand as a predictor for overall survival (OS) ($P = 0.016$); I: The nomogram model including T/N/Residual tumor, vascular invasion, body mass index and LINC01767; J: The calibration curve showed that there was good concordance between the predicted and observed values of 1-year and 3-year OS; K: Area under the curve (AUC) of 3-year accuracy was 0.71 [95% confidence interval (CI): 0.82-0.60], the AUC of 5-year accuracy was 0.67 (95%CI: 0.79-0.55) with a good prediction performance; L: Showed the high-risk score group of TCGA having a worse prognosis with median survival time 2398 d comparing 2752 d in low risk group with hazard ratio = 2.71 (95%CI: 1.51-4.89, $P = 5.6e-4$); M-O: The nomogram model showed a better DCA performance than sole LINC01767 with greater than line 0. TCGA: The Cancer Genome Atlas; FC: Fold change; lncRNA: Long non-coding RNA.

LASSO Cox regression analysis was performed with LINC01767 related genes. The model identified 9 genes: S100A9, RRM2, COL6A1, CXCL8, KCNH2, SLC7A11, TP53, PPARGC1A, and CXCL2. The ROC curve demonstrated that the 9-gene model had better performance in predicting OS of liver cancer patients compared to the 5-gene model. The best cut-off value for LASSO risk score was calculated to be 0.68567, dividing patients into high- and low- risk groups. Prognostic differences between the groups were analyzed using the log-rank test, showing a significant difference ($P = 3.4e-14$). Additionally, using a 5 genes signature, the optimal cutoff was 0.875627, with a significant prognostic difference between groups ($P = 7.9e-14$) (Supplementary Figure 1).

Drug sensitivity prediction of LINC01767 and the related genes based on GDCS and GSCALite web in HCC

A previous study developed a lncRNA profile to forecast drug responsiveness in TCGA HCC patients[24]. Upon re-evaluation, it was found that LINC01767 exhibited a negative correlation with the sensitivity to Rucapanib ($R = -0.37$, $P = 0.017$) as shown in Figure 4A, lapatinib ($R = -0.38$, $P = 0.014$) as shown in Figure 4B, and erlotinib ($R = -0.38$, $P = 0.013$) as shown in Figure 4C. Figure 4D displayed the correlation analysis between the mRNA expression of LINC01767 and the IC_{50} values of the candidate drugs.

Recent research has indicated that the immunophenoscore (IPS), which is based on immunogenicity, can be used to predict how patients will respond to immunotherapy. The correlation between IPS and LINC01767 expression was examined. We used IPS, IPS-CTLA4-programmed cell death ligand 1 (PDL1)+, IPS-CTLA4+-PDL1-, IPS-CTLA4+-PDL1+ to evaluate the potential role of LINC01767 prediction of immune checkpoint inhibitor. The levels of IPS, IPS-PD1/PD-L1/PD-L2, and IPS-CTLA4 were notably elevated in the high LINC01767 group compared to the low LINC01767 groups (all $P < 0.05$) as shown in Figure 4E. The spearman correlation analysis between LINC01767 mRNA and PDL1 mRNA from TCGA was conducted, the results showed LINC01767 was negatively correlated with PDL1, $P = 0.0026$ (Figure 4F).

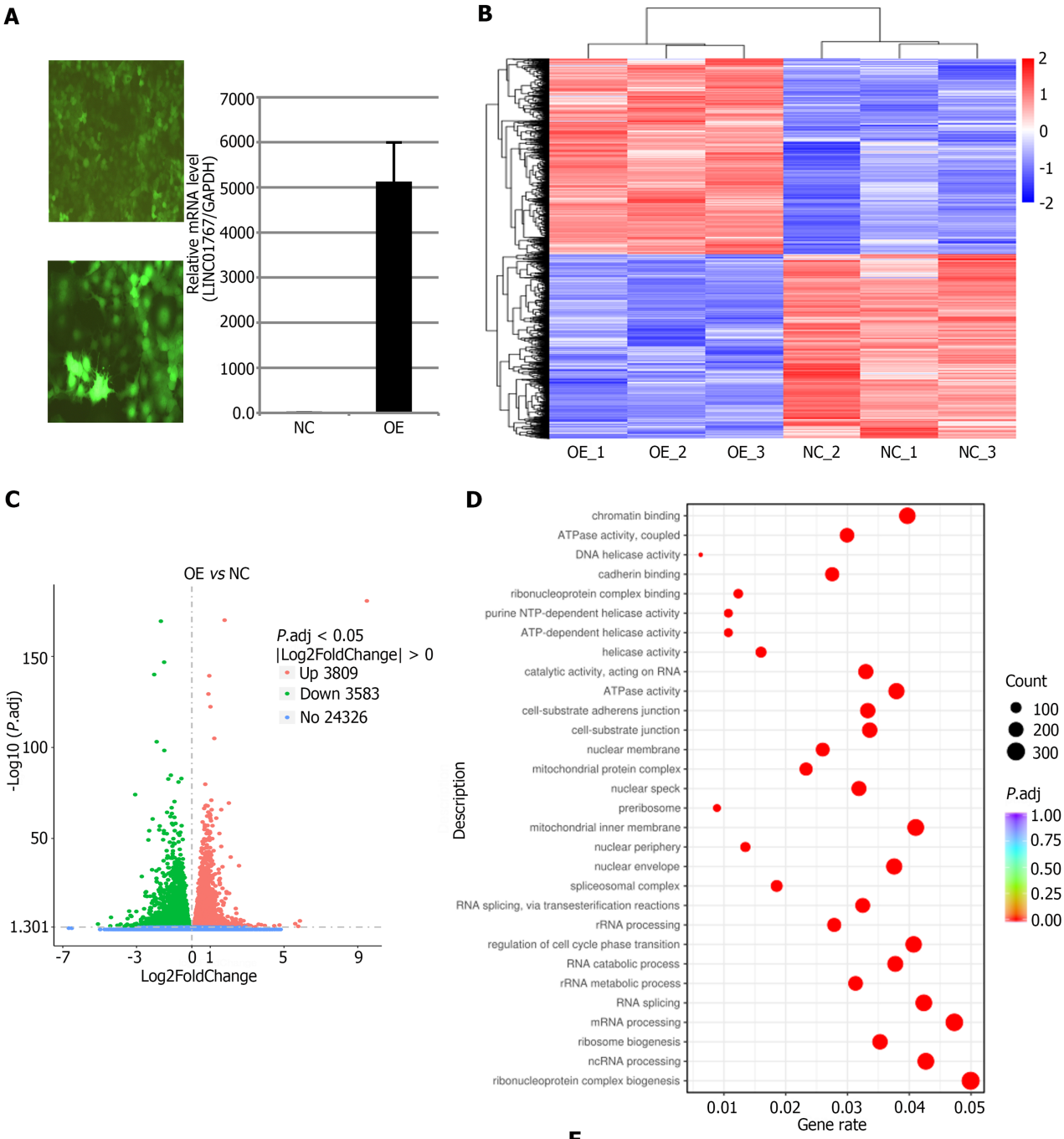
Additionally, we examine the possible role of LINC01767 in liver cancer immune cells using ImmReg (hrbmu.edu.cn), with a correlation coefficient of over 0.5 and a $P < 0.05$. The findings indicate a positive association between LINC01767 and Macrophage, and a negative relationship with B cell, CD4+ cell, neutrophil, myeloid dendritic cell, and macrophage M2 cell. What's more, it was negatively correlated with PDL1 rather than CTLA4, $P = 0.0026$, $R = -0.16$ (Supplementary Figure 2).

The in vitro experiments in HCC cell line Huh7

The RT-qPCR of tumor tissue and para-tumor tissue from HCC patients in our cohort showed that the LINC01767 was down regulated obviously in tumor cases (Figure 4G). The LINC01767 was down regulated in cancer cell line comparing with LO2 (Figure 4H). The down-regulation of LINC01767 in LO2 cell showed the growth of the LO2 was impeded (Figure 4I); Huh7 was transfected with LINC01767 over expression lentivirus, and then MTT method and formation of clones was conducted, the over expression of LINC01767 inhibit cell proliferation (Figure 4J) and impede the clone formation of Huh7 (Figure 4K and L). The results of *in vitro* experiment showed the LINC01767 has no significant influence on the cell migration/invasion/cell cycle or apoptosis in Huh7 cell (Supplementary Figure 3).

DISCUSSION

We firstly identified the down regulated non-coding RNA LINC01767 in HCC based on TCGA data , GEO data and our patient cohort. And we firstly analyze the diagnostic and prognostic performance of LINC01767 in HCC *via* bio-informatics analysis and its correlation with clinical characteristics. The underlying mechanism of LINC01767 in HCC was explored and displayed *via* RNA sequencing and bioinformatics analysis. LINC01767 was correlated with the cancer stem cell in HCC as well as in pan-cancer. The gene regulation net of LINC01767 was firstly identified, as following, E2f, TNFSF12, C1QTNF12, COL6A1, COL6A2, COL9A3, CXCL2, CXCL8, JAG2, KCNH2, MTHFD1, NOTCH3, PPARGC1A, RRM2, S100A9, SLC2A4, SLC7A11, and TP53. Additionally, a nomogram was developed using LINC01767 and a LASSO model with genes related to LINC01767 to accurately predict OS, demonstrating strong predictive capabilities. Previous studies were used to predict the drug sensitivity of HCC, revealing a negative association between LINC01767 and the sensitivity of rucapanib, lapatinib, and erlotinib, as well as a negative correlation with the immune score IPS and PDL1. The *in vitro* experiment showed the LINC01767 negatively regulated the cell growth ability and clone formation ability. All the above evidence draws out that LINC01767 was an important and pivotal tumor suppressor gene in progression of HCC.


A
Relative mRNA level (LINC01767/GAPDH)
7000
6000
5000
4000
3000
2000
1000
0.0
NC
OE
B
2
1
0
-1
-2
OE_1
OE_2
OE_3
NC_2
NC_1
NC_3
C
OE vs NC
P.adj < 0.05
|Log2FoldChange| > 0
Up 3809
Down 3583
No 24326
-Log10 (P.adj)
150
100
50
1.301
-7
-3
0
1
5
9
Log2FoldChange
D
chromatin binding
ATPase activity, coupled
DNA helicase activity
cadherin binding
ribonucleoprotein complex binding
purine NTP-dependent helicase activity
ATP-dependent helicase activity
helicase activity
catalytic activity, acting on RNA
ATPase activity
cell-substrate adherens junction
cell-substrate junction
nuclear membrane
mitochondrial protein complex
nuclear speck
preribosome
mitochondrial inner membrane
nuclear periphery
nuclear envelope
spliceosomal complex
RNA splicing, via transesterification reactions
rRNA processing
regulation of cell cycle phase transition
RNA catabolic process
rRNA metabolic process
RNA splicing
mRNA processing
ribosome biogenesis
ncRNA processing
ribonucleoprotein complex biogenesis
Description
0.01
0.02
0.03
0.04
0.05
Gene rate
Count
100
200
300
P.adj
1.00
0.75
0.50
0.25
0.00
E
Enrichment plot:
Positive_regulation_of_lymphocyte_apoptotic
_process (GO:0070230)
Enrichement score (ES)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
Ranked list metric (Signal2Noise)
5.0
2.5
0.0
-2.5
'OE' (positively correlated)
Zero cross at 17619
'NC' (negatively correlated)
10000
20000
30000
40000
50000
60000
Rank in ordered dataset
Enrichment profile
Hits
Ranking metric scores
F
Enrichment plot: Nucleotide_kinase_activity
(GO:0019201)
Enrichement score (ES)
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
Ranked list metric (Signal2Noise)
5.0
2.5
0.0
-2.5
'OE' (positively correlated)
Zero cross at 17619
'NC' (negatively correlated)
0
10000
20000
30000
40000
50000
60000
Rank in ordered dataset
Enrichment profile
Hits
Ranking metric scores

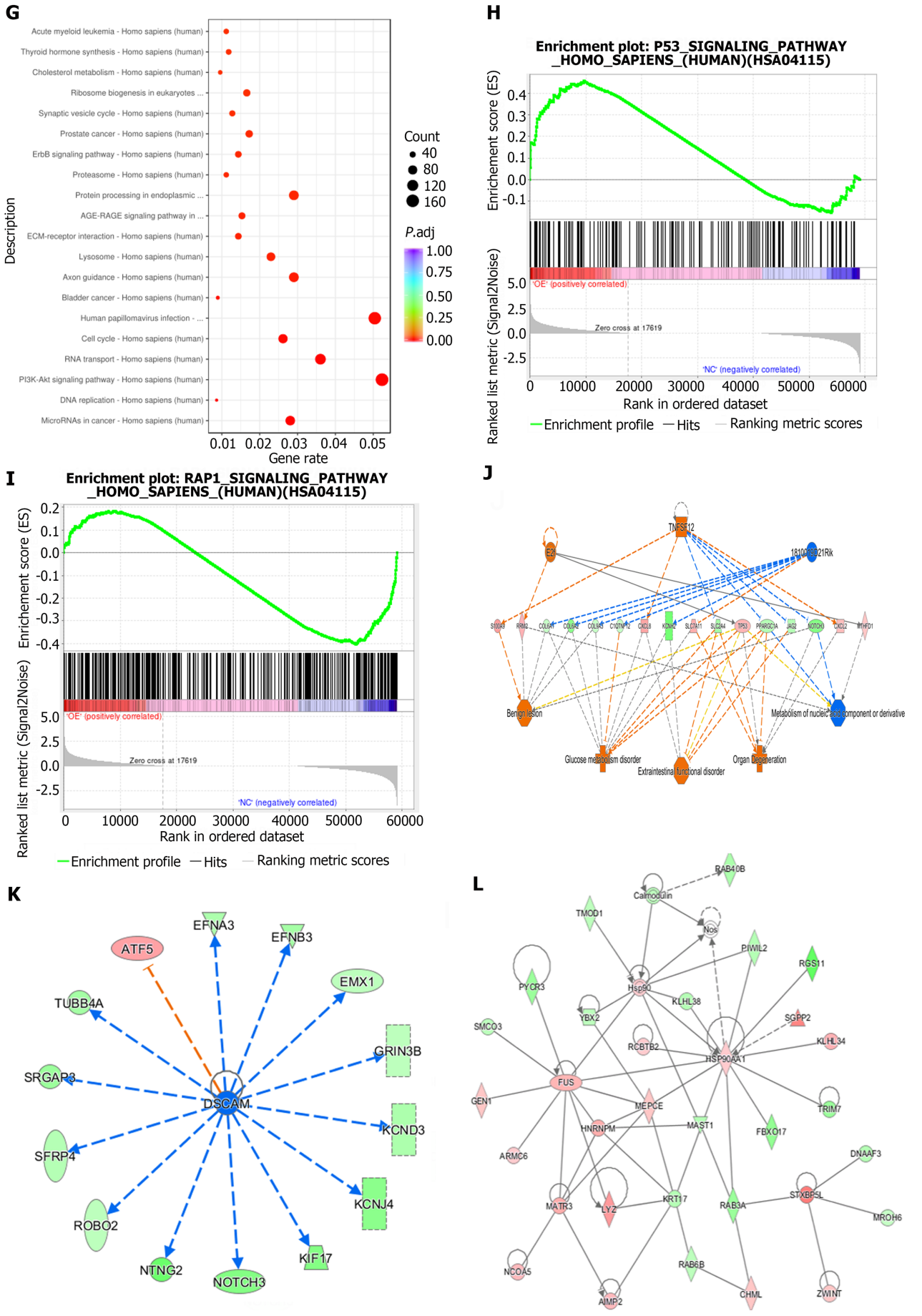


Figure 3 RNA-sequencing and bioinformatic analysis in Huh7 with LINC01767 over expression. A: Over expression of LINC01767 in Huh7 cells; B:

The differential expression heatmap is presented in using the log fold change (FC) > 1.5, and $P < 0.05$; C: The differential expression volcano plot is presented in using the logFC > 1.5, and $P < 0.05$; D: The top 10 most significantly enriched Gene Ontology terms; E and F: Gene Set Enrichment Analysis (GSEA) analysis showed that go term of LINC01767 involved in positive regulation of lymphocyte apoptosis, nucleotide kinase activity and so on; G: The top 10 most significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) terms; H and I: GSEA analysis showed KEGG pathway showed LINC01767 was involved in P53 signaling, RAP1 signaling and other important pathways; J: The top-ranked regulatory network in this regulatory effect analysis shows that the regulation net involved in regulators 1810019D21Rik, E2f, TNFSF12 through C1QTNF12, COL6A1, COL6A2, COL9A3, CXCL2, CXCL8, JAG2, KCNH2, MTHFD1, NOTCH3, PPARGC1A, RRM2, S100A9, SLC2A4, SLC7A11, TP53; K: The updream regulatory network analysis shows the DSCAM and ATF5 and so on involved in the regulatory net; L: Molecular network showed it can interact with HSP90, LYZ, FUS, and so on.

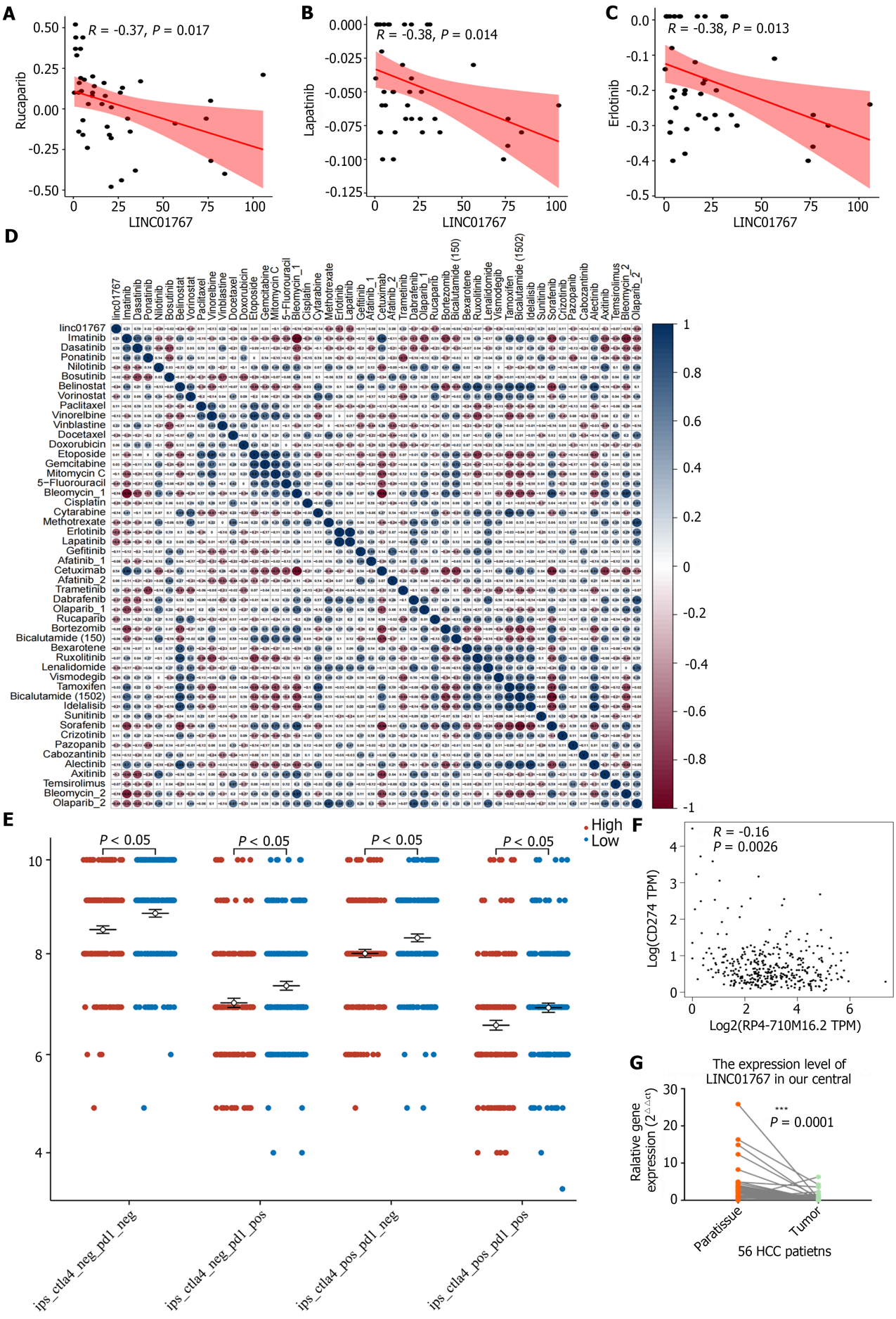
A prior study demonstrated that LINC01767 (ENSG00000223956, RP4-710M16.2), referred to as CRLM1, was found to be increased in metastatic CRC, leading to the inhibition of apoptosis in CRC cells and the promotion of liver metastasis in BALB/C nude mice. The CRLM1 facilitates liver metastasis by physically interacting with the hnRNPK protein and promoting its nuclear localization. CRLM1 significantly increases binding to the hnRNPK promoter and controls the activation of a group of genes related to metastasis[25]. We here add another potential mechanism from the single cell sequencing analysis showing the LINC01767 participates in the cancer stemness which may participate in metastasis.

AFP serves as the diagnostic and screening indicator for HCC. However, approximately 30%-40% of all patients with HCC exhibit AFP levels within the normal range, which is less than 20 ng/mL. AFP has a sensitivity of 41%-65% and specificity of 80%-90%, while DCP has a sensitivity of 56%-77% and specificity of 82%-87%. AFP-L3 has a sensitivity of 28%-56% and specificity of 92%-97% for diagnosing HCC[26]. Other tumor markers like GP73, GPC-3, PIVKAII, and certain microRNAs have been suggested, but none have demonstrated adequate sensitivity and/or specificity for early HCC diagnosis. We here demonstrated higher LINC01767 was correlated with higher AFP, LINC01767 could be a potentially diagnostic marker for HCC with sensitivity[27,28] better than AFP. GSE78160 showed that the serum LINC01767 was up regulated in HCC patients. However, why the level of LINC01767 down regulated in the tumor tissue but up regulated in serum was unclear, which was very interesting subject calling for further and deep exploration. This may be explained by the tumor heterogeneity or tumor cell subgroup heterogeneity, such as cluster7 which stands for the tumor stem cell was with a higher level of LINC01767 and it may secret LINC01767 into serum *via* exosome then demonstrating a higher level of serum LINC01767. It can be detected in urine as well, which gives the possible diagnosis HCC *via* the urine test as a non-invasive testing.

LINC01767 was a good predictor for OS rather than DFS. And the Cox regression verified the good performance of it in OS of HCC. A predictive nomogram was created using key factors identified in the Cox analysis to forecast the outcome of HCC, such as T/N/Residual tumor, vascular invasion, BMI, and LINC01767. After identified the LINC01767 related genes *via* next generation sequencing and bioinformatics analysis, we further construct a prognostic model *via* LASSO Cox regression analysis. The LASSO model's performance was evaluated using the ROC curve, indicating that the 9-genes model successfully predicted the OS of liver cancer patients, achieving an AUC greater than 0.75. This verified the prognostic performance of LINC01767, and also provide the potential mechanism of it in HCC initiation and progression.

The underlying mechanism how lncRNAs participate in the initiation and progression in HCC remains unexplored, we here provide an RNA-sequencing after over expression of LINC01767 and bioinformatics analysis hoping to intriguer the interest in this specific lncRNA[29,30]. We conducted potential co-related coding genes of LINC01767 *via* RNA-sequencing and IPA analysis, the results showed the related genes E2f, TNFSF12 through C1QTNF12, COL6A1, COL6A2, COL9A3, CXCL2, CXCL8, JAG2, KCNH2, MTHFD1, NOTCH3, PPARGC1A, RRM2, S100A9, SLC2A4, SLC7A11, TP53 participating in multiple process in cancers. The regulatory genes showed that the FMR1 was predicted to be strongly activated with 10 uniformly activated genes of LINC01767 (not shown). FMR1, also known as FMRP, has the ability to attach to RNA and is linked to polysome, showing high levels of expression in HCC tissues. Knocking down FMRP effectively inhibits HCC metastasis both *in vitro* and *in vivo*. The encoded protein plays a role in transporting mRNA from the nucleus to the cytoplasm. Fragile X syndrome is caused by an expansion of 55-230 repeats of a trinucleotide sequence (CGG) in the 5′ untranslated region (UTR), which is typically found at 6-53 copies[31]. FMRP aids in the localization of *STAT3* mRNA to protrusions by interacting with its 3′ UTR. FMRP could promote the interleukin-6-mediated translation of STAT3 *via* phosphorylation at serine 114 of FMRP. Although some potential target genes or regulatory genes did not show aberrant expression in HCC, such as KCNH2 which was involved in lipid metabolism[32]. Our research adds a fundamental evidence for the potential regulatory genes involved in the metabolism indicating a role of LINC01767 in HCC[33].

The findings from the *in vitro* study indicated that LINC01767 does not have a notable impact on cell migration, invasion, cell cycle, or apoptosis in Huh7 cells. Besides, the difference on the growth of cell after knockdown or over expression of LINC01767 was not so obvious. This may result from that only the over expression of LINC01767 in specific cell subgroup such as tumor stem cell can influence the function of tumor cell significantly, another potential hypothesis was that the LINC01767 exosome derived from tumor cell will function as the signal to influence other cells, such as immune cell. Thus, the role of LINC01767 in immune microenvironment was analyzed. The results showed the LINC01767 was positively correlated with the macrophage, and negatively with the B cell, CD4+ cell, neutrophil, myeloid dendritic cell, and macrophage M2 cell, what's more, it was negatively correlated with PDL1 rather than CTLA4; and the differential IPS in high-and low-LINC01767 group verified the correlation of LINC01767 with immunity in HCC. Besides, the sole dysregulation of LINC01767 may be rescued too fast in cell to show an obvious influence thus a gene set influenced by LINC01767 warrants further exploration in the future.


A
R = -0.37, P = 0.017
Rucaparib
LINC01767
B
R = -0.38, P = 0.014
Lapatinib
LINC01767
C
R = -0.38, P = 0.013
Erlotinib
LINC01767
D
E
P < 0.05
High
Low
ips_ctla4_neg_pd1_neg
ips_ctla4_neg_pd1_pos
ips_ctla4_pos_pd1_neg
ips_ctla4_pos_pd1_pos
F
R = -0.16
P = 0.0026
Log(CD274 TPM)
Log2(RP4-710M16.2 TPM)
G
The expression level of LINC01767 in our central
P = 0.0001
Ralative gene expression (2^-△△ct)
Paratissue
Tumor
56 HCC patietns

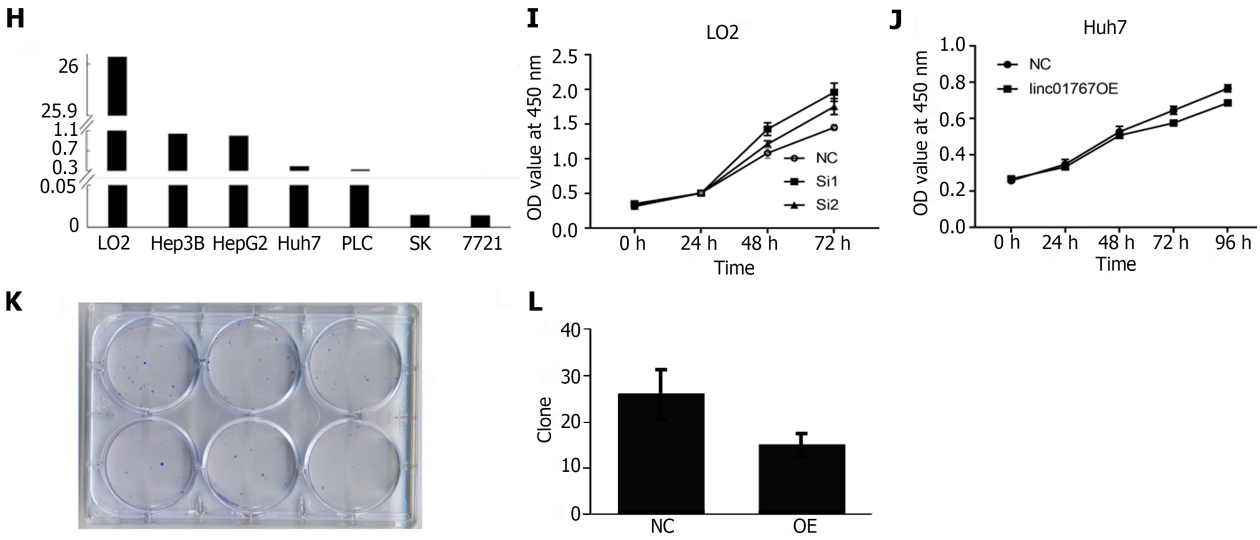


Figure 4 The role of LINC01767 of predicting drug sensitivity in hepatocellular carcinoma and the invitro experiment to valid the role of LINC01767 in Huh7. A: The re-analysis results of the previous research showed LINC01767 was negatively with the sensitivity of rucapanib (R = -0.37, P = 0.017); B: Lapatinib (R = -0.38, P = 0.014); C: Erlotinib (R = -0.38, P = 0.013); D: Sperman correlation analysis was used to evaluate the mRNA level of LINC01767 and IC_{50} of the potential drugs; E: The immunophenoscore (IPS), IPS-programmed cell death protein 1 (PD1)/programmed cell death ligand 1 (PDL1)/PD-L2 and IPS-CTLA4 were significantly lower higher in different between LINC01767 high group than in low groups (all P < 0.05); F: The spearman correlation analysis showed LINC01767 was negatively correlated with PDL1, P = 0.0026; G: The quantitative real time polymerase chain reaction of tumor tissue and para-tumor tissue from hepatocellular carcinoma (HCC) patients in our cohort showed that the LINC01767 was down regulated obviously in tumor; H: The cell lines of HCC and normal cell LO2 showed that the LINC01767 was down regulated in cancer cell line comparing with LO2; I: The down regulation of LINC01767 in LO2 cell showed the growth of the LO2 was impeded; J: MTT method and formation of clones was conducted to observe cell proliferation showing the overexpression of LINC01767 inhibit the cell proliferation; K and L: LINC01767 over expression impede the clone formation of Huh7. HCC: Hepatocellular carcinoma; NC: Normal control; OE: Over expression.

Our research has constraints. Our study is lacking biological research on the underlying mechanisms for specific conditions. Additionally, the initial examination of clinical features was conducted without additional analysis in a separate cohort due to the absence of public data sets and the lack of prognosis information in our cohort. Additionally, the mechanism or pathways was not investigated. Further investigation and testing are needed to confirm the effectiveness of diagnostic tests and treatments based on LINC01767. Furthermore, the impact of deregulation of LINC01767 on cancer diversity and evaluation of intratumoral clonality is still unclear. The therapeutic development the verification of *in vivo* bioactivity and cancer-cell-specific delivery of oligonucleotides.

CONCLUSION

LINC01767 is an important suppressor gene in HCC with good diagnostic and prognostic performance.

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FOOTNOTES

Author contributions: Zhang L and Cui TX contributed equally to this work and should be considered co-first authors. Wang WQ conceived this study and implemented the experiments; Li XZ and Liu C collected and preprocessed the data; Zhang L and Wang WQ drafted and revised the manuscript.

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SCIENTOMETRICS

Mapping the global research landscape on nonalcoholic fatty liver disease and insulin resistance: A visualization and bibliometric study

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Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is a liver condition that is prevalent worldwide and associated with significant health risks and economic burdens. As it has been linked to insulin resistance (IR), this study aimed to perform a bibliometric analysis and visually represent the scientific literature on IR and NAFLD.

AIM

To map the research landscape to underscore critical areas of focus, influential studies, and future directions of NAFLD and IR.

METHODS
This study conducted a bibliometric analysis of the literature on IR and NAFLD indexed in the SciVerse Scopus database from 1999 to 2022. The search strategy used terms from the literature and medical subject headings, focusing on terms related to IR and NAFLD. VOSviewer software was used to visualize research trends, collaborations, and key thematic areas. The analysis examined publication type, annual research output, contributing countries and institutions, funding agencies, journal impact factors, citation patterns, and highly cited references.

RESULTS
This analysis identified 23124 documents on NAFLD, revealing a significant increase in the number of publications between 1999 and 2022. The search retrieved 715 papers on IR and NAFLD, including 573 (80.14%) articles and 88 (12.31%) reviews. The most productive countries were China (n = 134; 18.74%), the United States (n = 122; 17.06%), Italy (n = 97; 13.57%), and Japan (n = 41; 5.73%). The leading institutions included the Università degli Studi di Torino, Italy (n = 29; 4.06%), and the Consiglio Nazionale delle Ricerche, Italy (n = 19; 2.66%). The top funding agencies were the National Institute of Diabetes and Digestive and Kidney Diseases in the United States (n = 48; 6.71%), and the National Natural Science Foundation of China (n = 37; 5.17%). The most active journals in this field were *Hepatology* (27 publications), the *Journal of Hepatology* (17 publications), and the *Journal of Clinical Endocrinology and Metabolism* (13 publications). The main research hotspots were "therapeutic approaches for IR and NAFLD" and "inflammatory and high-fat diet impacts on NAFLD".

CONCLUSION
This is the first bibliometric analysis to examine the relationship between IR and NAFLD. In response to the escalating global health challenge of NAFLD, this research highlights an urgent need for a better understanding of this condition and for the development of intervention strategies. Policymakers need to prioritize and address the increasing prevalence of NAFLD.

Key Words: Nonalcoholic fatty liver disease; Insulin resistance; Bibliometric; Visualization

Core Tip: Although numerous bibliometric studies have investigated insulin resistance (IR) and nonalcoholic fatty liver disease (NAFLD), few studies have explored the correlation of these two conditions. As explained here, understanding the interplay between IR and NAFLD is crucial for enhancing patient health outcomes. Consequently, this study aimed to perform a bibliometric analysis and visually represent the scientific literature pertaining to IR and NAFLD. Through mapping the research landscape, the study also aimed to emphasize key areas of focus, influential works, and potential future directions.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide[1]. NAFLD is defined as a spectrum of disorders that, in the absence of a secondary cause (*e.g.*, excessive alcohol consumption), share the characteristic of hepatic stenosis. A 2022 study concluded that NAFLD impacts the lives of more than 47 individuals per 1000 people, with another study from the same year projecting that NAFLD will affect more than half the population by 2040[1,2]. Taken together, these findings indicate that NAFLD is a significant public health concern that necessitates immediate action as uncontrolled NAFLD can progress to severe conditions, such as hepatocellular carcinoma, and is coupled with an increase in the risk of cardiovascular diseases that substantially increases the risk of mortality[3,4]. The effect of NAFLD is not limited to an increased risk of mortality. The direct and indirect costs of managing the condition alongside the loss of productivity of its patients are substantial[5]. In the United States alone, NAFLD expenditures can reach 103 billion USD; this excludes its complications, which, if considered, can increase the costs to more than 908 billion USD[6,7].

Other conditions, such as type 2 diabetes mellitus, metabolic syndrome, and obesity, have also been on the rise over the last few decades. This is not surprising given that these conditions are not independent; rather they are associated with one another in various ways[8,9]. For example, patients with NAFLD and obesity often have increased intraabdominal fat, which is hypothesized contributes to insulin resistance (IR). This resistance may lead to increased delivery of free

fatty acids to the liver, stimulating lipogenesis and aggravating hepatic steatosis. Conversely, in rat models, fat accumulation in the liver might be the primary cause of hepatic IR[10]. Furthermore, hepatic insulin sensitivity is improved in individuals who consume a diet intended to reduce hepatic fat[11].

Diet, lifestyle, and inflammatory cytokines all have major effects on IR. Currently, insulin sensitizers and lifestyle alterations are essential for managing and treating this illness. The use of insulin receptor cascade regulators and fresh perspectives on the prevention and treatment of primary IR are examples of future therapeutic targets. However, new discoveries have led to an improved understanding of insulin signaling and the mechanisms underlying IR. Additionally, sedentary lifestyles, high-fat and refined-carbohydrate diets, and inflammatory cytokines have all been connected to IR [12-14]. Among the endocrine disorders associated with this illness are obesity, type 2 diabetes, hypercholesterolemia, hypertriglyceridemia, and arterial hypertension[12]. An ideal lifestyle and, in the case of obesity, weight loss is the most crucial treatment component[14,15]. For instance, it has been clinically demonstrated that metformin causes insulin sensitization in high-risk individuals. With the use of laboratory concepts related to insulin and glucose, the disease was discovered and confirmed to be a significant entity in metabolism. This work is currently being conducted and put into practice[16]. Research has investigated the relationship between IR and the development of cardiometabolic disorders[17, 18]. Realistic therapeutic targets were identified after a thorough analysis of insulin signaling and IR mechanisms[19]. Additionally, as possible therapeutic targets for the treatment of IR, researchers are investigating the actions of insulin receptor cascade regulators[19]. Research is being conducted to learn more about the functions of the intestinal microbiota, peroxisome proliferator-activated receptors, autonomic nervous system, endocrine hormones, and vitamin D deficiency in the prevention and management of IR[17].

There is currently no Food and Drug Administration-approved treatment for NAFLD. However, management strategies have been proposed. One popular strategy is weight loss, with studies showing that a reduction of up to 5% in body weight improves liver fat content and fibrosis[4,20]. Some pharmacological agents have been suggested, but all remain under investigation. For example, metformin has yielded varying results in improving the liver conditions associated with NAFLD. Some studies have reported improved liver function[21,22], but others have shown no significant changes[23,24]. Another proposed class of pharmacological agent is thiazolidinediones, which have demonstrated the potential to improve liver function and decrease hepatic fat content in nonalcoholic steatohepatitis patients[25-27]. Other treatments, such as statins, omega-3 fatty acids, and antioxidant therapies, have shown mixed results[28-31].

It is essential to review and analyze existing studies to understand the current trends and gaps in research on the relationship between IR and NAFLD. A bibliometric analysis, which uses statistical methods to examine research publications, can help. This approach provides an overview of significant developments, major contributors, and notable papers. In addition, visualization techniques can reveal research and collaboration patterns and central themes resulting from the bibliometric analysis[32-34].

Numerous studies have examined research trends within the microbiota and liver, encompassing various diseases[35-42]. While there have been several studies on IR[43-45] and NAFLD[46-49], few have explored the relationship between them. Understanding the relationship between IR and NAFLD is essential for improving patient health outcomes. Thus, this study aimed to conduct a bibliometric analysis and to visualize the scientific literature on IR and NAFLD. By mapping the research landscape, this study highlights critical areas of focus, influential works, and future directions.

MATERIALS AND METHODS

Study design

This comprehensive cross-sectional analysis used bibliometric methods to analyze publications addressing IR and NAFLD that were published between 1999 and 2022.

Database used

The progression and expansion of the literature concerning IR and NAFLD were examined in publications retrieved from the SciVerse Scopus database. The use of this database is justified for several reasons[50-55]. First, Scopus includes more than 30000 indexed journals across health and the social, life, and physical sciences. Second, SciVerse entirely includes PubMed journals and surpasses the Web of Science the number of indexed journals. Third, Scopus offers various features that streamline bibliometric analysis. It is important to note that this database is biased toward publications from English-speaking countries and English-language journals[56]. As, the Web of Science faces a similar bias issue, Scopus was the most pragmatic choice. For these reasons, Scopus is widely regarded as exceptionally comprehensive because it encompasses publications from both PubMed and Web of Science[53,54].

Search strategy

A comprehensive literature search was conducted using the Scopus database to identify relevant studies investigating the association between IR and NAFLD. The search included publications up to December 31, 2022. It was conducted on November 17, 2023 to ensure that all relevant research items were retrieved and to reduce bias from database updates. Multiple broad terms and phrases were used to ensure that all IR and NAFLD research was retrieved.

The data for this study were extracted according to the following steps. Step 1: A group of search terms related to NAFLD was used. The terms were extracted from existing systematic reviews, meta-analyses, and medical subject headings from PubMed. For article retrieval, the terms were then inserted into the "Article Title" field of the Scopus search engine. Step 2: The retrieved publications were further refined by restricting the scope to those containing "IR and

associated terms" within their titles. Terms pertaining to IR were meticulously selected from a collection of previous systematic reviews and meta-analyses[57-59]. Subsequently, the terms "IR" and "insulin sensitivity" were used as search criteria within the article titles. Step 3: The research deliberately restricted its focus to scientific journal articles, meticulously excluding nonscholarly publications such as books, book chapters, retracted articles, and errata.

Validation of the search query

The investigation substantiated the search query by applying two distinct criteria. Two experts specializing in bibliometric sciences assessed the top 50 most common documents and documents with even numerical identifiers (55, 60, 65, 70, *etc*) in the retrieved document list. The experts scrutinized the documents in an Excel spreadsheet to identify false-positive results. In instances of disagreement, the principal investigator adjudicated the final judgment. The absence of false-positive results served as the validity indicator, and the author iteratively refined the search query until both reviewers concurred on its absence.

After this validation, the experts were instructed to compare the publication counts of the top 20 active authors with the number of articles attributed to each author, as documented in their respective Scopus profiles. The results obtained by both methods underwent correlation testing to assess significance and correlation. The correlation test revealed a robust correlation coefficient ($r = 0.976$), and the statistical significance ($P < 0.001$) emphasized the precision of the search query. This dual-method approach aimed to confirm the absence of false-negative outcomes, drawing inspiration from established bibliometric studies[60,61]. Notably, the inclusion of keywords in the title search, as opposed to a title/abstract search, was employed to enhance the reliability of the approach. Consequently, the title search method emerged as reliable, with minimal retrieval of false-positive documents. In contrast, the title/abstract search method[62-65] yielded numerous false positives that did not have a specific focus on IR and NAFLD.

Bibliometric indicators

This study systematically assigned the fundamental bibliometric indicators to four main classifications following established norms used in previous bibliometric analyses[66-69]. The four main classifications were: (1) Analysis of the research output and publication progress concerning IR and NAFLD, which included metrics such as the total number of articles, the year of publication, and the trends of the publications; (2) Identification of origin and study patterns in publications, which focused on identifying the countries that contributed substantially to the collective body of research; (3) Evaluation of publication productivity, which investigated the top prolific institutions and funding agencies actively involved in this field; and (4) Assessment of the quality of publication output, which examined the most frequently cited articles, citation patterns, the Hirsch index (h-index), and most prolific journals and their corresponding impact factors. The impact index per article was displayed for the top 10 highly cited papers sourced from the Reference Citation Analysis (RCA) database. RCA, an open multidisciplinary citation analysis database, is owned by Baishideng Publishing Group Inc. headquartered in Pleasanton, CA 94566, United States[70-72].

Visualization analysis

VOSviewer software (version 1.6.20; Van Eck & Waltman, www.vosviewer.com) was used to construct visual representations illustrating collaborations among countries *via* network maps[73-75]. To generate a term co-occurrence map in VOSviewer, only terms occurring at least 20 times in the title and abstract were considered under binary counting. The terms with the highest relevance scores were selected to construct a term map for network visualization. The algorithm was designed to ensure that terms with more frequent co-occurrences manifested as larger bubbles and that similar terms were positioned close together. A unique color was assigned to each term to visually represent the frequency of occurrence of each in the overlay. In contrast to yellow and green, which denote keywords that appeared more recently, blue was used to indicate keywords that appeared earlier in the time course[73-75].

RESULTS

General description of the retrieved publications

A global search of publications between 1973 and 2022 revealed a substantial collection of 23124 NAFLD-related documents, as indicated in their titles. Among these scholarly works, 715 examined NAFLD and its association with IR from 1999 to 2022. Articles were the most prevalent form of NAFLD and IR literature, accounting for 573 publications (80.14%). A total of 88 reviews were found, representing 12.31% of the total. The remaining five publication types, which included letters, notes, editorials, meeting minutes, and brief surveys, accounted for 54 documents, or 7.56% of the total.

Growth and productivity trends

The volume of publications on IR and NAFLD has steadily increased from 1999 to 2022. This growth can be divided into two distinct phases. The first phase, spanning from 1999 to 2010, was characterized by rapid growth, and the second phase, from 2011 to 2022, had a more stable and consistent increase. Reflecting this trend, the average annual publication rate increased from 16.33 per year in the first phase to 43.25 per year in the second phase. Statistical analysis using linear regression confirmed this observation, revealing a modest positive correlation ($R^2 = 0.7865$, $P < 0.001$) between the annual publication count and the corresponding year of publication (Figure 1).

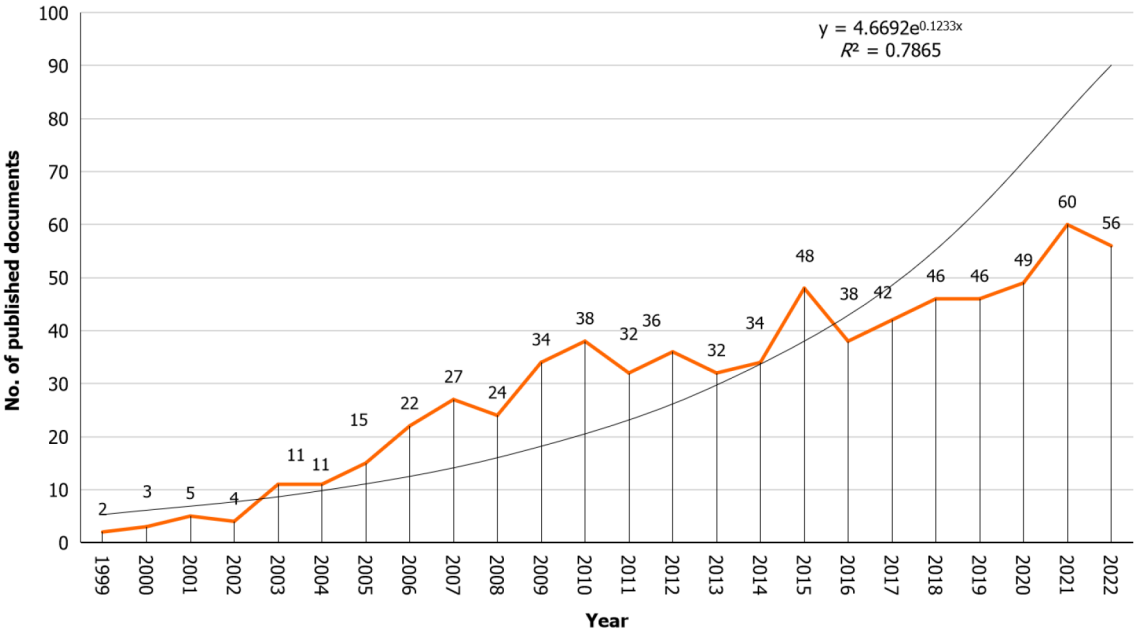

Figure 1 Growth trend of publications on insulin resistance and nonalcoholic fatty liver disease from 1999 to 2022.

Top active countries

Between 1999 and 2022, more than 60 countries actively conducted and published studies on NAFLD and its correlation with IR. The top 10 countries collectively authored 524 articles, constituting 73.28% of all the contributions in this field (Table 1). Notably, China was the foremost contributor, with 134 articles (18.74%), followed by the United States, with 122 articles (17.06%); Italy, with 97 articles (13.57%); and Japan, with 41 articles (5.73%). Furthermore, the United States and China led in international collaboration, boasting the greatest number of publications involving scholars from various countries. For a visual representation of international research collaborations on IR and NAFLD from 1999 to 2022, refer to Figure 2, which shows a network map of the key participating countries.

Top 10 active institutions

Table 2 shows the 10 most productive institutes with publications on NAFLD and its association with IR from 1999 to 2022. These institutions collectively accounted for 12.30% (n = 88) of the published articles. The Università degli Studi di Torino in Italy was the most prolific contributor, with 29 articles (4.06%), followed by the Consiglio Nazionale delle Ricerche in Italy, with 19 articles (2.66%), and the Alma Mater Studiorum Università di Bologna in Italy, with 16 articles (2.24%). Italy had seven institutions on the list, the United States had two, and South Korea had one.

Top 10 funding agencies

A total of 275 publications, accounting for 38.5% of the retrieved articles, were supported by funding. Table 3 lists the top 10 funding agencies associated with NAFLD and its correlation with IR from 1999 to 2022. These 10 agencies collectively contributed 19.86% (n = 142) of the published articles. The National Institute of Diabetes and Digestive and Kidney Diseases in the United States (n = 48; 6.71%) emerged as the most active funding agency in the field, followed by the National Natural Science Foundation of China (n = 37; 5.17%), and the National Institutes of Health in the United States (n = 31; 4.34%).

Top 10 most active journals

Table 4 shows that the leading 10 journals/sources contributed 19.18% (n = 137) of the overall research publications focused on NAFLD and its correlation with IR. *Hepatology* [impact factor (IF) 14.0, 2022] had the most publications, with 27, followed by the *Journal of Hepatology* (IF 25.7, 2022), with 17, and the Journal of *Clinical Endocrinology and Metabolism* (IF 5.8, 2022), with 13.

Analysis of citations

The collected articles had received a total of 42590 citations, with an average of 59.57 and an h-index of 99. Among these articles, 71 were not cited, and 96 had more than 100 citations. The citation count for these articles varied from 0 to 1826. Table 5 shows the top 10 publications on NAFLD and its association with IR, and they account for 9149 citations. The number of citations for these publications ranged from 545 to 1826[10,11,76-83]. The impact index per article of the 10 most cited articles ranged from 23.9 to 116.0 (Table 5).

Co-occurrence analysis

VOSviewer was used to perform a co-occurrence analysis of the title and abstract content of the publications, explicitly

Table 1 Top 10 countries leading in research on insulin resistance and nonalcoholic fatty liver disease: Publications spanning 1999 to 2022

Ranking[1]	Country	Number of documents	%
1st	China	134	18.74
2nd	United States	122	17.06
3rd	Italy	97	13.57
4th	Japan	41	5.73
4th	South Korea	41	5.73
6th	Iran	35	4.90
7th	Spain	32	4.48
8th	Turkey	32	4.48
9th	Germany	23	3.22
10th	United Kingdom	23	3.22

[1]Gap is left in the next ranking number when specific countries are given the same number.

Table 2 Top 10 most productive institutions in research related to insulin resistance and nonalcoholic fatty liver disease from 1999 to 2022

Ranking[1]	Institute	Country	No. of documents	%
1st	Università degli Studi di Torino	Italy	29	4.06
2nd	Consiglio Nazionale delle Ricerche	Italy	19	2.66
3rd	Alma Mater Studiorum Università di Bologna	Italy	16	2.24
4th	Sapienza Università di Roma	Italy	13	1.82
5th	IRCCS Ospedale Pediatrico Bambino Gesù	Italy	12	1.68
6th	Istituto di Fisiologia Clinica del CNR	Italy	11	1.54
6th	SKKU School of Medicine	South Korea	11	1.54
6th	Yale School of Medicine	United States	11	1.54
9th	Università degli Studi di Milano	Italy	10	1.40
10th	University of Florida	United States	9	1.26

[1]Gap is left in the next ranking number when specific institutes are given the same number.

emphasizing IR and NAFLD (Figure 3). The resulting co-occurrence network revealed three distinct clusters representing the primary research priorities concerning IR and NAFLD. Each cluster was distinguished by different colors (red for cluster 1, green for cluster 2, and blue for cluster 3), and terms associated with specific research topics were included. Cluster 1 includes publications on "population health implications", addressing IR and NAFLD's significant population health ramifications in light of the global increase in obesity and metabolic disorders. Ongoing discussions center on public health strategies for prevention and management. Cluster 2 focused on "inflammation and high-fat diets", exploring the potential contribution of high-fat diets, particularly those imbalanced in omega-3 and omega-6 fatty acids and those rich in highly saturated and trans fats, to inflammation, which is linked to IR and NAFLD. Cluster 3 publications addressed "therapeutic approaches", emphasizing the challenge of developing effective therapeutic strategies for IR and NAFLD through clinical trials. The exploration included lifestyle interventions, pharmacological treatments, and novel therapies to manage and potentially reverse these conditions. Notably, the terms within each cluster have robust connections, underscoring the coherence of the research within these thematic areas.

Analysis of future research directions

The term co-occurrence analysis (Figure 4) revealed evolving trends in publications on NAFLD and IR. Notably, recent studies (post-2015) investigated the effects of inflammation and a high-fat diet on the development of IR and NAFLD, as well as therapeutic approaches. These investigations underscore the challenge of devising effective therapeutic strategies, emphasizing the shift from earlier publications (pre-2015) that primarily focused on "population health implications".

Table 3 Top 10 most productive funding agencies for research on insulin resistance and nonalcoholic fatty liver disease from 1999 to 2022

Ranking[1]	Funding agency	Country	No. of documents	%
1st	National Institute of Diabetes and Digestive and Kidney Diseases	United States	48	6.71
2nd	National Natural Science Foundation of China	China	37	5.17
3rd	National Institutes of Health	United States	31	4.34
4th	National Center for Research Resources	United States	18	2.52
5th	Japan Society for the Promotion of Science	Japan	17	2.38
6th	National Center for Advancing Translational Sciences	United States	13	1.82
7th	Seventh Framework Programme	European Union's	10	1.40
8th	Horizon 2020 Framework Programme	European Union's	9	1.26
9th	Instituto de Salud Carlos III	Spain	8	1.12
9th	National Heart, Lung, and Blood Institute	United States	8	1.12
9th	United States Department of Veterans Affairs	United States	8	1.12

[1]Gap is left in the next ranking number when specific funding agencies are given the same number.

Table 4 Top 10 journals with the largest volume of research on insulin resistance and nonalcoholic fatty liver disease, 1999-2022

Ranking[1]	Journal/source title	No. of documents	%	IF[2]
1st	*Hepatology*	27	3.78	14.0
2nd	*Journal of Hepatology*	17	2.38	25.7
3rd	*Journal of Clinical Endocrinology and Metabolism*	13	1.82	5.8
3rd	*Nutrients*	13	1.82	5.9
5th	*Scientific Reports*	11	1.54	4.6
6th	*Liver International*	10	1.40	6.7
6th	*Chinese Journal of Hepatology*	10	1.40	NA
8th	*American Journal of Gastroenterology*	9	1.26	10.2
8th	*Plos One*	9	1.26	3.7
8th	*World Chinese Journal of Digestology*	9	1.26	NA
8th	*World Journal of Gastroenterology*	9	1.26	4.3

[1]Gap is left in the next ranking number when specific journals are given the same number.
[2]Impact factor based on Clarivate Analytics Journal Citation Reports 2022.
IF: Impact factor.

These earlier works addressed the significant population health implications of IR and NAFLD in the context of the global rise in obesity and metabolic disorders.

DISCUSSION

This is the first bibliometric analysis to provide an overview of the NAFLD and IR research landscape. The study covered research published between 1999 and 2022, including research trends, collaborations, and main thematic areas. Additionally, publication type, annual research output, contributing countries and institutions, funding agencies, journal impact factors, citation patterns, and highly cited references were analyzed.

The analysis revealed that after 2011, there was a substantial increase in publication output, which could signify that this point marked the beginning of significant interest in the topic. This increase might also indicate that at that time, the scientific community began to recognize the urgency of understanding the relationship between NAFLD and IR. A significant positive correlation was found between the year of publication and the publication output using linear

Table 5 The 10 most cited articles on insulin resistance and nonalcoholic fatty liver disease from 1999 to 2022

Ref.	Title	Year	Source title	Cited by	Impact index per article[1]
Sanyal *et al*[76], 2001	Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities	2001	*Gastroenterology*	1826	62.4
Marchesini *et al* [77], 1999	Association of nonalcoholic fatty liver disease with insulin resistance	1999	*American Journal of Medicine*	1331	42.0
Jung and Choi[78], 2014	Obesity and its metabolic complications: The role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease	2014	*International Journal of Molecular Sciences*	1265	116.0
Samuel *et al*[10], 2004	Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease	2004	*Journal of Biological Chemistry*	1062	47.1
Pagano *et al*[79], 2002	Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: Further evidence for an etiologic association	2002	*Hepatology*	659	23.9
Utzschneider and Kahn[11], 2006	Review: The role of insulin resistance in nonalcoholic fatty liver disease	2006	*Journal of Clinical Endocrinology and Metabolism*	657	32.6
Musso *et al*[80], 2003	Dietary habits and their relations to insulin resistance and postprandial lipemia in nonalcoholic steatohepatitis	2003	*Hepatology*	627	24.2
Gaggini *et al*[81], 2013	Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease	2013	*Nutrients*	594	49.7
Bugianesi *et al*[82], 2005	Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: Sites and mechanisms	2005	*Diabetologia*	583	26.2
Birkenfeld and Shulman[83], 2014	Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 Diabetes	2014	*Hepatology*	545	49.8

[1]The impact index per article is presented based on reference citation analysis [Source: Baishideng Publishing Group Inc (Pleasanton, CA 94566, United States)].

regression, further corroborating these findings.

The growth in publications on this subject was gradual and consistent with the increased interest in the topic. Some of those publications include Watt *et al*'s[84] analysis, of the effect of impaired liver lipid metabolism in NAFLD on the release of liver proteins, metabolites, and miRNAs, which influence metabolism in other tissues and lead to IR. Subsequently, elevated levels of IR, as described in a study by Angelico *et al*[85], more commonly coincide with severe stenosis. This relationship between NAFLD and IR was also observed by Kumashiro *et al*[86], who posited that the hepatic diacylglycerol content is the most accurate marker of IR.

These studies collectively emphasize that NAFLD is a threat to global health, highlighting the lack of a comprehensive understanding of NAFLD pathogenesis. Studies have suggested using preclinical models for more extensive research on this condition to mitigate this lack of understanding, and some have proposed combining herbal remedies with conventional lifestyle interventions and pharmacological management strategies for better NAFLD control[31,87-90].

A global effort can be observed in NAFLD and IR research involving more than 60 countries, with China and the United States in the number of publications, consistent with their longstanding leadership in biomedical research[91,92]. This notable publication lead in the United States can be attributed to substantial funding, advanced research facilities, and a solid scientific culture. Additionally, the United States' focus on these areas may stem from increasing rates of obesity and metabolic syndrome[93]. Financial support from agencies such as the National Institute of Diabetes and Digestive and Kidney Diseases and the National Institutes of Health drove this research, as indicated by our findings and those of other studies[94-97].

China is also seeing more funding and an increased publication output in this field, partly due to substantial research investments and the increased number of metabolic syndrome cases caused by rapid urbanization[98-100]. The National Natural Science Foundation of China is the most influential funding source for this research, as shown in other studies [101-103] and corroborated by our findings. Josol *et al*'s[90] bibliometric analysis shows that this phenomenon is not exclusive to China but is also apparent in Southeast Asia. Research productivity is linked to socioeconomic factors, particularly economic status and research and development spending. In addition, NAFLD research in the region has focused on topics such as metabolic syndrome, diagnostics, treatment, and epigenetics.

In addition to China and the United States, countries such as Italy, Japan, South Korea, Iran, Spain, Turkey, Germany, and the United Kingdom are significant contributors to NAFLD research, driven by global interest in NAFLD and the goal of finding its treatment. This diversity of research locations can also be observed in the top institutional contributions, with Italian institutions such as the Università degli Studi di Torino and the Consiglio Nazionale delle Ricerche leading in publications. The research interest of these institutions in the subject is demonstrated by the establishment of

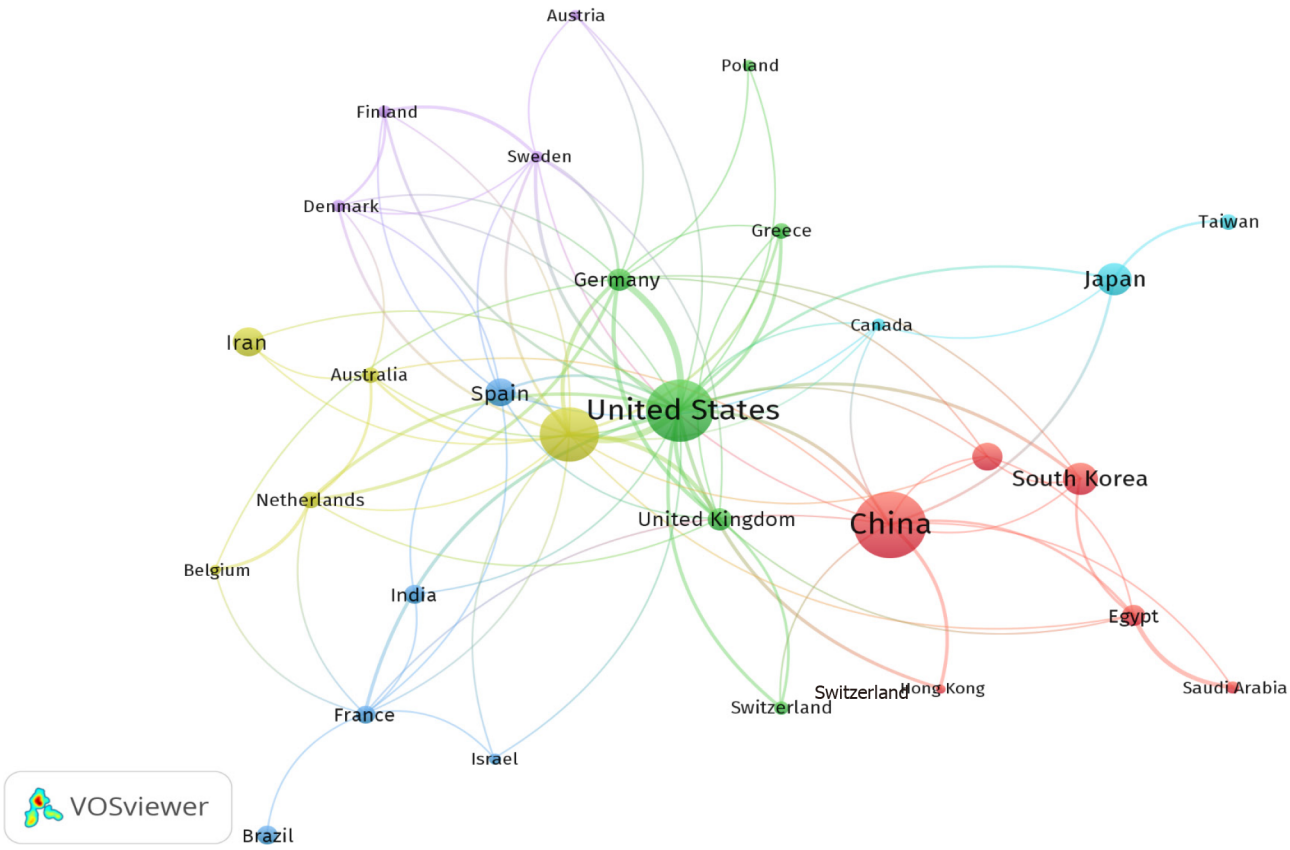

Figure 2 Visualization of global research collaborations in insulin resistance and nonalcoholic fatty liver disease: A network map spanning 1999 to 2022. Nodes indicate countries with significant collaborations (minimum of five publications per country) among 60 active nations. Size indicates the publication count.

the "NAFLD Study Group" at the Università degli Studi di Torino and the contributions of the Consiglio Nazionale delle Ricerche to various NAFLD-related projects[104,105].

The analysis of publication journals revealed that journals such as *Hepatology*, the *Journal of Hepatology*, and the *Journal of Clinical Endocrinology and Metabolism* are leading in publishing high-impact research. Citation analysis further highlights the impact of the research in this field. A high average citation count and h-index highlight the impact. Of NAFLD-IR research impact and its ongoing relevance[11,76,79]. Co-occurrence analysis revealed distinct research clusters that have evolved over time. The shift from discussing population health implications in early publications to a more recent focus on inflammation and high-fat diets and therapeutic approaches mirrors the shifting global focus on lifestyle factors and therapeutic advancements in understanding and managing NAFLD and IR[106-111].

Strengths and limitations

This study had several limitations. First, it relied solely on Scopus, possibly overlooking studies not in this database. Second, the approach may have missed discussions of the relationship between NAFLD and IR in other types of articles. Third, inaccuracies could have arisen in identifying research origins based on affiliations. Fourth, the focus was on NAFLD and IR, excluding factors such as genetics. Fifth, only English-language publications were considered. Finally, the study is primarily descriptive and did not evaluate the quality or impact of individual studies in NAFLD research.

CONCLUSION

This was the first bibliometric analysis of NAFLD and IR research. The increase in publications, especially after 2011, indicates growing interest in this field. The significant topics of interest included lifestyle impact, treatment strategies, and metabolic disorders. Notable contributions came from several countries, with China and the United States being the most prominent. Given the urgency and worldwide impact of NAFLD, it is now necessary for global policymakers and health authorities to pay attention to this topic.

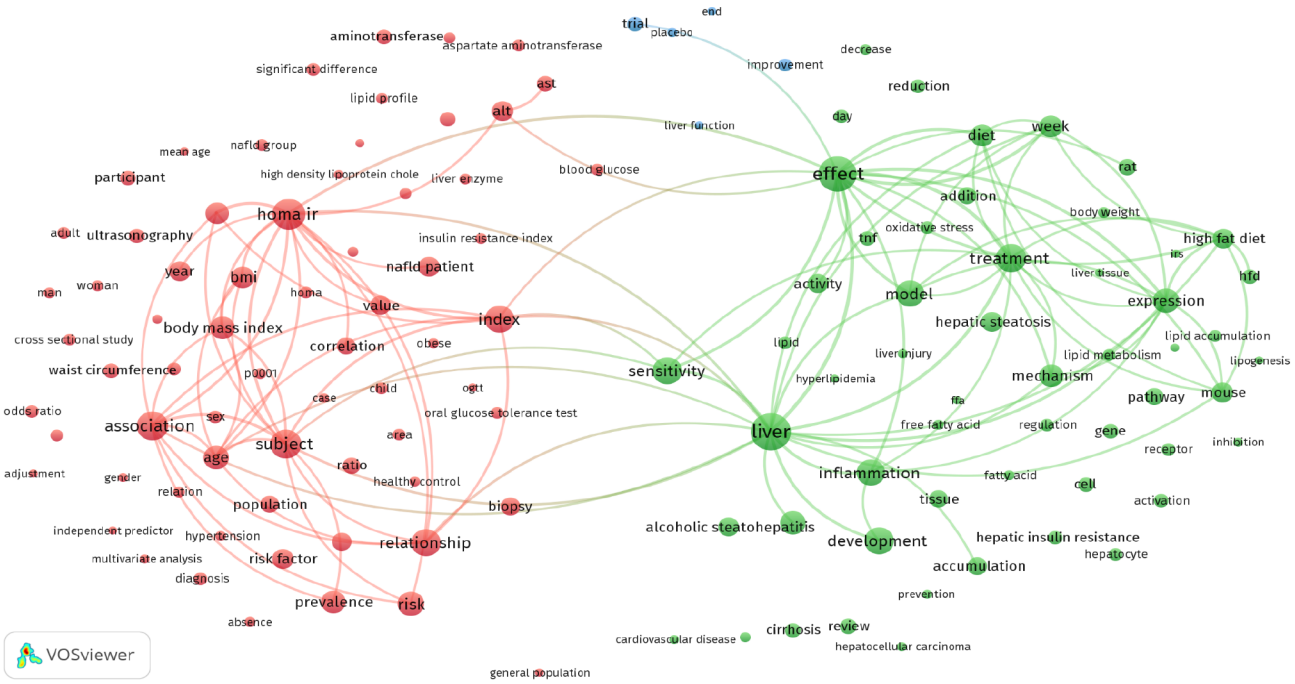

Figure 3 Visualization map of term networks in the titles and abstracts of publications on insulin resistance and nonalcoholic fatty liver disease between 1999 and 2022. The map was generated with a minimum term occurrence set at 20 times, resulting in 211 terms meeting this criterion among 34784 in the field. The terms are organized into three clusters, each shown in a different color. The size of each node corresponds to the number of publications utilizing that particular term.

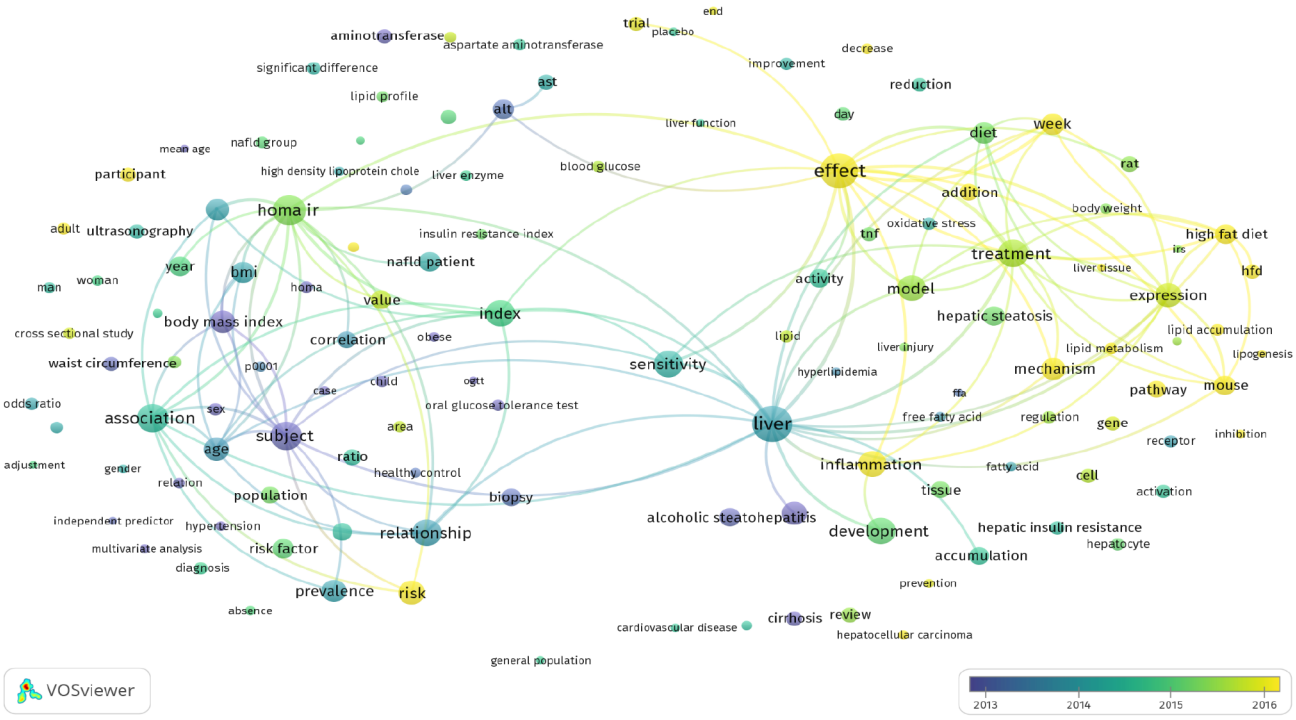

Figure 4 Visualization map of the network of terms in the title/abstracts of publications on insulin resistance and nonalcoholic fatty liver disease (1999-2022) with analysis of their distribution by mean frequency. Chronological emergence: Blue terms first, followed by yellow and green terms.

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FOOTNOTES

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CASE REPORT

Successful treatment of severe hepatic impairment in erythropoietic protoporphyria: A case report and review of literature

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Abstract

BACKGROUND

Erythropoietic protoporphyria (EPP) is a rare genetic disorder stemming from ferrochelatase gene mutations, which leads to abnormal accumulation of protoporphyrin IX primarily in erythrocytes, skin, bone marrow and liver. Although porphyria-related severe liver damage is rare, its consequences can be severe with limited treatment options.

CASE SUMMARY

This case study highlights a successful intervention for a 35-year-old male with EPP-related liver impairment, employing a combination of red blood cell (RBC) exchange and therapeutic plasma exchange (TPE). The patient experienced significant symptom relief and a decrease in bilirubin levels following multiple PE sessions and an RBC exchange.

CONCLUSION

The findings suggest that this combined approach holds promise for managing severe hepatic impairment in EPP.

Key Words: Erythropoietic protoporphyria; Red blood cell exchange; Plasma exchange; Delta-aminolevulinic acid; Ferrochelatase; Case report

Core Tip: Erythropoietic protoporphyria (EPP) is a rare autosomal recessive disorder, with few reported cases of associated hepatic injury, posing significant diagnostic challenges. Conventional therapies frequently fall short in severe cases, leading to the necessity for liver transplantation. Here, we report the case of a 35-year-old patient with EPP experiencing progressive liver dysfunction, unresponsive to standard medical care. A novel intervention comprising combined red blood cell exchange and plasma exchange therapies was administered. This approach resulted in a marked improvement in the patient's liver function, highlighting a potentially effective alternative treatment for serious hepatic manifestations in EPP.

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INTRODUCTION

Porphyria comprises a cluster of metabolic disorders arising from deficiencies in specific enzymes within the heme biosynthesis pathway[1]. This deficiency leads to elevated concentrations of porphyrins or their precursors, such as delta-aminolevulinic acid (δ-ALA) and porphobilinogen, culminating in their abnormal accumulation within tissues and subsequent cellular damage[2]. Porphyria is a relatively rare genetic disorder, with prevalence ranging from 0.5 to 10 per 100000 in different populations[3,4]. High levels of porphyrins can cause significant problems, primarily affecting the nervous system and skin[5].

Based on the main site of porphyrin intermediate metabolite accumulation, porphyria can be divided into erythropoietic protoporphyria (EPP, OMIM 177000) and hepatic porphyria. EPP is a rare autosomal recessive genetic disorder caused by mutations in the ferrochelatase (*FECH*) gene[6]. FECH catalyzes the final step in the heme biosynthetic pathway by chelating ferrous iron with protoporphyrin IX (PPIX). *FECH* deficiency leads to the abnormal accumulation of PPIX, predominantly in erythrocytes, skin, bone marrow, and the liver[7]. EPP is the most common form of porphyria in pediatric patients, and has a prevalence ranging from 1:200000 to 1:75000[8]. The symptoms of EPP include the formation of non-blistering skin lesions within minutes of exposure to sunlight, which begins in early childhood and persists throughout the individual's lifetime, significantly reducing their quality of life[9].

Approximately 10% of EPP patients experience liver damage. Although severe liver damage in EPP is rare, the implications for patient health are, nevertheless, significant[10]. The clinical manifestations of liver damage in porphyrias are varied and can range from mild liver enzyme disturbances to severe acute cholestatic hepatitis with hepatic failure. Furthermore, owing to the rarity of this condition, it can easily be overlooked by clinicians. Currently, the treatment options for severe liver damage in porphyria are extremely limited, and patients often ultimately require liver transplantation. Here, we present a successful case of treatment of severe hepatic injury in EPP through a combination of red blood cell (RBC) exchange and therapeutic plasma exchange (TPE). The results suggest that the combined intervention involving RBC and plasma exchange is a promising strategy for treating severe hepatic complications in EPP.

CASE PRESENTATION

Chief complaints

A 35-year-old Chinese male patient with a 32-year history of photosensitivity dermatitis presented with severe abdominal pain, jaundice, and gastrointestinal symptoms, including nausea, vomiting, cessation of bowel movements, and cessation of flatus, at the Department of Infectious Diseases of our hospital on March 10, 2023.

History of present illness

The patient had been experiencing recurrent skin erythema, swelling, pain, and itching within minutes of sunlight exposure since childhood, that typically resolved spontaneously after 2 to 3 d. This had been ongoing for the last 32 years. Eight years prior to this admission, the patient had experienced recurrent symptoms including right upper abdominal pain, poor appetite, aversion to fatty foods, and fatigue. Seeking medical attention at local hospitals, the patient underwent routine gastrointestinal decompression, treatment with ursodeoxycholic acid (UDCA) and glycyrrhizin to promote bile excretion and protect hepatocytes, and high glucose-load therapy to inhibit δ-ALA synthase. Although these treatments resulted in the alleviation of the mentioned symptoms, the condition recurred. One month before this admission, the patient's aforementioned digestive symptoms gradually worsened, leading to the decision to seek inpatient treatment at our hospital.

History of past illness

The patient's medical history showed no significant past illnesses such as viral hepatitis or drug allergies.

Personal and family history

The patient denied any history of tobacco smoking, alcohol consumption, or illicit drug use, reported no remarkable family history of similar illnesses nor three-generation inheritance of genetic disorders or psychiatric illnesses. Genetic screening of the patient's siblings did not reveal any significant abnormalities.

Physical examination

The patient presented with conjunctival icterus and generalized jaundice. Mild tenderness was observed on palpation in the left subxiphoid region.

Laboratory examinations

Following admission, the patient's auxiliary examinations revealed elevated liver enzymes (aspartate aminotransferase: 91 U/L, alanine aminotransferase: 149 U/L) and increased bilirubin levels (total bilirubin: 141.8 mmol/L, direct bilirubin: 104.3 mmol/L). Additionally, vitamin D deficiency was identified (25-hydroxyvitamin D total: 2.47 ng/mL, 25-hydroxyvitamin D2: 1.22 ng/mL, 25-hydroxyvitamin D3: 1.25 ng/mL), although serum ferritin concentrations were found to be unremarkable (139.08 ng/mL).

Imaging examinations

Abdominal X-ray revealed multiple air-filled intestine loops and fecal material accumulation. Further examination through abdominal magnetic resonance imaging demonstrated liver inflammation, hepatic iron deposition, chronic cholecystitis, and splenomegaly. Liver cirrhosis was detected through abdominal ultrasonography.

Histopathological examination and genetic screening

Liver biopsy confirmed chronic inflammatory liver injury (G3S3) with brownish pigment deposition and birefringent particles, suggestive of porphyria (Figure 1A and B). Genetic testing identified mutations in the *FECH* gene (heterozygous p.C202Y and homozygous c.315-48T>C intronic mutations) (Figure 1C and D).

FINAL DIAGNOSIS

The final diagnosis for the presented case is erythropoietic porphyria with severe hepatic involvement and paralytic ileus.

TREATMENT

Upon admission, the patient received high-glucose-load therapy, cimetidine, and arginine heme to inhibit ALA synthase [2]. Simultaneously, cholestyramine and UDCA were administered to enhance bile excretion. However, following initial treatment, the patient's symptoms worsened, with an increase in bilirubin levels and deterioration of liver function. When conventional treatments prove ineffective, the challenge of exploring the next steps in treatment arises. After extensive literature review and considering the pathogenesis of EPP[11-13], our attention shifted towards RBC exchange and TPE. TPE was subsequently performed on multiple occasions (March 20, 22, 24, and 28, 2023), involving the replacement of 1800–2000 mL of fresh frozen plasma during each session. During the red cell exchange (RCE) procedure, where erythrocytes were subjected to ABO-RhD matching, approximately 6 units of RBCs were transfused on March 21, 2023. The detailed procedures for TPE and RCE are presented in Table 1.

OUTCOME AND FOLLOW-UP

The interventions led to a gradual decrease in bilirubin levels (from 273.8 μmol/L to 68.6 μmol/L) (Figure 2), accompanied by the return of anal exhaust, defecation, and relief from abdominal pain. With continued improvement, the patient was discharged from the hospital.

DISCUSSION

EPP is a rare genetic disorder and liver damage associated with porphyria is even more rare, often leading to clinical misdiagnosis and oversight. Currently, treatment options for EPP are limited and primarily encompass the following approaches: First, there is an emphasis on promoting bile secretion, wherein UDCA is administered to enhance the excretion of protoporphyrin in bile[14,15]. However, its efficacy in EPP remains controversial. Second, efforts are directed towards reducing the synthesis of protoporphyrin precursors. Hemoglobin, through feedback inhibition, can suppress the activity of ALA synthase, consequently decreasing the production of protoporphyrin[1,16]. Cimetidine has also been shown to inhibit ALA synthase, thus reducing the protoporphyrin load in patients with EPP, and may also possess antihistamine effects that ameliorate pruritus associated with the condition[17,18]. Disruption of the enterohepatic

Table 1 Procedure details of therapeutic plasma exchange and red cell exchange

Parameter	RCE	TPE
Blood type	A+, RhD+	A +, RhD+
Th name of the apheresis machine or blood cell separator	COM.TEC	JAFRON
Total blood volume measured by the equipment	4.1 L	NA
Blood volume processed	2270 mL	NA
Calcium dose	12 mL (10% Calcium Gluconate)	10 mL (10% Calcium Gluconate)
Red cell replacement	6 units (approx. 750 mL)	NA
Plasma volume targeted	NA	1800-2000 mL
Replacement fluid volume	NA	1800-2000 mL
Blood flow rate	30 mL/min	80-120 mL/min
Adverse reactions	None reported	None reported

NA: Not available (indicating missing or not applicable values); TPE: Therapeutic plasma exchange; RCE: Red cell exchange.

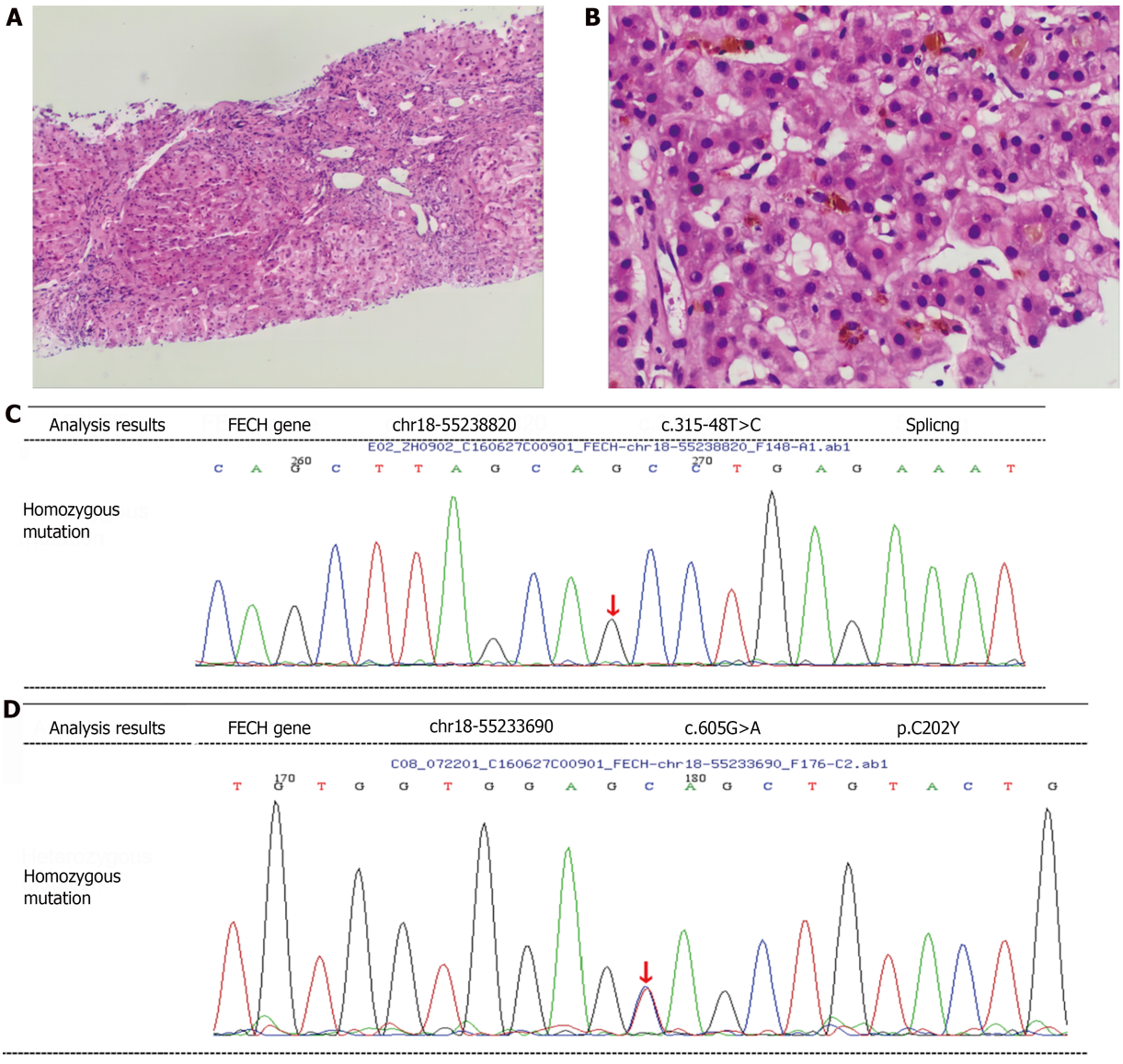

Figure 1 Hepatic pathology and genetic screening. A and B: Liver histopathology demonstrates brown-yellow granule deposits in hepatocytes, capillary

ducts, and Kupffer cells within the hepatic sinusoids. Enlarged portal area with increased lymphocyte and neutrophil infiltration, fibrous tissue hyperplasia, and hyperplasia of small bile ducts consistent with G3S3 chronic inflammatory liver injury; C and D: Genetic analysis reveals *ferrochelatase* gene mutations: homozygous intron c.315-48T>C mutation and heterozygous p.C202Y mutation. FECH: *Ferrochelatase*.

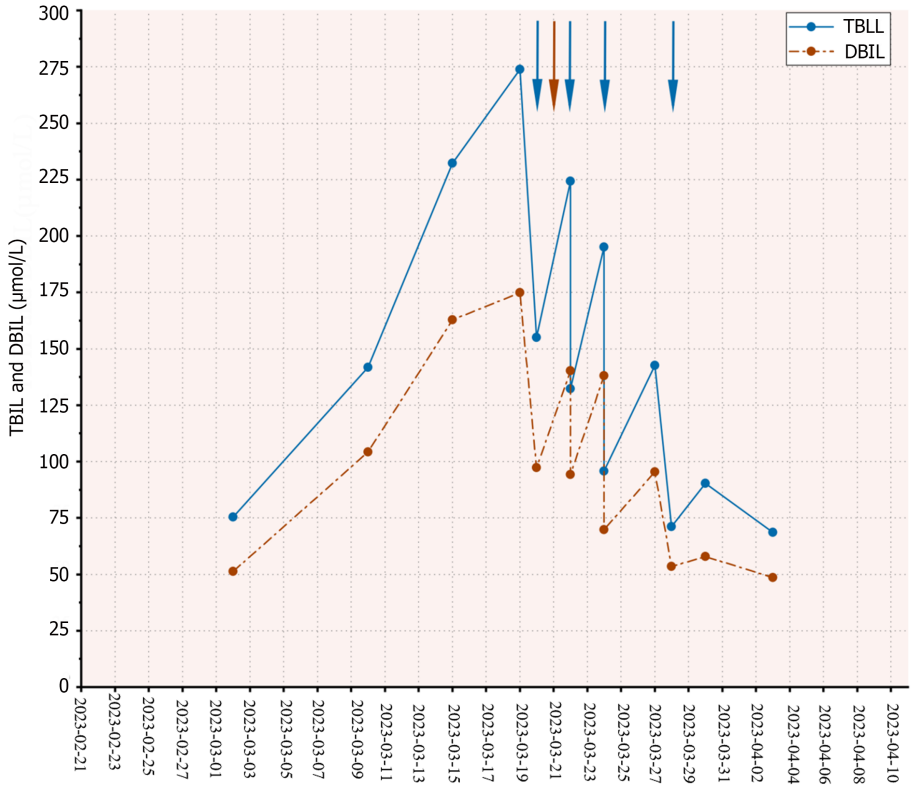

Figure 2 Dynamic changes in total bilirubin and direct bilirubin levels in the patient. Plasma exchange treatments were conducted on March 20, 22, 24, and 28, 2023 (involving the replacement of 1800-2000 mL of fresh frozen plasma during each session), and a red blood cell exchange treatment was performed on March 21, 2023 (approximately 6 units of red blood cells were transfused). Blue arrows represent plasma exchange procedures while brown arrow denotes red blood cell exchange treatments. TBIL: Total bilirubin; DBIL: Direct bilirubin.

circulation of protoporphyrin is achieved using agents such as cholestyramine and activated charcoal, which can bind to protoporphyrin, facilitating its elimination through feces[19-21]. Additionally, measures are taken to protect hepatocytes from toxic damage, using reducing agents such as β-carotene, cysteine, and vitamin C to clear reactive oxygen species[9,22,23]. Circulating protoporphyrin levels are lowered through techniques such as plasma exchange[24]. Nevertheless, it is crucial to note that the effectiveness of these measures in EPP has not been conclusively confirmed. Similarly, in this case, despite adequate conventional treatment, the patient's bilirubin levels continued to progressively rise and his liver function deteriorated.

Severe EPP-related liver damage can have significant consequences, often necessitating liver transplantation. However, post-transplantation relapse is possible because of the continuous release of the erythrocyte precursor protoporphyrin from the bone marrow[25]. Currently, on the options for effectively managing EPP-related liver damage are limited, posing a challenging clinical problem in the field of porphyria-associated liver diseases.

The exact cause of liver damage in EPP is not fully understood. It is believed that the deposition of protoporphyrin in the bile canaliculi and subsequent oxidative stress play a role[26]. Impaired bile excretion leads to further protoporphyrin accumulation, causing cholestatic liver disease, inflammation, fibrosis, and end-stage liver disease[27]. Cholestatic liver failure is a critical complication of EPP that can rapidly progress to a fatal condition called EPP hepatic crisis[28]. Intensive treatment, including TPE and RBC exchange, is necessary to rapidly reduce protoporphyrin levels and promote liver recovery. Given the concentration of free protoporphyrin in RBCs is approximately 10 times higher than in plasma [29], TPE alone may not be adequate. However, RBC exchange can increase circulating hemoglobin levels, triggering negative feedback inhibition of ALA synthase and avoiding iron overload. Therefore, the inclusion of RBC exchange becomes necessary.

However, the therapeutic efficacy of RBC in combination with TPE for conditions such as EPP-related liver disease is currently a subject of debate, primarily because of the paucity of robust clinical evidence. According to the American Society for Apheresis guidelines for TPE[30], the recommendation for employing TPE/RBC exchange in these conditions is modest (Category II, Grade 2C), reflecting reliance on lower-quality evidence or the presence of divergent opinions among experts. This cautious stance is further underscored by the existence of contradictory case reports—some suggest that this treatment modality may improve liver function, while others have not demonstrated a significant reduction in

protoporphyrin levels, thus casting doubt on its clinical benefit[11]. Notably, in this case, a regimen of four TPE sessions and one RBC exchange session was associated with a marked decrease in bilirubin levels and alleviation of clinical symptoms, without a significant rebound upon follow-up. These findings suggest that TPE/RBC exchange may hold therapeutic promise in the management of EPP.

CONCLUSION

In summary, treatment options and evidence-based medicine for EPP-related liver disease are currently very limited. RBC exchange combined with TPE holds potential for managing EPP-related liver damage. However, owing to the rarity of the disease, there is a continued need to gather clinical evidence in order to further validate its effectiveness.

FOOTNOTES

Author contributions: Zeng T contributed to writing the original draft, methodology, software development, and data curation; Chen SR was involved in methodology, data curation, and visualization; Liu HQ contributed to investigation and software development; Chong YT provided supervision and methodology; Li XH was responsible for conceptualization and supervision. All authors have read and approved the final manuscript.

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